Restoration of Cognitive Function in Aged Individuals

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Abstract

Background/Aims

The healthy brain in aging humans is characterized by reduced perceptual speed and increased difficulty in acquiring new memories. The ability to form new neural networks and change through growth and reorganization is referred to as brain plasticity. Reduction in brain plasticity and cognitive function affects quality of life and may result in older individuals being downgraded in their jobs or dropping out of the workforce. The aim of this study is to create a protocol that restores measurable phenotypes of brain aging, such as perceptual speed, of healthy individuals over the age of 65 years to that of an average 20-year-old.

Methods

Age-associated changes in perceptual speed and memory formation in the hippocampus result from reduced growth and density of synaptic sprouts and dendritic spines. These features are largely affected by a single signaling pathway, BDNF-TrkB, with known inhibitors and agonists, and by more general measures of cellular fitness such as redox imbalances and dysregulated proteostasis. In this study, age-associated changes to the hippocampus are identified and the efficacy of small molecules that modulate those changes are assessed. Dosages are determined from self-tests. Trail Making Test A (TMT-A) is used to measure the effect on perceptual speed.

Results

For a 67-year-old subject, TMT-A was not available until 12 weeks into this study and an initial baseline was not established. Notwithstanding, published results for one treatment, glutathione precursors termed GlyNAC, showed an 18% improvement in TMT-A scores after 12 weeks [153]. After the first 12 weeks of this study, a TMT-A baseline was then established for the subsequent treatments (7,8-DHF, hesperidin, lion's mane mushroom extract and TUDCA), which were found to give an additional 25% improvement. Together, the TMT-A time of a 67-year-old improved from 28 seconds (the 17th percentile of 20-year-old college students, measured after 12 weeks of treatment) to 21 seconds (the 55th percentile). Consistent with mouse studies, levels of anxiety and depression were reduced.

Conclusion

In healthy individuals, between the ages of 20 and 70 years perceptual speed slows on average by 76% as measured by TMT-A. By the age of 85, the decline is 138% [299]. This decline was completely abrogated in a 67-year-old by readily available supplements that modulate and restore age-associated changes to the BDNF-TrkB pathway, redox imbalances and dysregulated proteostasis.

Supporting Materials

Printable Trail Making Tests and a calculator for relating Trail Making Test scores to percentiles by age can be downloaded from Quantifying the results of this protocol - Trail Making Tests.

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Protocol for Restoring Age-Associated Decline in Cognitive Function

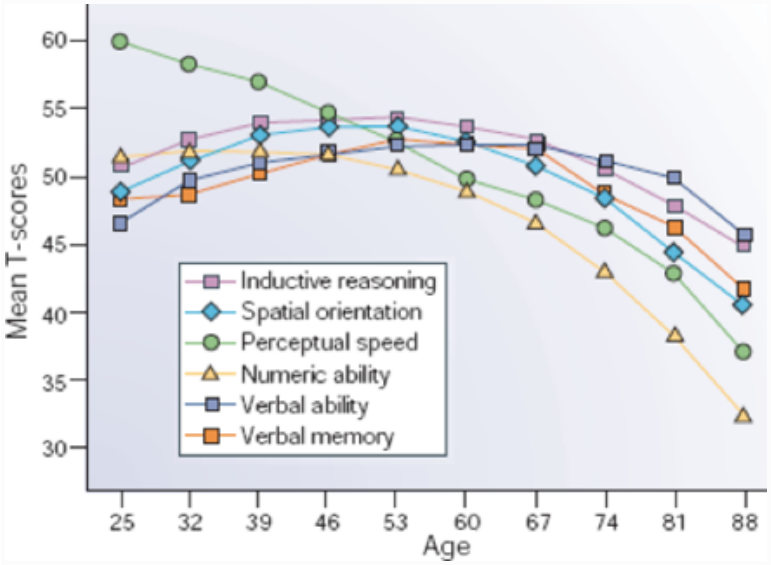
What does restoring age-related decline in cognitive function mean, and who might benefit?

Cognitive function declines naturally as people age with symptoms of

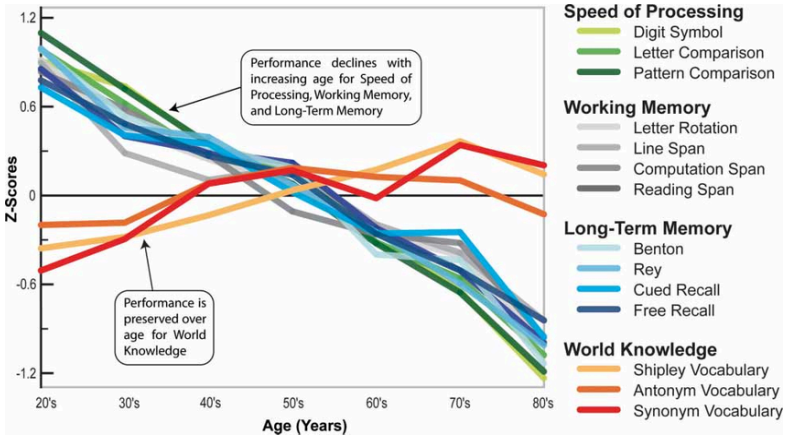
- entrenched daily routines,
- increased difficulty in acquiring new knowledge or breaking old habits, and
- slower perceptual and mental calculation speeds.

This decline is prevalent in people over 65-years-old. A large cross-section study found that about 30% of people over 65 in the US exhibit either dementia or mild cognitive impairment [298]. While dementia is a disease state, mild cognitive impairment is often a natural consequence of aging.

As we age, it is increasingly difficult to form new memories. Creative abilities decline, and the mind works more slowly. This decline in mental abilities with age is characterized in the landmark Seattle Longitudinal Study [148]. Major take-aways from this study are the steady decrease in perceptual speed throughout adult life and a decline in mental abilities after the ages of about 55 to 65 years. Other studies have shown that processing speed and working memory are closely related.



Longitudinal estimates of participant age-changes on the latent ability constructs (from 7-year longitudinal data). “The Seattle Longitudinal Study: Relationship Between Personality and Cognition” (2004) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1474018/



Processing speed and working memory are closely related and decline with age. Adapted from “Models of visuospatial and verbal memory across the adult lifespan” (2002) https://doi.org/10.1037/0882-7974.17.2.299

The object of this protocol is the sustained restoration of the cognitive abilities (such as perceptual speed as measured by Trail Making Tests) that are lost due to age [282], chronic stress or depression.

Evidence supporting this protocol is discussed in the "Background - Selected Research Findings" section of this document and summarized in Table 1. Broadly, **it is hypothesized that in healthy individuals the age-associated decline in cognitive abilities is restored by repairing accumulated damage, reducing inhibitory factors and modulating the BDNF-TrkB signaling pathway** through the following interventions:

1. Increasing levels of brain derived neurotrophic factor (BDNF). Promoting the BDNF-related pathways increases the growth of excitatory synapses and dendritic spines and improves impaired memory and cognition. BDNF is a key growth factor involved in plastic changes related to learning and memory.
2. Reducing inflammatory factors TNF-α and IL-6, which increase with age and are known to inhibit brain plasticity.
3. Reducing age-related accumulation of senescent cells.
4. Reducing the burden of subclinical amyloid β plaque and tau protein aggregates.
5. Reducing the accumulation of misfolded protein aggregates and lipofuscin.
6. Restoring ATP/AMP energy production and GSH/GSSG and NAD+/NADH redox balances.

Aged, healthy individuals exhibiting the natural decline in forming new declarative memories or slower perceptual speed might benefit from this protocol, as might those with subclinical accumulations of amyloid β plaque and tau protein aggregates.

It is well-documented that chronic elevation of the stress hormone cortisol can damage neurons in the hippocampus. Additionally, severely depressed patients may suffer from hippocampal atrophy. In these contexts, individuals who are chronically stressed, or depressed, or prone to anxiety might benefit from this protocol.

There is no evidence that memory and cognition can be improved above the basal levels of a healthy, youthful state. Younger individuals are unlikely to find much if any benefit from this protocol. The age at which this protocol may begin to show benefit is expected to be about 45- to 55-years-old, based on Trail Making Tests score results and the precipitous declines in the ATP/AMP and GSM/GSSG ratios at about that age as described later in this document.

Strategy and protocol

Much of the research concerning age-related cognitive decline is conducted in the context of diseases such as Alzheimer's, while little directly addresses the needs of aging in healthy individuals. Research data does point to reasons for reduced brain plasticity including age-related reduction in BDNF, increased systemic inflammation, dysregulated autophagy, and increased protein aggregation and byproducts of oxidative stress.

Generally, loss of neurons is not central to the decline in brain plasticity, and restoration of brain plasticity is not achieved by stimulating the growth of new neurons. Instead, the decline in brain plasticity is principally caused by synaptic pruning and a reduced ability to form new synaptic connections. The formation of new synaptic connections is regulated in part by BDNF, whose production declines with age. Contributing to the decline in plasticity is an age-related increase in inflammatory factors such as TNF-α, which have an inhibitory influence on BDNF and the formation of new synaptic connections.

Table 1 lists selected interventions that reportedly improve age-related decline in memory and cognition with an emphasis on BDNF, TNF-α, amyloid-β plaque and tau aggregates. These interventions include standouts among many supplements, especially the following:

- Tropoflavin (7,8-DHF or 7,8-Dihydroxyflavone) is nearly unique among phytosubstances in its ability to potently activate the BDNF pathways in the central nervous system. Multiple research publications support the ability of this flavone to promote synaptic and dendritic spine outgrowths, and to improve memory and cognition in rats and mice.
- Hericium erinaceus (lion's mane mushroom) has been shown to reduce amyloid-β plaque accumulation, promote neural growth in the peripheral (PNS) and central nervous systems (CNS), and to improve cognition in humans and mice.
- Hesperiden, naringenin and diosmetin are produced in the skin of citrus fruits by the same synthetic pathway, are similar in structure and have similar biological effects. Hesperidin potently inhibits TNF-α, and remarkably reduces

amyloid-β plaque accumulation and tau pathology. Hesperidin affects serotonin receptors and reduces anxiety comparably to Prozac.

From personal experience, I can recommend the following protocol to restore age-associated decline in cognitive function. Effects will be noticed within a few days, and benefits will continue to improve over weeks and months. What the long term effects of this protocol are, whether tolerance is acquired, and what dosage works best for other individuals, are open questions.

This basic protocol has three main components: hesperidin primarily counters metabolic decline and systemic inflammation, lion's mane mushroom promotes neurogenesis, and 7,8-DHF stimulates synaptic and dendritic outgrowths.

7,8-DHF is the "big gun" of the protocol for stimulating synapse and dendritic spine sprouting in the hippocampus. For an older person, this flavone has a profound effect on cognition, but too much of it can also cause a low-level headache and restless sleep.

Lion's mane mushroom extract is effective in promoting neurogenesis and improving cognition. Lion's mane appears to be safe as few side effects have been reported [193].

Hesperidin has wide ranging benefits ranging from reduction of TNFα systemic inflammation to improving glucose and Ca2+ homeostasis, improving mitochondrial function, and reducing amyloid β plaque accumulation. Remarkably, 20-month-old mice (equivalent to 60-year-old humans) that were fed 100 mg/kg (equivalent to about 500 mg/day in humans) for 6 months restored function and gene expression in cardiac and skeletal muscle similar to that of 3-month-old mice [270].

Hesperidin has antidepressant-like effects similar to Prozac. Taking too much may impair judgment while stopping abruptly may cause feelings of depression. It is best to ramp up the dose when starting and wean off if discontinuing.

As a side note, the therapies described here repair damage and modulate metabolic pathways, but there is no evidence that they make one younger in the sense of reversing the aging process. By repairing damage, one might live healthier and longer, but underlying gene expression and one's appearance are expected to be about the same.

Dosages - Daily

- 100 mg hesperidin 1x/day in the morning. Inhibits inflammatory factors [1,59,60,90,114,115,149]. This low dose should be sufficient to reduce a burden of amyloid β plaque and tau aggregates if taken over an extended period [51,90]. Larger doses may destabilize one's mood, or may give greater antidepressant effects, but also may impair judgment. I have found that 250 mg 1x/day over several months causes brain fog. More…Hesperidin reduces TNF-α and restores age-related decline in memory and cognition
- 25 mg tropoflavin (7,8-DHF) 1x/day in the morning. 7,8-DHF promotes BDNF and the growth of dendritic spines [54,55,63,64,67,120], and it improves impaired memory and cognition [36,54,55,64,67,80]. More…7,8-DHF potently promotes BDNF, sprouts dendritic spines and improves learning
- 800 mg lion's mane mushroom extract powder 1x/day in the morning (1 cc scoop of loose powder or a bit less than 1/2 tsp). Lion's mane mushroom stimulates neurogenesis primarily in the peripheral nervous system by activating NGF/TrkA, MEK/ERK and PI3K/Akt pathways [25,28,186]. Lion's mane mushroom has been reported to improve impaired memory and cognition with few side effects [24,26,28,47,157,176,183]. More…Lion's mane mushroom (Hericium erinaceus) improves cognitive impairment in elderly humans

Ramping up and discontinuation

Gradually increase (reduce) dosages over 3 days when starting (discontinuing) to avoid possible side effects of unstable mood, depression or headaches.

Recommended additions to the basic protocol - Daily

Much of the decline in cognitive function with age can be explained by the causal relationships between the decline in ATP production, cellular concentrations of NAD+ and GSH, and the accumulation of senescent cells (SnCs) as described in the "The bigger picture" section of this report. The interrelationships between BDNF and these factors suggest that in order to fully restore synaptic plasticity, systemic inflammation must be reduced, SnCs must be eliminated, and ATP/AMP and GST/GSSG deficits must be corrected. Simultaneous treatment of all proximate causes of the decline in BDNF is expected to work synergistically for greater effect. To that end, the following additions to the basic protocol are recommended.

1. Walk daily. After the age of 50 one cannot stay healthy without exercise. After 65-years-old, walking 6000 to 8000 steps per day (3 to 4 miles) is essential for better overall health, increasing BDNF and promoting cognitive function [108,109,159]. More…Walking helps maintain BDNF in individuals over 65 years old
2. Correct glutathione deficiency. For individuals older than 50 or so years, supplementation with 1000 mg each of the glutathione precursors (N-acetyl-cysteine (NAC) and glycine) 2x/day will correct glutathione deficiency and redox balance in individuals experiencing oxidative stress. Restoring age-related deficiencies in cysteine and glutathione significantly restores BDNF, perceptual speed and physical function in older humans [153], and has been shown to increase the medium lifespan in mice by 24% [152-154]. In those over 70-years-old, the beneficial effects of this supplementation increase over 6 months or more, which suggests a gradual reduction in lipid peroxidation damage and replacement of oxidation-damaged somatic cells by more fit precursor cells. To avoid stomach upset, NAC and glycine are best taken with food. More…Glutathione precursors (GlyNAC) improve GSH/GSSG, BDNF and physical function in older individuals
3. Reduce ER stress and clear accumulated protein aggregates. TUDCA is an endoplasmic reticulum (ER) chaperone that has been found to safely increase ATP and the ATP-to-AMP ratio, alleviate ER stress, increase glucose sensitivity, and increase the neural stem cell (NSC) pool in a mitochondrial ROS/redox state manner. In humans, 500 mg 2x/day for 3 months appears to be a safe and effective dose for decreasing Aβ plaque and tau aggregates, and presumably protein aggregates in general [268]. As a side note, decreased ATP production leads to some of the most conspicuous features of the extreme age, such as decreased activity and less body heat generation. In my view, steps that can be taken to restore ATP production and reduce redox imbalances to youthful levels should be a high priority. More…Cellular protein aggregates and TUDCA
4. Limit consumption of alcohol. Alcohol decreases BDNF in the cortex and dysregulates BDNF homeostasis [297]. Drinking every day is discouraged, and if one does drink, limit consumption to one glass of wine or equivalent. Furthermore, under this protocol more than one drink of alcohol will cause a headache and brain fog.
5. Test for and remedy any possible vitamin B12 deficiency. Vitamin B12 deficiency is common in older individuals. It is linked to impaired cognition and memory along with a sensation of tingling and numbness, all outcomes of poor myelination and oxidative stress [265,266].

Recommended additions to the basic protocol for those over 60 - 1x to 2x/Year

1. Senescent cell removal. With age, our immune system declines and fails to effectively remove senescent cells (SnCs). SnCs accumulate significantly in the brain, and their paracrine signaling inhibits neurogenesis in the hippocampus. Removal of senescent neural precursors enhances hippocampal neurogenesis, dendritic spines and spatial memory in adult mice and rats [142,146]. Furthermore, removal of SnCs reduces systemic inflammation (TNF-α, IL-1β, IL-6, …), amyloid β plaques and tau protein aggregates, and, in part, restores cognitive performance in mice and rats [106,107,146,128,130,139]. More generally, the accumulation of SnCs impairs regeneration in older people while removing them has significant, wide ranging benefits. SnCs are readily removed by a 3-day treatment of grape seed extract and fisetin, as described in detail in "Protocol for Restoration of Physical Function in Aged Individuals by Removal of Senescent Cells".

Contraindication - Alcohol with this protocol can cause headaches, brain fog and depression

This protocol is incompatible with alcohol. Taking more than one drink of alcohol per day, such as one glass of wine, together with these supplements can cause next-day brain fog, and possibly a mild to severe headache or depression in an alcohol dose-dependent manner.

For me, taking 7,8-DHF and hesperidin in the morning followed by a glass of wine in the evening invariably results in brain fog and possibly a mild headache the following morning. Two or three glasses of wine leads to depression. Blogger Muninn's experience from New Year's Eve excess was also dramatic: he had severe brain fog and a headache for nearly two days afterwards. These experiences suggest that this protocol is incompatible with alcohol. It can cause mild to severe headaches, brain fog and feelings of depression in an alcohol dose dependent manner. Research on rats supports this relationship between alcohol and 7,8-DHF [267].

Safety, contraindications and side effects

The pharmacokinetics of hesperidin and 7,8-DHF are listed in Table 2. Note that hesperidin potently suppresses CYP450. CYP450 is a major group of enzymes involved in detoxification and metabolism [C,D]. Its suppression affects the clearance and activity of many drugs. Check the medications you may be taking for interactions with CYP450 or warnings against grapefruit juice.

Do not take hesperidin or 7,8-DHF in the afternoon or evening. Doing so can cause restless sleep.

Hesperidin interacts with serotonin receptors and gives effects comparable to fluoxetine (Prozac) [10,11,15,57]. A 250 to 500 mg dose gives a positive outlook, but larger doses could lead to impaired judgment or feeling unfocused.

From my personal experience, an effective dose appears to be 12 to 25 mg 7,8-DHF in combination with 1000 mg hesperidin. 1x/day in the morning. However, the combination of 25 mg 7,8-DHF + 1000 mg hesperidin + 2000 mg lion's mane mushroom as a starting dose is too much. It leads to very restless sleep, at least in the week of treatment. Dosages may have to be adjusted to suit individual preferences, but I caution against large day-to-day changes as that will affect mood and can cause feelings of depression for a couple of days.

As for effects on brain function, for me time seems to go slower and days seem longer. Subjectively, I feel quicker, more alert and notice more nuanced detail. My memory has improved to the extent that it is noticeably easier to memorize lists of items than a year ago, and recall of older memories has improved.

Among other benefits, autophagy is upregulated or normalized by hesperidin [87,88,89,92]. With use, I have noticed a reduction in the hyperpigmented spots on my hands and arms. Hesperidin could also be prophylactic against Alzheimer's disease; 10-day treatment of hesperidin reduces the burdens of amyloid-β plaque and tau aggregation in the brains of mice [51].

For hypertensive individuals, hesperiden lowers systolic blood pressure (SBP) by 9%, possibly through a decrease in inflammatory factors, modulation of nitric oxide (NO) metabolites, and suppression of angiotensin [135,140,271]. For reference, those with a SBP of 140 mm Hg and higher have significantly higher risk of stroke or cardiovascular death. Those higher risk individuals should target their SBP to 130 mm Hg or less [136].

Disclaimer

I am not a doctor or qualified health professional. The contents of this document do not substitute for medical advice and are not intended and should not be construed as medical advice, nor is the information a substitute for professional medical expertise or treatment. The use of any information provided is solely at your own risk. If you have any concerns or questions about your health, you should consult with a physician or other health-care professional. If you follow this protocol and experience adverse reactions, you must stop immediately. If you think you may have a medical emergency, call your doctor, go to the nearest hospital emergency department, or call emergency services immediately.

Purchase sources and cost

The supplements described in this protocol are readily available on Amazon and other sources. I have no affiliation with any of these companies and am not making recommendations.

A) Hesperidin 90% Extract Powder can be purchased from https://nootropicsdepot.com/hesperidin-powder/

60 g costs $19.99 USD. A 500 mg/day dose is $0.17/day.
Hesperidin is available from other suppliers on Amazon in capsule and bulk powder forms.

B) 7,8-Dihydroxyflavone (7,8-DHF) capsules can be purchased from https://nootropicsdepot.com/7-8-dihydroxyflavone-capsules/
60 capsules of 25 mg costs $29.99 USD. A 25 mg/day dose is $0.50/day.

C) Lion's mane mushroom extract powder, 31% beta glucan is available on Amazon. The Freshcap brand contains a 1 cc scoop. 1 heaping scoop of loose powder is about 1000 mg. 60 g costs $30 USD. 1000 mg comes to $0.50/day. Note that while this alcohol and water extract is effective, from my experience unconcentrated mushroom powder appears not to work.

D) NAC (N-acetyl-cysteine) is available at Walmart.com and other online sources but not Amazon. Because of a regulatory quirk, the FDA categorized this acetylated amino acid as a drug and not a supplement. The FDA has stated that it will not enforce this distinction, but because of this unfortunate classification Amazon does not sell NAC.

E) Glycine is a simple amino acid that is available on Amazon in 1000 mg capsule form.

F) TUDCA is available as a supplement on Amazon.

Self-tests: assessing dosages for long term use

Previous self-testing relied on recommended or guessed-at doses of hesperiden, 7,8-DHF and lion's mane mushroom extract. During that time I took various dosages, eventually settling in on (1000 mg hesperidin + 800 mg lion's mane extract + 25 mg 7,8-DHF) 1x/day + GlyNAC 2x/day. Then, after a couple of months I began experiencing persistent brain fog. I stopped all supplementation for 4 weeks and began this round of systematic self-testing to determine dosages that are consistent with long term use. The outcomes follow for me, aged 67 years.

<u>7,8-DHF, 25 mg 1x/day + GlyNAC 2x/day</u>

2022-07-05 Start - I have not taken any supplements other than GlyNAC for 4 weeks. GlyNAC supplementation is 1000 mg each of glycine and N-Acetyl-Cysteine (NAC).
2022-07-07 The day after the first dose I feel clear headed and productive today, without apparent side effects. Last night my dreams were more vivid than usual.
2022-07-08 I sleep fewer hours now, 6 to 7 instead of 8 to 9, and get up wide awake instead of groggy.
2022-07-09 Last night I slept poorly, very lightly. My mind is too keyed-up and tense.

- Conclusions: This short experiment confirmed my previous experiences that a 25 mg 1x/day dose of 7,8-DHF is too high, at least for me. I suspect that for long term use this dose should be cut in half.

<u>12.5 mg 7,8-DHF 1x/day + GlyNAC 2x/day</u>

2022-07-09 Start - reduced dose of 7,8-DHF to 12.5 mg.
2022-07-24 Some headaches over the last week, possibly associated with alcohol consumption and eye strain.
2022-07-25 Mild headache associated with alcohol consumption. One glass of wine seems OK but more causes a headache the next morning.

- Conclusions: 7,8-DHF is reasonably tolerated for long term use at this dosage. The milder headaches and subjective mental clarity suggest that 7,8-DHF at 12.5 mg 1x/day is effective in increasing BDNF.

<u>(800 mg lion's mane extract + 12.5 mg 7,8-DHF) 1x/day + GlyNAC 2x/day</u>

2022-07-25 Start - added 800 mg lion's mane extract .
2022-07-26 Slept lightly with a racing mind and vivid dreams last night. Lion's mane mushroom appears to be synergistic with 7,8-DHF. This morning my mind is quick and clear.
2022-07-29 My mind continues to race. I sleep lightly every night and dream vividly. I have not experienced headaches since starting this combination.
2022-08-11 My mind has been active, alert and clear with this combination of supplements. Every night I experienced vivid dreams.

- Conclusions: Lion's mane mushroom extract (LMM) is clearly bioactive. LMM and 7,8-DHF appear to be synergistic and have few side effects at this dosage. Missing a single dose of either DHF or LMM does not appear to have

consequences of mood or clarity. During this trial, I have been productive, and my working memory and speed seem improved. Moderate alcohol consumption (1 drink) does not have side effects, but 2 drinks results in a mild headache and mental fogginess the next morning.

(500 mg hesperidin + 800 mg lion's mane extract + 12.5 mg 7,8-DHF) 1x/day + GlyNAC 2x/day

2022-08-12 Start - added 500 mg hesperidin.
2022-08-14 Woke up at 3:00 AM with a headache following less than 2 drinks in the evening.
2022-08-15 One drink last night, no headache this morning. Felt extremely anxious this AM, which improved mid-afternoon to near normal.
2022-08-16 I slept poorly last night. My mind raced and I woke every hour or two.
2022-08-17 My response to this dosage appears to have settled down. Last night I slept soundly. Yesterday and today I experienced a more acute sense of smell. Shower water seemed hotter too. Subjectively, the PNS and CNS seem energized.
2022-08-18 Yesterday I was extraordinarily productive in my academic work. Time will tell if this dosage gives sustainable results. Today I have been productive but felt edgy most of the day.
2022-08-19 Restless night's sleep again last night. Stable mood, clear minded, and very productive today.
2022-08-20 Persistent low-level headache today. I am sick today (not COVID) and can't distinguish between the effects of the supplements and this illness.
2022-08-21 Feeling better today with no headache and only minor fatigue and stomach cramps, which I attribute to passing illness.
2022-08-22 Feeling better today with no headache.
2022-08-28 I have continued to have mild headaches, probably associated with a single glass of wine in the evenings. For the last couple of weeks on most days I have experienced an eye twitch. My mind has been very clear and I have been productive in my work.

- Conclusions: I like much of what hesperidin has to offer, but in combination with the other nootropics it is too much. The good news is that this experience supports my hypothesis that hesperidin would work synergistically with 7,8-DHF and lion's mane mushroom.

(250 mg hesperidin + 800 mg lion's mane extract + 12.5 mg 7,8-DHF) 1x/day + GlyNAC 2x/day

- Concept: 250 mg hesperidin is a bit less than 1/16 tsp. Studies of the effects of 113-227 mg human equivalent doses of hesperidin and naringin on mice and rats support the idea that this 250 mg dose should be at least marginally effective as a stand-alone long term treatment [14,48,141,211]. In combination with GlyNAC, this dose of hesperidin should be sufficient in its primary role of reducing TNFα.

2022-08-29 Start - reduced hesperidin to 250 mg.
2022-08-30 No headache yesterday. Time seemed to go very slowly all day.
2022-08-31 No headache yesterday or today. Time still drips by slowly. My mood is stable and anxiety levels are low.
2022-09-03 The world continues to move in slow motion. Since my last entry, I have not experienced a headache and my mood has been stable.
2022-09-07 This combination of supplements is close to a sweet spot in improved cognition. Subjectively, I am thinking quickly and am observant of more detail. For example, when listening to the radio, I am aware of the Instrumentalization, not just the melody. I remain a bit keyed up, and occasionally my eyes have a small twitch, but these symptoms are much less than with the previous trial. I have not experienced a headache.
2022-09-11 Today I missed taking lion's mane extract and noticed that I am not as keyed up as I sometimes (often) get with this protocol. That may be another variation to explore.
2022-09-18 Yesterday I was out of town and missed taking this treatment for a day. There were no negative side effects. My mood continues to be stable and my mind sharp if a bit keyed up.

- Conclusions: The addition of 250 mg hesperidin to the stack is well tolerated, I have a stable mood and am alert. As for measurable benefits, my Trail Making Test A (TMT-A) score is now 23 seconds compared to 28 seconds on 4/16/2022. This 23 second score puts me in the 40 percentile of 20-year-old college students and may be about the best I can achieve at my age (67-years-old). However, this difference (17%) is not much different than my score in 5/2022 and could be accounted for by learning. Note that I do not have a pretreatment baseline for TMT-A. I took my first TMT-A test on 4/2022 after 12 weeks of (1000 mg hesperiden + 25 mg 7,8-DHF) + 6 weeks of 1 g/ea 1x/day GlyNAC.

(300 mg centrophenoxine + 250 mg hesperidin + 800 mg lion's mane extract + 12.5 mg 7,8-DHF) 1x/day + GlyNAC 2x/day

- Concept: Centrophenoxine is thought to reduce lipofuscin deposits in neurons and speed their activity on old or impaired cohorts [239,240,241,242,263,264]. Remarkably, centrophenoxine restored total RNA synthesis in the brain cortex of old (26-mo) rats to that of adult (13-mo) rats - about a 50% increase in total RNA in the brain cortex and about a 15% increase in the liver [280]. A dosage of 300 to 500 mg 1x/day for 60 days is expected to be effective.

2022-09-19 Start - added 300 mg centrophenoxine to the stack.
2022-09-24 Thus far, I have not experienced side effects from the addition of centrophenoxine to the stack.
2022-09-28 I have experienced feelings of depression for the last 2 days. Centrophenoxine rapidly decomposes into DMAE and 4-CPA. DMAE acts to increase choline levels throughout the body. In the brain, higher choline levels are linked to depression symptoms. While I like the promise of centrophenoxine in reducing lipofuscin and improving RNA synthesis rates, this is not a supplement I can take. I will discontinue this treatment after today.

- Conclusions: Centrophenoxine causes depression symptoms in me by increasing choline levels. I cannot take this supplement for more than 5 days. Others might be able to tolerate it better.

(500 mg 2x/day TUDCA) + (250 mg hesperidin + 800 mg lion's mane extract + 12.5 mg 7,8-DHF) 1x/day + GlyNAC 2x/day

- Concept: The bile acid tauroursodeoxycholic acid (TUDCA) has potent ability to inhibit apoptosis, attenuate oxidative stress, increase autophagy and reduce endoplasmic reticulum (ER) stress. As an effective ER stress inhibitor, it has been found to safely reverse age-related changes in the levels of synaptic plasticity proteins, increase neuron proliferation and preserve dendritic spine numbers when compensating for oxidative and other cellular stresses [246-253]. In humans, 500 mg 2x/day appears to be a safe and effective dose [W].

2022-09-29 Start - added 500 mg 2x/day TUDCA to the stack and removed centrophenoxine.
2022-10-04 TUDCA has not caused noticeable side effects, nor any effects so far that I can determine. I have to trust the research for the benefits of this ER chaperone. Recently, I have felt that my mind was not as active as I would like, and I upped my dosage of 7,8-DHF to 25 mg. This change is unquestionably positive. My mind and speech have been much quicker and without effort or jumpiness. More self-testing is needed to determine if this should be a permanent change to the protocol.

- Conclusions: It is too soon to tell if TUDCA is having an effect. However, the change in 7,8-DHF from 12.5 to 25 mg is unquestionably positive.

(500 mg 2x/day TUDCA) + (250 mg hesperidin + 800 mg lion's mane extract + 25 mg 7,8-DHF) 1x/day + GlyNAC 2x/day

- Concept: I felt like the 12.5 mg dosage of 7,8-DHF was too low to be effective and changed it to 25 mg on the assumption that the headaches and brain fog I experienced earlier were due to excessive hesperidin.

2022-10-05 Start - changed 7,8-DHF from 12.5 to 25 mg. Otherwise, all else is the same.
2022-10-09 This stack continues to be effective. I am quick and productive in my studies and without the low grade headache that I experienced in the past. I am, however, a bit impatient and edgy.
2022-10-17 There has been substantial reduction of lipofuscin in the largest pigmented age spot on the skin of my left hand. This spot has been there for many years, and now it and the others are faded to the point of being almost gone. Since nothing else has changed in this self test, the addition of TUDCA must be the cause even if it is not reported in the literature. GlyNAC or maybe hesperidin could contribute to this effect in combination with TUDCA.
2022-10-25 Age spots continue to fade and are hardly noticeable now. I am not experiencing side effects such as headaches or edginess.
2022-11-01 I have had no alcohol for the last week. My mind is clear and my mood is stable and positive.

- Conclusions: TUDCA is a beneficial addition to the protocol. I plan to continue taking it for a total of 3 months to ensure removal of accumulated protein aggregates [268]. At this point it is not clear whether I should take TUDCA on a daily basis or to cycle it once or twice a year. Regardless, TUDCA belongs in this protocol.
- My scores on today's (2022-11-01) batch of Trail Making Tests A were all within 1 sec, and I did them relaxed and without effort. My average score was 21 seconds, which is the 55th percentile for 18- to 22-year-old college students and about the 3σ level for my age. This protocol meets my goal of restoring perceptual speed to that of an average 20-year-old.

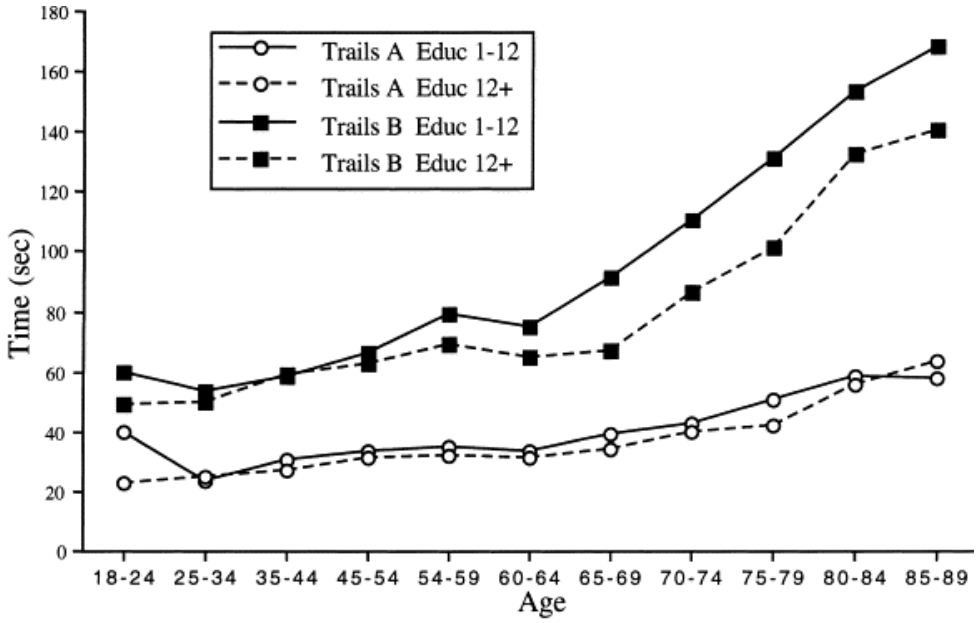
Quantifying the results of this protocol - Trail Making Tests

Trail Making Tests readily capture the cognitive speed and task switching flexibility aspects of cognitive function.

Trail Making Tests (TMTs) A and B are simple connect-the-dot tasks that purportedly measure several functions, particularly cognitive flexibility, alternating attention, sequencing, visual search, and motor speed. While these tests are widely used for cognitive impairment, they also capture the decline in perceptual speed, attention and task switching that are associated with age as shown in the figure below.

Trails A requires the connection in sequence of 25 dots labeled by numbers without lifting the pencil. Trails B requires the connection in sequence of 25 dots labeled by alternating numbers and letters (1–A–2–B–3–C-...). Results for both TMT-A and -B are reported as the number of seconds required to complete the task; therefore, higher scores reveal greater impairment. The calculated difference between TMT-B and TMT-A times (TMT-B minus TMT-A), is considered a measure of cognitive flexibility, relatively independent of manual dexterity.

If this protocol is truly effective, it should restore the perceptual speed and executive function of educated, older individuals to about that of an average 20-year-old college student. Support for this assertion comes from the partial restoration by GlyNAC supplementation for 24 weeks, where GlyNAC treatment increased BDNF and improved Trails Making Tests perceptual speed and executive function of 75-year-olds to about that of 45-year-olds [153].



Performance on Trail Making Tests A and B as a function of 11 age groups and 2 education levels [299]. “Trail Making Test A and B: Normative data stratified by age and education” (2004) https://doi.org/10.1016/S0887-6177(03)00039-8

Practice improves TMT scores. For example, one learns that it is better to keep one’s hand above the paper so numbers are not obscured. Also, number-letter associations like “8-H, 9-I” are learned, significantly reducing TMT-B times. Because of learning, TMT-A is a more reliable and consistent measure of perceptual speed and working memory than TMT-B.

Learning affects TMT-A scores too. This effect can be compensated for by increasing score times by 16%, where the 16% is taken from a study of possible cognitive benefits of astaxanthin on 51-year-olds. That study used a similar test of perceptual speed and found a 16% improvement in both the placebo and test groups [210].

Incidentally, one or fewer drinks a day has been shown in a large-scale study to have little effect on perceptual speed, as measured by Trail Making Test A. However, more than one drink a day leads to a measurable decline in perceptual speed and working memory [269].

Results of various treatments - my TMT scores							
		My average scores after discarding outliers §				18-24 year-old students *	
Test Date	Age	Trails A	Trails B	Percentile A	Percentile B	Percentile A	Percentile B
4/16/2022 (1)	67	28 sec	58 sec	79%	88%	17%	24%
5/06/2022 (2)	67	23 sec	28 sec	90%	90%	40%	90%
8/01/2022 (3)	67	25 sec	---	87%	---	30%	---
9/19/2022 (4)	67	23 sec	---	90%	---	40%	---
11/01/2022 (5)	67	21 sec	---	90%	---	55%	---

§ Scores are taken from an average of 4 tests after outliers were discarded. Higher percentiles correspond to shorter test times.
* Percentiles are interpolated from LMS fit equations of data taken from [299] "Trail Making Test A and B: Normative data stratified by age and education" (2004) https://doi.org/10.1016/S0887-6177(03)00039-8
(1) After 12 weeks of the 1000 mg hesperiden + 25 mg 7,8-DHF 1x/day + 1 g/ea GlyNAC 1x/day during the last 6 weeks. No pre-protocol baseline was established, but I had practiced these tests when writing the software to generate them.
(2) After 15 weeks of 1000 mg hesperiden + 25 mg 7,8-DHF 1x/day + 1 g/ea GlyNAC 1x/day during the last 9 weeks. Learning contributed to the significant reduction in Trails B scores but had less effect on my Trails A times.
(3) After 12.5 mg 7,8-DHF + 500 mg hesperiden + 800 mg lion's mane extract 1x/day + GlyNAC 2x/day during the last 7 weeks.
(4) After 12.5 mg 7,8-DHF + 250 mg hesperiden + 800 mg lion's mane extract 1x/day + GlyNAC 2x/day during the last 7 weeks.
(5) After 25 mg 7,8-DHF + 250 mg hesperiden + 800 mg lion's mane extract 1x/day + GlyNAC + 500 mg TUDCA 2x/day during the last 6 weeks. Note - my scores on this batch of TMT-A were all within 1 sec, and I did them relaxed and without effort.

Age-stratified statistics for Trail Making Tests		
Age group 18–24 (university students; n = 155)		
	Mean (S.D.)	Median
Age	20.17 (1.48)	20.00
Trails A (processing speed) Wiki	22.93 (6.87)	21.70
Trails B (executive function) Wiki	48.97 (12.69)	47.00

Age-stratified statistics for Trail Making Tests		
Age group 18–24 (university students; n = 155)		
Percentile	Trail A	Trail B
90	16	35
80	17	38
70	19	41
60	20	44
50	**22**	**47**
40	23	49
30	25	54
20	27	61
10	31	66

A calculator for relating Trail Making Test times to percentiles by age can be downloaded from the following link.

- Trail Making Test calculator_2022-05.xlsm
  https://drive.google.com/file/d/1SNqggvpHR1VWrzxTK03ldfEsXthBvT0Z/view?usp=sharing

Printable Trail Making Tests can be downloaded from the following sources.

1. TrailsMakingTestsA+B_20tests_Set01.pdf
   https://drive.google.com/file/d/106bPgnLuySsaUFuQur2HjVJInS9nDdaW/view?usp=sharing
2. TrailsMakingTestsA+B_20tests_Set02.pdf
   https://drive.google.com/file/d/1d2C-hOz8noDwxj9Q5MY_GqA9NYy-LrJd/view?usp=sharing
3. TrailsMakingTestsA+B_20tests_Set03.pdf
   https://drive.google.com/file/d/1NeQtst8I5im7CtJuzrMlj9rsdnmEuavo/view?usp=sharing
4. TrailsMakingTestsA+B_20tests_Set04.pdf
   https://drive.google.com/file/d/14qUn6nqpJmIJoFxcbi_7XEm7YU4nhs9i/view?usp=sharing
5. TrailsMakingTestsA+B_20tests_Set05.pdf
   https://drive.google.com/file/d/1dQ4FOeamPKpkFEja9y1jLyD2p27pfK1j/view?usp=sharing
6. TrailsMakingTestsA+B_21tests_Set06.pdf
   https://drive.google.com/file/d/1B87jAjJjKZnO7rcJJgXsQqJlG0Y4-_1r/view?usp=sharing
7. "Trail Making Test (TMT) Parts A & B" http://apps.usd.edu/coglab/schieber/psyc423/pdf/IowaTrailMaking.pdf
8. "Trail-Making Test for Screening, Part A and B - Administration Instructions"
   http://safemobilityfl.com/pdfs/CliniciansGuide/Trail%20Making%20Test%204th%20Edition.pd

Table 1-A. Effects of selected interventions on memory, cognition and related factors

	Effect on systemic inflammation (TNFα, IL6)	Effect on estradiol, estrogen receptors	Effect on NGF/TrkA pathways	Effect on GDNF*	Effect on choline compounds	★ Effect on BDNF-TrkB pathways [43]	Induces neuron growth (PNS, CNS)	Restores synapse outgrowth	Restores dendritic spine outgrowth	Restores impaired memory & cognition‡	Removes or inhibits amyloid β, tau deposits	Comparison to Prozac§	Trail Making Test A Time‡
Hesperidin†	↘↘↘ [1,59,60,90,114, 115,149,211,273]	↗↗↗ [40,92, 149]	↗ [7,149,211]	↗ [7]	↕ [211]	↗↗ [7,11,12,149,211]	↗ [61]	↗↗ [16,213]		↗↗ [16,211,213.234, 275]	↗↗ [51,90,275] ≈ [234]	>≈ [10,11,15, 57]	
Naringin†	↘↘↘ [1,27,49,59, 60,81,82]	↗↗↗ [30]	--- [7]	↗↗ [7,53]		↗↗ [8,13,29]	↗ [49]	↗↗ [27,58,70]		↗↗ [27,49,50, 58,71]	↗↗ [49,50]	<≈ [9,13,14]	
Diosmetin†	↘↘↘ [2,18,19]	↗↗↗ [41]		↗ [53]		↗ [32]				↗↗ [17]	↗↗ [66]		
7,8-DHF	↘ [3,4]	↗↗↗ [35]	--- [32]		↕ [214]	↗↗↗↗ [3,4,32,33,34,35,37, 38,54,55,63,67,85, 86,116,119,151,183, 202,287]		↗↗↗ [4,67]	↗↗ [36,54,55,63, 64,67,120, 192]	↗↗↗ [36,54,55, 64,67,80,192]	↗ [214,287]	≈ [34,151]	
Lion's mane mushroom extract‡	↘ [5,25,51]		↗↗↗↗ [23,28,158]	↗ [25]	↕ [212]	↗↗ [25,28,69,158, 162,186]	↗↗↗ [21,22,24, 28,157,162]	↗↗ [157,162, 186]	↗↗ [157]	↗↗↗ [24,26,28,47, 157,176,183]	↗ [79,118,157, 176]		
Exercise, walking [43,108]						↗↗ [43,68,109,159]	↗ [43,137]	↗ [43,147]	↗↗ [65]	↗ [62,68,137]			
Senescent cell clearance [104]	↘↘↘ [104,105, 106,107]						↗↗ [105]		↗ [146]	↗↗ [106,142,146]	↗↗ [106,107]		
Fisetin (senolytic)	↘↘↘ [131,132.133]	↗↗ [7,31]	↗↗ [7]	--- [7]		↗↗ [123,124,130]				↗↗ [128,130,139,164]	--- [128]	≈ [129]	
N-acetyl-cysteine + glycine (GlyNAC)‡	↘↘↘ [153,276]					↗↗↗ [153]				↗↗↗ [153]			↘↘↘ [153]
TUDCA‡	↘↘ [268,294]						↗ [257]		↗↗ [250,252]	↗↗ [246,252,268]	↗↗ [256,268]		
Centrophenoxine‡					↗↗↗↗ [240]					↗↗ [239,240,241,242]			
NSI-189						↗↗ [77]	↗↗↗ [73-77,110]			↗↗ [77]			
Luteolin	↘↘ [45,84,112]	↗↗↗ [7,31]	↗↗↗ [7]	↗↗↗ [7]		↗↗ [7,52,83]	↗ [52]	↗↗ [83]		↗ [44,84,111,172]	↗ [111]		
Fasting Mimicking Diet‡							↗↗ [78,284]			↗↗ [78,284]	↗↗ [284]		
Healthy sleep									↗ [103]	↗ [103]			

A blank cell indicates no published reference found; "---" means no effect is observed; ↘ and ↗ arrows indicate an inhibition or promotion effect; ↕ indicates regulation; ≈ means approximately equivalent.

★ BDNF declines with age, is associated with age-related decline in hippocampal volume, and promotes the growth of synapses and dendritic spines [42,43].
§ Antidepressant drugs such as Prozac act in part by binding to TrkB receptors and inducing TrkB signaling.
† Hesperidin, naringin and diosmetin are produced in the skin of citrus fruits by the same synthetic pathway, are similar in structure, and have similar biological effects.
‡Memory and cognition improvements are in most studies compensative of oxidative stress, a deficiency or an injury, with a return to basal levels by the involvement of corrective treatments.
* GDNF promotes GABA, a major inhibitory neurotransmitter. An increase in GDNF is associated with less pain sensation and sedative-like activity.

Table 1-B. Effects of selected interventions on blood markers and oxidative stress factors

							Effect on oxidatively stressed cells‡						
	hsCRP	**Improves insulin sensitivity**	**Neuro-protective**	**Senolytic**	**Effect on lipofuscin Accum-ulation**	**Regulates or promotes autophagy**	**8-hydroxy deoxyguanosine (8-OHdG, DNA oxidation marker)**	**Lipid peroxidation (MDA or TBARS markers)**	**GSH (reduced glutathione)**	**GSSG (oxidized glutathione)**	**Δψm (mito membrane potential)**	**ATP pro-duction**	**Increases BCL2/BAX ratio (promotes neuron survival)**
Hesperidin†	↘↘↘ [166]	↗↗ [1,10,59,60,90, 91,163,165,270]	↗↗ [39,173,199]			↕↕ [87,88,89]	↘↘ [165]	↘↘ [141,165,173, 199]	↗↗ [141,173,199, 234,275]		↗↗ [173,199]	↗↗ [173,292,2 93]	↗ [173]
Naringin†		↗↗ [1,6,59,60,71]	↗↗ [27,29,30,48, 50,58]			↕↕ [93,94,95, 96,97,98]							
Diosmetin†		↗↗ [41,46]	↗↗ [18]			↕↕ [99,100]							
7,8-DHF			↗↗↗ [32,117,119, 193,201,202]			↕ [122]	↘↘ [178,179]	↘↘↘ [181,183]	↗↗ [182,183]		↗↗ [200]		↗↗ [191,202,287]
Lion's mane mushroom extract			↗↗ [69,72,121]				↘↘ [176]	↘↘ [176,185,245]	↗↗ [5,175,203, 245]		↗ [203]		↗↗ [175,203]
Exercise, walking [43,108]												↗↗↗	
Senescent cell clearance [104]				↗↗↗↗ [104,105,125, 126,127,138, 142,146]									
Fisetin (senolytic)				↗↗↗↗ [125,126,127]		↗↗ [133,134]							
N-acetyl-cysteine + glycine (GlyNAC)‡	↘↘↘ [153, 276]	↗↗↗ [153,276]					↘↘↘↘ [153,276]	↘↘↘↘ [153,276]	↗↗↗↗ [153,276]	--- [153,276]		↗↗ [154]	
TUDCA‡		↗↗↗ [247,249,259]	↗↗↗ [252,260,268]			↗↗ [256,261]		↘↘↘ [294]	↗↗↗ [294] ≈ [258,260]			↗↗ [251]	↗↗ [252,260,261]
Centrophenoxine‡			↗↗↗ [277,278]		↘↘,≈ [240,241]	↗↗↗ [242,243]		↘↘ [239,242,264,279]					
NSI-189													
Luteolin			↗↗ [111]			↗↗ [101,102]							
Fasting Mimicking Diet‡		↗↗ [78]				↗↗↗ [284]		↘ [300]	↗ [300]				

A blank cell indicates no published reference found; "---" means no effect is observed; ↘ and ↗ arrows indicate an inhibition or promotion effect; ↕ indicates regulation; ≈ means approximately equivalent.

† Hesperidin, naringin and diosmetin are produced in the skin of citrus fruits by the same synthetic pathway, are similar in structure, and have similar biological effects.
‡ Improvements are in most studies compensative of oxidative stress, a deficiency or an injury, with a return to basal levels by the involvement of corrective treatments.
* Nitric oxide synthase is implicated in synaptic plasticity and is enriched in the dendrites of CA1 hippocampal neurons.

Table 2. Characterization of age-related changes to the hippocampus

	TNFα	BDNF	Neural progenitor cells*	Neuro-genesis	Axon length	Neural density	Synapse density	Dendritic spine density	Dendritic spine length	Dendritic spine width	dsDNA damage (γ-H2AX)	Cytoplasmic dsDNA	Epigenetic regulators†	Senescent cell accum-ulation§	Aggre-somes	Aβ plaque accum-ulation
Characterization																
Mice, healthy Old vs Young	↗↗↗↗ [227]	≈ [220]	↘↘↘ [231]	↘↘ [222]				↘↘↘ [55,104]	↘↘↘ [104,231]					↗↗ [142]		
Monkeys, healthy Old vs Young	↗↗↗↗ [215]		↘↘ [215]	↘ [215]		↘≈ [215]		↘↘ [215,221,225]	↘↘ [221,225]		↗↗↗ [215]	↗↗↗ [215]	↘↘ [215]	↗↗ [215]	↗↗↗ [215]	↗↗ [215]
Humans, healthy Old vs Young		↘↘ [217]	↘↘ [224]	↘ [223,224]				↘↘↘ [104,219]	↘↘ [219]							↗↗ [216]
Intervention																
Hesperidin (healthy cohorts)							↗↗↗ [16]									
Hesperidin‡ (impaired cohorts)	↘↘↘ [Table 1A]	↗↗ [Table 1A]	↗↗ [233]	≈ [231,233]				≈ [231,233]	≈ [233]							↘↘ [51,90] ≈ [234]
7,8-DHF (young mice, healthy)						≈ [235]		↗↗ [235]								
7,8-DHF (old mice, healthy)			↗≈ [231]					↗↗↗ [55,231]	↗↗↗ [231]							
7,8-DHF‡ (old mice and impaired)	↘ [3,4]	↗↗↗↗ [Table 1A]	↗≈ [214]			≈ [235]	↗↗↗ [67]	↗↗↗ [36,38,55,67, 214,235]	↗↗↗ [4,55,67]							↘≈ [38,67,214]
Lion's mane mushroom extract‡ (old or impaired)		↗↗ [Table 1A]	↗↗↗ [24]	↗↗↗ [24,236]	↗↗ [158]											
Senescent cell clearance [104] (old)	↘↘↘ [Table 1A]		↗↗↗ [142]	↗↗↗ [142]									† [104]	↘↘↘↘ [138]		↘ [38]
TUDCA‡			↗↗↗ [251,253]	↗↗ [251]			↗↗↗ [250,252]									↘↘ [256]
Centrophenoxine																
Fasting Mimicking Diet			↗↗ [284]	↗↗ [78,284]												↘↘ [284]
Psilocybin** (healthy mice,pigs, humans)		↗↗↗↗↗ [238]						↗↗↗↗↗ [228,229,230] Persists 3 wks?	↗↗↗↗↗ [228]	↗↗↗↗ [228]						
LSD** (healthy mice,humans)		↗↗↗↗ [237,238]					↗↗↗↗↗ [244]	↗↗↗↗↗ [244] Persists 1 wk?	↗↗↗↗↗ [244]							

‡ Improvements are in most studies compensative of oxidative stress, a deficiency or an injury, with a return to basal levels by the involvement of corrective treatments.
† Changes in epigenetic regulators (H3K9me3, HP1γ, etc) are modulated in part by senescent cell SASP [104].
* Neural precursors are identified as DCX+.
§ Senescent cells are identified as SA-β-gal+.
** Psychoplastogens affect serotonin 5-HT receptors in addition to the BDNF-TrkB pathway, and cause greater dendrite and spine growth. In contrast, BDNF induces a noticeably larger, more rapid increase in synaptogenesis [244].

Table 3. Oral dosages of hesperidin and naringin reported in published studies

	Oral dose mg/kg body weight	Human or human equivalent dose (HED)*	Dose or HED for a 70 kg human	Comments
Hesperidin				
Hesperidin increased vascular flow in patients with metabolic syndrome [90]			500 mg (human)	Hesperidin increased vascular flow-mediated dilation and reduced concentrations of inflammatory biomarkers (hsC-reactive protein, serum amyloid A, soluble E-selectin).
Hesperidin caused an antidepressant effect comparable to fluoxetine (Prozac) [11]	100 mg/kg (rats)	16.2 mg/kg	1135 mg	The effects may have been partly mediated by its amelioration of hyperglycaemia, anti-inflammatory activities, the enhancement of neurogenesis, and changes in the levels of monoamines in the brain.
Amyloid β plaque clearance and cognitive improvement in APP/PS1 mice [51]	100 mg/kg (mice)	8.1 mg/kg	567 mg	10 days hesperidin treatment restored deficits in nesting ability and social interaction. β-amyloid deposition was significantly attenuated.
8.7% increase in lifespan, reduced fat, restored cardiac and skeletal muscle [270]	100 mg/kg (mice)	8.1 mg/kg	567 mg	4 mo hesperidin supplementation to 20-mo-old mice significantly restored functional gene transcription and muscle function, apparently through activation of Cisd2.
Exercise-induced immune alterations were improved [115]	200 mg/kg (rats)	32.4 mg/kg	2270 mg	Leukocytosis induced by exhaustion was prevented and the proportion of T helper cells was increased by administration of hesperidin.
Improved memory in oxidative stress model [141]	20 mg/kg (rats)	3.2 mg/kg	227 mg	Behavioral tests showed improvement on memory retrieval and recognition memory consolidation in an AD model exposed to oxidative stress.
Inhibition of inflammatory cytokines TNF-α, and IL-6 in rats fed a high fat diet [59]	55 mg/kg (rats)	8.9 mg/kg	624 mg	Hesperidin improved insulin resistance via down-regulation of inflammatory responses.
Inhibited IL-6, increased BDNF, regulated acetylcholinesterase [211]	50 mg/kg (mice)	4.1 mg/kg	183 mg	The main targets of hesperidin are pro-inflammatory cytokine modulation, helping to maintain brain plasticity and acetylcholinesterase activity regulation in a model of depression.
Naringin				
An antidepressant effect resulted from increased hippocampal serotonin (5-HT), norepinephrine (NE) and GR levels [14]	20 mg/kg (mice)	1.6 mg/kg	113 mg	Naringenin was administered for 14 days. Its efficacy increased over time.
Amyloid β plaque clearance and cognitive improvement in APP/PS1 mice [50]	100 mg/kg (mice)	8.1 mg/kg	567 mg	3 mo naringin treatment improved learning and memory retention. β-amyloid deposition was significantly attenuated.
Inhibition of inflammatory cytokines TNF-α, and IL-6 [80]	100 mg/kg (mice)	8.1 mg/kg	567 mg	Cytokine production was dampened by lysosome function regulation.
Protection against cognitive dysfunction from oxidative damage [48]	40 mg/kg (mice)	3.2 mg/kg	227 mg	Naringenin was administered daily for a period of 25 days protected against colchicine-induced cognitive impairment.
Inhibition of inflammatory cytokines TNF-α, and IL-6 in rats fed a high fat diet [71]	100 mg/kg (mice)	8.1 mg/kg	567 mg	Naringin improved insulin resistance via down-regulation of inflammatory responses.

* Human equivalent dose (HED) can be related to mouse dosage by the following formula
HED (mg/kg) = Animal Dose (mg/kg) x [Animal K_m / Human K_m] where the scaling factor K_m = 3 in mice, 6 in rats, and 37 in humans.
"Dose translation from animal to human studies revisited" (2008) http://www.fasebj.org/content/22/3/659.full.pdf+html

Table 4. Pharmacokinetics of selected supplements (high dose)

	Elimination Half-life T1/2 (hr)	Time to Peak Concentration Tmax (hr)	Oral Bioavailability	Effect on CYP450 ‡	Oral LD50 † (mg/kg)
Hesperidin *	2.2 ± 0.8 (humans) [B]	5.4 ± 1.6 (humans) [B]	8.5 ± 2.3% (rats) [B]	↘↘↘ [D]	4837.5 (rats) [P]
Naringin *	0.74 ± 0.78 (rats) [A] 2.51 ± 1.58 (humans) [A] 2.2 ± 0.1 (humans) [B] 4 ± 1 (rabbits) [I]	2.85 ± 4.06 (rats) [A] 1.66 ± 1.0 (humans) [A] 4.8 ± 1.1 (humans) [B]	4% (rabbits) [I]	↘↘↘ [C]	
Luteolin	6.57 (rats) [F]	0.25 (rats) [F]	4.1% (rats) [F]		> 5000 (rats) [R]
Fisetin			30% (mice) [S]		1700 (mice) [S]
7,8-DHF	2.23 (mice) [G,J] 4 to 8 (monkeys) [T]	0.17 (mice) [G,J] 4 (monkeys) [T]	4.6% (mice) [G,J]	↘↘↘ [E]	
4'-DMA-7,8-DHF §	1.5 (mice) [G,J]	0.17 (mice) [G,J]	Less than 7,8-DHF (mice) [J]		
Lion's Mane mushroom	7.3 ± 0.2 (rats) [M]	4.5 ± 1.2 (rats) [M]	13.1% (rats) [M]		> 5000 (rats) [Q]
NSI-189	17.4–20.5 (humans) [L]	0.13 (mice) [O]			
Astaxanthin	15.9 ± 5.3 (humans) [U]	8 (humans) [U]	4% to 34% (humans) [U]		> 20,000 (rats) [V]
TUDCA	1 ± 2 (humans) [W]	0.8 (humans) [W]			

A blank cell indicates no published reference found.
"---" means no effect is observed; ↘ and ↗ arrows indicate an inhibition or promotion effect; ≈ means approximately equivalent.

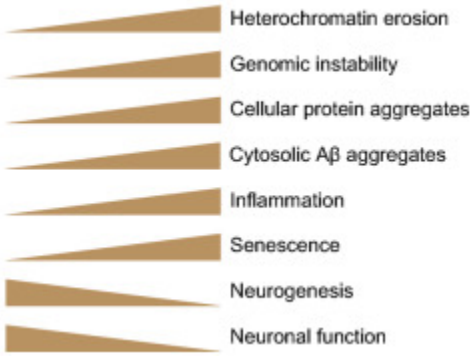
* Hesperidin, naringin and diosmetin have similar structures and are expected to have similar bioavailability and pharmacokinetics. However, published pharmacokinetics of each of these flavones varies widely.
§ 4'-DMA-7,8-DHF is a synthetic alternative to the flavone 7,8-DHF with greater potency and similar pharmacokinetics [J].
† LD50 = the amount of a material, given all at once, which causes the death of 50% of a group of test animals.
‡ CYP450 is a key enzyme class involved in the breakdown and clearance of compounds in the liver. Inhibition of CYP450 can affect drug pharmacokinetics, particularly clearance, and can cause drugs to persist longer in the bloodstream than they are intended.

Characterization and Restoration of the Aged Brain

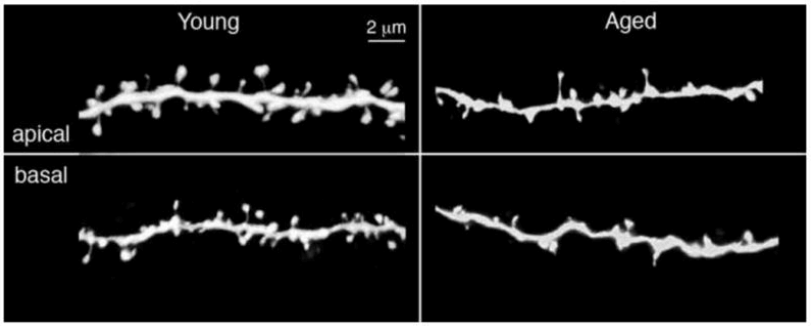
The aged hippocampus has decreased dendritic spines and accumulated cellular damage

The hippocampus plays a crucial role in learning and memory, and it progressively deteriorates with age. Studies of non-human primates (cynomolgus monkeys) have shown that in comparing the hippocampus between the young and the old [215]:

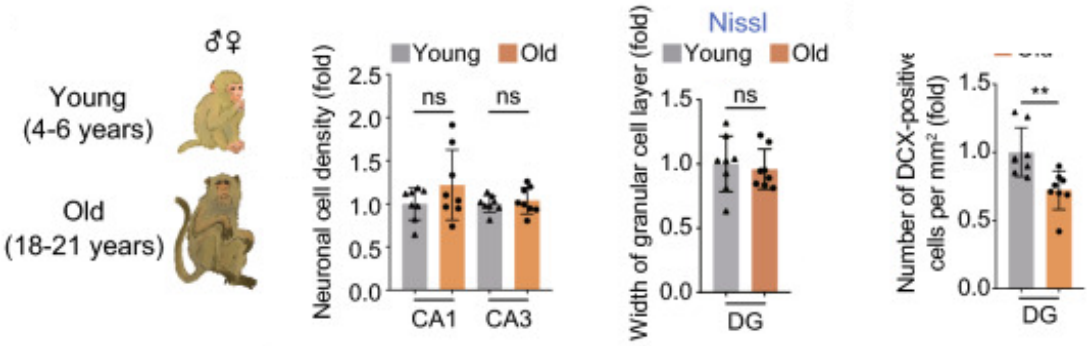
- The neural density is about the same
- The width of the granular cell layer (a measure of the size of the hippocampus) remains about the same
- The density of dendritic spines markedly decreases. (Dendritic spines of neurons are where the majority of excitatory synaptic signaling occurs in the brain. Dendritic spines increase the surface area of a neuron and this allows more connections to be made with other neurons.)
- DNA damage (marked by γ-H2AX foci formation) increases
- dsDNA release from the nucleus to the cytoplasm increases (results in increased inflammation).
- Epigenetic regulators H3K9me3 and HP1γ decrease.



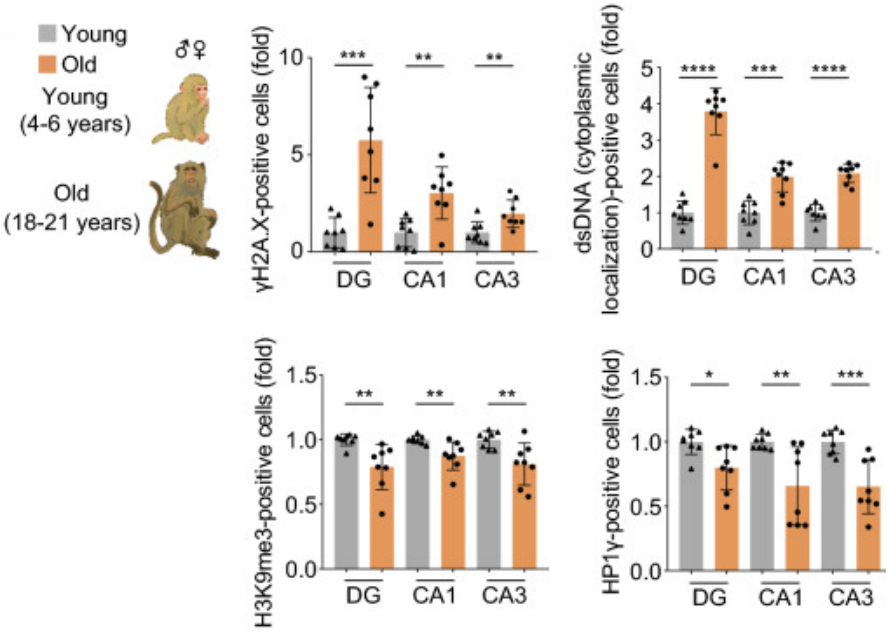
A schematic illustration showing the phenotypic and transcriptomic signatures of cynomolgus monkey hippocampal aging [215]. “Single-nucleus transcriptomic landscape of primate hippocampal aging” (2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8403220/



Representative dendritic segments and spines from layer 3 pyramidal neurons from PFC of young (left) and aged (right) rhesus monkeys [225]. “Dendritic spine changes associated with normal aging” (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3654095/



Aging-related phenotypes of the cynomolgus monkey hippocampus. Young monkeys are equivalent to ~16 years-old humans and old are equivalent to ~60 years-old. It is seen that the neural density of young and old is about the same, as is the size of the endoplasmic reticulum (Nissl stain). However, there is a marked decrease in dendritic spines as indicated by significantly lower DCX expression. DG = dentate gyrus, and CA1 and CA3 designate regions of the hippocampus. Young, n = 8; old, n = 8 monkeys; "ns" = not significant; *P < 0.05; **P < 0.01. [215] "Single-nucleus transcriptomic landscape of primate hippocampal aging" (2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8403220/



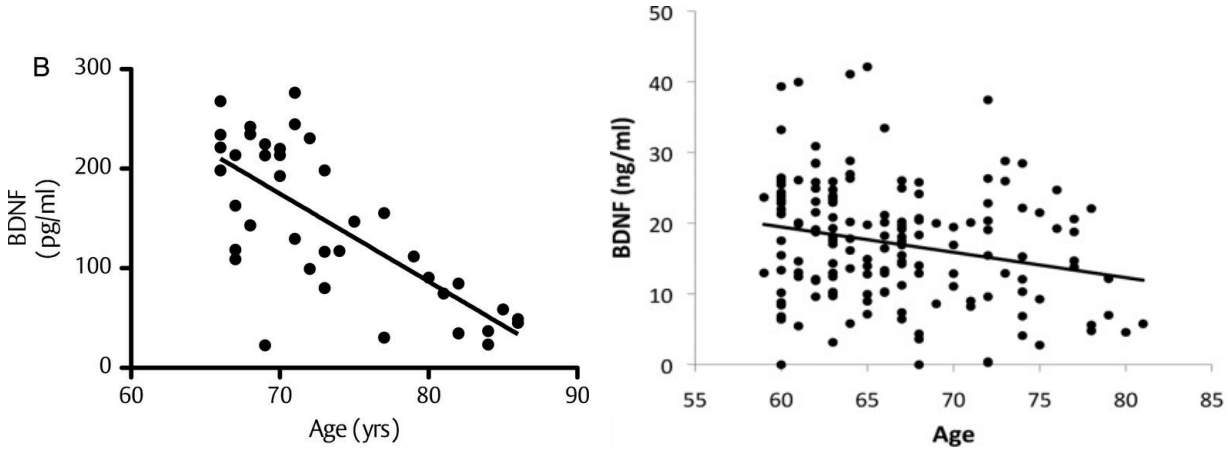
Aging-related loss of genomic and epigenomic stability in the monkey hippocampus. γH2A.X is a sensitive molecular marker of DNA damage and repair. Double-strand DNA (dsDNA) release from the nucleus to the cytoplasm induces an inflammatory response and is another indicator of DNA damage. H3K9me3 and HP1γ are epigenetic regulators. DG = dentate gyrus, and CA1 and CA3 designate regions of the hippocampus. Young monkeys are equivalent to ~16 years-old humans and old are equivalent to ~60 years-old.; *P < 0.05; **P < 0.01; ***P < 0.001. [215] "Single-nucleus transcriptomic landscape of primate hippocampal aging" (2022)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8403220/

BDNF levels decline in older individuals; modulates dendritic spine density

Dendritic spines decline substantially in size and numbers with age. This decline is the major cause of age-related changes in cognitive function and neural plasticity in healthy individuals. Dendritic spines are profoundly affected by brain-derived neurotrophic factor (BDNF) through TrkB receptors. BDNF is necessary and sufficient to induce long-lasting structural changes at dendritic spines that are associated with synaptic plasticity. Higher levels of the BDNF are associated with improved cognitive functioning, mental health, as well as short-term and long-term memory.

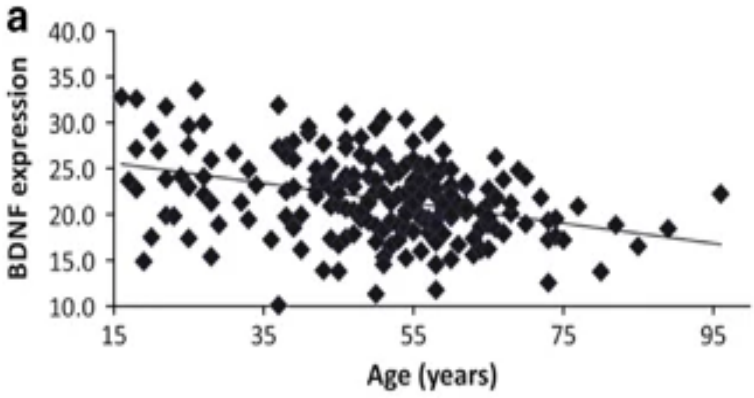
1. (Review) "Involvement of BDNF in Age-Dependent Alterations in the Hippocampus" (2010) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2952461/
2. "The Role of BDNF in Age-Dependent Changes of Excitatory and Inhibitory Synaptic Markers in the Human Prefrontal Cortex" (2016) https://www.nature.com/articles/npp2016126

In human adults, BDNF levels remain, on average, steady from the ages of 35 to 55 then decline. Restoration of BDNF levels in aged humans and laboratory animals is associated with improved memory and cognitive performance, while excessive levels are associated with ADHD in children.



Age-related reduction of serum BDNF ($p<0.05$).

1. "High-flavonoid intake induces cognitive improvements linked to changes in serum brain-derived neurotrophic factor: Two randomized, controlled trials" (2016) https://content.iospress.com/articles/nutrition-and-healthy-aging/nha1615
2. "Brain-Derived Neurotrophic Factor Is Associated with Age-Related Decline in Hippocampal Volume" (2010) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3069644/



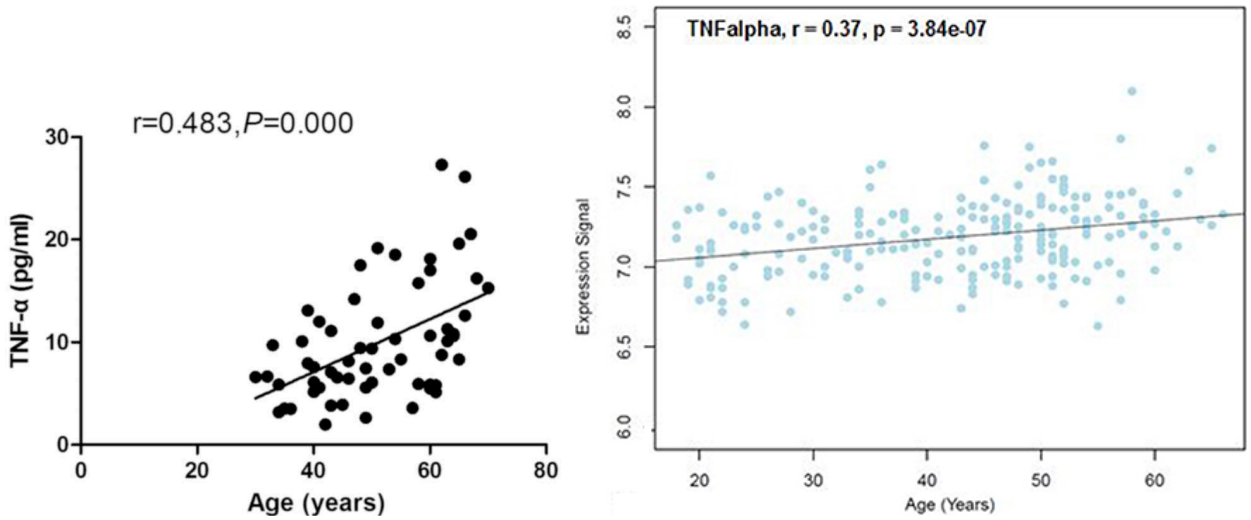
Age-related reduction of BDNF in the human frontal cortex ($r=-0.36$, $p<0.0000001$).
"The Role of BDNF in Age-Dependent Changes of Excitatory and Inhibitory Synaptic Markers in the Human Prefrontal Cortex" (2016) https://www.nature.com/articles/npp2016126

Inflammatory cytokine TNF-α increases with age, mediates synaptic plasticity, and impairs BDNF and memory

Systemic TNF-α increases with age, and its sustained elevation in the aging brain is linked to cognitive dysfunction and impaired memory. Significantly, BDNF and TNF-α are interactively associated in adults, and TNF-α has been found to have a role in mediating homeostatic synaptic plasticity (references listed below).

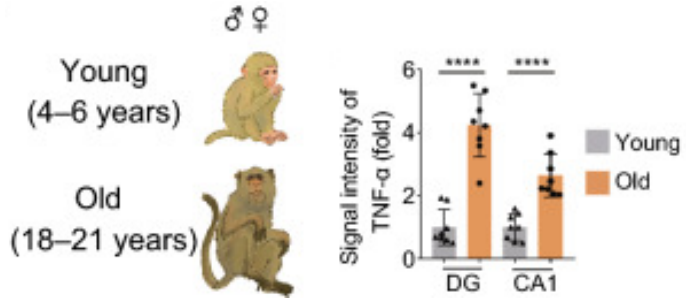
Increased levels IL-6 in aging humans is also associated with cognitive decline and memory performance. However, its relationship with neuroplasticity is not established.

Of note, aging mice showed a spontaneous decline in muscle strength, which is prevented by TNF-α blockade [226]. Citrus flavonoids such as hesperidin, naringin and diosmetin have been shown to potently lower TNF-α in the hippocampus and to be neuroprotective [1,59,60,90,114,115,149]. Removal of SnCs by senolytic drugs also lowers systemic inflammation and enhances hippocampal neurogenesis and spatial memory in adult mice.

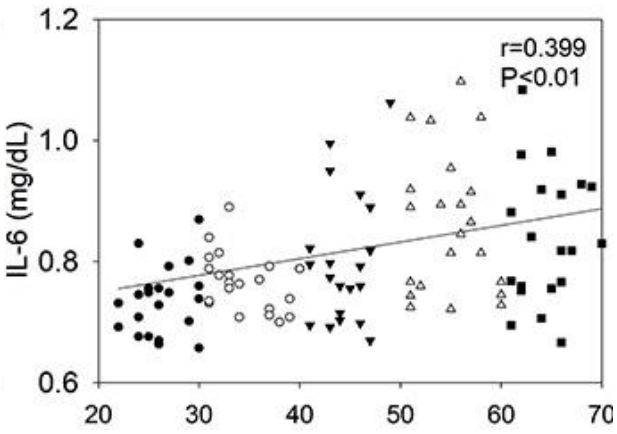


Serum TNF-α expression level in correlation with age (years).

1. "Age-related differences in serum MFG-E8, TGF-β1 and correlation to the severity of atherosclerosis determined by ultrasound" (2017) https://www.spandidos-publications.com/mmr/16/6/9741?text=fulltext
2. "Epigenetics of Delirium and Aging: Potential Role of DNA Methylation Change on Cytokine Genes in Glia and Blood Along With Aging" (2018) https://www.frontiersin.org/articles/10.3389/fnagi.2018.00311/full



TNF-α in the hippocampus from young and old monkeys. DG = dentate gyrus, and CA1 and CA3 designate regions of the hippocampus. Young monkeys are equivalent to ~16 years-old humans and old are equivalent to ~60 years-old.; ****P < 0.0001. [215] "Single-nucleus transcriptomic landscape of primate hippocampal aging" (2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8403220/



Pro-inflammatory cytokine interleukin 6 (IL-6) of the 110 volunteers. •: G21–30; ○: G31–40; ▾: G41–50; Δ: G51–60; ▪: G61–70. "Effects of natural aging and gender on pro-inflammatory markers" (2019) https://www.scielo.br/j/bjmbr/a/DG4PY93XphmjMDdX3qNKMdf/?lang=en

Tumor Necrosis Factor Alpha (TNF-α)

1. "TNF-Mediated Homeostatic Synaptic Plasticity: From in vitro to in vivo Models" (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7556297/
2. "TNF-alpha inhibition prevents cognitive decline and maintains hippocampal BDNF levels in the unpredictable chronic mild stress rat model of depression" (2015) https://doi.org/10.1016/j.bbr.2015.05.062
3. "Neuroinflammatory TNFα Impairs Memory via Astrocyte Signaling" (2015) https://www.sciencedirect.com/science/article/pii/S0092867415015032
4. "The TNFα-Transgenic Rat: Hippocampal Synaptic Integrity, Cognition, Function, and Post-Ischemic Cell Loss" (2016) https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0154721
5. "Tumor Necrosis Factor Alpha: A Link between Neuroinflammation and Excitotoxicity" (2014) https://www.hindawi.com/journals/mi/2014/861231/
6. "Systemic TNF-α produces acute cognitive dysfunction and exaggerated sickness behavior when superimposed upon progressive neurodegeneration" (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5176008/
7. "Tumor necrosis factor-α triggers a cytokine cascade yielding postoperative cognitive decline" (2010) https://www.pnas.org/content/107/47/20518
8. "BDNF and TNF-α polymorphisms in memory" (2013) https://link.springer.com/article/10.1007/s11033-013-2648-6
9. "Diosmin improved cognitive deficit and amplified brain electrical activity in the rat model of traumatic brain injury" (2021) https://pubmed.ncbi.nlm.nih.gov/28738538/
10. "Protective effect of naringin, a citrus flavonoid, against colchicine-induced cognitive dysfunction and oxidative damage in rats" (2010) https://www.liebertpub.com/doi/10.1089/jmf.2009.1251
11. "Naringin Dihydrochalcone Ameliorates Cognitive Deficits and Neuropathology in APP/PS1 Transgenic Mice" (2018) https://www.frontiersin.org/articles/10.3389/fnagi.2018.00169/full

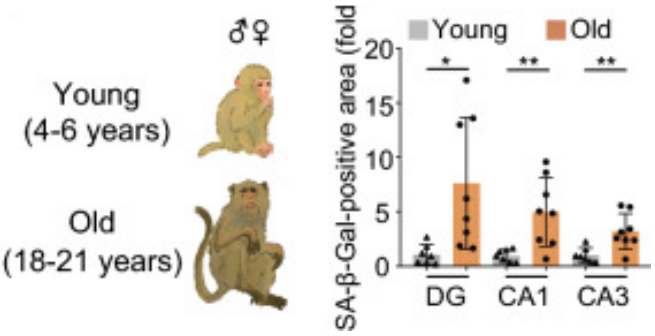
Interleukin-6 (IL-6)

1. "Pro-inflammatory interleukin-6 signaling links cognitive impairments and peripheral metabolic alterations in Alzheimer's disease" (2021) https://www.nature.com/articles/s41398-021-01349-z
2. "Interleukin-6 is both necessary and sufficient to produce perioperative neurocognitive disorder in mice" (2018) https://www.sciencedirect.com/science/article/pii/S0007091217541793
3. "Association of Peripheral Interleukin-6 with Global Cognitive Decline in Non-demented Adults: A Meta-Analysis of Prospective Studies" (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5766662/
4. "Interleukin-6 and Memory Functions of Encoding and Recall in Healthy and Depressed Elderly Adults" (2012) https://www.ajgponline.org/article/S1064-7481(12)61992-7/fulltext

Senescent cells accumulate with age; removal restores physical function and enhances memory acquisition

The phenotypes of brain aging, similar to that of other organs, are in part caused by the influences of senescent cells (SnCs). Targeting SnCs alleviates some of those effects [104]. Research on whole body clearance of SnCs in mice supports this contention [138].

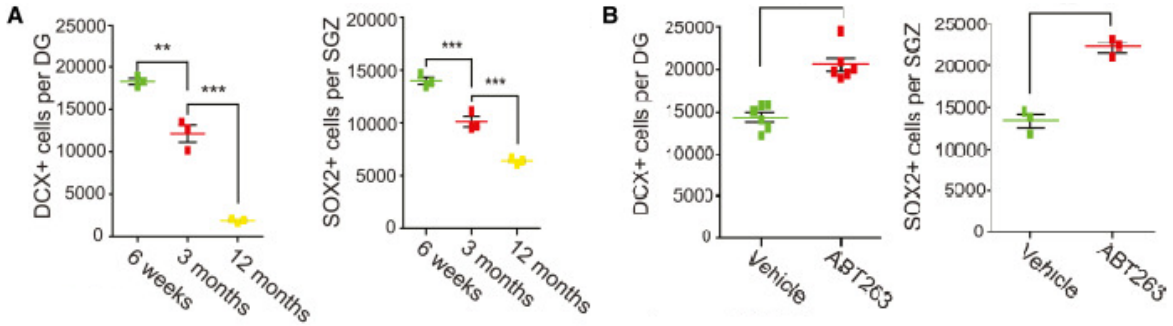
In cynomolgus monkeys, a model for aged primates including humans, senescent cells accumulate significantly in the hippocampus. This accumulation of SnCs is a fundamental driver of neurodegenerative disease, systemic inflammation, and the accumulation of amyloid β plaques and tau aggregates in the brain. It is causally linked to cognition-associated neuronal loss [106,107].



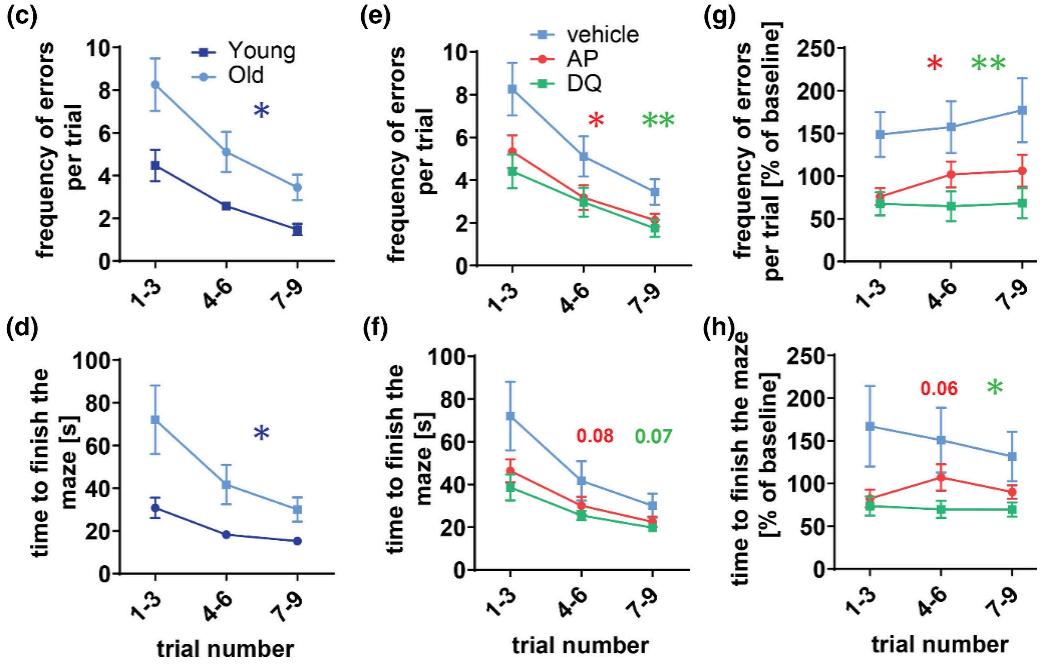
SA-β-Gal staining of senescent cells in the indicated regions of the hippocampus from young and old monkeys. DG = dentate gyrus, and CA1 and CA3 designate regions of the hippocampus. Young monkeys are equivalent to ~16 years-old humans and old are equivalent to ~60 years-old. *P < 0.05; **P < 0.01. “Single-nucleus transcriptomic landscape of primate hippocampal aging” (2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8403220/

Clearance of SnCs reduces associated inflammation, amyloid β plaques and tau aggregates, increases dendritic spines, and in part restores cognitive performance in mice [106,107]. The age-related accumulation of senescent cells (SnCs) is readily reduced by a combination of grape seed extract and fisetin, as described in detail in “Protocol for Restoration of Physical Function in Aged Individuals by Removal of Senescent Cells” https://docs.google.com/document/d/1ndfwj3mBvIZceP6A2e6dliieJNM7kxFYf-afuP67KGA/edit?usp=sharing.

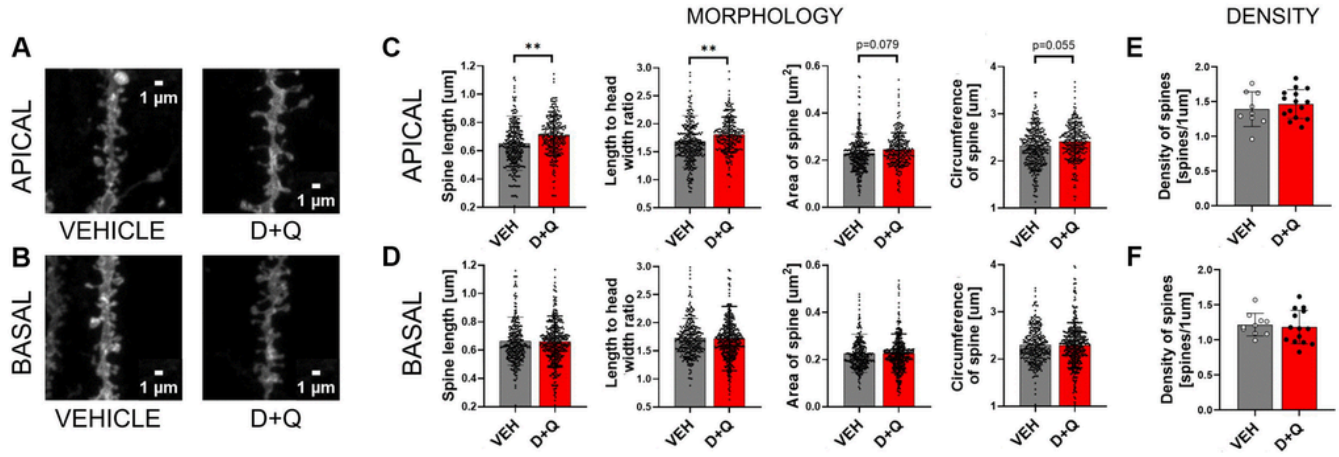
In mice at age 12 months (equivalent to a 45-50 year old human), 22% of hippocampal neurons (SOX2+) are SA-β-gal+ senescent, and proliferation of neural progenitors (DCX+) is reduced 85% compared to a 3 month old mice. Removing senescent brain cells substantially increased hippocampal neurogenesis by 30% and improved spatial memory [142, 146]. These experiments establish that SnCs accumulate in the hippocampus, that they impede neurogenesis, and that their removal partially restores the capacity to acquire new memories.



(A) 22% of hippocampal neurons (SOX2+) are SA-β-gal+ senescent, and proliferation of neural progenitors (DCX+) is reduced 85% in 12-month compared to 3-month-old mice. (B) Removing senescent brain cells increased hippocampal neurogenesis by 30% [142]. “Restoration of hippocampal neural precursor function by ablation of senescent cells in the aging stem cell niche” (2021) https://www.sciencedirect.com/science/article/pii/S2213671121006494?via%3Dihub



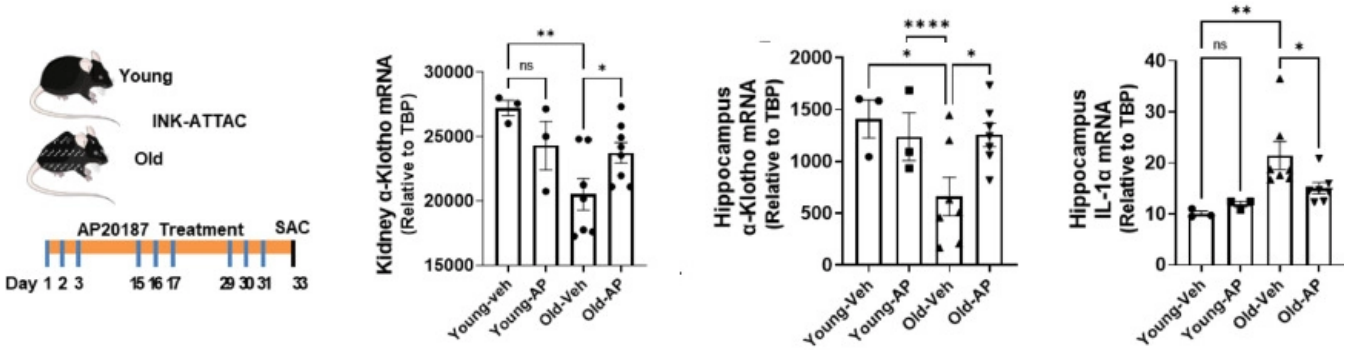
Whole-body senescent cell clearance alleviates age-induced spatial memory dysfunction. 4-month and 25 to 29-month old mice were split into three groups and assigned to vehicle (v), Dasatinib and Quercetin (D + Q), or AP20187 (AP) treatments [138]. "Whole-body senescent cell clearance alleviates age-related brain inflammation and cognitive impairment in mice" (2021) https://onlinelibrary.wiley.com/doi/full/10.1111/acel.13296



D+Q treatment changes synaptic plasticity in the apical dendrites of neurons of the CA1 region of the hippocampus. Representative images of DiI stained dendrites (A and B). DiI stained hippocampal slices from an aged vehicle (25 mo, gray bars) or D+Q (red bars) treated rats were used for the analysis of synaptic plasticity. Different parameters of the dendritic spine shape (length, length to width ratio, area, circumference C and D) and spine density (E and F) were analyzed in the CA1 region of the hippocampus. The analysis was done for two dendritic arbors of stratum pyramidale, namely basal (BASAL) (D and F) and apical (APICAL) dendrites (C and E) [146]. "Combination of dasatinib and quercetin improves cognitive abilities in aged male Wistar rats, alleviates inflammation and changes hippocampal synaptic plasticity and histone H3 methylation profile" (2022) https://www.aging-us.com/article/203835/text

α-Klotho declines with ageing in mice and humans. α-Klotho is a geroprotective factor that exerts anti-physiological stress effects and protects against oxidative damage, hypoxia, and cytotoxic drugs. Several preclinical studies have implicated α-Klotho as a molecule that impacts lifespan, health-span, and renal and cognitive function. Soluble α-Klotho is an endocrine protein that regulates multiple biological processes, including phosphate homeostasis, mineral metabolism, and signaling by insulin-like growth

factor 1 (IGF-1), mammalian target of rapamycin (mTOR), cyclic adenosine monophosphate (cAMP), p53/ p21CIP1, and Wnt proteins. The brain and kidneys are primary sites of α-Klotho production. Senolytics increase α-Klotho protein in the cerebellum and choroid plexus as well as α-Klotho mRNA in whole brains of old mice in which α-Klotho decreases with ageing [291].



Genetic clearance of highly p16Ink4a-expressing cells increases α-Klotho in old (27-29-month-old) vs young (8-month-old) mice. Mice were treated every 20 days with D+Q, F, or vehicle by oral gavage for 3 consecutive days in each of 3 cycles over 2 months (9 doses in total) for old animals starting at the age of 26–27 months. *p = 0.03, **p < 0.01, ***p < 0.001, ****p < 0.0001; n = 7 old + vehicle; n = 7 old + AP20187 [291]. “Orally-active, clinically-translatable senolytics restore α-Klotho in mice and humans” (2022) https://doi.org/10.1016/j.ebiom.2022.103912

Misfolded proteins accumulate in the aged brain; reduction improves cognition

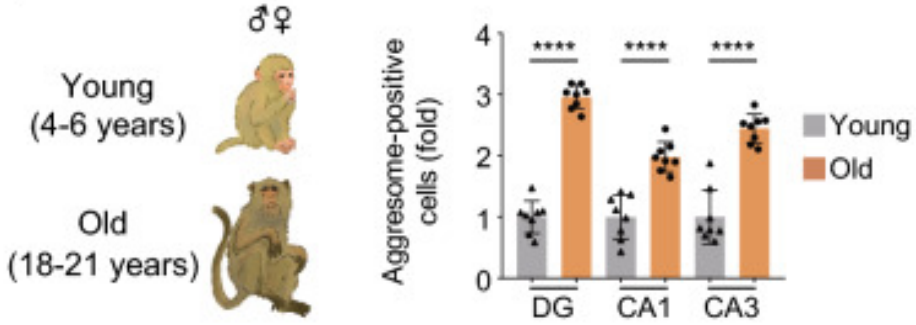
Cellular protein aggregates and TUDCA

Cellular protein homeostasis, or proteostasis, refers to the dynamic regulation of protein synthesis, folding, trafficking, and degradation. When proteostasis is impaired, the failure to refold or degrade unfolded proteins causes aggregated proteins (aggresomes) to accumulate within cells. Chronic expression and accumulation of aggresomes contributes to the development of some age-related pathologies, such as Alzheimer's disease and Parkinson's disease.

The complex processes involved in proteostasis are sensitive to disruption by many factors, in particular ATP insufficiency and age-associated oxidative stress in the endoplasmic reticulum (ER). The protein folding process is dependent on redox homeostasis, and its disruption by oxidative stress results in an increase in the production of misfolded proteins.

1. "The Hallmarks of Aging" (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3836174/
2. "New hallmarks of ageing: a 2022 Copenhagen ageing meeting summary" (2022) https://doi.org/10.18632/aging.204248
3. "Pathways of cellular proteostasis in aging and disease" (2017) https://doi.org/10.1083/jcb.201709072
4. "Aging and Rejuvenation of Neural Stem Cells and Their Niches" (2020) https://doi.org/10.1016/j.stem.2020.07.002
5. "Reducing ER stress with chaperone therapy reverses sleep fragmentation and cognitive decline in aged mice" (2022) https://onlinelibrary.wiley.com/doi/10.1111/acel.13598
6. "Redox signaling and unfolded protein response coordinate cell fate decisions under ER stress" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6859529/
7. "Proteasome Failure Promotes Positioning of Lysosomes around the Aggresome via Local Block of Microtubule-Dependent Transport" (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3993571/

The accumulation of misfolded proteins is a particular concern in nondividing, long-lived cells such as neurons. This accumulation is seen in the hippocampus of aged cynomolgus monkeys [215].



Aggresome (aggregation of misfolded proteins) staining in the indicated regions of the hippocampus from young and old monkeys. DG = dentate gyrus, and CA1 and CA3 designate regions of the hippocampus. Young monkeys are equivalent to ~16 years-old humans and old are equivalent to ~60 years-old. ****P < 0.0001. Single-nucleus transcriptomic landscape of primate hippocampal aging" (2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8403220/

A common feature of neurodegenerative diseases is the accumulation of misfolded proteins in the brain. The aggregation of abnormal proteins can perturb cellular structure and function, lead to neuronal loss, and promote the pathogenesis of Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS).

1. "Endoplasmic Reticulum Stress-Mediated Hippocampal Neuron Apoptosis Involved in Diabetic Cognitive Impairment" (2013) https://doi.org/10.1155/2013/924327
2. "Elimination of endoplasmic reticulum stress and cardiovascular, type 2 diabetic, and other metabolic diseases" (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3581057/
3. "Protein lifetimes in aged brains reveal a proteostatic adaptation linking physiological aging to neurodegeneration" (2022) https://www.science.org/doi/10.1126/sciadv.abn4437

Periodic fasting improves proteostasis.

1. "Dietary restriction improves proteostasis and increases life span through endoplasmic reticulum hormesis" (2019) https://www.pnas.org/doi/10.1073/pnas.1900055116

2. (Review) “Nutrition, longevity and disease: From molecular mechanisms to interventions” (2022) https://doi.org/10.1016/j.cell.2022.04.002

Short duration liposomal trehalose supplementation is effective in reducing ER stress and inducing autophagy by competitively interfering with glucose metabolism and activating of autophagy [262]. However, there is a danger that this treatment may over-stimulate autophagy and cause rhabdomyolysis. A guide for preparing liposomal trehalose with dosages is available upon request.

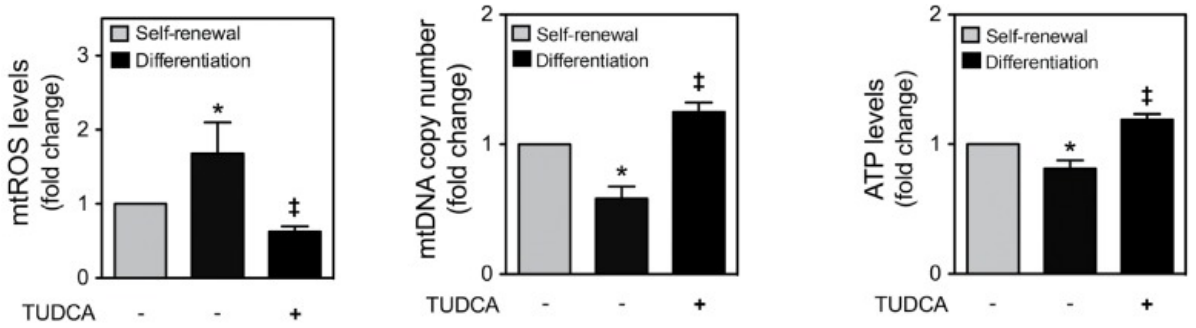
1. “Trehalose supplementation reduces hepatic endoplasmic reticulum stress and inflammatory signaling in old mice” (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5492939/
2. “Proteostasis in Huntington's disease: disease mechanisms and therapeutic opportunities” (2018) https://www.nature.com/articles/aps201811
3. ‘Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis” (2016) http://stke.sciencemag.org/content/9/416/ra21.full
4. “Trehalose induces autophagy via lysosomal-mediated TFEB activation in models of motoneuron degeneration” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6526812/
5. “Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation” (2015) https://www.sciencedirect.com/science/article/pii/S0969996110001658
6. “Trehalose ameliorates oxidative stress-mediated mitochondrial dysfunction and ER stress via selective autophagy stimulation and autophagic flux restoration in osteoarthritis development” (2017) https://www.nature.com/articles/cddis2017453
7. “Trehalose Alleviates Polyglutamine-Mediated Pathology in a Mouse Model of Huntington Disease” (2004) https://drive.google.com/open?id=1tbdSH6XGe_2ZGqvOeJA1VQqjn1Rs78Z

TUDCA

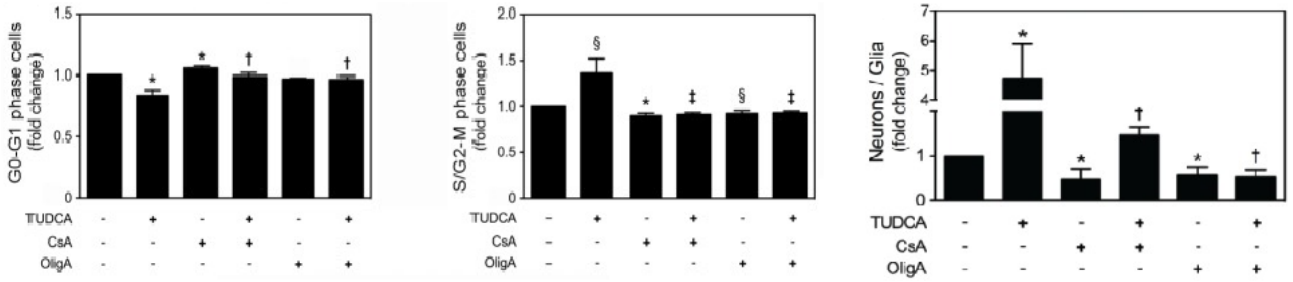
The bile acid tauroursodeoxycholic acid (TUDCA) is an ER chaperone that has been found to safely

- reverses age-related memory loss in old mice but not curiosity measured by exploration time [301]
- alleviate ER stress and promote cell survival [296]
- reverse age-related changes in the levels of synaptic plasticity proteins,
- increase neuron proliferation and preserve dendritic spine numbers when compensating for oxidative and other cellular stresses [246-253]
- Increase ATP and the ATP-to-AMP ratio [295,296]
- Increases the neural stem cell (NSC) pool in a mitochondrial redox state manner by biasing NSCs towards self-renewal instead of differentiation [251]
- decrease Aβ plaque and tau aggregates in the hippocampus and frontal cortex [268]

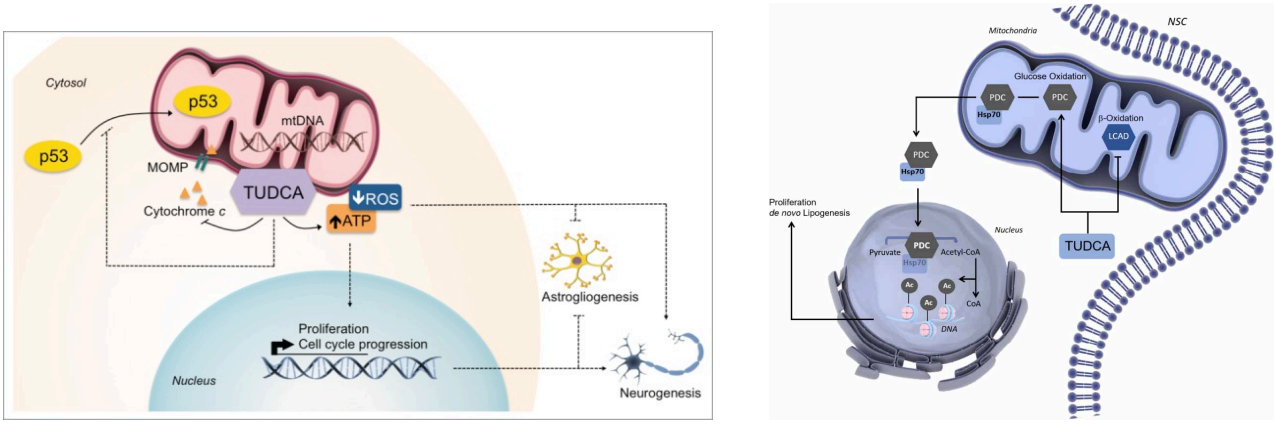
In humans, 500 mg 2x/day for 3 months appears to be a safe and effective dose for decreasing Aβ plaque and tau aggregates [268, W].



TUDCA reduces mtROS to modulate NSCs towards differentiation instead of self-renewal [253]. “Tauroursodeoxycholic Acid Enhances Mitochondrial Biogenesis, Neural Stem Cell Pool, and Early Neurogenesis in Adult Rats” (2018) https://www.ncbi.nlm.nih.gov/pubmed/28534273

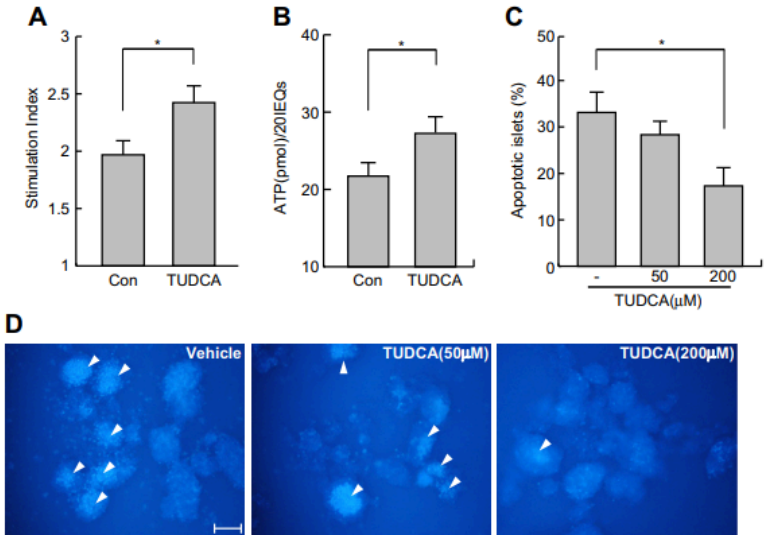


Quantification of G0-G1 and S/G2-M phase NSCs. TUDCA biases differentiation of NSCs towards neurons rather than glial cells [253]. “Tauroursodeoxycholic Acid Enhances Mitochondrial Biogenesis, Neural Stem Cell Pool, and Early Neurogenesis in Adult Rats” (2018) https://www.ncbi.nlm.nih.gov/pubmed/28534273



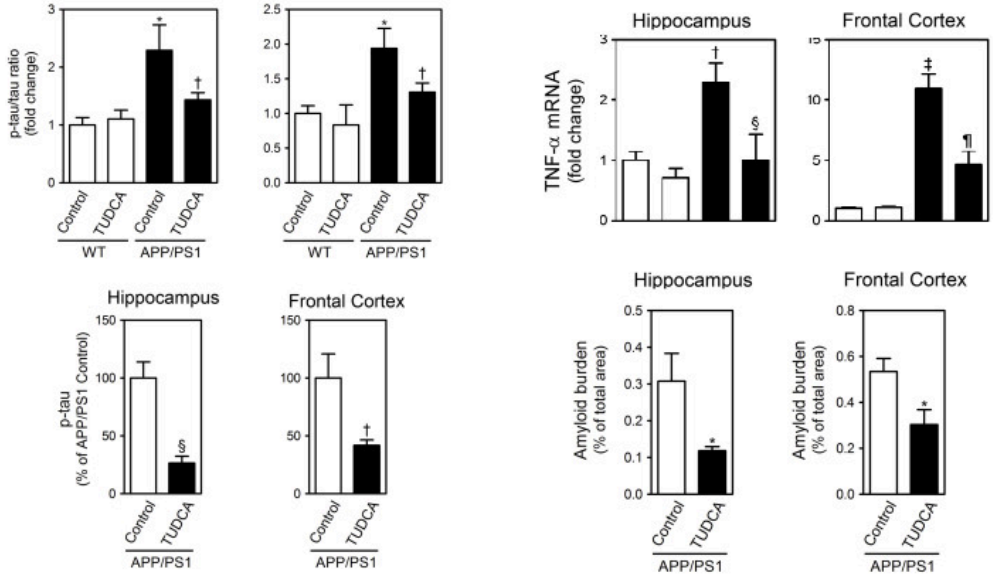
TUDCA mediates mitochondria-cell cycle retrograde signals to regulate NSC fate. The NSC modulatory properties of TUDCA result from inhibition of differentiation-induced mitochondrial apoptotic events by the bile acid, and from subsequent decrease in ROS and ATP mitochondrial levels. This, in turn, contributes to the enhancement of both NSC proliferation and neuronal rather than astroglial conversion of differentiating NSCs. In early-differentiating NSCs, TUDCA increases de novo lipogenesis and proliferation by inducing a metabolic shift from FA to glucose catabolism that facilitates NSC cell cycle-associated H3 acetylation. Importantly, TUDCA-mediated effects in increasing NSC pool and lineage determination occur in a mitochondrial redox state- and ATP-dependent manner [251].

1. [251] "Tauroursodeoxycholic acid increases neural stem cell pool and neuronal conversion by regulating mitochondria-cell cycle retrograde signaling" (2014) https://doi.org/10.4161/15384101.2014.962951
2. “Reprogramming of Lipid Metabolism as a New Driving Force Behind Tauroursodeoxycholic Acid-Induced Neural Stem Cell Proliferation” (2020) https://doi.org/10.3389/fcell.2020.00335



Effect of TUDCA on ATP contents, insulin secretion and viability of islets. Cells were treated in a two hundred micromolar solution of TUDCA for 24 h immediately after isolation for the analysis of insulin secretion index (n = 12) (A) and ATP content of islets (n = 9) (B). In addition, 10 lM of diamidino-2-phenylindole (DAPI) was treated to islets for 15 min and the photos of islets were taken under the fluorescence microscope (20) (C). Islets undergoing apoptosis were counted and presented as relative percentage (n = 4) (D). Arrowhead indicates apoptotic islets. Blue, incorporated DAPI. The values are mean ± SEM values. *p < 0.05. "Tauroursodeoxycholate (TUDCA), chemical chaperone, enhances function of islets by reducing ER stress" (2010) https://doi.org/10.1016/j.bbrc.2010.06.022

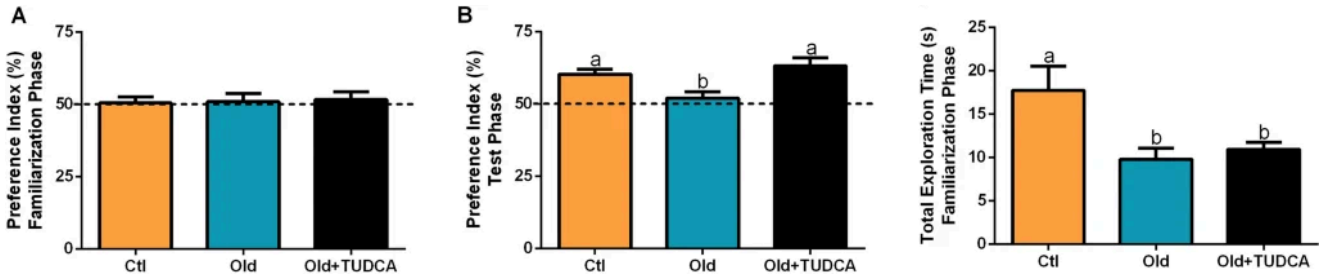
Moreover, TUDCA was found to significantly decrease Aβ plaque and tau aggregates in the hippocampus of mice when IP injected at 500 mg/kg every 3 days for 3 months (≈ 2800 mg for a 70 kg individual) [268]. This is evidence that TUDCA may be effective in reducing the accumulation of other types of protein aggregates.



TUDCA significantly decreased Aβ plaque and tau aggregates in the hippocampus of 7-month-old mice compared to same-age controls when IP injected at 500 mg/kg every 3 days for 3 months (≈ 2800 mg for a 70 kg individual) [268]. "Amyloid-β pathology is attenuated by tauroursodeoxycholic acid treatment in APP/PS1 mice after disease onset" (2015) https://doi.org/10.1016/j.neurobiolaging.2014.08.034

A mechanism by which TUDCA decreases Aβ plaque is by increasing gene expression of insulin-degrading enzyme (IDE) and consequently, insulin clearance. IDE is known to degrade amyloid beta plaque. By improving glucose tolerance and insulin

sensitivity. The resulting improvement in glucose-insulin homeostasis in old mice is accompanied by a reduction in adiposity, associated with adipocyte hypertrophy, and lipids accumulation in the liver (i.e., results in weight loss) [301].



TUDCA reverses age-related memory loss but not interest in exploration (novel object recognition test) in 18-mo old mice ($P \leq 0.05$, n=7-10). [301].

Supporting references

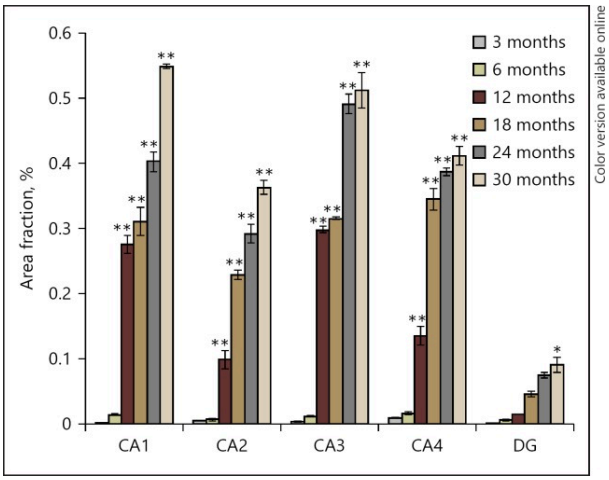
1. [301] "The bile acid TUDCA reduces age-related hyperinsulinemia in mice" (2022) https://doi.org/10.1038/s41598-022-26915-3
2. [246] "Cognitive Impairment and Endoplasmic Reticulum Stress Induced by Repeated Short-Term Sevoflurane Exposure in Early Life of Rats" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6083612/
3. [247] "Chemical Chaperones Reduce ER Stress and Restore Glucose Homeostasis in a Mouse Model of Type 2 Diabetes" (2006) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4741373/
4. [248] (Review) "Tauroursodeoxycholic acid: a potential therapeutic tool in neurodegenerative diseases" (2022) https://translationalneurodegeneration.biomedcentral.com/articles/10.1186/s40035-022-00307-z
5. [249] (Review) "Elimination of endoplasmic reticulum stress and cardiovascular, type 2 diabetic, and other metabolic diseases" (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3581057/
6. [250] "Tauroursodeoxycholic acid suppresses amyloid β-induced synaptic toxicity in vitro and in APP/PS1 mice" (2012) https://doi.org/10.1016/j.neurobiolaging.2012.04.018
7. [251] "Tauroursodeoxycholic acid increases neural stem cell pool and neuronal conversion by regulating mitochondria-cell cycle retrograde signaling" (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4613652/
8. [252] "Protective effects of tauroursodeoxycholic acid on lipopolysaccharide-induced cognitive impairment and neurotoxicity in mice" (2017) https://doi.org/10.1016/j.intimp.2019.03.065
9. [252] "Mitochondria: Major Regulators of Neural Development" (2015) https://doi.org/10.1177/1073858415585472
10. [253] "Tauroursodeoxycholic Acid Enhances Mitochondrial Biogenesis, Neural Stem Cell Pool, and Early Neurogenesis in Adult Rats" (2018) https://www.ncbi.nlm.nih.gov/pubmed/28534273
11. [254] "Anti-inflammatory effect of Tauroursodeoxycholic acid in RAW 264.7 macrophages, Bone marrow-derived macrophages, BV2 microglial cells, and spinal cord injury" (2018) https://www.nature.com/articles/s41598-018-21621-5
12. [255] "TLR4 promoted endoplasmic reticulum stress induced inflammatory bowel disease via the activation of p38 MAPK pathway" (2022) https://doi.org/10.1042/BSR20220307
13. [256] "Tauroursodeoxycholic acid (TUDCA) supplementation prevents cognitive impairment and amyloid deposition in APP/PS1 mice" (2012) https://doi.org/10.1016/j.nbd.2012.09.003
14. [258] "Tauroursodeoxycholic Acid Protects Hepatocytes From Ethanol-Fed Rats Against Tumor Necrosis Factor–Induced Cell Death by Replenishing Mitochondrial Glutathione" (2001) DOI: 10.1053/jhep.2001.28510
15. [259] "Bile acid TUDCA improves insulin clearance by increasing the expression of insulin-degrading enzyme in the liver of obese mice" (2017) https://www.nature.com/articles/s41598-017-13974-0
16. [260] "Tauroursodeoxycholic Acid Alleviates H2O2-Induced Oxidative Stress and Apoptosis via Suppressing Endoplasmic Reticulum Stress in Neonatal Rat Cardiomyocytes" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6052504/
17. [261] "Tauroursodeoxycholic acid mediates endoplasmic reticulum stress and autophagy in adrenocortical carcinoma cells" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6888259/
18. [268] "Amyloid-β pathology is attenuated by tauroursodeoxycholic acid treatment in APP/PS1 mice after disease onset" (2015) https://doi.org/10.1016/j.neurobiolaging.2014.08.034

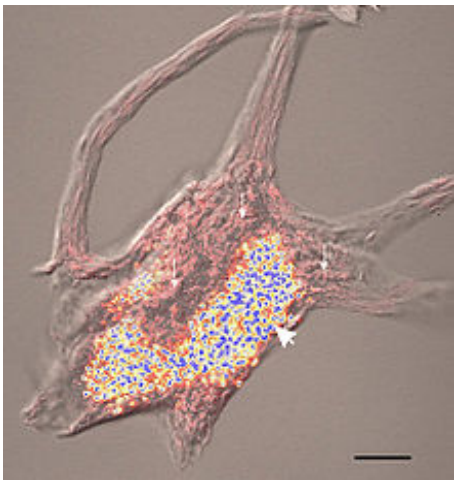
19. (Review) “Effects of bile acids on neurological function and disease” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5067249/
20. “TUDCA Promotes Phagocytosis by Retinal Pigment Epithelium via MerTK Activation” (2015) https://doi.org/10.1167/iovs.14-15962
21. [295] “Tauroursodeoxycholic Acid Mitigates High Fat Diet-Induced Cardiomyocyte Contractile and Intracellular Ca2+ Anomalies” (2017) https://doi.org/10.1371/journal.pone.0154907
22. [296] “Tauroursodeoxycholate (TUDCA), chemical chaperone, enhances function of islets by reducing ER stress” (2010) https://doi.org/10.1016/j.bbrc.2010.06.022

Lipofuscin accumulation, ALCAR and centrophenoxine

Lipofuscin is the fine brownish fatty pigments of lipid-containing residues of incomplete lysosomal digestion. It is identified by its distinct yellowish auto-fluorescence. Numerous studies indicate that the formation of lipofuscin is due to the oxidative alteration of macromolecules by oxygen-derived free radicals generated in reactions catalyzed by redox-active iron of low molecular weight. The accumulation of lipofuscin within cells is associated with aging and is a recognized hallmark of aging. https://en.wikipedia.org/wiki/Lipofuscin

The removal of lipofuscin from non-dividing cells, such as neurons and cardiomyocytes, by an exocytosis transport mechanism to capillaries has been observed in mouse cardiomyocytes. "Observation of the Transport and Removal of Lipofuscin from the Mouse Myocardium using Transmission Electron Microscope" (2020) https://www.biorxiv.org/content/10.1101/2020.03.10.985507v1.full





(Left) Quantitative data of percent area fraction of lipofuscin accumulation in hippocampal subregions of different age groups of rats. Values are presented as mean ± SEM (n = 3). **p < 0.001, *p < 0.050 for comparison of the increase in the rate of lipofuscin between various age groups. "A Sequential Study of Age-Related Lipofuscin Accumulation in Hippocampus and Striate Cortex of Rats" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6470335/
(Right) Confocal image of a spinal motor neuron showing stained lipofuscin granules in blue and yellow. https://en.wikipedia.org/wiki/Lipofuscin

The reduction of accumulated lipofuscin is a complex topic with mixed experimental results. Summary points are listed below.

Centrophenoxine (meclofenoxate)

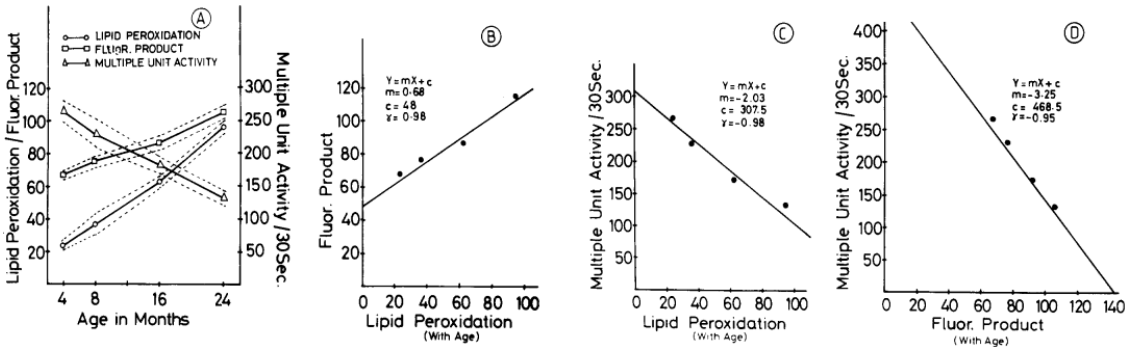
1. In some studies, centrophenoxine was shown to reduce lipofuscin deposits in the hippocampus and prefrontal cortex.
    a. 11-12 month-old mice injected intraperitoneally 80 mg/kg centrophenoxine for 3 months showed a 20% reduction of lipofuscin [242]. "Centrophenoxine: effects on aging mammalian brain" (1978) https://doi.org/10.1111/j.1532-5415.1978.tb02544.x
    b. 24-26 month-old rats were injected intraperitoneally 80 mg/kg centrophenoxine for 8 weeks. Lipofuscin accumulation was reduced by about 37% over 8 weeks compared to young (8-9 mo) rats [243]. "Effects of centrophenoxine on the lipofuscin pigments in the nervous system of old rats" (1974) https://doi.org/10.1016/0006-8993(74)90864-6

2. However, other studies showed no significant reduction in lipofuscin by centrophenoxine.
    a. "An Overview of the Role of Lipofuscin in Age-Related Neurodegeneration" (1986) https://doi.org/10.1016/0197-4580(86)90148-X
    b. "Centrophenoxine Slows Down, but Does Not Reverse, Lipofuscin Accumulation in Cultured Cells" (2009) https://www.liebertpub.com/doi/10.1089/rej.1.1999.2.265

3. Rats injected with dose 100 mg/kg centrophenoxine showed significant reduction in aluminum accumulation in the brain. Aluminum is also known as a potent neurotoxicant that accumulates in the brain and causes cognitive deficits [278].

"Evidence for centrophenoxine as a protective drug in aluminum induced behavioral and biochemical alteration in rat brain" (2006) DOI: 10.1007/s11010-006-9125-7

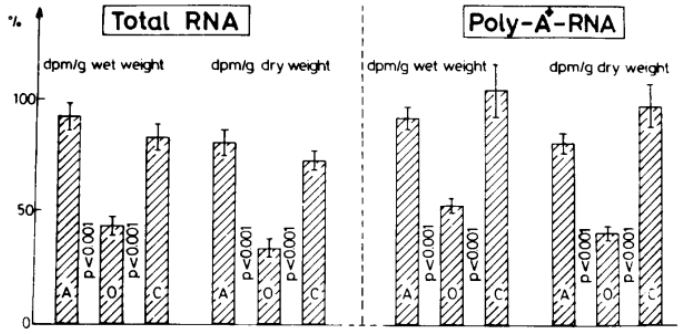
4. Centrophenoxine was shown to restore age-related decline in MUA (Multiple Unit Action potential, a measure of neuronal activity by firing rate). Rats injected with dose 80 mg/kg centrophenoxine showed significant reductions in lipofuscin and lipid peroxidation over 10, 20, and 30 days in old rats (16-24 months). There was a corresponding significant increase in MUA [239]. Another study of rats injected with centrophenoxine showed a 50% increase in MUA after 30 days at 120 mg/kg or after 45 days at 80 mg/kg, after which time MUA plateaued [264].



Age-related changes in lipid peroxidation, fluorescent products (i.e., lipofuscin), and multiple unit activity (MUA) [264]. "Age-related decline in multiple unit action potentials of CA3 region of rat hippocampus: correlation with lipid peroxidation and lipofuscin concentration and the effect of centrophenoxine." (1993) https://doi.org/10.1016/0197-4580(93)90117-T

5. When chronically administered orally (60 mg/day in water), mice treated from the age of 8 months had a 29% increase in medium lifespan (p=0.039) [263]. The human equivalent dose (HED) of this treatment is 340 mg/day for a 70 kg individual. "EFFECT OF DIMETHYLAMINOETHYL p-CHLOROPHENOXYACETATE ON THE LIFE SPAN OF MALE SWISS WEBSTER ALBINO MICE " (1973) https://doi.org/10.1016/0531-5565(73)90024-7

6. Total RNA synthesis was restored in the brain cortex of old (26-mo) rats to that of adult (13-mo) rats by intraperitoneal injection with centrophenoxine, 100 mg/kg body weight/day for 2 months [280]. This result is consistent with the membrane hypothesis of aging [280].



The rates of total and polyA+RNA synthesis in the brain cortex of 6 adult (A), 7 old (O), and 3 centrophenoxine treated, old (C) rats [280]. "CENTROPHENOXINE INCREASES THE RATES OF TOTAL AND mRNA SYNTHESIS IN THE BRAIN CORTEX OF OLD RATS: AN EXPLANATION OF ITS ACTION IN TERMS OF THE MEMBRANE HYPOTHESIS OF AGING" (1983) DOI: 10.1016/0531-5565(84)90035-4

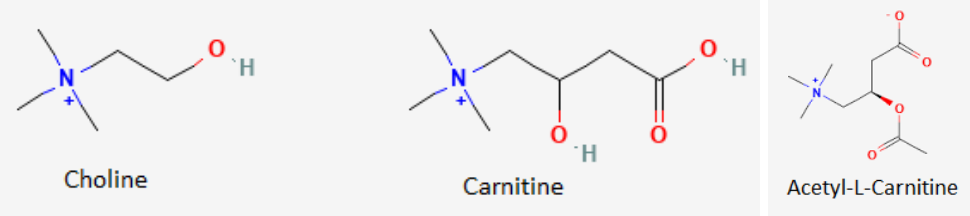
7. A small trial on older individuals (70 to 72-years-old), 600 mg 2x/day centrophenoxine boosted memory and increased alertness [281]. "The differential effects of meclofenoxate on memory loss in the elderly" (1977) https://doi.org/10.1093/ageing/6.2.123

8. From these results, it can be inferred that a 30 to 60 day treatment of 300 - 500 mg 1x/day centrophenoxine is needed for effective reduction of lipofuscin and aluminum accumulations, and for the restoration of RNA synthesis, in older individuals.

9. The DMAE moiety of centrophenoxine can enter the choline synthesis cycle and consequently increase the brain's acetylcholine supply. This may lead to feelings of depression in some individuals. "Centrophenoxine improves chronic cerebral ischemia induced cognitive deficit and neuronal degeneration in rats" (2005) https://pubmed.ncbi.nlm.nih.gov/15569402/
    a. **In my case, 300 mg/day centrophenoxine led to depression within a week, probably through an increase in choline levels. I discontinued taking it after 10 days.**
    b. Separately, a literature review found that the purported benefits of centrophenoxine to be mixed and marginal. https://brain.forever-healthy.org/display/EN/Centrophenoxine

Acetyl-L-Carnitine (ALCAR)

An alternative to centrophenoxine is acetyl-L-carnitine (ALCAR). ALCAR has been shown to reduce lipofuscin deposits in hippocampal neurons in a dose-dependent manner [285]. Whereas the mechanism of centrophenine's effects is through its DMAE moiety causing an increase in the synthesis choline, in comparison, ALCAR has a chemical structure that is similar to choline, but unlike choline, carnitine is not a neurotransmitter and has fewer reported side effects. "Anti-aging Effects Of Acetyl-L-Carnitine" (2000) https://www.lifeextension.com/magazine/2000/5/cover2

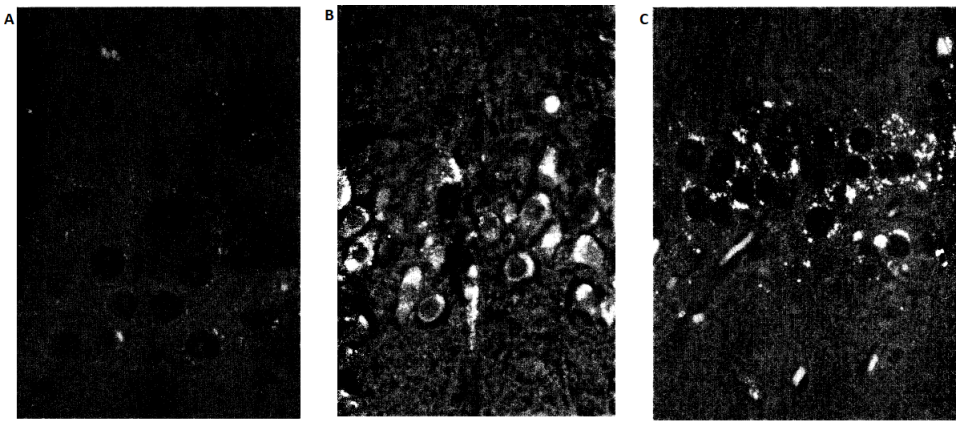


Similar to centrophenoxine, ALCAR has been shown to reduce lipofuscin in the brain and increase neuron firing rates (MUA). Summary points are:

1. 20 month-old rats fed 100 mg/kg ALCAR for 3 months showed a 46% decrease in hippocampal lipofuscin compared to a 23-month-old control group [285]. However, lipofuscin levels were still significantly higher than those of 5-month-old rats [285]. "Effects of Acetyl-L-Carnitine on the Brain Lipofuscin Content and Emotional Behavior in Aged Rats" (1988) https://doi.org/10.1254/jjp.48.365

Age	Dose (mg/kg/day)	N	Brain regions		
			Frontal cortex	Hippocampus	Nucleus oliva
23-month-old	0	6	11.71±0.43	7.29±0.25	18.26±0.83
	50	8	10.44±0.42	6.42*±0.20	17.45±0.64
	100	7	7.31**±0.41	3.92**±0.26	17.07±0.86
5-month-old	0	8	0.23±0.05	0.13±0.03	3.34±0.26

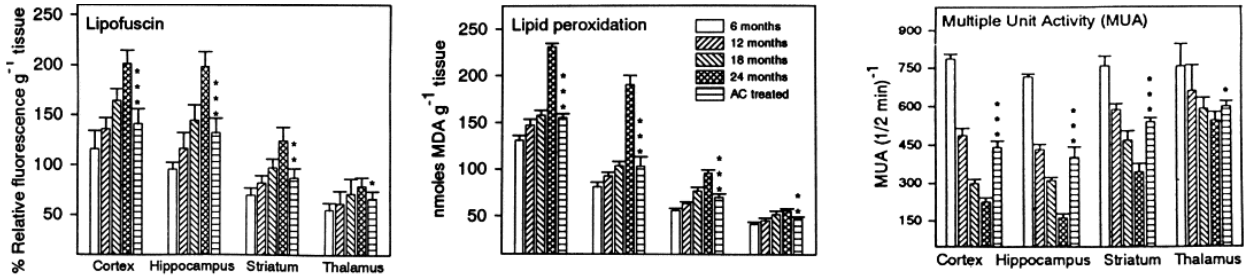
Lipofuscin deposition in nerve cells after dosing with ALCAR for 3 months;*p<0.05, **p<0.01 [285]. "Effects of Acetyl-L-Carnitine on the Brain Lipofuscin Content and Emotional Behavior in Aged Rats" (1988) https://doi.org/10.1254/jjp.48.365



Effect of acetyl-L-carnitine on the lipofuscin deposition in the hippocampus of (A) 5-month-old untreated rats, (B) 23-month-old untreated rats, and (C) 23-month-old rats after dosing with 100 mg/kg ALCAR for 3 months [285]. “Effects of Acetyl-L-Carnitine on the Brain Lipofuscin Content and Emotional Behavior in Aged Rats” (1988) https://doi.org/10.1254/jjp.48.365

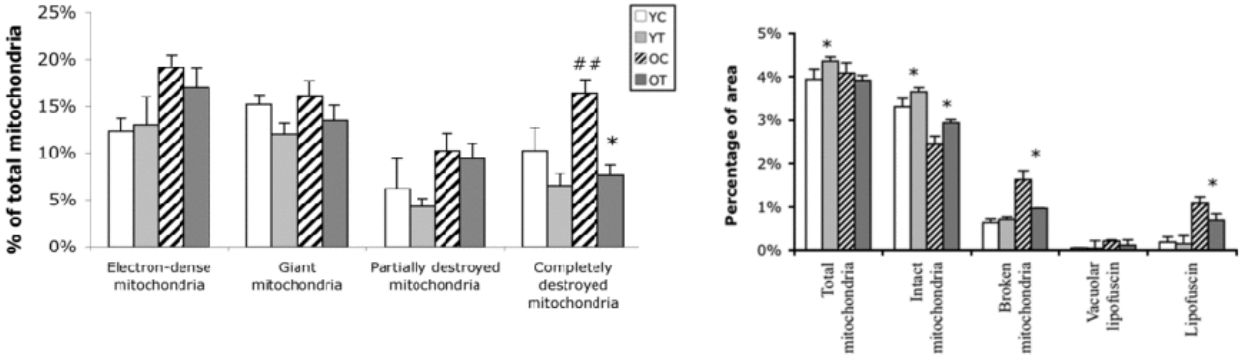
2. 20 month-old rats fed 50 mg/kg ALCAR from the age of 11 months showed 20% less hippocampal lipofuscin than untreated rats. Young, 3-month-old rats, had undetectable lipofuscin levels [288]. "Reduced lipofuscin accumulation in senescent rat brain by long-term acetyl-L-carnitine treatment" (1989) https://doi.org/10.1016/0167-4943(89)90035-6

3. ALCAR reduced or limited the levels of lipofuscin and lipid peroxidation in old (24 month-old) rats to that of middle-aged rats after IP injected with 75 mg/kg ALCAR for 3 months [289]. “Acetyl-l-carnitine enhances Na+, K+-ATPase glutathione-s-transferase and multiple unit activity and reduces lipid peroxidation and lipofuscin concentration in aged rat brain regions” (2001) https://doi.org/10.1016/S0304-3940(01)01576-2



Effects of ALCAR on ageing parameters from 6 to 24 months [289]. “Acetyl-l-carnitine enhances Na+, K+-ATPase glutathione-s-transferase and multiple unit activity and reduces lipid peroxidation and lipofuscin concentration in aged rat brain regions” (2001) https://doi.org/10.1016/S0304-3940(01)01576-2

4. 21-month-old rats given 0.2% ALCAR in drinking water for 3 months showed increased the proliferation of intact mitochondria and reduced the density of mitochondria associated with vacuoles and lipofuscin [290]. The human equivalent dose is about 2100 mg/day. “Neuronal mitochondrial amelioration by feeding acetyl-L-carnitine and lipoic acid to aged rats” (2009) https://doi.org/10.1111/j.1582-4934.2008.00324.x



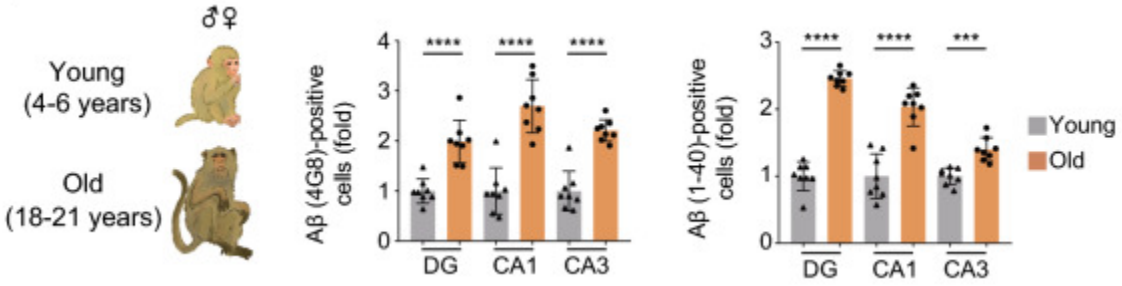
The effect of ALCAR + LA dietary supplementation on the distribution of the different types of mitochondria. YC, young control non-treated rats; YT, young rats treated with ALCAR + LA dietary supplementation; OC, old control rats non-treated; OT, old rats treated with ALCAR + LA. *P < 0.05, ##P < 0.01 [290]. “Neuronal mitochondrial amelioration by feeding acetyl-L-carnitine and lipoic acid to aged rats” (2009) https://doi.org/10.1111/j.1582-4934.2008.00324.x

From these studies, I can infer that about 1000 mg/day ALCAR for 3 months may reduce hippocampal lipofuscin and improve neuron firing rates (MUA) in older or aged individuals (those older than about 65 years).

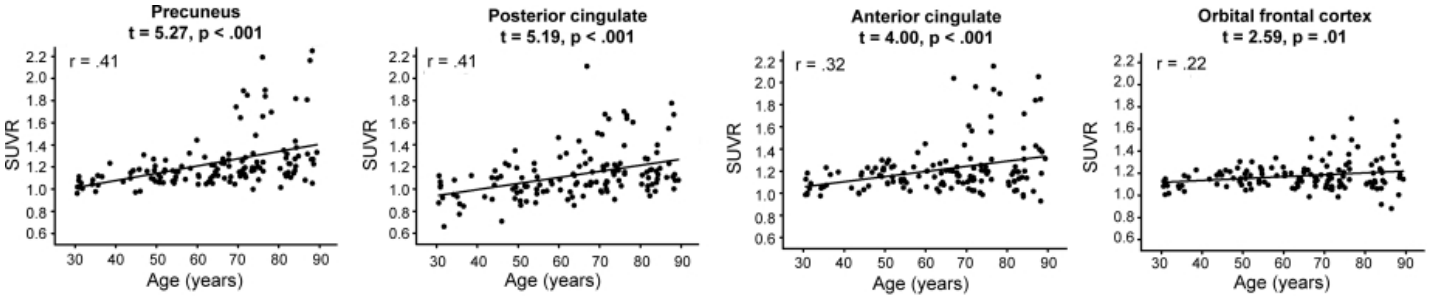
Amyloid β plaque accumulates in the aged brain; is associated with poorer processing speed and working memory

In healthy primates and humans, the burden of β-Amyloid plaque accumulates with age, particularly in APOE ϵ4 allele carriers. In humans, this accumulation is correlated with a decrease in mental processing speed, working memory and fluid reasoning. "β-Amyloid burden in healthy aging" (2012) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3280058/

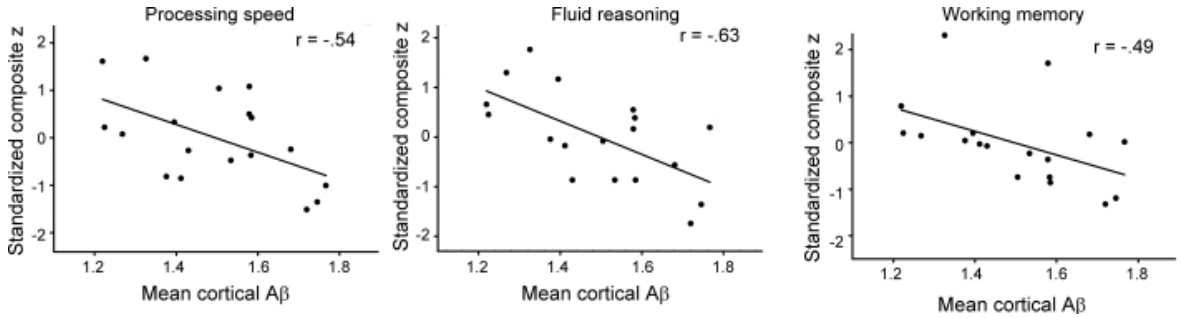
As discussed in the section on misfolded proteins and Cellular protein aggregates, in humans, 500 mg 2x/day for 3 months appears to be a safe and effective dose for decreasing Aβ plaque and tau aggregates [268, W].



Accumulation of amyloid-β types Aβ(4G8) and Aβ(1-40) in the indicated regions of the hippocampus from young and old monkeys. DG = dentate gyrus, and CA1 and CA3 designate regions of the hippocampus. Young monkeys are equivalent to ~16 years-old humans and old are equivalent to ~60 years-old. ***P < 0.001, ****P < 0.0001. "Single-nucleus transcriptomic landscape of primate hippocampal aging" (2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8403220/



Regional increases in β-amyloid deposition across the lifespan of healthy humans. "β-Amyloid burden in healthy aging" (2012) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3280058/



Effect of elevated β-amyloid (Aβ) deposition on processing speed, working memory, and fluid reasoning. "β-Amyloid burden in healthy aging" (2012) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3280058/

Hesperidin reduces TNF-α and restores age-related decline in memory and cognition

Hesperidin is a flavanone glycoside found in citrus fruits with biological effects that are similar to related compounds naringin and diosmetin. A combination of factors contribute to hesperidin's effects on the restoration of age-related decline in memory and cognition including suppression of inflammation, elevation of serotonin, and degradation of amyloid β plaque and tau protein aggregates [16,17,27,49,50,51,58,71,146], as reviewed in [271].

1. Hesperidin is a potent inhibitor of inflammatory cytokines TNF-α, IL-1β and IL-6 that may inhibit BDNF and neuroplasticity [1,59,60,90].
2. Hesperidin interacts with the serotonergic receptor 5-HT(1A) and elevates serotonin levels, thereby producing an antidepressant effect comparable to fluoxetine (Prozac) [10,11,15,57]
3. Hesperidin clears amyloid β plaque and tau protein aggregates [51]
4. Hesperidin reduces fat and improves glucose homeostasis [270]
5. Hesperetin slows down skeletal muscle aging [270]
6. Hesperetin slows down cardiac aging [270]
7. Hesperetin treatment results in a younger transcriptome pattern [270]
8. Hesperidin increases the lifespan of mice by 8.7% when fed ad libitum at 100 mg/kg, in part through activation of the CISD2 gene. CISD2 regulates autophagy, maintains Ca2+ homeostasis, mitochondrial function, and glucose homeostasis to mediate lifespan and healthspan [270].

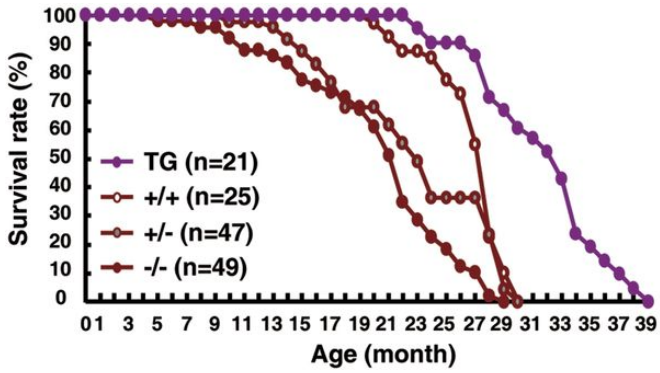
Additionally, hesperiden lowers systolic blood pressure (SBP) in in hypertensive individuals by 9%, possibly through a decrease in inflammatory factors, modulation of nitric oxide (NO) metabolites, and suppression of angiotensin [135,140,271]. For reference, those with a SBP of 140 mm Hg and higher have significantly higher risk of stroke or cardiovascular death. Those higher risk individuals should target their SBP to 130 mm Hg or less [136].

Hesperetin reduces degradation of neurons, increases CISD2 and shifts gene patterns towards a younger transcriptome

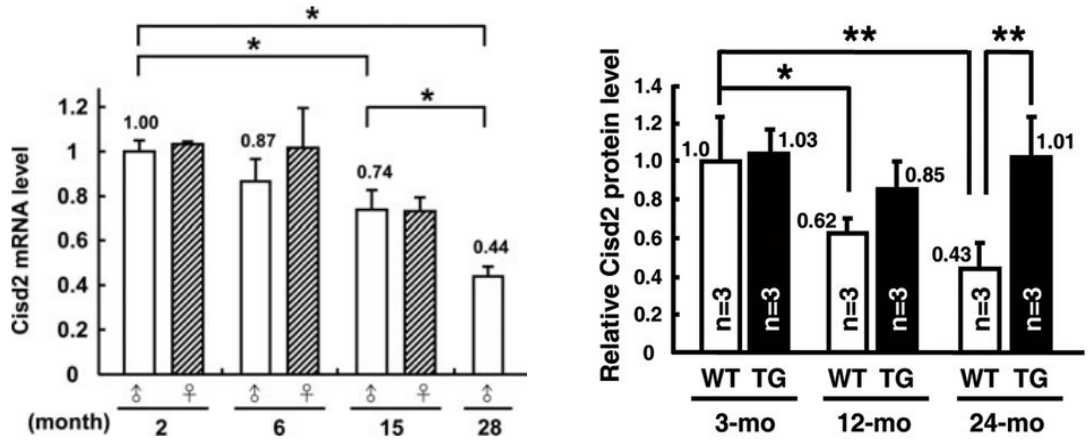
Hesperetin is an effective Cisd2 activator. The Cisd2 protein locates in the membranes of endoplasmic reticulum (ER) and mitochondria; protects mitochondria from age-associated damage and functional decline; maintains Ca2+ homeostasis; and significantly ameliorates age-associated degeneration of the skin, skeletal muscles and neurons [270,274].

Research has demonstrated that CISD2 expression decreases with age, and that engineering transgenic mice to overexpress Cisd2 significantly extends their medium and maximum lifespan by about 30% from 30 months to a remarkable 39 months. In contrast, hesperidin increases the medium lifespan of mice by 8.7% with a maximum of 33.6 months [270].

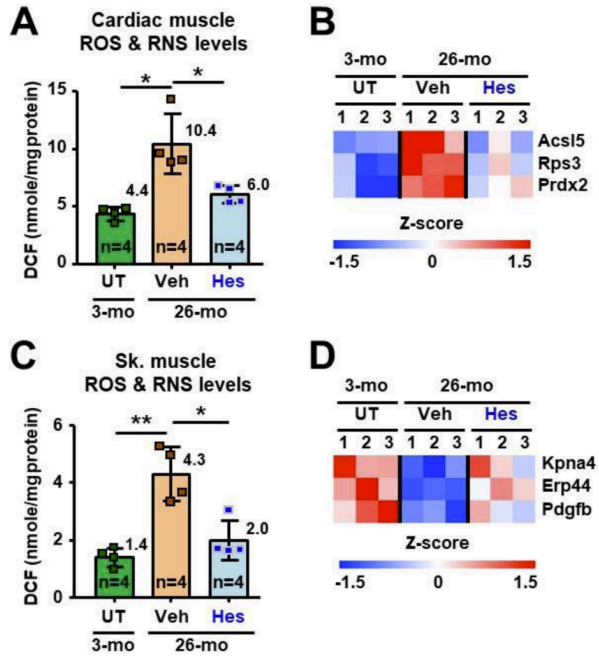
1. "A role for the CISD2 gene in lifespan control and human disease" (2010) https://doi.org/10.1111/j.1749-6632.2010.05619.x
2. "A persistent level of Cisd2 extends healthy lifespan and delays aging in mice" (2012) https://doi.org/10.1093/hmg/dds210
3. "Cisd2 mediates lifespan: is there an interconnection among Ca2+ homeostasis, autophagy, and lifespan?" (2014) https://doi.org/10.3109/10715762.2014.936431
4. [270] "Hesperetin promotes longevity and delays aging via activation of Cisd2 in naturally aged mice" (2022) https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-022-00838-7
5. [274] "Upregulation of Cisd2 attenuates Alzheimer's-related neuronal loss in mice maintains Ca2+ homeostasis" (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7065100/



Lifespan of Cisd2 knockout (+/-), (-/-); wild-type (+/+); and transgenic (TG) mice. "A persistent level of Cisd2 extends healthy lifespan and delays aging in mice" (2012) https://doi.org/10.1093/hmg/dds210



Age-dependent decrease of Cisd2 expression in the brain (left) and skeletal muscle (right) in naturally aged wild-type and Cisd2+/+ transgenic (TG) mice (*P < 0.05; **P < 0.005). "A role for the CISD2 gene in lifespan control and human disease" (2010) https://doi.org/10.1111/j.1749-6632.2010.05619.x; "A persistent level of Cisd2 extends healthy lifespan and delays aging in mice" (2012) https://doi.org/10.1093/hmg/dds210

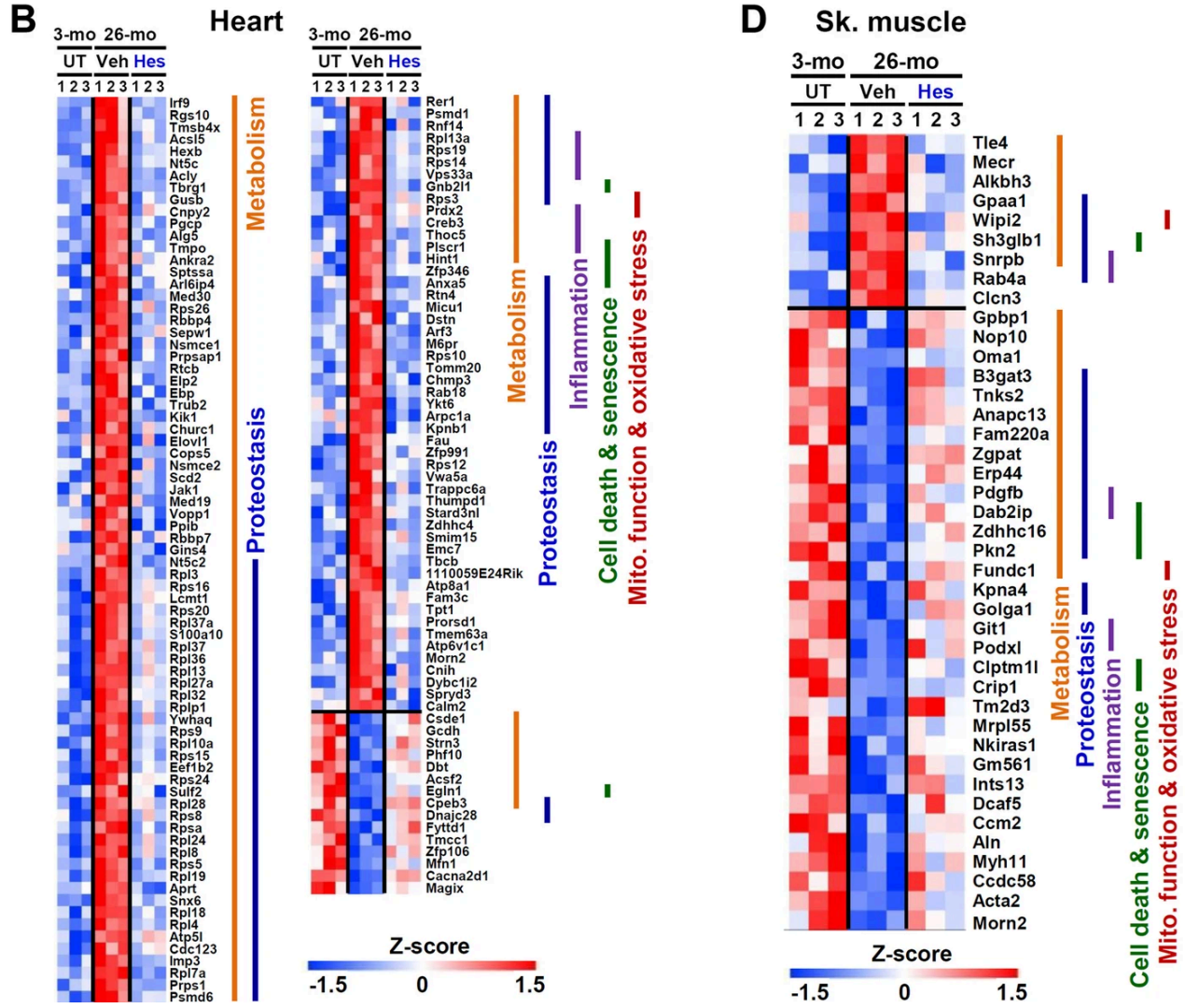


Gene	GO or KEGG terms	Functional description by GeneCards
Acsl5	Peroxisome (KEGG)	Catalyzes the conversion of long-chain fatty acids to their active form acyl-CoAs for both synthesis of cellular lipids, and degradation via beta-oxidation.
Rps3	Cellular response to hydrogen peroxide	When located in the mitochondrion, reduces cellular ROS levels and mitochondrial DNA damage
Prdx2	Cellular response to hydrogen peroxide. Regulation of ROS metabolic process	Thiol-specific peroxidase that catalyzes the reduction of hydrogen peroxide and organic hydroperoxides to water and alcohols, respectively.

Gene	GO or KEGG terms	Functional description by GeneCards
Kpna4	Response to oxidative stress	Functions in nuclear protein import as an adapter protein for nuclear receptor KPNB1.
Erp44	Cell redox homeostasis	Mediates thiol-dependent retention in the early secretory pathway, forming mixed disulfides with substrate proteins through its conserved CRFS motif.
Pdgfb	Regulation of ROS metabolic process	Growth factor that plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis.

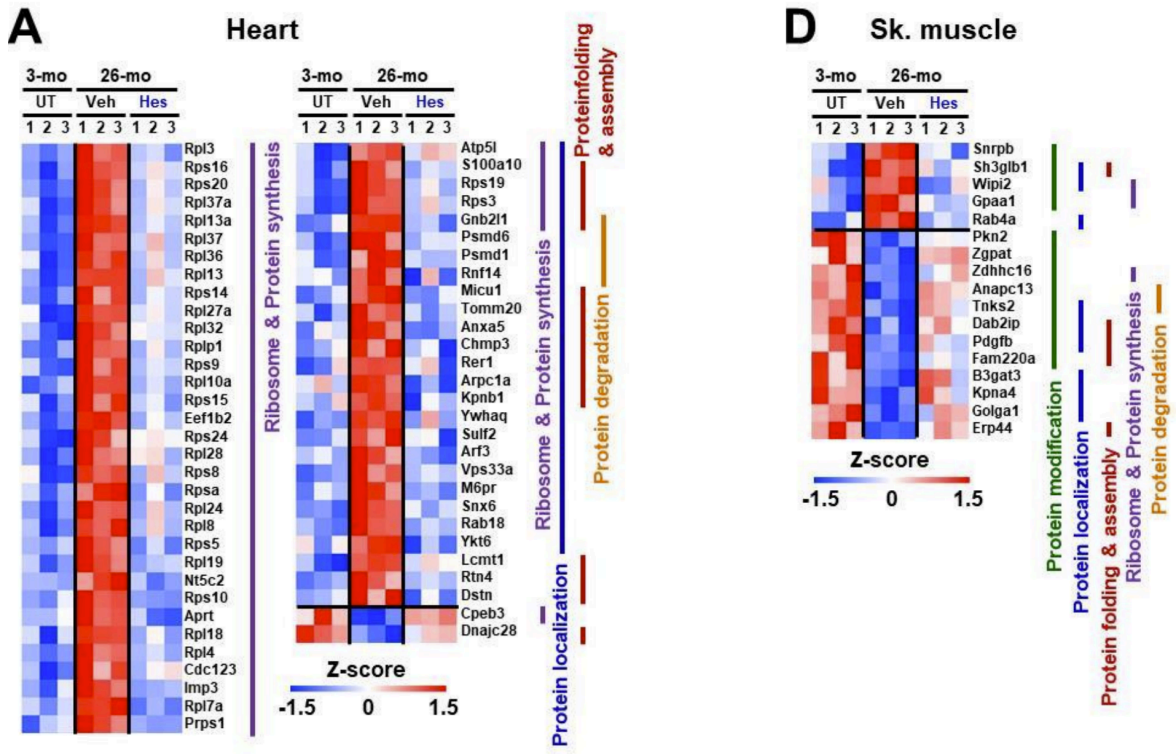
Hesperidin decreases levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and shifts the gene expression patterns of several ROS-related differentially expressed genes (DEGs) in healthy, aged hearts and skeletal muscles towards the patterns of young mice [270]. “Hesperetin promotes longevity and delays aging via activation of Cisd2 in naturally aged mice” (2022) https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-022-00838-7

100 mg/kg hesperidin feed to 20-month-old mice (equivalent to a 60-year-old human) for 6 months restored function and gene expression in cardiac and skeletal muscle similar to that of 3-month-old mice, in part through upregulation of Cisd2.



B) Transcriptome heatmap illustrating that a total of 141 differentially expressed genes (DEGs) are reversed by dietary hesperetin (126 up-regulated and 15 down-regulated genes; 26-month WT-Veh vs 3-month WT, FDR < 0.1) and the aged heart pattern is moved toward the pattern of a young heart.

D) Heatmap illustrating that all 41 DEGs that are reversed by dietary hesperetin (9 up-regulated and 32 down-regulated genes; 26-month WT-Veh vs 3-month WT, FDR < 0.1) and the aged skeletal muscle (gastrocnemius); this showing a movement toward the pattern present in young skeletal muscle [270]. A 26-month-old mouse is equivalent to an 85-year-old human. “Hesperetin promotes longevity and delays aging via activation of Cisd2 in naturally aged mice” (2022) https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-022-00838-7



Subgroups of proteostasis-related differentially expressed genes (DEGs) in the hearts and skeletal muscle of mice [270]. "Hesperetin promotes longevity and delays aging via activation of Cisd2 in naturally aged mice" (2022) https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-022-00838-7

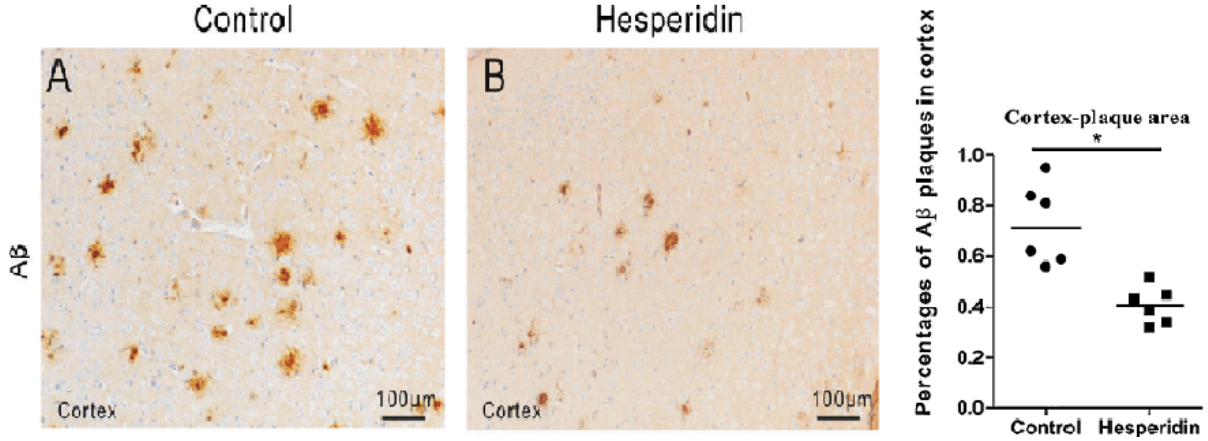
Human equivalent dose of hesperidin for reducing amyloid β plaque

100 mg/kg hesperidin administered by oral gavage for 10 days reduced amyloid β plaque in a mouse model of Alzheimer's disease [51]. Similar neuroprotective effects have been observed in related compounds naringin and diosmetin [49,50,66]. The human equivalent dose of hesperidin used in this study is calculated as [N]

HED (mg/kg) = Animal Dose (mg/kg) x [Animal K_m / Human K_m] where the scaling factor K_m = 3 in mice, 37 in humans.

(100 mg/kg in mice) (3/37) = 8.1 mg/kg in humans

For a 70 kg individual = 567 mg



Effect of hesperidin on amyloid β deposition. Representative micro-photos of the coronal section through cortex and hippocampus show the reduction of amyloid β deposition following 10 days of hesperidin treatment. In the cortex of control mice (A), more and relatively larger-size amyloid β plaques were observed compared to the hesperidin-treated mice (B). Area percentages show significant reduction (n = 6). *P < 0.05 [51]. "Hesperidin ameliorates behavioral impairments and neuropathology of transgenic APP/PS1 mice" (2015) https://doi.org/10.1016/j.bbr.2014.12.012

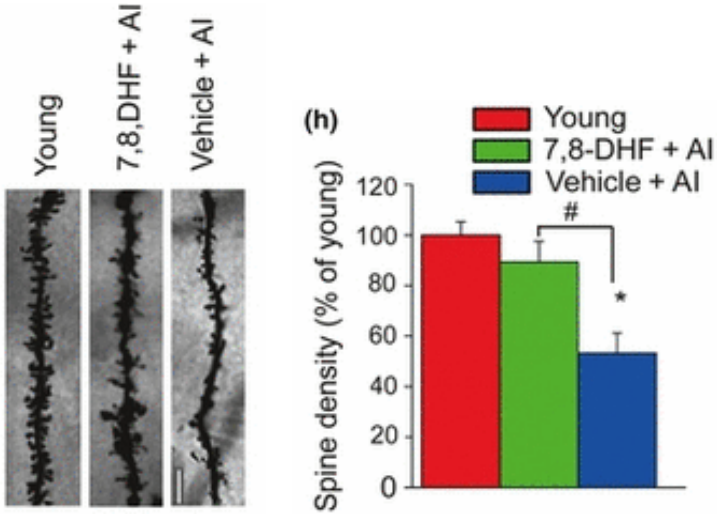
7,8-DHF potently promotes BDNF, sprouts dendritic spines and improves learning

7,8-dihydroxyflavone (7,8-DFH or tropoflavin) is a small molecule high affinity agonist of the BDNF receptor TrkB. It is found in nature in Godmania aesculifolia, Tridax procumbens, and primula tree leaves. Among phytosubstances, 7,8-DHF is rare in its ability to potently promote BDNF. https://en.wikipedia.org/wiki/Tropoflavin

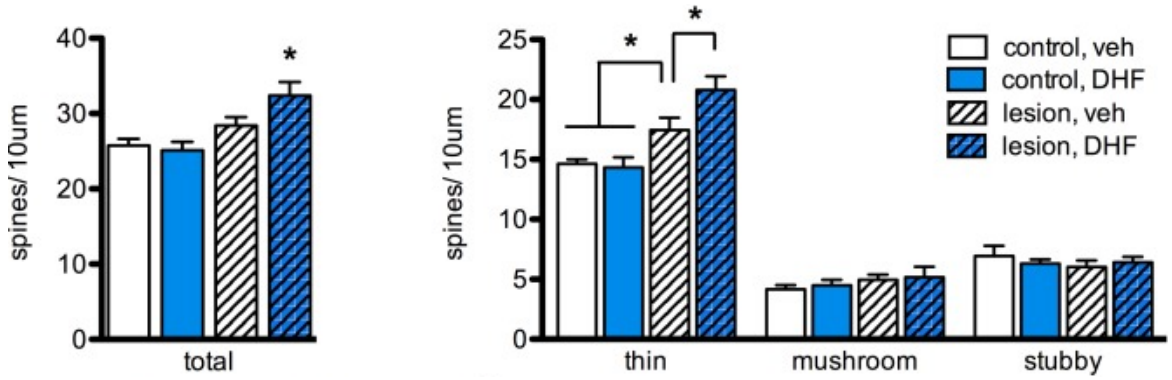
The structural changes to the brain induced by 7,8-DFH are summarized as [214]:

- Protected cortical neurons against reduced dendritic arbor complexity
- Promotes synapse outgrowth
- Promotes dendritic spine outgrowth
- No significant impact on the density of dendritic spines.
- Decreased cortical Aβ plaque deposition

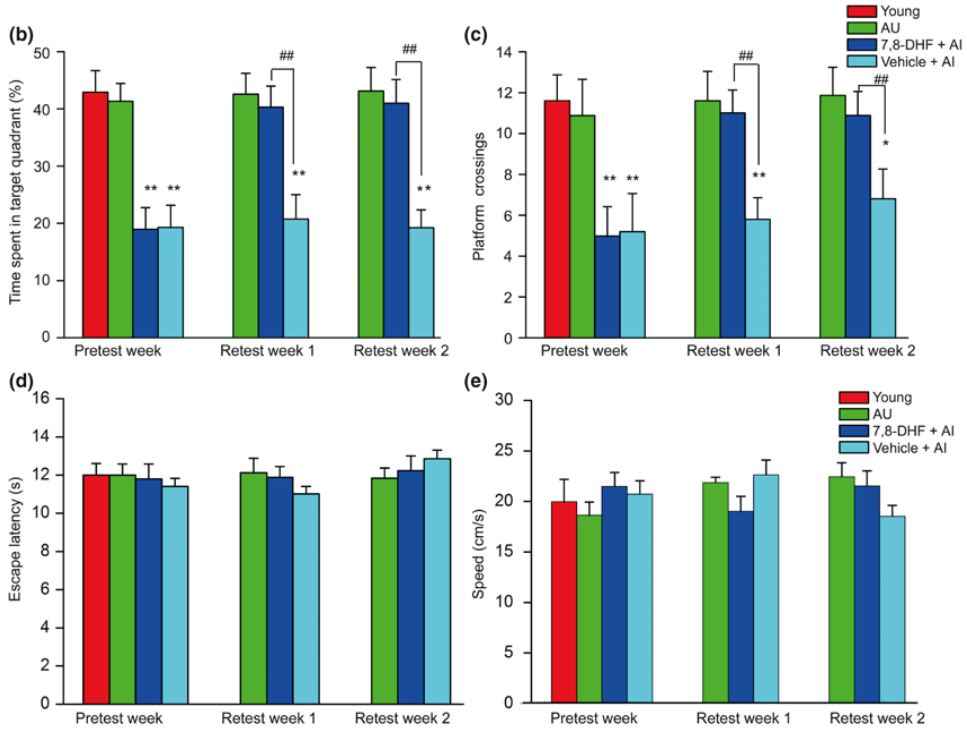
7,8-DHF has been studied in the context of alcoholism, where its effect on BDNF-TrkB signaling reduces alcohol-induced memory impairment in rats from a human equivalent dose of 50 mg/day [143-145].



7,8-DHF modifies dendritic spines and facilitates synaptic plasticity in the hippocampus of aged impaired rats (22-month-old). The 22 month old mice were separated into two groups: fast learners (AU) and slow learners (AI). Young controls = 3-month-old rats. AU = aged-unimpaired rats. AI = aged-impaired rats [55]. "7,8-dihydroxyflavone rescues spatial memory and synaptic plasticity in cognitively impaired aged rats" (2012) https://onlinelibrary.wiley.com/doi/10.1111/j.1471-4159.2012.07830.x



7,8-DHF treatment increases thin spine density on dendrites in CA1 pyramidal neurons, $p<0.01$ [36]. "7,8-Dihydroxyflavone, a Small Molecule TrkB Agonist, Improves Spatial Memory and Increases Thin Spine Density in a Mouse Model of Alzheimer Disease-Like Neuronal Loss" (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3948846/



7,8-DHF improves spatial learning and memory in 22-month-old aged impaired rats. The 22-month-old mice were separated into two groups: fast learners (AU) and slow learners (AI). Hidden platform test. (b) Probe trials. AU rats and 7,8-DHF-treated AI rats showed comparable task retention, while vehicle-treated AI rats showed retention deficit on two retests. (c). Platform crossings. Both AU rats and 7,8- DHF-treated AI rats displayed more platform crossings compared to the vehicle-treated AI rats on two retests. (d). The visible platform task. There were no significant differences between any groups for the latency to find the visible platform throughout the three test weeks. (e). The swimming speed. There was no significant difference in swimming speed among each animal group throughout the three test weeks.
Young controls = 3-month-old rats. AU = aged-unimpaired rats. AI = aged-impaired rats. 7,8-DHF was administered at 5 mg/kg by daily i.p. injections [55]. This dose is roughly equivalent to 50 mg for a 70 kg individual.

Lion's mane mushroom (Hericium erinaceus) improves cognitive impairment in elderly humans

Compounds derived from H. erinaceus have been shown to promote NGF synthesis and secretion and to act via the TrkA pathway involving MEK/ERK and PI3K/Akt activation. The NGF/TrkA pathway is known to affect neural growth in the peripheral nervous system (PNS), and H. erinaceus can be expected to offer downstream benefits [28]. Research results showing improvement in cognition in humans and mice suggest that the effects of lion's mane mushroom extend beyond the NGF/TrkA pathway in the CNS.

In a study involving 30 Japanese aged 50-80 who were diagnosed with mild cognitive impairment, a significant cognitive benefit was found from consumption of lion's mane mushroom. After 4 weeks post-trial the cognitive scores declined, suggesting that lion's mane must be taken on an ongoing basis for continued benefit [26]. Patients were instructed to consume 1 gram, 3 times daily of powdered lion's mane mushroom for a period of 16 weeks. Cognitive testing took place at weeks 0, 4, 8, 12, 16 and 4 weeks post-trial [26].

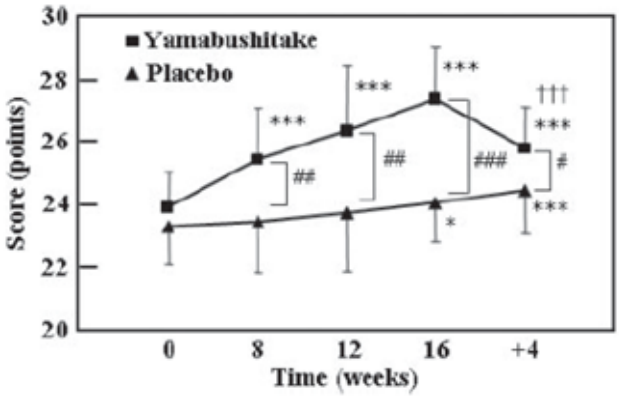
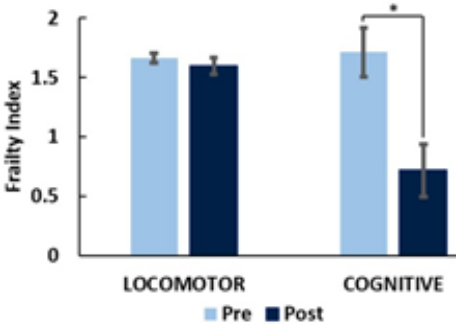


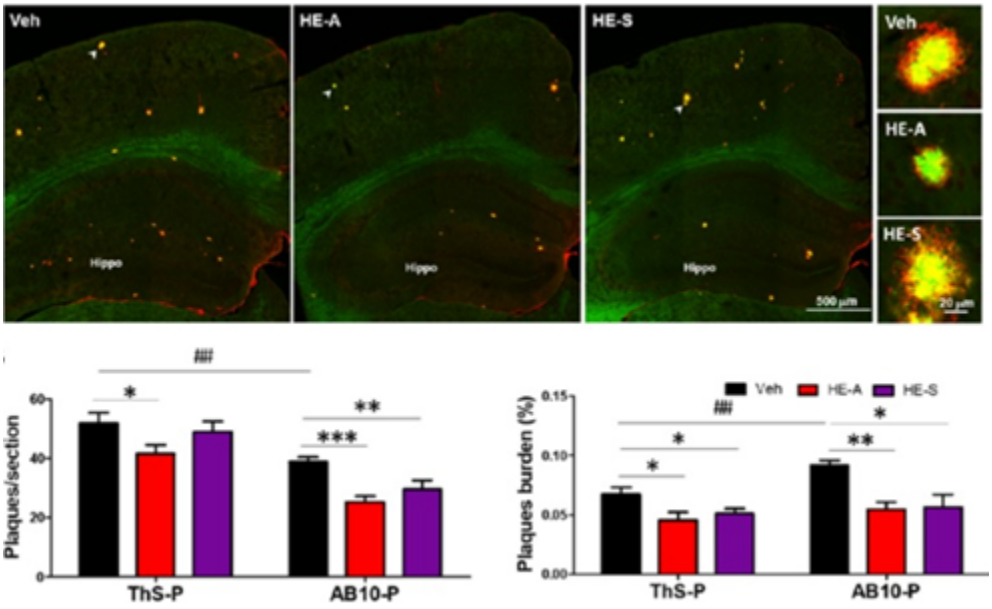
Figure 1. Score of the cognitive function scale. * $p < 0.05$, *** $p < 0.001$ vs week 0. ††† $p < 0.001$ vs week 16. #, ##, ### $p < 0.05$, 0.01, 0.001 Yamabushitake vs placebo at the same time.

A statistically significant benefit was noted among 50- to 80-year-olds diagnosed with mild cognitive impairment at weeks 8, 12 and 16 in the lion's mane group [26]. "Improving effects of the mushroom Yamabushitake (Hericium erinaceus) on mild cognitive impairment: a double-blind placebo-controlled clinical trial" (2008) https://doi.org/10.1002/ptr.2634

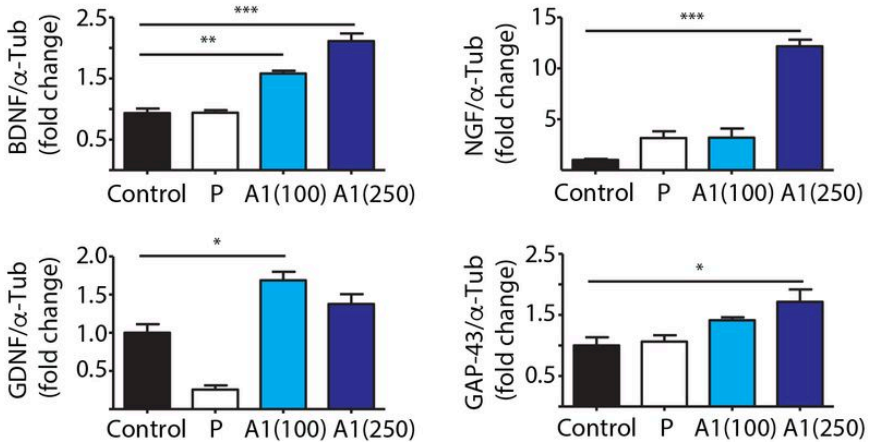


Senescent mice (21.5 weeks old) supplemented with the human equivalent of 1000 mg lion's mane mushroom per day for 2 weeks showed improvement in cognitive but not locomotor scores [24]. "Hericium erinaceus Improves Recognition Memory and Induces Hippocampal and Cerebellar Neurogenesis in Frail Mice during Aging" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6521003/

An extract of Lion's Mane mushroom, when administered at an extremely high dose equivalent to about 20 g/day in humans, has been shown to reduce amyloid plaque burden by about 40% ± 20% in a mouse model of Alzheimer's. Notably, extracts of Lion's Mane mushroom were shown to increase insulin-degrading enzyme (IDE) by 130%. IDE is known to degrade amyloid beta plaque. "Neurohealth Properties of Hericium erinaceus Mycelia Enriched with Erinacines" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5987239/



HE-A and HE-S reduce amyloid plaque burden and size in APP/PS1 mice. APP/PS1 transgenic mice orally administered with vehicle (Veh) and HE-A or HE-S for 30 days (n = 8 for each group). "The Cyanthin Diterpenoid and Sesterterpene Constituents of Hericium erinaceus Mycelium Ameliorate Alzheimer's Disease-Related Pathologies in APP/PS1 Transgenic Mice" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5855820/; "Neurohealth Properties of Hericium erinaceus Mycelia Enriched with Erinacines" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5987239/



Lion Mane mushroom A1 dietary treatment improves recognition memory and increases neurotrophic factors.
Mice were treated with A1 (100 or 250 mg/kg) and piracetam (PC, 400 mg/kg). Representative western blot levels of BDNF, NGF, GDNF and GAP-43 in the whole brain. α-tubulin was used as a loading control. GAP43 is described as a "growth" or "plasticity" protein because it is expressed at high levels in neuronal growth cones during development and axonal regeneration. All data are expressed as the mean ± S.E.M. (n = 3). *P < 0.05, **P < 0.01 and ***P < 0.001 [28]. "Hericerin derivatives from Hericium erinaceus exert BDNF-like neurotrophic activity in central hippocampal neurons and enhance memory" (2020) https://www.biorxiv.org/content/10.1101/2020.08.28.271676v1.full

<u>Human equivalent dose of Hericium erinaceus for upregulation of BDNF</u>

250 mg/kg oral supplementation of lion's mane mushroom exhibited upregulation of BDNF in mice [28]. The human equivalent dose is calculated as [N]

HED (mg/kg) = Animal Dose (mg/kg) x [Animal K_m / Human K_m] where the scaling factor K_m = 3 in mice, 37 in humans.
(250 mg/kg in mice) (3/37) = 20.2 mg/kg in humans
For a 70 kg individual = 1400 mg

I took 2000 mg Lion's Mane powder for several months without apparent effect. In contrast, I took 800 mg/day of extract powder, extracted by alcohol (triterpenes) and by water (beta-glucans), and standardized to 31% beta-glycans, and found the effects clearly bioactive. The alcohol-soluble terpenoids, erinacines A, B and C, are known to stimulate NGF synthesis [184].

1. "Hericerin derivatives from Hericium erinaceus exert BDNF-like neurotrophic activity in central hippocampal neurons and enhance memory" (2020) https://www.biorxiv.org/content/10.1101/2020.08.28.271676v1.full
2. "Hericium erinaceus enhances neurotrophic factors and prevents cochlear cell apoptosis in senescence accelerated mice" (2020) https://doi.org/10.1016/j.jff.2020.103832
3. "Hericium erinaceus Improves Recognition Memory and Induces Hippocampal and Cerebellar Neurogenesis in Frail Mice during Aging" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6521003/
4. "Improvement of cognitive functions by oral intake of Hericium erinaceus" (2019) https://doi.org/10.2220/biomedres.40.125
5. "Erinacine A-Enriched Hericium erinaceus Mycelium Produces Antidepressant-Like Effects through Modulating BDNF/PI3K/Akt/GSK-3β Signaling in Mice" (2018) https://www.mdpi.com/1422-0067/19/2/341/htm
6. "Improving effects of the mushroom Yamabushitake (Hericium erinaceus) on mild cognitive impairment: a double-blind placebo-controlled clinical trial" (2008) https://doi.org/10.1002/ptr.2634
7. "Effect of anexo-polysaccharide from the culture broth of Hericium erinaceus on enhancement of growth and differentiation of rat adrenal nerve cells" (2002) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3449638/
8. "The Cyanthin Diterpenoid and Sesterterpene Constituents of Hericium erinaceus Mycelium Ameliorate Alzheimer's Disease-Related Pathologies in APP/PS1 Transgenic Mice" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5855820/
9. "Erinacine S, a Rare Sesterterpene from the Mycelia of Hericium erinaceus" (2016) https://doi.org/10.1021/acs.jnatprod.5b00474
10. "Neurohealth Properties of Hericium erinaceus Mycelia Enriched with Erinacines" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5987239/

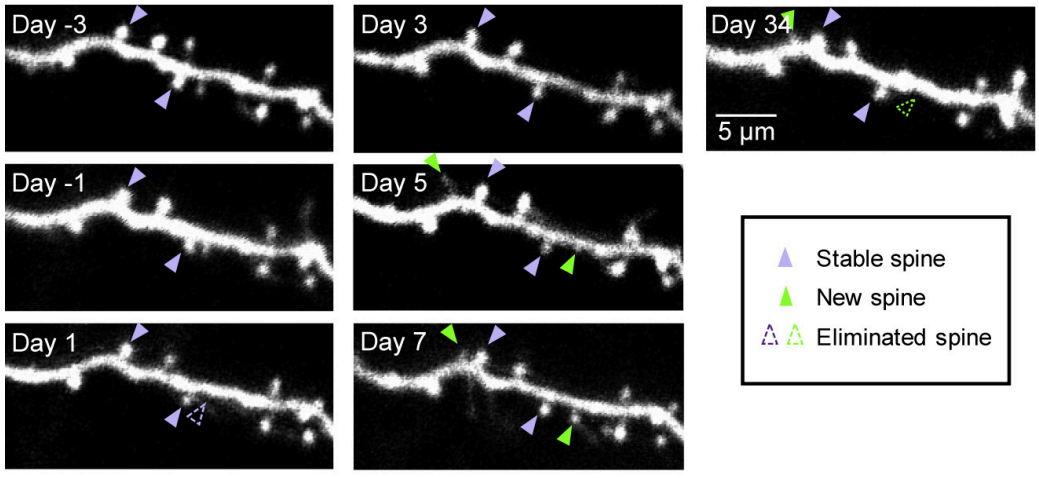
Psilocybin induces rapid growth of neural connections in the brain's frontal cortex

While I cannot endorse the use of an illegal substance, research results demonstrate that a single dose of psilocybin potently increases dendritic spine density and size with an accompanied increase in brain plasticity. The induced changes persist for 3 or 4 weeks. LSD has also been shown to enhance neural plasticity signaling pathways.

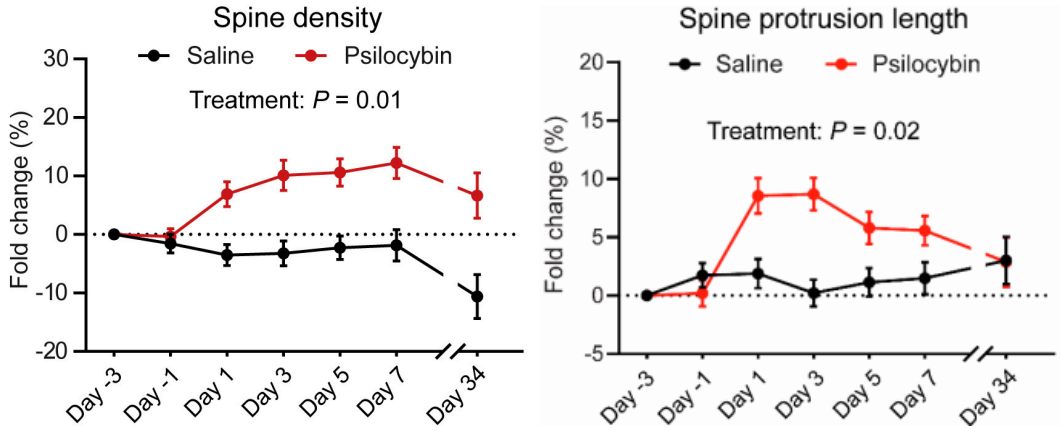
1. [228] "Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo" (2021) https://doi.org/10.1016/j.neuron.2021.06.008
    a. "Psychedelic spurs growth of neural connections lost in depression" (2021) https://www.eurekalert.org/news-releases/744231
    b. "We not only saw a 10% increase in the number of neuronal connections, but also they were on average about 10% larger, so the connections were stronger as well."
2. [229] "A Single Dose of Psilocybin Increases Synaptic Density and Decreases 5-HT2A Receptor Density in the Pig Brain" (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7830000/
3. "Transcriptional regulation in the rat prefrontal cortex and hippocampus after a single administration of psilocybin" (2020) https://doi.org/10.1177/0269881120959614
4. "Nootropic effects of LSD: Behavioral, molecular and computational evidence" (2022) https://www.sciencedirect.com/science/article/abs/pii/S001448862200173X
    a. "Neuroscience research suggests LSD might enhance learning and memory by promoting brain plasticity" (2022) https://www.psypost.org/2022/08/neuroscience-research-suggests-lsd-might-enhance-learning-and-memory-by-promoting-brain-plasticity-63701

Increased brain plasticity can allow for recovery from cycles of depression.

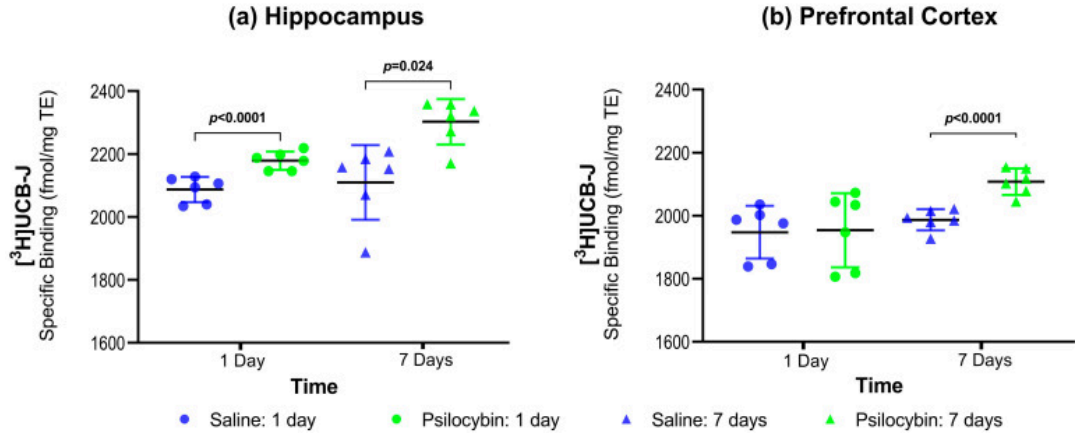
1. [230] "Prolonged epigenomic and synaptic plasticity alterations following single exposure to a psychedelic in mice" (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8582597/
2. "Psilocybin treatment for major depression effective for up to a year for most patients, study shows" (2022) https://medicalxpress.com/news/2022-02-psilocybin-treatment-major-depression-effective.html
3. "Efficacy and safety of psilocybin-assisted treatment for major depressive disorder: Prospective 12-month follow-up," (2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8864328/
    - "Psilocybin treatment for major depression effective for up to a year for most patients, study shows" (2022) https://medicalxpress.com/news/2022-02-psilocybin-treatment-major-depression-effective.html
4. "Increased global integration in the brain after psilocybin therapy for depression" (2022) https://www.nature.com/articles/s41591-022-01744-z
5. "Psychedelics, but Not Ketamine, Produce Persistent Antidepressant-like Effects in a Rodent Experimental System for the Study of Depression" (2020) https://pubs.acs.org/doi/10.1021/acschemneuro.9b00493
6. "Psilocybin targets a common molecular mechanism for cognitive impairment and increased craving in alcoholism" (2021) https://www.science.org/doi/10.1126/sciadv.abh2399
7. (Review) "Psychedelics and Neuroplasticity: A Systematic Review Unraveling the Biological Underpinnings of Psychedelics" (2021) https://doi.org/10.3389/fpsyt.2021.724606



Psilocybin increases the density, size and length of dendritic spines in the mouse medial frontal cortex. "Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo" (2021) https://doi.org/10.1016/j.neuron.2021.06.008



Psilocybin increases the density, size and length of dendritic spines in the mouse medial frontal cortex. "Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo" (2021) https://doi.org/10.1016/j.neuron.2021.06.008



Group-wise comparisons of synaptic vesicle protein 2A (SV2A) density (mean ± SD) in the hippocampus (a) and prefrontal cortex (PFC) (b). "A Single Dose of Psilocybin Increases Synaptic Density and Decreases 5-HT2A Receptor Density in the Pig Brain" (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7830000/

<u>Microdosing psilocybin does not make meaningful changes to dendritic spines or brain plasticity</u>

Microdosing psilocybin has subjective effects but lacks evidence for making meaningful changes to dendritic spines or brain plasticity. A 2022 placebo controlled study found that "*low doses of psilocybin mushrooms can result in noticeable subjective effects and altered EEG rhythms, but without evidence to support enhanced well-being, creativity and cognitive function*." https://www.wweek.com/news/state/2022/08/03/psilocybin-microdosing-shows-no-benefit-to-creativity-cognition-perception-in-new-scientific-study/

1. "Microdosing with psilocybin mushrooms: a double-blind placebo-controlled study" (2022) https://www.nature.com/articles/s41398-022-02039-0
    a. *…there was no effect of microdosing except for a few small changes towards cognitive impairment. According to our findings, low doses of psilocybin mushrooms can result in noticeable subjective effects and altered EEG rhythms, but without evidence to support enhanced well-being, creativity and cognitive function.*

2. "Microevidence for microdosing with psilocybin mushrooms: a double-blind placebo-controlled study of subjective effects, behavior, creativity, perception, cognition, and brain activity" (2021) https://www.biorxiv.org/content/10.1101/2021.11.30.470657v1.full
    a. *We investigated the effects of 0.5 g dried mushrooms on subjective experience, behavior, creativity, perception, cognition, and brain activity. The reported acute effects were significantly more intense for the active dose compared to the placebo, which could be explained by unblinding. For the other measurements, we observed either null effects or a trend towards cognitive impairment and, in the case of EEG, towards reduced theta band spectral power. Our findings support the possibility that expectation effects underlie at least some of the anecdotal benefits attributed to microdosing with psilocybin mushrooms.*
3. "Psilocybin microdosers demonstrate greater observed improvements in mood and mental health at one month relative to non-microdosing controls" (2022) https://www.nature.com/articles/s41598-022-14512-3
    a. "Psilocybin microdosers display mental health improvement during 1 month period compared to non-microdosers" (2022) https://www.psypost.org/2022/07/psilocybin-microdosers-display-mental-health-improvement-during-1-month-period-compared-to-non-microdosers-63566

Alleviating nausea

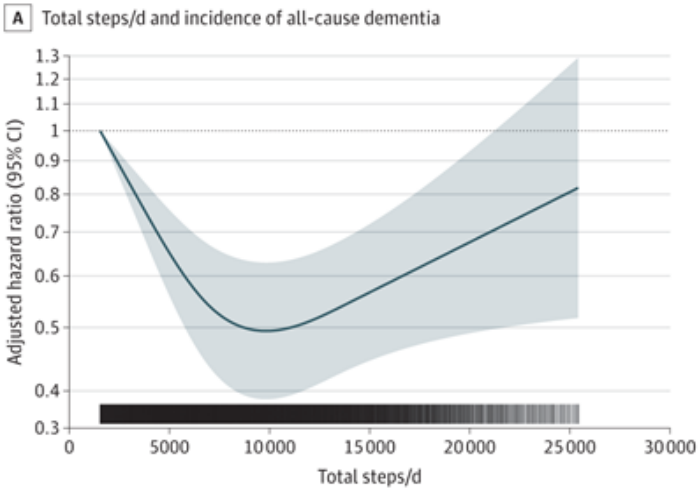
Feeling nauseous is reported to be a common side effect of psilocybin mushrooms. Multiple studies support alleviating nausea by taking 1000 mg to 1500 mg of fresh, raw ginger.

1. https://www.psilohealth.co/blog/combating-nausea
2. https://www.healthline.com/nutrition/ginger-for-nausea#effectiveness
3. "The Effectiveness of Ginger in the Prevention of Nausea and Vomiting during Pregnancy and Chemotherapy" (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4818021/
4. "Comparing the effects of ginger and metoclopramide on the treatment of pregnancy nausea" (2011) https://pubmed.ncbi.nlm.nih.gov/22545357/
5. "A randomized comparison of ginger and dimenhydrinate in the treatment of nausea and vomiting in pregnancy" (2007) https://pubmed.ncbi.nlm.nih.gov/22545357/
6. "A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting" (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3995184/

Walking helps maintain BDNF in individuals over 65 years old

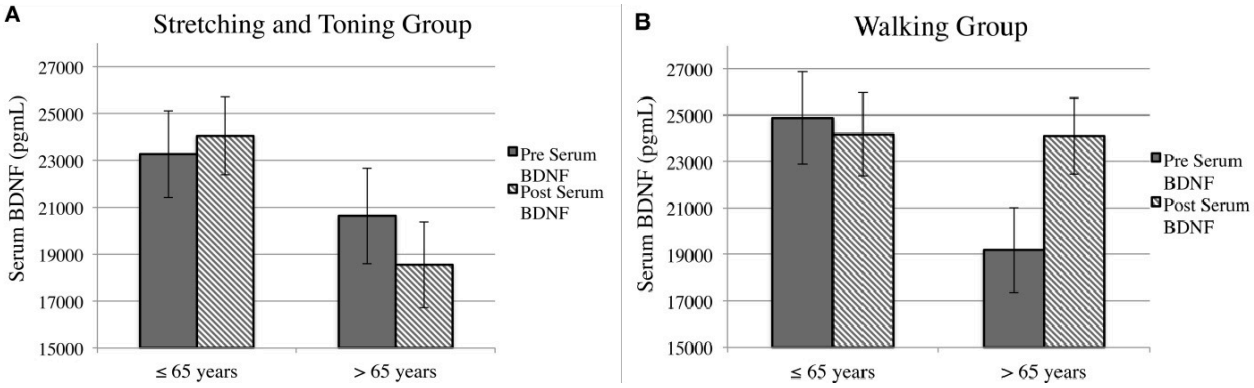
A 1-year walking intervention program in 90 older adults (mean age = 66.82 years) showed restoration of serum BDNF levels. Participants started by walking for 10 min and increased walking duration weekly by 5-min increments until a duration of 40 min was achieved at week 7. Participants walked for 40 min per session for the remainder of the program [109]. 40 minutes for the elderly is about 2 to 2.5 miles, or 4000 to 5000 steps.

More generally, a large-scale study of adults aged 61.1 ±7.9 years found that those that walked 9,000 to 10,000 steps per day had a 50% lower incidence of all-cause dementia compared to those who walked 3,800 steps per day after a 6.9 year follow-up period. Another study found a 300% difference in all-cause mortality between those who walked 4,000 versus 10,000 steps per day.



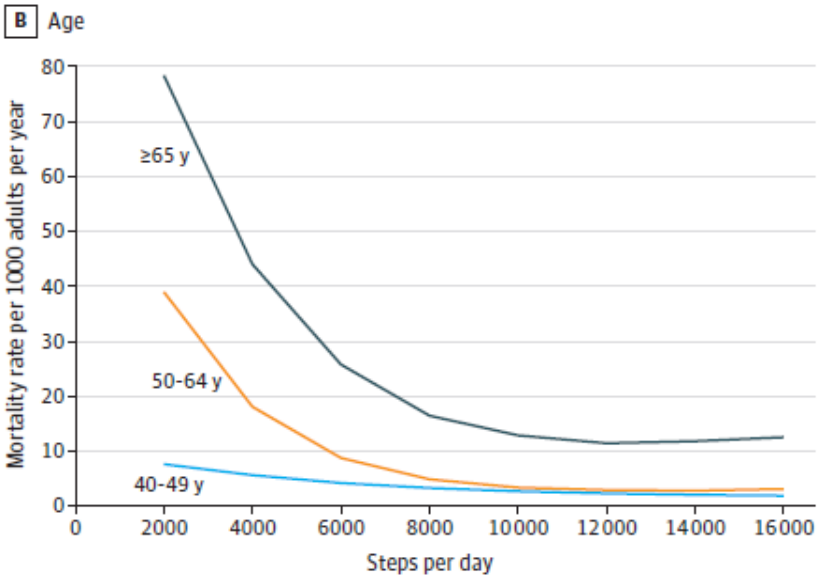
A Dose-Response Association Between Different Accelerometer-Measured Step-Based Metrics and Incidence of All-Cause Dementia. Shading indicates 95% CIs; solid lines are hazard ratios in logarithmic scale. "Association of Daily Step Count and Intensity With Incident Dementia in 78 430 Adults Living in the UK" (2022) https://jamanetwork.com/journals/jamaneurology/fullarticle/2795819

While moderate intensity exercise, such as 30 min of acute aerobic exercise, increases BDNF, mild intensity walking did not in a 58.0 (±12.8) year age group [150]. One mechanism associating exercise with increased BDNF appears to be an increase in the HDAC β-hydroxybutyrate. "Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body β-hydroxybutyrate" (2016) https://elifesciences.org/articles/15092



Moderating effect of age (stratified). Note: Effects of exercise on serum BDNF as a function of age. Age stratified using a median split. (A) Serum BDNF decreases following the intervention in the control group among adults >65 years. (B) Serum BDNF increases following 1 year of walking exercise, specifically for adults aged >65 years [109]. "BDNF mediates improvements in executive function following a 1-year exercise intervention" (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4263078/

More generally, physical exercise has been consistently shown to increase levels of BDNF mRNA and protein expression in the hypothalamus, striatum and other cortical areas. The increases in serum BDNF levels following exercise have been shown to be intensity-dependent. Further, shorter bouts of high intensity interval exercise have been shown to elevate BDNF levels above those following intense continuous exercise in healthy subjects. “Carbohydrate-restricted Diet and Exercise Increase Brain-derived Neurotrophic Factor and Cognitive Function: A Randomized Crossover Trial” (2019) https://doi.org/10.7759%2Fcureus.5604



Walking contributes to overall health and significantly reduces all-cause mortality in adults 65 years and older [108]. “Association of Daily Step Count and Step Intensity With Mortality Among US Adults” (2020) https://jamanetwork.com/journals/jama/article-abstract/2763292

The bigger picture: ATP insufficiency and redox imbalances are major contributors to the phenotypes of aging

The brain is an energy consuming "canary in the coal mine"

Pushing the analogy, if the brain's health (measured by the decline in perceptual speed) is the body's "canary in the coal mine", will interventions that repair the brain's age-related decline have the effect of reversing this and other phenotypes of aging? Published results from GlyNAC supplementation appear to suggest so, at least for older individuals.

Generally, a cell's energy production and work requirements are tightly coupled, without depleting ATP. Nowhere in the body is this tight coupling more apparent than in the brain, particularly as seen in the decline in perceptual speed and the ability to form new memories with age.

The average human brain weighs about three pounds, or about 2% of body weight. But, the brain receives ~15% of cardiac output at rest and ~20% of the body glucose consumption - facts that highlight its intensive energy requirements. Experimental evidence has shown that impaired brain metabolism and blood flow cause dendritic spine loss in hippocampal neurons.

1. "Brain metabolism in health, aging, and neurodegeneration" (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5452017/
2. "GLUT1 reductions exacerbate Alzheimer's disease vasculoneuronal dysfunction and degeneration" (2015) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4734893/

In the brain, the main use-dependent neurotrophin, BDNF, has a role in modulating complex I of the electron-transport chain (ETC). BDNF has been shown to increase the respiratory control index (RCI) of rat brain mitochondria (but not liver or heart) by 64% through an MEK-kinase mechanism via complex 1. While synaptic plasticity is inextricably linked to mitochondrial energy production, inflammatory cytokines can block the action of BDNF from increasing ATP. BDNF-induced positive effects on respiratory efficiency associated with complex I were inhibited by anti-BDNF antibody, MEK inhibitors and by IL-1β, indicating that the mitochondrial effects were mediated via the same MEK-Bcl2 pathway associated with neuroprotection.

1. "Changes in mitochondrial function are pivotal in neurodegenerative and psychiatric disorders: How important is BDNF?" (2013) https://bpspubs.onlinelibrary.wiley.com/doi/full/10.1111/bph.12531
2. "Astrocytic BDNF signaling within the ventromedial hypothalamus regulates energy homeostasis" (2022) https://doi.org/10.1038/s42255-022-00566-0

The interrelationships between BDNF, IL-1β and ATP production suggest that in order to restore synaptic plasticity (and many other phenotypes of aging), systemic inflammation must be reduced, SnCs must be eliminated, and ATP/AMP and GST/GSSG deficits must be corrected. Evidence presented throughout this document support the ideas that

1. The decline in expression levels of BDNF in the brain causes less synaptic and dendritic spine outgrowths, resulting in reduced brain plasticity and increasing difficulty in forming new memories.
2. BDNF levels are inhibited by interrelationships between systemic inflammation, SnC accumulation and NAD+ deficiency, all leading to ATP insufficiency.
3. While treating any one of the proximate causes of ATP insufficiency will improve BDNF levels and brain plasticity, single interventions will only perturb the interrelationships that lead to ATP insufficiency. Discontinuation of any single intervention results in cells' return to a previous, less fit state. These single treatments include
    a. Inhibiting TNF-α, IL-1β and IL-6 by supplementation of hesperidin or equivalent
    b. Periodic SnC clearance
    c. Increasing ATP and GSH by GlyNAC and possibly NMN supplementation.
4. While all of these interventions have the effect of restoring physical function restoration and BDNF in aged humans and mice through interrelated mechanisms, simultaneous treatment of all proximate causes are expected to work synergistically for greater effect. Possibly, by correcting all of these mechanisms, the feedback cycles that dampen cellular bioenergetics will be reset to a fitter, more youthful state, and many of the phenotypes of aging will be somewhat reversed.

ATP, NAD+ and GSH declines are strongly correlated with biological aging

Importantly, age-related declines in ATP, the reduced redox cofactors NAD+ and GSH, and the ratios ATP/AMP, oxidized-to-reduced glutathione (GSSG/GSH), and oxidized-to-reduced nicotinamide-adenine-dinucleotide (NAD+/NADH) are correlated with many aspects of biological aging as indicated below, where t is in years.

1. ATP content drops linearly by ~50% between 20-80 years (RBC) Ref. Explanation: NAD+ is a cofactor in the synthesis of ATP, and its levels in mitochondria drop linearly with age. Furthermore, complex changes in mitochondrial structure and function, including disorganization of mitochondrial structure, decline in the activity of enzymes involved in mitochondrial ATP synthesis, accumulation of mtDNA mutations, increased damage of mitochondrial proteins and lipids by reactive oxygen species are considered to contribute to the decrease in cellular ATP production in aged cells Ref.
2. NAD+ content drops linearly by ~75% between 20-80 years (cerebral tissue) Ref. Indeed, levels of many NAD+ metabolites change with age Ref. Explanation: CD38, which is expressed by immune cells, is a major consumer of NAD+. CD38 increases with age due to inflammation that is a consequence of SnC cytokines Ref.
3. GSH content drops linearly by ~70% between 20-80 years (RBC) Ref. Explanation: ATP is required to recycle GSSG to GSH, while GSH is consumed by hydrogen peroxide. Insufficient GSH also has bioenergetic consequences such as lower ATP production and mitochondrial dysfunction Ref.

Factors that decline linearly with age, like ATP, NAD+ and GSH, might be input parameters into exponential functions, such as those listed below. Many age-related changes in humans progress at essentially the same exponential rates.

- Decline in energy metabolism (ages 25-55, mononuclear cells) Ref, ATP/AMP $\propto e^{-0.04\,t}$
- Reduced to oxidized glutathione ratio (ages 25-55, red blood cells) Ref, GSH/GSSG(t) $\propto e^{-0.043\,t}$
    - Equivalently, GSSG/GSH(t) $\propto e^{0.043\,t}$
- Senescent cell accumulation (baboons, dermal fibroblasts) Ref, S(t) $\propto e^{0.046\,t}$
- Nuclear DNA damage rate, DSB (pH2AX) (ages 30-80, non-sun exposed skin, males) Ref, DNA(t) $\propto e^{0.042\,t}$
- Mitochondrial dysfunction accumulation (ages 25-90, hair cells) Ref, mtDNA(t) $\propto e^{0.042\,t}$
- Mitochondrial dysfunction accumulation (ages 30-85, intestinal crypts) Ref, Ref, mtDNA(t) $\propto e^{0.057\,t}$. Explanation: The exponential increase in somatic mtDNA mutations with age may be due to impaired mitophagy and a bias towards asymmetric stem cell (SC) division where one daughter remains within the SC pool. Symmetric division would instead result in less healthy SCs exiting a constant-size SC pool, thus improving the SC population Ref.
- Thymic T cell production rate Ref, R(t) $\propto e^{-0.044\,t}$
- Stem cell population decline
    - MSC stem cells per marrow cells (x 100,000) Ref, MSC(t) $\propto e^{-0.045\,t}$
    - Somatic cell turnover rate estimated from declining MSC stem cell population Ref, TrnOvr(t) $\propto e^{0.045\,t}$
    - Hair follicle melanocyte stem cell decline leading to gray hair (ages 25-90) Ref, Melan(t) $\propto e^{-0.039\,t}$

Most of these correlations are expected, given that they are affected by ATP availability. For example, ATP is needed to recycle oxidized glutathione (GSSG) back to its reduced form (GSH), which partially explains the decline in GSH/GSSG. Negative feedback re-enforces this decline. While ATP is needed to regenerate oxidized glutathione (GSSG), mitochondrial reduced GSH (mGSH) also regulates mitochondrial ATP production by modifying critical protein sulfhydryl redox states that consequently influence electron flow in the electron transport chain (ETC). Cysteine influx and efflux membrane transport also contribute as rate-limiting factors in GSH synthesis.

1. “L-cysteine efflux in erythrocytes as a function of human age: correlation with reduced glutathione and total antioxidant potential” (2013) doi/10.1089/rej.2012.1394;
2. “Mitochondrial Glutathione: Recent Insights and Role in Disease” (2020) https://www.mdpi.com/2076-3921/9/10/909/htm.

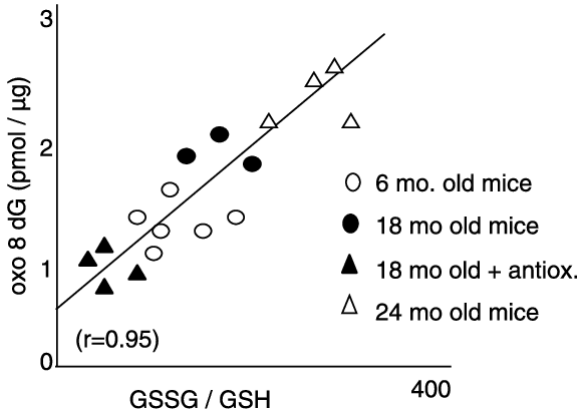
The consequences of oxidative stress to aging cells also contribute to these correlations. Redox imbalances cause elevated levels of reactive oxygen species (ROS), which is a well known cause of cellular damage such as lipid peroxidation and DNA oxidation. Less well known is the regulatory effect of ROS of stem cell fate: low levels of ROS lead to symmetric division of stem cells and stem cell self-renewal, while high levels of ROS leads to asymmetric division and an unmaintained stem cell pool (i.e., one stem

cell divides to become a stem cell and a differentiated cell). “Redox homeostasis: the linchpin in stem cell self-renewal and differentiation” (2013) http://www.nature.com/cddis/journal/v4/n3/full/cddis201350a.html

Another example of an age-related decline correlated to insufficient ATP is senescent cell (SnC) accumulation, which may be a consequence of adaptations for cell survival in an insufficient ATP/AMP state. Cytokines from accumulated SnCs stimulate CD38 on immune cells, which in turn consumes NAD+, a cofactor needed for ATP production. Lower ATP production then contributes to the increase in SnCs.

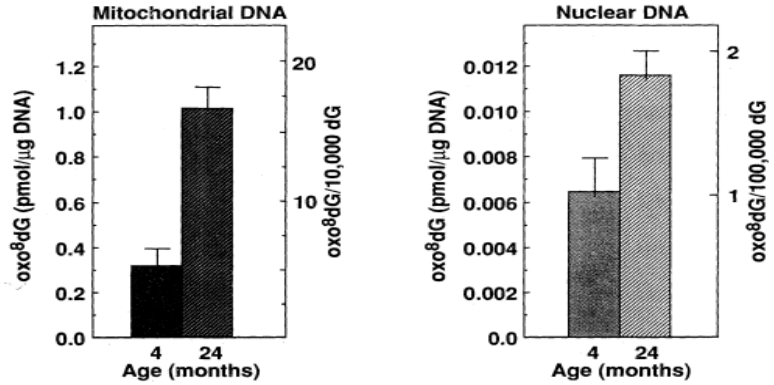
GSH deficiency causes oxidative stress, mitochondrial dysfunction, cellular senescence, systemic inflammation, and phenotypes of age

Guanosine (the “G” in DNA and RNA) is particularly vulnerable to oxidative damage due to its low redox potential. Glutathione is the major defense against its oxidation. Low levels of GSH result in higher levels of oxidized guanosine in DNA, the most common form of which is 7,8-dihydro-8 oxo-deoxyGuanine (8-oxo-G).



Relationship between DNA damage and glutathione oxidation in mice. Age-associated mitochondrial glutathione oxidation and mitochondrial DNA damage (estimated measuring the levels of 8- oxo-20 -deoxyguanosine) are directly correlated. “Role of mitochondrial oxidative stress to explain the different longevity between genders. Protective effect of estrogens“ (2007) https://doi.org/10.1080/10715760600952851

Oxidative damage to DNA accumulates with age, particularly in mitochondrial DNA (mtDNA) where the incidence of 8-oxo-dG is about 1000x higher than in nuclear DNA. Mitochondrial DNA is highly susceptible to oxidative damage because of the absence of histones, proximity to reactive oxygen, and poor repair mechanisms.



Steady-state oxidative DNA damage increases with age in the livers of young and old rats. “Oxidants, antioxidants, and the degenerative diseases of aging“ (1993) https://doi.org/10.1073/pnas.90.17.7915

Oxidized mtDNA results in system inflammation and is a player in age-related disease pathology. If oxidized mtDNA cannot be repaired, it is cleaved into fragments that may enter the cytosol and activate inflammatory responses. In autoimmune diseases these inflammatory responses may prompt the immune system to attack and destroy healthy cells and tissues.

1. "How Mitochondrial Damage Ignites the '"Auto-Inflammatory Fire'" (2022) https://today.ucsd.edu/story/how-mitochondrial-damage-ignites-the-auto-inflammatory-fire
2. "Oxidized DNA fragments exit mitochondria via mPTP- and VDAC-dependent channels to activate NLRP3 inflammasome and interferon signaling" (2022) https://doi.org/10.1016/j.immuni.2022.06.007

Guanosine oxidation in nuclear and mitochondrial DNA and RNA is significantly associated with damage, dysfunction, disease and the phenotypes of aging. Its effects are categorized as

1. Pro-mutagenic: This damage can manifest as point mutations due to its miscoding potential that instructs most DNA polymerases (Pols) to preferentially insert Adenine (A) opposite 8-oxo-G instead of the correct Cytosine (C). If uncorrected, A:8-oxo-G mispairs can give rise to C:G ⟶ A:T transversion mutations that could compromise the accuracy of transcription and translation.
2. Senescence induction: Oxidative damage to the telomeric regions of DNA is particularly harmful. The presence of 8-oxo-G in telomeres can cause senescence in the absence of telomere shortening.
    a. "Telomeric 8-oxo-guanine drives rapid premature senescence in the absence of telomere shortening" (2022) https://www.nature.com/articles/s41594-022-00790-y
        i. "Age Accelerating "Zombie Cells" – A New Study Sheds Light on These Unique Cells" (2022) https://scitechdaily.com/age-accelerating-zombie-cells-a-new-study-sheds-light-on-these-unique-cells/
    b. "Roles for the 8-Oxoguanine DNA Repair System in Protecting Telomeres From Oxidative Stress" (2021) https://www.frontiersin.org/articles/10.3389/fcell.2021.758402/full
3. Mitochondrial dysfunction induction: Oxidation of a single mtDNA nucleotide reduces the activity of complexes I and IV, leading to decreased ATP production and increased ROS levels.
    a. "Premature aging is associated with higher levels of 8-oxoguanine and increased DNA damage in the Polg mutator mouse" (2022) https://doi.org/10.1111/acel.13669
    In conclusion, these results provide multiple lines of evidence that mtDNA mutation associated with the accumulation of chronic oxidative stress is the center of aging by increasing nDNA and mtDNA strand breakage, and telomere shortening.
        i. "How Mitochondrial Dysfunction Leads to Premature Aging" (2022) https://www.technologynetworks.com/cell-science/news/how-mitochondrial-dysfunction-leads-to-premature-aging-364938
4. DNA transcription modification and epigenetic regulation: the presence of 8-oxo-G in DNA can influence gene transcription by acting as an epigenetic regulator.
    a. "Not breathing is not an option: How to deal with oxidative DNA damage" (2017) https://doi.org/10.1016/j.dnarep.2017.09.007
    b. "Epigenetics and Oxidative Stress in Aging" (2017) https://doi.org/10.1155/2017/9175806
5. RNA translational modification: Studies have shown that the predominantly oxidized nucleic acid is RNA. Oxidatively damaged RNA may interfere with correct base pairing and could compromise the accuracy of transcription and translation. These aspects may be particularly important in the case of highly metabolic cells such as neurons. As such, it may be that oxidative damage to RNA. It is well established that In the brain, increased levels of oxidized DNA and RNA are associated with impaired cognitive function and Alzheimer's disease.
    a. "RNA Oxidation Is a Prominent Feature of Vulnerable Neurons in Alzheimer's Disease" (1999) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6782583/
    b. "Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: Partial reversal by feeding acetyl-L-carnitine and/or R-α-lipoic acid" (2002) https://www.pnas.org/doi/10.1073/pnas.261709299

Oxidatively damaged RNA interferes with correct base pairing and compromises the accuracy of transcription and translation. This situation is particularly important in the case of highly metabolic cells such as neurons.

"Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation" (2011) https://doi.org/10.3945/ajcn.110.003483
Elevated oxidative stress has been linked to several aging-related illnesses, including the development of cataracts, macular degeneration, immune deficiencies, neurodegenerative diseases, and increased DNA damage. The ability of a cell to resist oxidant damage is determined by a balance between the generation of reactive oxygen species and the defensive capacity to produce antioxidants. Glutathione (γ-glutamylcysteinylglycine) is the most abundant endogenous intracellular antioxidant present in millimolar quantities within cells. Glutathione plays a central role in antioxidant defenses, and irreversible cell damage supervenes when the cell is unable to maintain intracellular glutathione concentrations.

Oxidative damage is repaired by repleting redox reaction factors

To what extent can oxidative damage in cells be repaired? It turns out that much of this damage is reversible. Restoring levels of glutathione and NAD+ enables glycosylase enzymes to do their jobs and repair oxidative damage. Invoking mitophagy for mitochondrial quality control and normal cellular turnover also play important roles.

Repair of oxidative damage		
Damage	**Location**	**Treatment/Evidence**
Protein and lipid peroxidation	- All bilipid membranes - Fatty acids - Misc cellular proteins	- GlyNAC supplementation, continuous. (3,4,5,8) - Fasting has a modest effect on reducing lipid peroxidation. (9)
Cross-linked glycoproteins	- Intra- and extra-cellular	- NAC, a glutathione precursor, efficiently breaks disulfide bonds (16)
DNA mutagenic damage by adenine insertion opposite 8-oxo-G	- Nuclear DNA - Mitochondrial DNA	- Adenine substitution in nuclear DNA is repaired during the cell cycle by 8-oxoguanine glycosylase (OGG1) coordinated with MUTYH glycosylase. - In mitochondria, this type of damage may also be cleared by mitophagy, induced by Urolithin A under the brand name Celltrient. (10,11)
8-oxoG of telomeric DNA resulting in senescence (1)	- Nuclear DNA	- Clear senescent cells periodically. (2) - GlyNAC supplementation restores redox levels necessary for 8-oxoguanine glycosylase (OGG1) to repair 8-oxoG in DNA. (12,13,14,15)
8-oxoG in RNA causing modification of translation to proteins	- Nuclear RNA - Mitochondrial RNA	- GlyNAC supplementation, continuous, to lower ROS. (6,7)
Epigenetic transcriptional regulation resulting from 8-oxoG in DNA	- Nuclear DNA - Mitochondrial DNA	- GlyNAC supplementation to restore redox levels necessary for 8-Oxoguanine DNA glycosylase (OGG1) to repair 8-oxoG in DNA. (12,13,14,15)

(1) "Telomeric 8-oxo-guanine drives rapid premature senescence in the absence of telomere shortening" (2022) https://www.nature.com/articles/s41594-022-00790-y
(2) "Protocol for Restoration of Physical Function in Aged Individuals by Removal of Senescent Cells" https://docs.google.com/document/d/1ndfwj3mBvIZceP6A2e6dliieJNM7kxFYf-afuP67KGA/edit?usp=sharing
(3) "Reversal of Methylmercury-Induced Oxidative Stress, Lipid Peroxidation, and DNA Damage by the Treatment of N-Acetyl Cysteine: A Protective Approach" (2014) DOI: 10.1615/JEnvironPatholToxicolOncol.2014010291
(4) "Glycine and N-acetylcysteine (GlyNAC) supplementation in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, insulin resistance, endothelial dysfunction, genotoxicity, muscle strength, and cognition: Results of a pilot clinical trial" (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8002905/
(5) "Supplementing Glycine and N-Acetylcysteine (GlyNAC) in Older Adults Improves Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Physical Function, and Aging Hallmarks: A Randomized Clinical Trial" (2022) https://doi.org/10.1093/gerona/glac135
(6) "RNA Oxidation Is a Prominent Feature of Vulnerable Neurons in Alzheimer's Disease" (1999) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6782583/
(7) "Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: Partial reversal by feeding acetyl-L-carnitine and/or R-α-lipoic acid" (2002) https://www.pnas.org/doi/10.1073/pnas.261709299
(8) "Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tert-butyl-alpha-phenylnitrone" (1991) https://doi.org/10.1073/pnas.88.9.3633

(9) "EFFECTS OF RAMADAN FASTING ON LIPID PEROXIDATION, SERUM LIPOPROTEINS AND FASTING BLOOD SUGAR" (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7380785/
(10) "The mitophagy activator urolithin A is safe and induces a molecular signature of improved mitochondrial and cellular health in humans" (2019) https://doi.org/10.1038/s42255-019-0073-4
(11) "Improvement of cognitive and motor performance with mitotherapy in aged mice'' (2020) https://www.ijbs.com/v16p0849.htm
(12) "Not breathing is not an option: How to deal with oxidative DNA damage" (2017) https://doi.org/10.1016/j.dnarep.2017.09.007
(13) "Redox Regulation of Human OGG1 Activity in Response to Cellular Oxidative Stress" (2006) https://journals.asm.org/doi/10.1128/MCB.00624-06
(14) "Redox Regulation of Human OGG1 Activity in Response to Cellular Oxidative Stress" (2006) https://journals.asm.org/doi/10.1128/MCB.00624-06
(15) "Disruption of 8-hydroxy-2'-deoxyguanosine DNA Glycosylase (OGG1) Antioxidant Response Capacity by Sodium Arsenite" (2013) https://scholarworks.umt.edu/etd/329/
(16) "N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why" (2018) https://doi.org/10.1080/10715762.2018.1468564

The brain is particularly susceptible to DNA and RNA guanine oxidation resulting from GSH deficiency

(Review) "The effects of stress and aging on glutathione metabolism" (2022) https://doi.org/10.1016/j.arr.2005.02.005

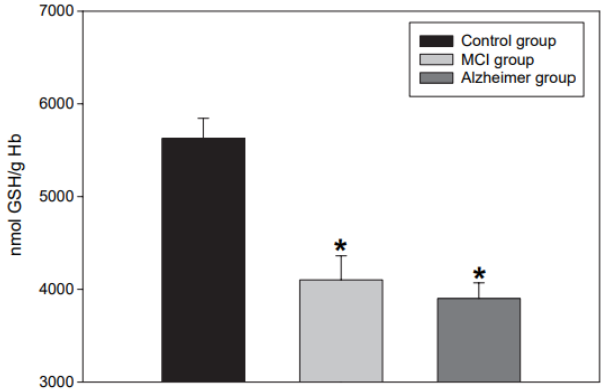
… during aging, glutathione levels appear to decline in a number of tissues, thereby putting cells at increased risk of succumbing to stress. The evidence for such a decline is strongest in the brain where glutathione loss is implicated in both Parkinson's disease and in neuronal injury following stroke.

"Oxidative stress predicts cognitive decline with aging in healthy adults: an observational study" (2018) https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-017-1026-z

- *Glutathione plays a key role in the antioxidant defense of neuronal cells, and circulating glutathione levels are reduced in Alzheimer's disease. Our findings show that the plasma glutathione levels predict future decline in cognition. Moreover, individuals who experience decreases in glutathione level over time also have a greater decline in their cognition.*
- *Lower levels of glutathione at baseline was associated with a decline in the executive domain over 4 years (covariate-adjusted relative risk (RR) for glutathione = 1.70 (95% CI = 1.02–2.85), p = 0.04). Furthermore, a longitudinal decline in glutathione level was associated with a faster decline in the executive domain (p = 0.03). None of the other oxidative stress markers or C-reactive protein were linked to cognitive decline over 4 years.*

"Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from Mild Cognitive Impairment" (2009) https://doi.org/10.1080/10715760701861373

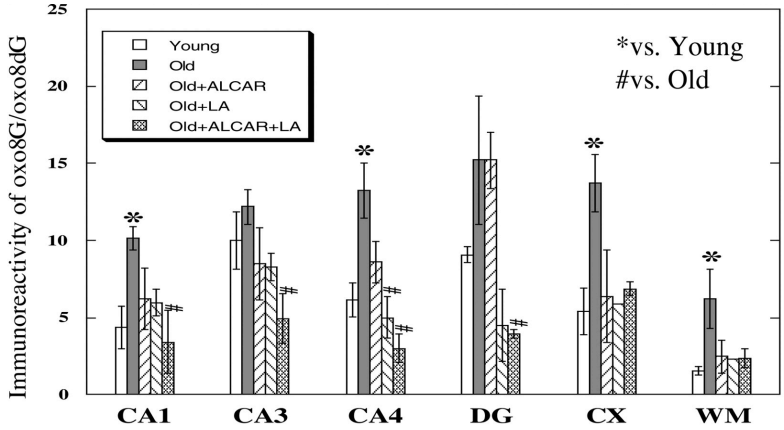
The present study shows that some peripheral markers of oxidative stress appear in mild cognitive impairment (MCI) with a similar pattern to that observed in Alzheimer's disease .



GSH levels in erythrocytes from control, MCI and Alzheimer's patients. (*p<0.05). "Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from Mild Cognitive Impairment" (2009) https://doi.org/10.1080/10715760701861373

"Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: Partial reversal by feeding acetyl-L-carnitine and/or R-α-lipoic acid" (2002) https://www.pnas.org/doi/10.1073/pnas.261709299

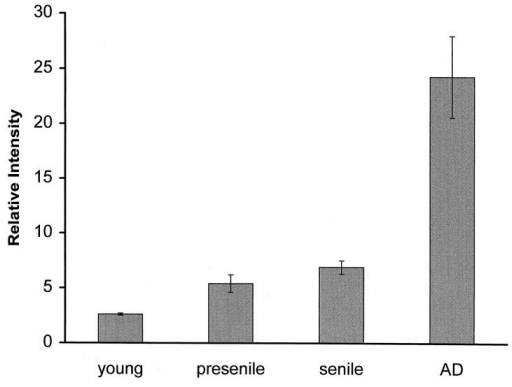
The effects on cognitive function, brain mitochondrial structure, and biomarkers of oxidative damage were studied after feeding old rats (24 mo) two mitochondrial metabolites, acetyl-L-carnitine (ALCAR) [0.5% or 0.2% (wt/vol) in drinking water], and/or R-α-lipoic acid (LA) [0.2% or 0.1% (wt/wt) in diet]. … Dietary administration of ALCAR and/or LA significantly reduced the extent of oxidized RNA, the combination being the most effective. Electron microscopic studies in the hippocampus showed that ALCAR and/or LA reversed age-associated mitochondrial structural decay. These results suggest that feeding ALCAR and LA to old rats improves performance on memory tasks by lowering oxidative damage and improving mitochondrial function.



Oxidized DNA or RNA (oxo8dG or oxo8G) is measured in various regions of the brain. Dietary administration of ALCAR and/or LA significantly reduced the extent of oxidized RNA, the combination being the most effective. "Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: Partial reversal by feeding acetyl-L-carnitine and/or R-α-lipoic acid" (2002) https://www.pnas.org/doi/10.1073/pnas.261709299

"RNA Oxidation Is a Prominent Feature of Vulnerable Neurons in Alzheimer's Disease" (1999) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6782583/

…Relative density measurement showed that there was a significant increase ($p < 0.0001$) in 8OHdG and 8OHG immunoreactivity with 1F7 in cases of AD (n = 22) as compared with senile (n = 13), presenile (n = 10), or young controls (n = 4). Surprisingly, the oxidized nucleoside was associated predominantly with RNA.



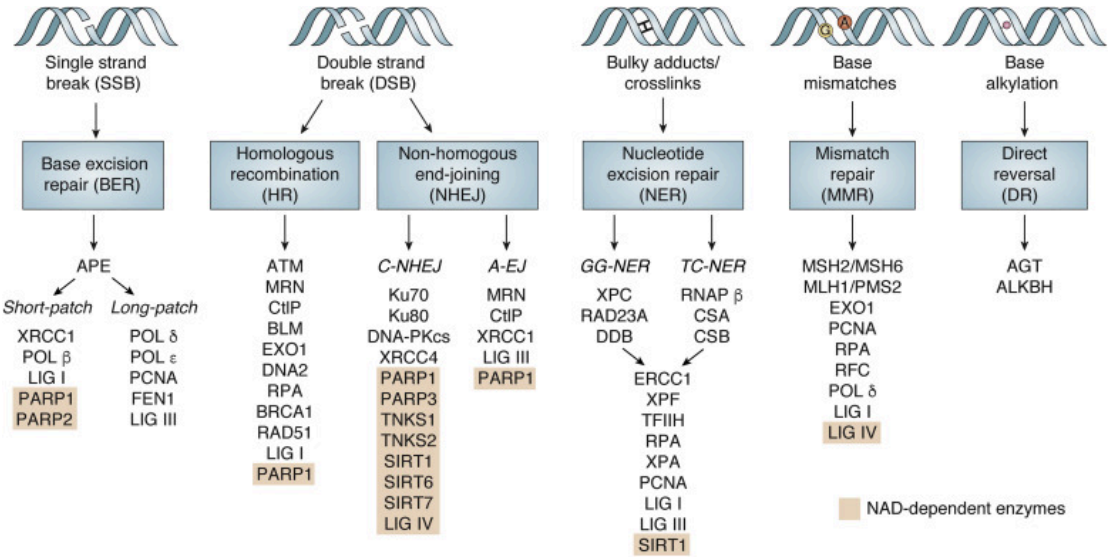
Relative scale of 8OHdG and 8OHG immunoreactivity with 1F7 antibody in prosubiculum neurons of 4 young controls, 10 presenile controls, and 13 senile controls, and in 22 cases of AD. Values shown are the means with SE. The difference among all controls and AD is significant by ANOVA ($p < 0.0001$), with post hocanalysis showing significant differences between young controls and AD ($p = 0.0015$), between presenile controls and AD ($p = 0.0001$), and between senile controls and AD ($p = 0.0001$). "RNA Oxidation Is a Prominent Feature of Vulnerable Neurons in Alzheimer's Disease" (1999) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6782583/

NAD+ deficiency contributes to phenotypes of aging

Besides glutathione, nicotinamide adenine dinucleotide (NAD) is one of the most important coenzymes within cells. It participates in over 500 redox reactions in cells, including those in DNA repair, glycolysis and most of the reactions in the Krebs cycle of cellular respiration.

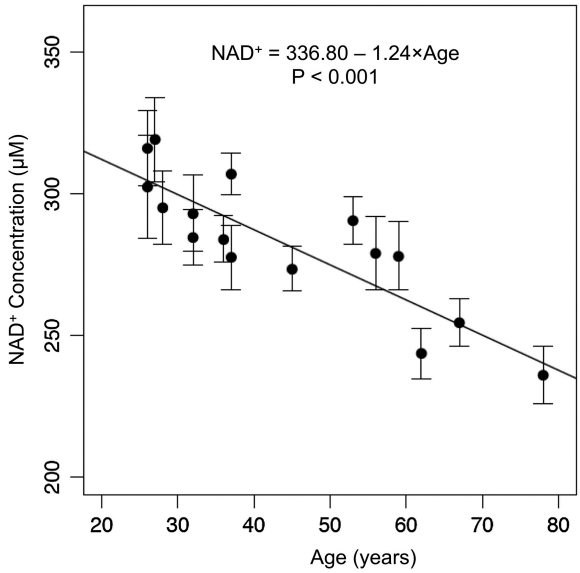
Furthermore, ATP-dependent NAD+ kinases (NADKs) in the cytosol (cNADK) and mitochondria (mNADK) phosphorylate NAD+ to NADP+, which together with its reduced form NADPH, is critical for the maintenance of cellular redox homeostasis. It regenerates molecules responsible for xenobiotic detoxification, for example, cytochrome P450, and eradication of reactive oxygen species (ROS), such as GSH, thioredoxin, or peroxiredoxin.

1. "Fueling genome maintenance: On the versatile roles of NAD+ in preserving DNA integrity" (2022) https://www.jbc.org/article/S0021-9258%2822%2900477-X/fulltext
2. "The Plasma NAD+ Metabolome Is Dysregulated in "Normal" Aging" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6482912/
3. "Single-Voxel 1H MR spectroscopy of cerebral nicotinamide adenine dinucleotide (NAD+) in humans at 7T using a 32-channel volume coil" (2019) https://onlinelibrary.wiley.com/doi/10.1002/mrm.27971



Major DNA repair pathways with NAD-dependent proteins highlighted. DNA repair occurs via different pathways involving multiple proteins, among them many are directly NAD+ dependent (shaded in orange). Repair of single-strand breaks (SSBs) through base excision repair (BER) involves NAD+-consuming PARP1 and PARP2. Double-strand breaks (DSBs) are repaired either through homologous recombination (HR) or nonhomologous end-joining (NHEJ) pathways, facilitated by multiple ARTDs and SIRTs. NAD+-dependent enzymes are also implicated in nucleotide excision repair (NER) as well as mismatch repair (MMR) pathways. A potential NAD+-dependent role of LIG IV is under discussion (see text for details). ARTD, diphtheria toxin–like ADP-ribosyltransferase; LIG IV, ligase IV; PARP, poly-ADP-ribose polymerase; SIRT, sirtuin.
"Fueling genome maintenance: On the versatile roles of NAD+ in preserving DNA integrity" (2022)
https://www.jbc.org/article/S0021-9258%2822%2900477-X/fulltext

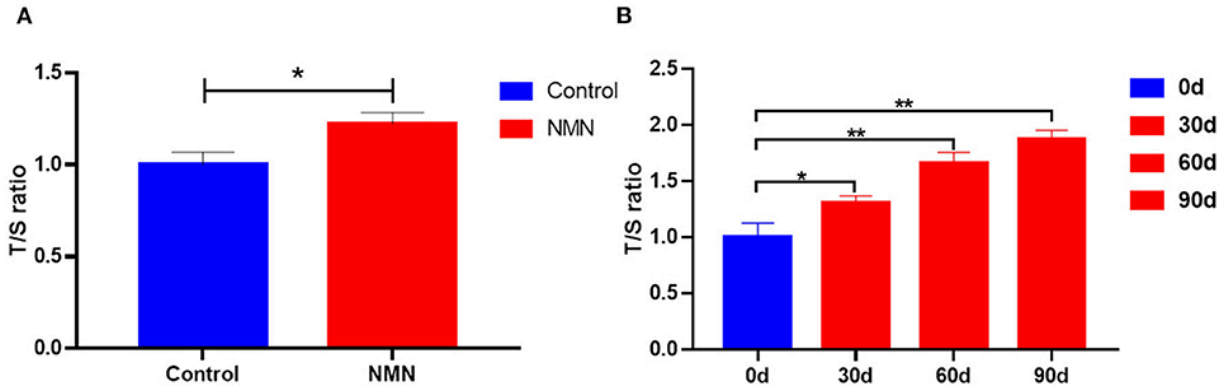
NAD+ levels decline with age as shown below. This decline is observed in serum and in the cytosolic, mitochondrial and nuclear compartments.



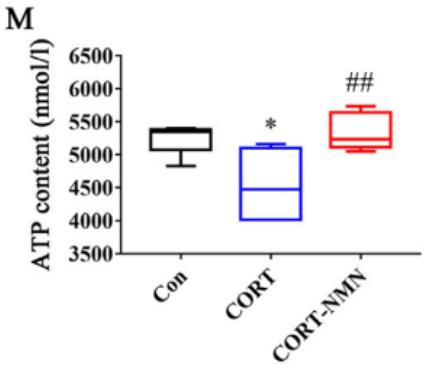
Decline of cerebral tissue NAD+ with aging. Individual NAD+ levels measured from the occipital cortex of healthy volunteers plotted against the age showing a linear decrease of NAD+ level with healthy aging. Filled circles represent within-subject means and error bars depict ±1 standard error. “Single-Voxel 1H MR spectroscopy of cerebral nicotinamide adenine dinucleotide (NAD+) in humans at 7T using a 32-channel volume coil” (2019) https://onlinelibrary.wiley.com/doi/10.1002/mrm.27971

Supplementation of a NAD+ precursor (NMN or NR) or injection of NAD+ has been shown to restore redox balances, ATP production and preserve youthful gene expression inside cells. Because NAD+ is compartmentalized within cells, it has been questioned whether serum levels or oral consumption of NAD+ precursors can raise mitochondrial NAD+ levels. This concern is convincingly disproven. NAD+ has been shown by multiple studies to increase ATP production in multiple tissues including the brain. Notably, the relationship between NAD+ and ATP production is strong. In a screening of approximately 2,400 drugs to find which restore ATP levels, the one with the most significant impact on the production of ATP is NAD+.

Notably, 300 mg per day oral NMN taken 30 minutes after breakfast over 3 months increased peripheral blood mononuclear cells (PBMC) telomere length by almost 100% in 48 to 58-year-old humans (n=8).



Supplementary effect of NMN on the telomere length of the peripheral blood mononuclear cell (PBMC) in (A) pre-aging mice (n = 6), and (B) pre-aging human volunteers (n = 8). *p < 0.05. **p < 0.01. “The Impacts of Short-Term NMN Supplementation on Serum Metabolism, Fecal Microbiota, and Telomere Length in Pre-Aging Phase” (2021) https://www.frontiersin.org/articles/10.3389/fnut.2021.756243/full

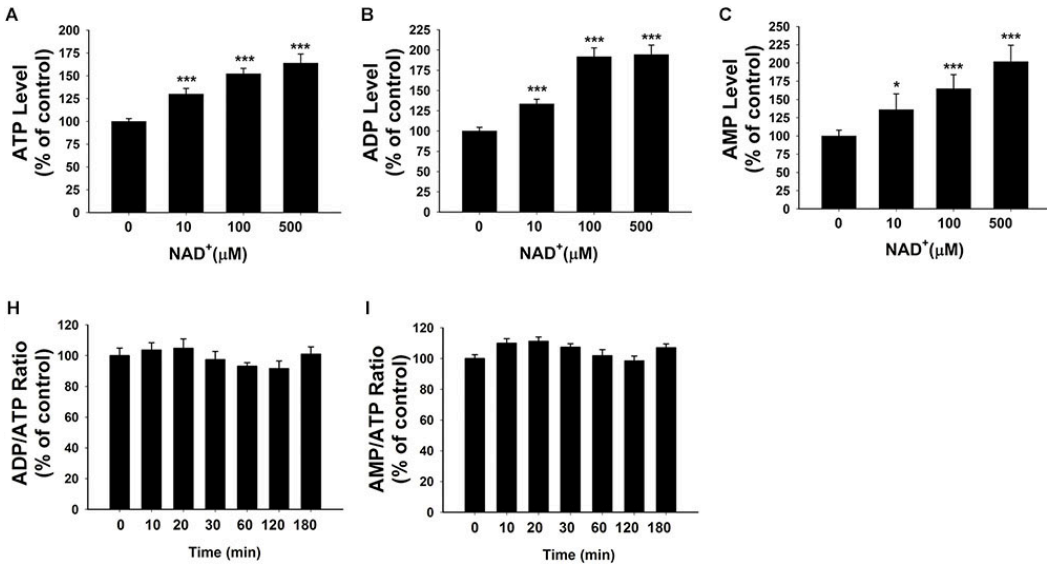


Oral NMN stimulates β-oxidation and glycolysis in mitochondria, and raises ATP levels in the hippocampus in a model of stressed mice. Corticosterone (CORT; 20 mg/kg) was injected each day for 6 weeks. NMN (300 mg/ kg) was administered by oral gavage in the last 2 weeks of the experiment. Male C57/BL6 mice (6 weeks old, n = 8 mice/group). *P < 0.05, **P < 0.01 vs. control group; #P < 0.05, ##P < 0.01 vs. CORT group. "Nicotinamide mononucleotide ameliorates the depression-like behaviors and is associated with attenuating the disruption of mitochondrial bioenergetics in depressed mice" (2020) https://pubmed.ncbi.nlm.nih.gov/31818774/

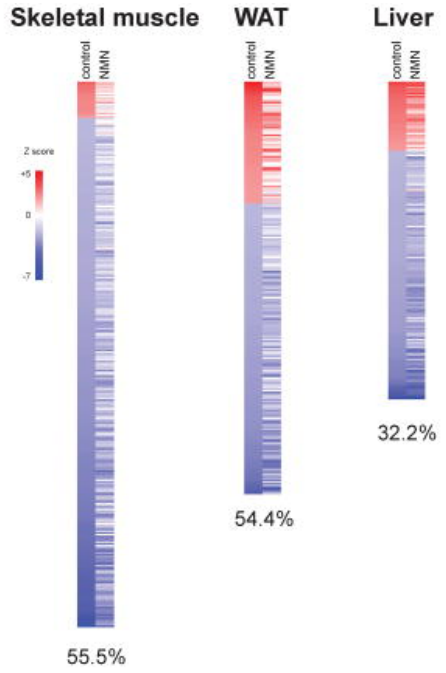
NAD+ metabolites in humans resulting from NR supplementation (500 mg, 2×/day).
"Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD+ in healthy middle-aged and older adults" (2018) https://www.nature.com/articles/s41467-018-03421-7

Metabolite	Median (range)		P-value
	Placebo	NR	
NAAD	0.0 (0.0−2.3)	0.0 (0.0−8.7)	0.018*
NAD+	7.7 (0.0−27.4)	12.2 (4.7−67.8)	0.048*
NADP	6.1 (3.3−17.9)	6.3 (2.7−42.7)	0.267
NaM	257.6 (109−411)	278.6 (171−1357)	0.065
NMN	0.0 (0.0−5.5)	0.0 (0.0−11.9)	0.179
ATP	1592 (363−3446)	2205 (763−5459)	0.032‡

All values expressed as pmol per mg protein.
* represents unadjusted P < 0.05 significance level.
‡ ATP represents secondary outcome assessed at Bonferroni-adjusted P < 0.006.

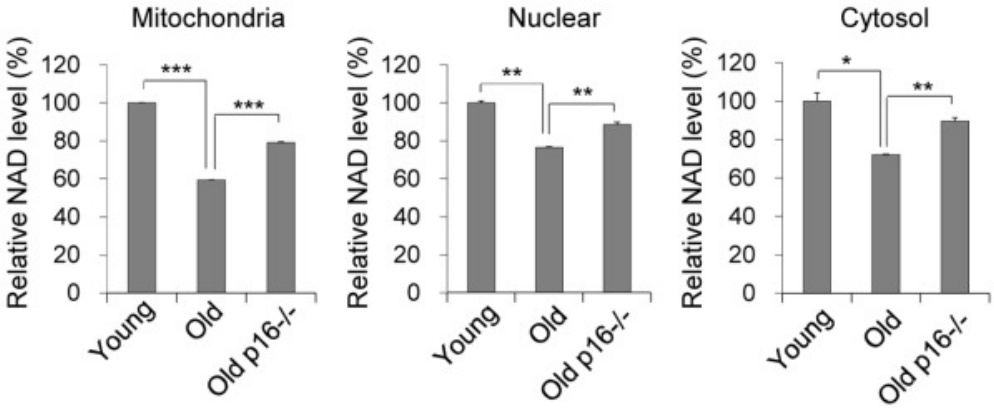


NAD+ injected into the bloodstream elevates ATP inside cells. "Extracellular Degradation into Adenosine and the Activities of Adenosine Kinase and AMPK Mediate Extracellular NAD+-Produced Increases in the Adenylate Pool of BV2 Microglia Under Basal Conditions" (2018) https://www.frontiersin.org/articles/10.3389/fncel.2018.00343/full



Gene expression profiles of skeletal muscle, white adipose tissue (WAT), and the liver in middle-aged (5 to 17 months) control and NMN-administered mice after 12 months of NMN administration. NMN administration inhibits age-induced changes in pathways of skeletal muscle (55.5% of 299 pathways), WAT (54.4% of 226 pathways) and liver (32.2% of 174 pathways). “Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5668137/

Another point of interest is the observation that removing SnCs significantly increases NAD+ levels and consequently partially restores ATP production. The effect of SnC removal on NAD+ levels is shown in the figure below where p16 is a marker of cellular senescence and p16-/- refers to inducibly cleared p16 cells. Since NAD+ is a cofactor in the Krebs cycle, increasing NAD+ results in more ATP.



Mitochondrial NAD+ levels were lower in tail-tip fibroblasts (TTFs) collected from old (22 month) wild type (WT) mice versus those collected from young (2 month) WT or old (22 month) p16 KO mice in early-stage reprogramming. Reprogramming intermediates from young WT, old WT, and old p16−/− TTFs on D8 were fractionated into mitochondrial, nuclear, and cytosolic fractions. The NAD+ level in each lysate was quantified. The data are shown as the mean ± SE of duplicate samples for NAD assays and triplicate samples for PCR analyses. *, $p < .05$; **, $p < .01$; ***, $p < .001$ (Student's t test). “Restoration of Mitochondrial NAD+ Levels Delays Stem Cell Senescence and Facilitates Reprogramming of Aged Somatic Cells” (2016) https://stemcellsjournals.onlinelibrary.wiley.com/doi/full/10.1002/stem.2460

However, study results on NR and NMN are mixed. A small clinical trial involving 71-year-old men showed that 250 mg NMN per day for 12 weeks is insufficient to make a material difference in many phenotypes of aging in older individuals. “Chronic nicotinamide mononucleotide supplementation elevates blood nicotinamide adenine dinucleotide levels and alters muscle function in healthy older men” (2022) https://www.nature.com/articles/s41514-022-00084-z

- Blood NAD+ levels increased
- Skeletal muscle strength and gait speed increased
- Mass mass did not change
- Liver and visceral fat mass are not affected
- Vascular functions, such as assessed blood pressure and flow-mediated dilation, were unchanged
- Cognitive function was not affected

Supporting references

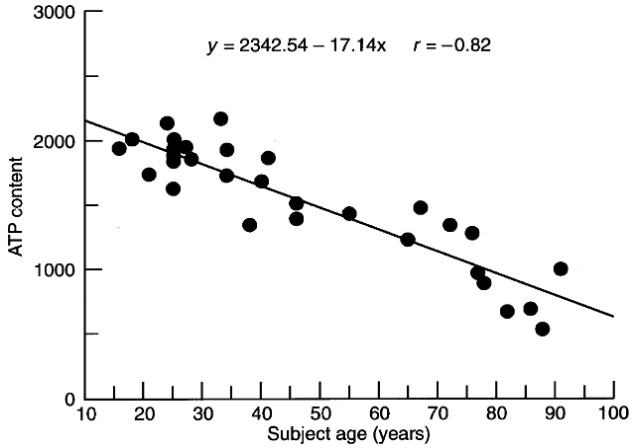
1. “A Screen Using iPSC-Derived Hepatocytes Reveals NAD+ as a Potential Treatment for mtDNA Depletion Syndrome” (2018) https://www.sciencedirect.com/science/article/pii/S2211124718316127?via%3Dihub
2. “Age-Associated Changes In Oxidative Stress and NAD+ Metabolism In Human Tissue” (2012) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3407129/
3. “Impaired nicotinamide adenine dinucleotide (NAD+) metabolism in diabetes and diabetic tissues: Implications for nicotinamide-related compound treatment” (2020) https://onlinelibrary.wiley.com/doi/10.1111/jdi.13303
4. “Nicotinamide mononucleotide ameliorates the depression-like behaviors and is associated with attenuating the disruption of mitochondrial bioenergetics in depressed mice” (2020) https://pubmed.ncbi.nlm.nih.gov/31818774/
5. “Extracellular Degradation into Adenosine and the Activities of Adenosine Kinase and AMPK Mediate Extracellular NAD+-Produced Increases in the Adenylate Pool of BV2 Microglia Under Basal Conditions” (2018) https://www.frontiersin.org/articles/10.3389/fncel.2018.00343/full
6. “NAD+ metabolism governs the proinflammatory senescence-associated secretome” (2019) https://doi.org/10.1038/s41556-019-0287-4
7. “Nicotinamide Mononucleotide: A Promising Molecule for Therapy of Diverse Diseases by Targeting NAD+ Metabolism” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198709/
8. “Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5668137/
9. “Nicotinamide mononucleotide ameliorates the depression-like behaviors and is associated with attenuating the disruption of mitochondrial bioenergetics in depressed mice” (2020) https://pubmed.ncbi.nlm.nih.gov/31818774/

ATP production declines with age, causes age-related ATP/AMP deficits

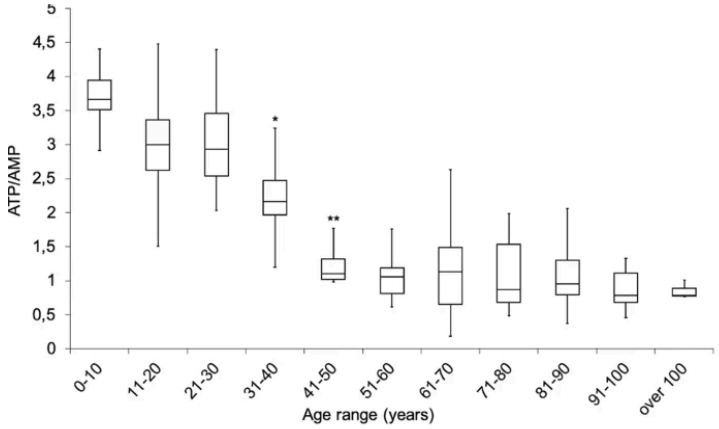
Support for the idea that a decline in ATP production is the major cause of age-related ATP/AMP deficits comes from studies of gene expression changes with age. In addition to pathways that are related to up-regulation of inflammation, the most prominent transcriptional pathways that change with aging across multiple tissues are mitochondrial: the downregulation of oxidative phosphorylation, respiratory electron transport and biological oxidation. “Age-Related Gene Expression Signature in Rats Demonstrate Early, Late, and Linear Transcriptional Changes from Multiple Tissues” (2019) https://www.cell.com/cell-reports/fulltext/S2211-1247(19)31091-5

Multiple factors contribute to the decline in ATP production with age including (but not limited to)

1. Age-associated decline in the redox factor NAD+ results in lower ATP production.
2. The decline in ATP is in part causally related to the increase in nuclear and mitochondrial DNA damage, and the exponential decline in somatic cell turnover rates, stem cell populations, etc.
3. Lower ATP levels feedback to cause even lower ATP production through increased damage, lipid peroxidation, decreased transport, and multiple other ATP-dependent cellular processes that relate to the cell’s fitness. A simple feedback model for these processes is a first order differential equation which gives an exponential response.
4. Causal relationships between the decline in ATP production, senescent cell (SnC) accumulation, and the subsequent decline in NAD+ concentrations.
5. Reduced ATP levels lead to cellular adaptations through epigenetic regulation that preserve cells’ survival during stress and damage at the cost of health and proliferative ability.



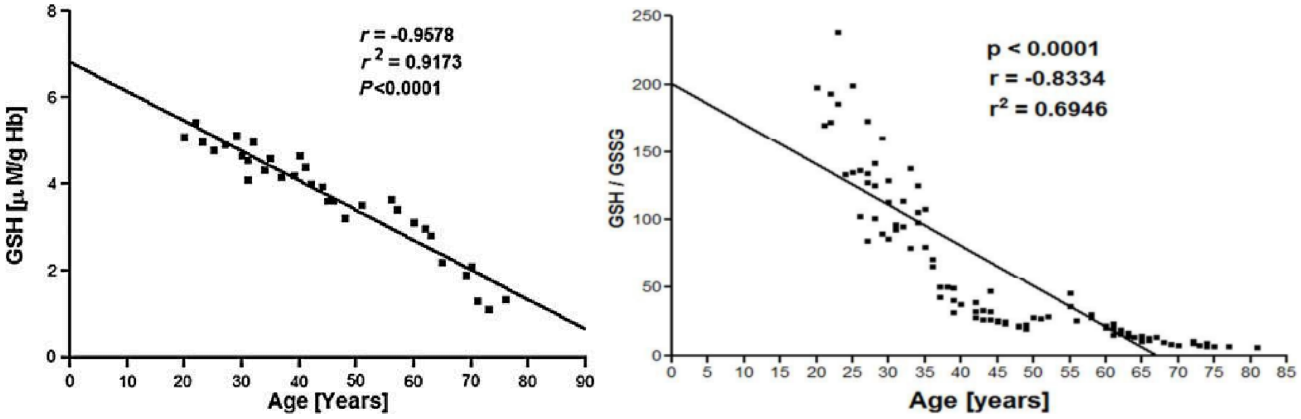
ATP content in red blood cells. “Diabetes mellitus and subjects' ageing: a study on the ATP content and ATP-related enzyme activities in human erythrocytes” (1997) http://dx.doi.org/10.1046/j.1365-2362.1997.1130652.x



Changes of ATP/AMP ratio, a marker of the energy status, in mononuclear cells obtained from a healthy population between ages of 5 to 106 years. * or ** indicate, respectively, a significant difference for $p<0.05$ or $p<0.01$ between the marked decade and the previous decade “Discrete Changes in Glucose Metabolism Define Aging” (2019) https://www.nature.com/articles/s41598-019-46749-w

Glutathione precursors (GlyNAC) improve GSH/GSSG, BDNF and physical function in older individuals

Glutathione is the major endogenous antioxidant in our bodies and is essential for metabolic and biochemical reactions such as energy metabolism and mitochondrial dysfunction, DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation. In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH), with the remainder in the disulfide form (GSSG). As we age glutathione levels decrease, resulting in wide ranging cellular adaptations and dysfunction including a decline in BDNF.



Erythrocyte glutathione (GSH) and GSH/GSSG plotted as a function of human age for normal, healthy, non-smoker subjects of both sexes between the ages of 20-81 years, where GSH and GSSG are reduced and oxidized glutathione, respectively. GSH drops linearly between 20-80 years. An exponential trendline gives a decline of GSH/GSSG (t) $\propto e^{-0.043\,t}$ from reference (1) or $\propto e^{-0.067\,t}$ from reference (2) where t is in years. (1) "L-cysteine efflux in erythrocytes as a function of human age: correlation with reduced glutathione and total antioxidant potential" (2013) doi/10.1089/rej.2012.1394; (2) "Multi-target detection of oxidative stress biomarkers in quercetin and myricetin treated human red blood cells" (2016) DOI:10.1039/C6RA05121A

Why does GSH/GSSG drop with age? The possible answers include reduced uptake of precursors (via the Na+ cystine/glutamate antiporter), reduced biosynthesis (rate limited by cysteine), increased degradation (via glutathione reductase), or increased consumption of GSH (which is consistent with an increase in ROS production by the mitochondria with age). Research has found that *" glutathione deficiency in aged humans occurs in large part because of a markedly diminished supply of the precursors glycine and cysteine."* Moreover, it was also found that supplementation with glutathione precursors restored GSH in aged humans to the levels of young one, as shown in the table below. I confirmed this finding by supplementation with glycine and NAC, as shown below.

1. "Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation" (2011) https://doi.org/10.3945/ajcn.110.003483
2. (Review) "The effects of stress and aging on glutathione metabolism" (2022) https://doi.org/10.1016/j.arr.2005.02.005
3. (Review ★) "Glutathione: A Samsonian life-sustaining small molecule that protects against oxidative stress, ageing and damaging inflammation" (2022) https://doi.org/10.3389/fnut.2022.1007816

While cellular glycine is generally available, it is sometimes that case that it is not present in the surplus needed for glutathione synthesis. Research supports the idea of supplementing with glycine in addition to NAC. "Dietary Glycine Is Rate-Limiting for Glutathione Synthesis and May Have Broad Potential for Health Protection" (2018) https://www.ochsnerjournal.org/content/ochjnl/18/1/81.full.pdf

Directly consuming or intravenously injecting glutathione does not give the benefits of consuming its precursors N-acetyl-cysteine and glycine. This difference is explained in part by the age-related deficit in cysteine. As we age, the mean plasma cysteine/cystine redox status of human subjects shows a significant oxidative shift between the third and the ninth decade of life [156]. Since cysteine availability is a limiting factor for glutathione synthesis, compensatory supplementation of NAC helps restore glutathione levels.

Supplementation of NAC without glycine gives mixed results in numerous studies. In one clinical trial involving 20-77 year olds (average about 50), supplementation of 500 mg of NAC 2x/day without glycine did not significantly change IL6, CRP or BDNF levels [155], possibly because many of the subjects were too young to have significant deficiencies.

Red blood cell concentrations in young control (40-years-old, unsupplemented) and elderly (70-years-old, before and after 14 day supplementation with glutathione precursors) subjects.
"Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation" (2011) https://doi.org/10.3945/ajcn.110.003483

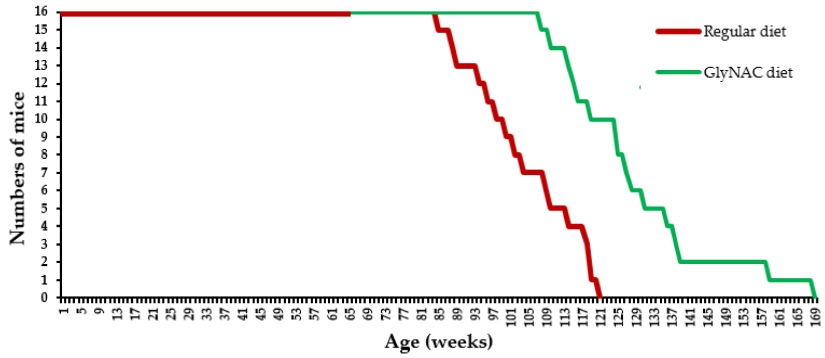
Variable	Young control subjects (n = 8)	Elderly subjects (n = 8)	
		Before supplementation	After 14 days supplementation
Glycine (µmol/L)	486.7 ± 28.32	218.0 ± 23.73	528.6 ± 33.5
Cysteine (µmol/L)	26.2 ± 1.44	19.8 ± 1.35	30.6 ± 2.2
Glutamate (µmol/L)	463.1 ± 69.0	464.0 ± 115.3	—
GSSG (mmol/L)	0.11 ± 0.044	0.15 ± 0.05	0.13 ± 0.05
GSH:GSSG ratio	18.9 ± 2.12	7.4 ± 2.36	16.1 ± 4.3

Effect of GlyNAC supplementation (1000 mg/ea/day NAC and glycine) on my serum Total Glutathione (GST) in whole blood (age 65) .

	10/2019	5/2020	10/2020	6/2022	8/2022		Reference*
Glutathione, Total (uM)	323	556	569	NA	NA		373-838 µM
Epigenetic Age‡ (difference from chrono-age, years)	NA	-10.5	-10.3	-14.7	-11.1		
Supplements; exercise	Little exercise	GlyNAC 1x/day; Little exercise	Inconsistent GlyNAC 1x/day; 4000 steps/day	GlyNAC 2x/day; Walking 4000 steps/day	GlyNAC 2x/day; Walking 4000 steps/day; 500mg NMN 2x/day		

* Quest Diagnostics assay. The reference range depends on test type and cell type.
‡ TruMe Labs assay

Oral supplementation of 1000 mg/day each of its precursors glycine and N-acetylcysteine (NAC) restores age-related glutathione deficiencies to near-youthful levels after eight to 24 weeks [153,154]. In addition to glutathione, this supplementation restores age-related decline in BDNF in humans, and gives systemic benefits to cognition and physical function, as listed in the table below. Medium lifespan in mice is increased by 24% [152-154]. Of note, this increase in lifespan is one of the largest reported for any intervention. Its effect is similar in magnitude to calorie restriction, senescent cell removal, and autophagy activation.
"Lifespan Increase in Mice From Various Interventions" (2022)
https://docs.google.com/document/d/1Z1nMkllVrSPFdgq1x6gg-8bYE18UsV7nMLZVdQddJ1g/edit?usp=sharing



Regular diet vs. GlyNAC-supplemented diet on length of life in C57BL/6J mice from the age of 65-weeks (equivalent to about a 50-year-old human). "GlyNAC (Glycine and N-Acetylcysteine) Supplementation in Mice Increases Length of Life by Correcting Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Abnormalities in Mitophagy and Nutrient Sensing, and Genomic Damage" (2022) https://www.mdpi.com/2072-6643/14/5/1114/htm

Concentrations of total glutathione, reduced glutathione, oxidized glutathione (GSSG), and TBARS in the liver of five mice/group of young control mice (Y), old control mice (OC) and old mice supplemented with GlyNAC (OG) [154].
"GlyNAC (Glycine and N-Acetylcysteine) Supplementation in Mice Increases Length of Life by Correcting Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Abnormalities in Mitophagy and Nutrient Sensing, and Genomic Damage" (2022) https://doi.org/10.3390/nu14051114

	Young mice (8-20 weeks)	**Older mice (65 weeks§)**	**Older mice fed GlyNAC (65 weeks§)**
Medium lifespan (increased 24%, both sexes)		104 weeks	128 weeks
		$p < 0.0001$	$p < 0.0001$
Total GSH‡ (μmol/kg, = GSH + GSSG) (master antioxidant; cofactor in many reactions)	3.3 ± 0.1	1.2 ± 0.0	3.2 ± 0.1
		$p < 0.0001$	$p < 0.0001$
GSH‡ (μmol/kg) (reduced glutathione)	2.9 ± 0.1	1.0 ± 0.1	2.8 ± 0.1
		$p < 0.0001$	$p < 0.0001$
GSSG‡ (μmol/kg) (oxidized glutathione)	0.4 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
		$p < 0.001$	$p = 0.08$
Plasma TBARS‡ μM) (an index of plasma lipid peroxidation)	7.1 ± 0.2	14.8 ± 0.6	8.3 ± 0.2
		$p < 0.001$	$p < 0.001$
PGC1α/β-actin‡ (master regulator of mitochondrial biogenesis)	1.0	0.6	1.1
	$p < 0.01$	$p < 0.01$	$p < 0.01$
PPARα/β-actin‡ (major regulator of lipid metabolism)	0.75	0.5	0.8
	$p < 0.05$	$p < 0.05$	$p < 0.05$
ATP5A/β-actin‡ (ATP synthase catalyzes ATP synthesis)	1.0	0.5	0.8
	$p < 0.01$	$p < 0.01$	$p < 0.01$
PINK1/β-actin‡ (regulator of mitophagy)	1.0	0.6	0.95
	$p < 0.05$	$p < 0.01$	$p < 0.01$
SirT3/β-actin‡ (mitochondria-located regulator of nutrient sensing and metabolism)	1.1	0.45	1.2
	$p < 0.05$	$p < 0.05$	$p < 0.05$
pH2AX/β-actin‡ (DNA damage marker)	0.4	1.0	0.5
	$p < 0.05$	$p < 0.05$	$p < 0.05$

‡ Liver. β-actin is a "housekeeper" gene whose expression is used as a reference level.
§ A 65-week old mouse is equivalent in age to a 50 year old human.

Effects of GlyNAC supplementation and withdrawal on humans. Values± SD. Older adults (OA, 71–80 years) received GlyNAC supplementation for 24 weeks, and GlyNAC was stopped for 12 weeks from week 24 to week 36 [153].
"Glycine and N-acetylcysteine (GlyNAC) supplementation in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, insulin resistance, endothelial dysfunction, genotoxicity, muscle strength, and cognition: Results of a pilot clinical trial" (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8002905/

Cognition and physical function (see the paper for more markers)	Young adults (24 ± 1 years): 0-week	Older adults (71–80 years): 0-week	Older adults: after 12-weeks on GlyNAC	Older adults: after 24-weeks on GlyNAC	Older adults at 36-weeks: (12-weeks after stopping GlyNAC)
Blood markers					
RBC Total GSH (mmol/L.RBC) (RBC = red blood cells)	1.8 ± 0.3	0.8 ± 0.4	1.0 ± 0.3	1.6 ± 0.2	0.9 ± 0.3
		p = 0.0005	p = 0.0045	p = 0.0004	p = 0.0029
RBC GSH/GSSG	10.1 ± 2.9	1.1 ± 0.8	3.4 ± 2.7	4.7 ± 3.0	1.1 ± 0.4
		p = 0.0003	p = 0.095	p = 0.022	p = 0.011
RBC GSH (mmol/L.RBC) (reduced glutathione)	1.7 ± 0.2	0.4 ± 0.2	0.7 ± 0.4	1.2 ± 0.1	0.4 ± 0.2
		p = 0.0000	p = 0.043	p = 0.0000	p = 0.0001
RBC GSSG (mmol/L.RBC) (oxidized glutathione)	0.2 ± 0.1	0.4 ± 0.3	0.4 ± 0.4	0.4 ± 0.2	0.4 ± 0.2
		p = 0.026	p = 0.91	p = 0.96	p = 0.73
Plasma TBARS (µM/L) (an index of lipid peroxidation)	2.4 ± 0.4	22.7 ± 3.5	14.1 ± 6.0	5.7 ± 1.8	18.2 ± 2.2
		p = 0.0000	p = 0.0008	p = 0.0000	p = 0.0000
Plasma BDNF (ng/ml)	36.3 ± 10.6	21.1 ± 4.2	25.3 ± 4.6	31.8 ± 5.6	27.8 ± 5.1
		p = 0.005	p = 0.0002	p = 0.0000	p = 0.048
TNFα (pg/ml)	45.3 ± 9.4	97.9 ± 13.9	80.4 ± 10.6	58.7 ± 8.3	72.6 ± 11.1
		p = 0.0000	p = 0.0000	p = 0.0000	p = 0.0003
IL6 (pg/ml)	0.5 ± 0.1	4.8 ± 0.4	2.7 ± 0.4	1.1 ± 0.4	2.0 ± 0.4
		p = 0.0000	p = 0.0000	p = 0.0000	p = 0.0001
High sensitivity C-reactive protein (hsCRP, ng/ml)	2.4 ± 0.4	4.9 ± 0.6	3.5 ± 0.5	3.2 ± 0.5	3.9 ± 0.5
		p = 0.0000	p = 0.0002	p = 0.0000	p = 0.0004
Plasma insulin (mU/L)	8.4 ± 2.4	47.8 ± 6.3	36.1 ± 4.1	21.4 ± 3.8	33.5 ± 6.5
		p = 0.0000	p = 0.0000	p = 0.0000	p = 0.0004
Fasting respiratory quotient (RQ) (CO_2 produced/oxygen consumed) Wiki	0.77 ± 0.01	0.84 ± 0.03	0.79 ± 0.04	0.77 ± 0.03	0.83 ± 0.03
		p = 0.0008	p = 0.02	p = 0.0000	p = 0.0003
Fasting mitochondrial fatty-acid oxidation (mg/kgLM/min)	1.7 ± 0.2	0.8 ± 0.3	1.4 ± 0.2	1.5 ± 0.2	
		p = 0.0004	p = 0.0018	p = 0.0002	
Fasting mitochondrial carbohydrate oxidation (mg/kgLM/min)	1.3 ± 0.5	2.0 ± 0.5	1.7 ± 0.8	1.2 ± 0.5	
		p = 0.034	p = 0.26	p = 0.0026	
8-hydroxy deoxyguanosine (pg/ml) (Oxidized DNA) [160,161] Wiki	23.9 ± 3.8	106.9 ± 30.1	59.7 ± 16.2	36.9 ± 11.6	50.1 ± 17.6
		p = 0.000095	p = 0.0002	p = 0.0002	p = 0.026
Physical function					
Gait speed (m/s)	1.4 ± 0.2	1.0 ± 0.1	1.3 ± 0.2	1.5 ± 0.2	1.2 ± 0.2
		p = 0.0061	p = 0.0007	p = 0.0002	p = 0.035
Grip strength, dominant hand (kg)	35.3 ± 5.7	27.0 ± 9.7	29.7 ± 8.7	30.9 ± 9.2	27.3 ± 9.4
		p = 0.014	p = 0.045	p = 0.0056	p = 0.018
Cognition					
Trails Making Test A (sec) (perceptual speed)	25.2 ± 8.4	45.2 ± 15.1	37.3 ± 10.6	32.1 ± 8.2	33.4 ± 10.4
		p = 0.004	p = 0.012	p = 0.003	p = 0.58
Trails Making Test B (sec) (perceptual and task switching speed)	36.4 ± 8.7	71.0 ± 26.5	51.5 ± 15.2	48.6 ± 13.4	53.2 ± 23.3
		p = 0.007	p = 0.023	p = 0.016	p = 0.38
Verbal fluency test (detection of mild cognitive impairment)	52.0 ± 6.7	44.0 ± 10.8	51.4 ± 10.7	52.1 ± 9.7	51.8 ± 13.4
		p = 0.059	p = 0.0129	p = 0.013	p = 0.90
Digit symbol substitution test (% completion)	60.9 ± 9.1	41.3 ± 8.3	42.4 ± 8.2	43.6 ± 8.0	42.1 ± 7.1
		p = 0.006	p = 0.38	p = 0.013	p = 0.013
Digit symbol substitution test (% accuracy)	99.4 ± 0.9	96.0 ± 3.1	98.7 ± 2.5	99.6 ± 1.2	97.5 ± 1.4
		p = 0.018	p = 0.0038	p = 0.015	p = 0.0075

A placebo controlled clinical trial of 100 mg/kg/d each of glycine and NAC in 21-40-year-olds vs 61-80-year-olds found that GlyNAC (and not the placebo) supplementation in OA improved GSH deficiency, OxS, mitochondrial dysfunction (with defective molecular regulation), inflammation, endothelial dysfunction, IR, multiple aging hallmarks, impaired physical function, increased waist circumference, and systolic blood pressure, as shown in the following tables [276] "Supplementing Glycine and N-Acetylcysteine (GlyNAC) in Older Adults Improves Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Physical Function, and Aging Hallmarks: A Randomized Clinical Trial" (2022) https://doi.org/10.1093/gerona/glac135

Glutathione, Oxidative Stress, and Mitochondrial Function (100 mg/kg/d each of glycine and NAC)
"Supplementing Glycine and N-Acetylcysteine (GlyNAC) in Older Adults Improves Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Physical Function, and Aging Hallmarks: A Randomized Clinical Trial" (2022) https://doi.org/10.1093/gerona/glac135

	Young (21-40 y) GlyNAC		Old (61-80 y) GlyNAC		Week 16 vs 0, Old vs Young		Old Placebo	
Outcome Measure	**YAG-0w (N = 11)**	**YAG-2w (N = 11)**	**OAG-0w YA-0w vs OAG-0w (N = 12)**	**OAG-2w (N = 12)**	**OAG-16w OAG-0w vs OAG-16w; OAG-16w vs YA-0w (N = 11)**	**OAP-0w YA-0w vs OAP-0w; OAP-0w vs OAG-0w (N = 12)**	**OAP-2w (N = 12)**	**OAP-16w OAP-0w vs OAP-16w (N = 12)**
Glutathione and oxidative stress								
Muscle-glutathione (mmol/kg muscle)	7.0 ± 1.3	7.0 ± 1.3	2.4 ± 0.6 p < .001	5.3 ± 0.9 p < .001	6.3 ± 1.2 p < .001 p = .32	2.4 ± 0.8 p < .001 p > .99	2.3 ± 0.8 p > .99	2.4 ± 0.8 p > .99
RBC-total glutathione (tGSH; mmol/L.RBC)	1.4 ± 0.2	1.5 ± 0.2	0.4 ± 0.1 p < .001	1.2 ± 0.1 p < .001	1.4 ± 0.1 p < .001 p = .84	0.5 ± 0.1 p < .001 p = .84	0.5 ± 0.1 p = .96	0.5 ± 0.1 p = .96
RBC-reduced glutathione (rGSH; mmol/L.RBC)	1.4 ± 0.2	1.4 ± 0.2	0.4 ± 0.1 p < .001	1.1 ± 0.1 p < .001	1.3 ± 0.1 p < .001 p = .86	0.4 ± 0.1 p < .001 p = .86	0.5 ± 0.1 p = .86	0.4 ± 0.1 p = .86
RBC-oxidized glutathione (GSSG; mmol/L.RBC)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0 p > .99	0.1 ± 0.0 p = .45	0.1 ± 0.1 p > .99 p > .99	0.1 ± 0.0 p > .99 p > .99	0.1 ± 0.0 p > .99	0.1 ± 0.0 p > .99
RBC-GSH/GSSG	24.6 ± 14.7	25.2 ± 16.3	7.5 ± 4.8 p = .001	14.7 ± 7.6 p = .38	27.6 ± 18.2 p < .001 p > .99	9.0 ± 6.7 p = .003 p > .99	9.6 ± 5.4 p > .99	9.5 ± 5.3 p > .99
Plasma TBARS (μM/L)	4.2 ± 1.0	4.1 ± 0.8	22.1 ± 3.3 p < .001	12.7 ± 3.3 p < .001	6.2 ± 1.9 p < .001 p = .28	21.9 ± 3.6 p < .001 p = .90	21.4 ± 2.3 p = .88	22.9 ± 3.5 p = .74
Plasma F2-isoprostane (pg/mL)	53.9 ± 2.9	53.0 ± 2.9	216.0 ± 26.9 p < .001	119.9 ± 37.6 p < .001	59.7 ± 4.0 p < .001 p > .99	215.8 ± 19.4 p < .001 p > .99	214.2 ± 13.6 p > .99	219.8 ± 13.9 p > .99
Mitochondrial function								
Fasting respiratory quotient (RQ)	0.79 ± 0.01	0.78 ± 0.02	0.85 ± 0.03 p < .001	0.82 ± 0.03 p < .001	0.77 ± 0.02 p < .001 p = .27	0.84 ± 0.02 p < .001 p = .27	0.84 ± 0.03 p > .99	0.84 ± 0.03 p > .99
Fasting mitochondrial fatty-acid oxidation (mg/kgLM/min)	1.2 ± 0.2	1.2 ± 0.2	0.7 ± 0.3 p < .001	0.9 ± 0.3 p < .001	1.2 ± 0.2 p < .001 p > .99	0.8 ± 0.2 p < .001 p = .47	0.8 ± 0.2 p > .99	0.8 ± 0.2 p > .99
Fasting mitochondrial glucose oxidation (mg/kgLM/min)	0.9 ± 0.6	0.9 ± 0.4	2.2 ± 0.7 p < .001	1.5 ± 0.6 p < .001	0.8 ± 0.3 p < .001 p > .99	2.0 ± 0.5 p = .001 p > .99	2.1 ± 0.6 p > .99	2.1 ± 0.8 p > .99
Rate of palmitate oxidation (μmol/kgLM/min)	146.8 ± 25.5	151.6 ± 19.2	119.0 ± 11.9 p = .003	139.7 ± 24.3 p = .007	157.1 ± 31.7 p < .001 p = .65	122.9 ± 10.6 p = .010 p > .99	125.0 ± 12.5 p > .99	121.8 ± 9.9 p > .99
Rate of acetate oxidation (μmol/kgLM/min)	227.4 ± 42.8	228.4 ± 40.6	258.4 ± 40.3 p > .99	254.8 ± 52.8 p > .99	249.9 ± 44.5 p > .99 p > .99	259.2 ± 72.4 p > .99 p > .99	258.3 ± 43.5 p > .99	259.4 ± 51.7 p > .99
Energy expenditure (kcal/d)	1 348 ± 246	1 363 ± 269	1 516 ± 223 p = .75	1 420 ± 191 p = .28	1 438 ± 214 p > .99 p > .99	1 384 ± 258 p > .99 p > .99	1 434 ± 233 p > .99	1 365 ± 279 p > .99 p > .99

Physical Function, Body Composition, and Blood Pressure (100 mg/kg/d each of glycine and NAC) [276]
"Supplementing Glycine and N-Acetylcysteine (GlyNAC) in Older Adults Improves Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Physical Function, and Aging Hallmarks: A Randomized Clinical Trial" (2022) https://doi.org/10.1093/gerona/glac135

	Young (21-40 y) GlyNAC		Old (61-80 y) GlyNAC		Week 16 vs 0, Old vs Young		Old Placebo	
	YAG-0w (N = 11)	**YAG-2w (N = 11)**	**OAG-0w YA-0w vs OAG-0w (N = 12)**	**OAG-2w (N = 12)**	**OAG-16w OAG-0w vs OAG-16w; OAG-16w vs YA-0w (N = 11)**	**OAP-0w YA-0w vs OAP-0w; OAP-0w vs OAG-0w (N = 12)**	**OAP-2w (N = 12)**	**OAP-16w OAP-0w vs OAP-16w (N = 12)**
Physical function								
Gait speed (m/sec)	1.36 ± 0.14	1.42 ± 0.11	1.13 ± 0.19 p = .031	1.32 ± 0.36	1.34 ± 0.28 p = .032 p > .99	1.11 ± 0.19 p = .019 p > .99	1.17 ± 0.22	1.16 ± 0.21 p > .99 p = .11
Grip strength, dominant hand (kg)	34.8 ± 10.7	35.1 ± 12.1	32.3 ± 10.0 p > .99	34.2 ± 10.1	36.7 ± 10.6 p = .004 p > .99	31.4 ± 9.8 p > .99 p > .99	30.8 ± 12.7	29.3 ± 10.9 p = .14 p > .99
Grip strength, nondominant hand (kg)	31.7 ± 10.5	32.5 ± 12.0	29.6 ± 9.2 p > .99	30.7 ± 10.5	33.9 ± 10.5 p = .004 p > .99	30.7 ± 10.6 p > .99 p > .99	30.1 ± 11.7	27.8 ± 11.7 p = .009 p > .99
Chair-rise test (sec)	16.5 ± 1.8	15.4 ± 1.9	25.6 ± 8.9 p = .005	21.8 ± 5.2	18.8 ± 6.6 p = .002 p = .86	22.6 ± 4.0 p = .10 p = .86	22.1 ± 3.2	20.7 ± 3.8 p = .86 p = .31
Six-minute rapid walk test (m)	635.4 ± 71.6	645.6 ± 78.7	522.5 ± 62.5 p < .001	534.8 ± 70.1	564.8 ± 68.9 p = .053 p = .039	518.3 ± 52.9 p < .001 p = .99	532.3 ± 67.1	527.1 ± 66.0 p = .99 p = .003
Body composition								
Weight (kg)	69.3 ± 10.9	69.1 ± 10.6	89.5 ± 14.8 p = .005	88.4 ± 13.8	87.4 ± 14.2 p = .063 p = .007	84.5 ± 12.2 p = .037 p > .99	84.5 ± 12.2	84.6 ± 11.5 p > .99 p = .034
Body mass index	23.8 ± 2.9	23.8 ± 2.9	31.5 ± 4.0 p < .001	31.1 ± 3.7	30.9 ± 3.8 p = .09 p < .001	31.0 ± 2.2 p < .001 p > .99	31.0 ± 2.1	31.1 ± 2.4 p > .99 p < .001
Fat mass (kg)	16.0 ± 5.5	15.7 ± 5.3	29.9 ± 8.1 p < .001	29.5 ± 8.1	27.8 ± 7.0 p > .99 p < .001	31.2 ± 7.2 p < .001 p > .99	31.2 ± 7.4	31.2 ± 8.1 p > .99 p < .001
Waist circumference (m)	0.91 ± 0.04	0.91 ± 0.04	1.06 ± 0.09 p < .001	1.04 ± 0.08	1.03 ± 0.09 p = .002 p = .003	1.02 ± 0.07 p = .009 p = .33	1.01 ± 0.06	1.01 ± 0.08 p = .43 p = .018
Lean mass (kg)	50.8 ± 11.6	51.0 ± 11.5	57.2 ± 10.2 p > .99	56.8 ± 10.0	57.6 ± 10.8 p > .99 p > .99	51.2 ± 13.0 p > .99 p > .99	51.0 ± 13.4	51.1 ± 12.8 p > .99 p > .99
Blood pressure								
Systolic blood pressure (mmHg)	110.8 ± 7.9	107.4 ± 11.7	132.0 ± 16.8 p = .01	131.4 ± 19.5	124.4 ± 17.0 p = .043 p = .18	129.5 ± 16.0 p = .026 p > .99	127.8 ± 14.3	127.6 ± 14.3 p > .99 p = .043
Diastolic blood pressure (mmHg)	70.0 ± 5.2	67.0 ± 3.5	79.2 ± 6.0 p = .01	76.1 ± 5.6	75.9 ± 8.6 p = .61 p = .32	78.1 ± 7.2 p = .027 p > .99	76.7 ± 9.6	75.8 ± 8.1 p = .91 p = .32

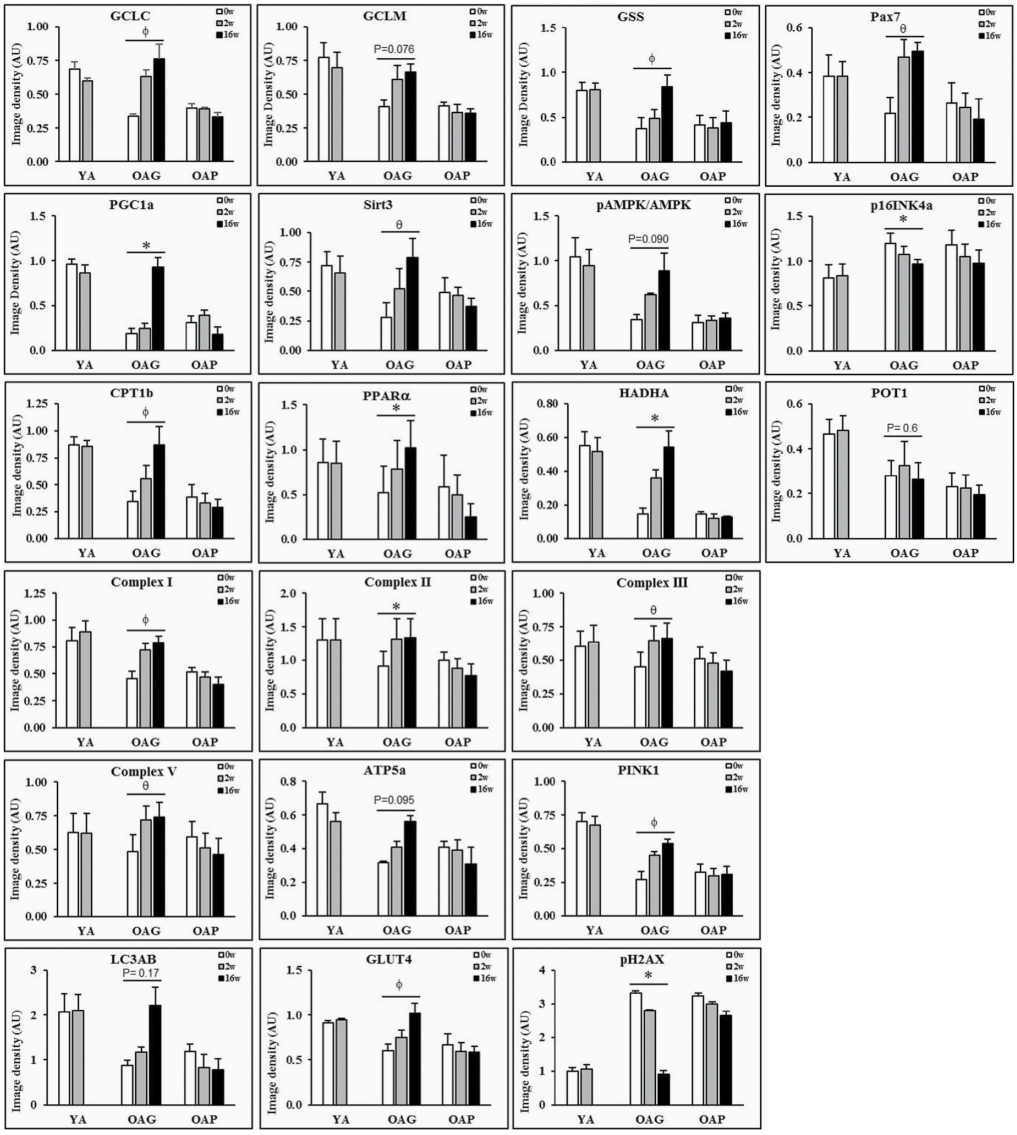
Mitochondrial Function, Inflammation, Endothelial Function, Glycemic Indices, and Genomic Damage (100 mg/kg/d each of glycine and NAC) [276] "Supplementing Glycine and N-Acetylcysteine (GlyNAC) in Older Adults Improves Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Physical Function, and Aging Hallmarks: A Randomized Clinical Trial" (2022) https://doi.org/10.1093/gerona/glac135

	Young (21-40 y) GlyNAC		Old (61-80 y) GlyNAC		Week 16 vs 0, Old vs Young		Old Placebo	
	YAG-0w (N = 11)	YAG-2w (N = 11)	OAG-0w YA-0w vs OAG-0w (N = 12)	OAG-2w (N = 12)	OAG-16w OAG-0w vs OAG-16w; OAG-16w vs YA-0w (N = 11)	OAP-0w YA-0w vs OAP-0w; OAP-0w vs OAG-0w (N = 12)	OAP-2w (N = 12)	OAP-16w OAP-0w vs OAP-16w (N = 12)
Plasma inflammatory cytokines								
High-sensitivity interleukin-6 (IL-6, pg/mL)	0.5 ± 0.1	0.5 ± 0.1	4.6 ± 1.4 p < .001	2.0 ± 0.5 p < .001	1.0 ± 0.3 p < .001 p > .99	4.1 ± 1.6 p < .001 p > .99	4.0 ± 1.5 p > .99	4.2 ± 1.5 p > .99
Tumor necrosis factor alpha (TNFα, pg/mL)	33.3 ± 6.3	32.4 ± 8.4	103.4 ± 14.7 p < .001	72.8 ± 10.4 p < .001	47.1 ± 11.3 p < .001 p = .018	102.4 ± 12.8 p < .001 p = .84	98.7 ± 9.4 p = .23	107.8 ± 12.1 p = .09
High-sensitivity C-reactive protein (CRP, ng/mL)	2.2 ± 0.5	2.0 ± 0.4	4.4 ± 0.5 p < .001	3.3 ± 0.4 p < .001	2.6 ± 0.4 p < .001 p = .47	4.3 ± 0.6 p < .001 p > .99	4.3 ± 0.6 p > .99	4.5 ± 0.6 p = .16
Interleukin-10 (IL-10, pg/mL)	3.9 ± 0.7	4.1 ± 0.5	1.8 ± 0.6 p < .001	2.7 ± 0.6 p < .001	3.5 ± 0.6 p < .001 p = .67	2.0 ± 0.6 p < .001 p > .99	2.1 ± 0.6 p > .99	1.9 ± 0.6 p > .99
Plasma markers of endothelial function								
Soluble intercellular adhesion molecule-1 (sICAM1, ng/mL)	255.5 ± 125.9	261.8 ± 111.4	948.3 ± 171.8 p < .001	692.4 ± 153.6 p < .001	407.8 ± 147.3 p < .001 p = .102	885.3 ± 147.9 p < .001 p = .88	909.5 ± 158.0 p = .88	903.6 ± 155.0 p = .88
Soluble vascular cell adhesion molecule-1 (sVCAM1, ng/mL)	463.0 ± 136.5	466.8 ± 114.8	1 037.6 ± 261.8 p < .001	814.9 ± 212.0 p < .001	582.1 ± 144.1 p < .001 p = .68	1 000.0 ± 208.2 p < .001 p > .99	1 005.4 ± 214.3 p > .99	1 023.9 ± 212.2 p > .99
Glycemic parameters								
Insulin resistance (HOMA-IR)	2.4 ± 0.5	2.3 ± 0.4	10.8 ± 2.5 p < .001	7.4 ± 1.8 p < .001	3.9 ± 1.1 p < .001 p = .28	11.1 ± 2.5 p < .001 p = .72	10.7 ± 2.7 p = .61	11.6 ± 3.5 p = .61
Glucose (mmol/L)	4.8 ± 0.4	4.8 ± 0.5	5.6 ± 0.7 p = .018	5.7 ± 0.5 p = .51	5.6 ± 0.6 p > .99 p = .013	5.4 ± 0.5 p = .10 p > .99	5.3 ± 0.5 p > .99	5.5 ± 0.6 p > .99
Insulin (mU/L)	11.3 ± 2.6	10.8 ± 1.9	44.5 ± 11.8 p < .001	29.4 ± 7.8 p < .001	15.8 ± 4.8 p < .001 p = .57	46.6 ± 10.7 p < .001 p > .99	45.1 ± 10.1 p = .86	47.5 ± 12.5 p > .99
Area under the curve (oral glucose tolerance test)	66.6 ± 37.1	54.9 ± 26.6	117.3 ± 57.5 p = .18	109.3 ± 49.3	102.9 ± 47.9 p = .21 p = .54	118.9 ± 61.7 p = .18 p = .94	119.6 ± 58.2	127.8 ± 60.9 p = .54
Genomic damage								
8-hydroxy deoxyGuanosine (8-OHdG, pg/mL)	36.5 ± 10.5	31.8 ± 8.2	158.0 ± 54.8 p < .001	67.3 ± 20.3 p < .001	42.6 ± 11.8 p < .001 p > .99	154.9 ± 38.7 p < .001 p > .99	145.6 ± 33.4 p > .99	157.0 ± 55.2 p > .99

GlyNAC supplementation effects on epigenetics, ATP, DNA repair and autophagy

Taking GlyNAC supplementation 2x/day (1000 mg each of the glutathione precursors (N-acetyl-cysteine (NAC) and glycine) plus 500 mg NMN 2x/day for 5 months did not change my epigenetic age as measured by https://trumelabs.com/.

However, GlyNAC has been shown to affect a variety of proteins involved in ATP production, DNA repair, autophagy, and the rate-limiting step in glutathione biosynthesis (GCLC, GCLM).



Western blot protein expression results. GCLC and GCLM = Glutamate cysteine ligase catalytic and modifier subunits; GSS = Glutathione synthetase; PGC1α = PPARG coactivator 1-alpha; SirT3 = Sirtuin 3; pAMPKα = phosphorylated AMP-activated protein kinase α subunit; AMPKα = total AMP-activated protein kinase α subunit; CPT1B = Carnitine palmitoyltransferase 1B; PPARα = Peroxisome proliferator-activated receptor α; HADHA = hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit α; C-I, II, III, V = mitochondrial complexes I, II, II, V; ATP5A = mitochondrial ATP synthase F1 subunit alpha; PINK1 = PTEN-induced kinase 1; LC3AB = Microtubule-associated protein light chain 3 A and B; GLUT4 = Glucose transporter type 4; pH2AX = phospho-H2A histone family member X; PAX7 = Paired box protein 7; p16INK4α = p16; POT1 = Protection of telomeres protein 1. (3 participants per group of YA, OAP and OAG. * = $p < .05$; ϕ = $p < .01$; θ = $p < .001$. GlyNAC = combination of glycine and N-acetylcysteine; YA = young adults; OAG = older adults receiving GlyNAC; OAP = older adults receiving placebo.) [276] "Supplementing Glycine and N-Acetylcysteine (GlyNAC) in Older Adults Improves Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Physical Function, and Aging Hallmarks: A Randomized Clinical Trial" (2022) https://doi.org/10.1093/gerona/glac135

GlyNAC supplementation is effective in older individuals (>60 years) experiencing high levels of oxidative stress

In a trial involving a cohort of healthy 60- to 70-year-old adults, daily doses of 1.2 g NAC + 1.2 g glycine 2x/day improved whole blood levels of total glutathione (GSH-T) only in those exhibiting higher levels of oxidative stress, as measured by malondialdehyde (MDA), a reliable marker for circulating levels of lipid peroxides. There was large individual variability.

In this study, GlyNAC supplementation did not significantly change plasma levels of GSH or GSH/GSSG levels in whole blood. In comparison, earlier studies on mice and 70- to 80-year-old humans showed a significant increase in GSH and a statistically insignificant change in GSSG in red blood cells, which are known to express glutathione at high levels. Apparently, GlyNAC supplementation does not change circulating levels of glutathione, and its effects are cell type and degree of deficiency specific. "A Randomized Controlled Clinical Trial in Healthy Older Adults to Determine Efficacy of Glycine and N-Acetylcysteine Supplementation on Glutathione Redox Status and Oxidative Damage" (2022) https://www.frontiersin.org/articles/10.3389/fragi.2022.852569/full

Data points related to GlyNAC supplementation are

- In the GlyNAC human trial [153], glycine and N-acetyl-cysteine was supplemented at high doses equivalent to 3500 mg glycine and 4750 mg NAC 2x/day for a 70 kg individual to to 71-80 year olds with impressive results, as shown in Table 5. "Glycine and N-acetylcysteine (GlyNAC) supplementation in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, insulin resistance, endothelial dysfunction, genotoxicity, muscle strength, and cognition: Results of a pilot clinical trial" (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8002905/
- In the GlyNAC mouse study [154], the human equivalent doses of glycine and NAC are 1450 mg/day for a 70 kg individual. The results are shown in Table 6. "GlyNAC (Glycine and N-Acetylcysteine) Supplementation in Mice Increases Length of Life by Correcting Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Abnormalities in Mitophagy and Nutrient Sensing, and Genomic Damage" (2022) https://doi.org/10.3390/nu14051114
- Oral supplementation of N-acetylcysteine (100 mg/kg) and glycine (100 mg/kg) to 60- to 75-year-olds restored glutathione concentrations by 94.6% within 14 days to those seen in 40-year-olds. For a 70 kg individual, these doses are 7000 mg/day. "Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation" (2011) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3155927/
- GlyNAC supplementation was found to improve GST redox imbalances in older individuals experiencing oxidative stress, but not in others. "A Randomized Controlled Clinical Trial in Healthy Older Adults to Determine Efficacy of Glycine and N-Acetylcysteine Supplementation on Glutathione Redox Status and Oxidative Damage" (2022) https://pubmed.ncbi.nlm.nih.gov/33783984/
- In a study of workers in their 40's, administration of NAC at doses of 200 mg and 400 mg 2x/day elevated erythrocyte GSH concentrations by 5% and 6%, respectively. "The administration of N-acetylcysteine reduces oxidative stress and regulates glutathione metabolism in the blood cells of workers exposed to lead" (2013) https://www.ncbi.nlm.nih.gov/pubmed/23731375
- My self test of 1000 mg, 1x/day of each glycine and NAC at age 65 years confirmed that this dose restores age-related glutathione deficiency to the normal range of a younger person. The results are shown in Table 4.
- Oral supplementation of 1,000 mg glutathione over four weeks failed to increase red blood cell stores of glutathione in otherwise healthy humans aged 21–62 years (mean=40.7±11.8). "Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers" (2011) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3162377/
- Compared to age-matched controls, hospitalized COVID-19 patients have significantly worse markers for oxidative stress, which could be remedied by GlyNAC supplementation. "Severe Glutathione Deficiency, Oxidative Stress and Oxidant Damage in Adults Hospitalized with COVID-19: Implications for GlyNAC (Glycine and N-Acetylcysteine) Supplementation" (2022) https://doi.org/10.3390/antiox11010050

From this limited data, I can infer that

1. Oral supplementation of glutathione or glutathione precursors will be ineffective for those younger than about 40 years.
2. Oral supplementation of glutathione precursors gives benefit for those older than 40 years who may be experiencing oxidative stress from redox imbalances.
3. 1000 mg, 1x/day of each glycine and NAC restores glutathione levels in healthy 65+ year-olds with deficiencies (N=1). Subjectively, I feel like I have more energy.
4. Taking GlyNAC 2x/day gives additional benefit to older individuals compared to 1x/day.

References

Pharmacokinetics

[A] “Pharmacokinetics and Metabolism of Naringin and Active Metabolite Naringenin in Rats, Dogs, Humans, and the Differences Between Species” (2020) https://www.frontiersin.org/articles/10.3389/fphar.2020.00364/full

[B] “Plasma Kinetics and Urinary Excretion of the Flavanones Naringenin and Hesperetin in Humans after Ingestion of Orange Juice and Grapefruit Juice” (2001) https://academic.oup.com/jn/article/131/2/235/4686993

[C] “In vitro and in vivo effects of naringin on cytochrome P450-dependent monooxygenase in mouse liver” (1999) DOI: 10.1016/s0024-3205(99)00528-7

[D] “Flavonoids Diosmetin and Hesperetin are Potent Inhibitors of Cytochrome P450 2C9-mediated Drug Metabolism in vitro” (2010) https://doi.org/10.2133/dmpk.DMPK-10-RG-044

[E] “Interactions of 7,8-Dihydroxyflavone with Serum Albumin as well as with CYP2C9, CYP2C19, CYP3A4, and Xanthine Oxidase Biotransformation Enzymes” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6920897/

[F] “Pharmacokinetics of Luteolin and Metabolites in Rats” (2008) https://journals.sagepub.com/doi/pdf/10.1177/1934578X0800301218#:~:text=The%20low%20bioavailability%20(F)%20of,%CE%BCg%2FmL%20at%200%20h.&text=Luteolin%20was%20found%20to%20have,distribution%20and%20a%20high%20clearance.

[G] "7,8-Dihydroxyflavone and 7,8-substituted flavone derivatives, compositions, and methods related thereto" (2015) https://patents.google.com/patent/US20150274692

[H] “Simultaneous determination of diosmin and diosmetin in human plasma by ion trap liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry: Application to a clinical pharmacokinetic study” (2010) https://doi.org/10.1016/j.jpba.2009.09.012

[I] “Comparison of metabolic pharmacokinetics of naringin and naringenin in rabbits” (2002) https://doi.org/10.1016/S0024-3205(01)01491-6

[J] “O-Methylated Metabolite of 7,8-Dihydroxyflavone Activates TrkB Receptor and Displays Antidepressant Activity” (2013) https://www.karger.com/Article/FullText/346920

[K] “7,8-dihydroxyflavone, a small molecular TrkB agonist, is useful for treating various BDNF-implicated human disorders” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4702337/

[L] “A Phase 1B, randomized, double blind, placebo controlled, multiple-dose escalation study of NSI-189 phosphate, a neurogenic compound, in depressed patients” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5030464/

[M] “Absolute Bioavailability, Tissue Distribution, and Excretion of Erinacine S in Hericium erinaceus Mycelia” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6514545/

[N] “Dose translation from animal to human studies revisited” (2008) http://www.fasebj.org/content/22/3/659.full.pdf+html

[O] “Amelioration of Both Central and Peripheral Neuropathy in Mouse Models of Type 1 and Type 2 Diabetes by the Neurogenic Molecule NSI-189” (2019) https://diabetes.diabetesjournals.org/content/diabetes/68/11/2143.full.pdf

[P] “Acute and sub-chronic oral toxicity studies of hesperidin isolated from orange peel extract in Sprague Dawley rats” (2019) DOI: 10.1016/j.yrtph.2019.04.001

[Q] “Evaluation of the toxicological safety of erinacine A-enriched Hericium erinaceus in a 28-day oral feeding study in Sprague-Dawley rats” (2014) https://www.sciencedirect.com/science/article/pii/S0278691514002221?via%3Dihub

[R] “Therapeutic anti-inflammatory effects of luteolin on endotoxin-induced uveitis in Lewis rats” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5053947/

[S] “Anti-inflammatory effects of kaempferol, myricetin, fisetin and ibuprofen in neonatal rats” (2016) DOI: 10.4314/tjpr.v16i8.10

[T] “Neuroprotective Effects of 7,8-dihydroxyflavone on Midbrain Dopaminergic Neurons in MPP+-treated Monkeys” (2016) https://doi.org/10.1038/srep34339

[U] “Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations” (2003) https://doi.org/10.1016/S0928-0987(03)00135-0

[V] “Safety assessment of astaxanthin from Haematococcus pluvialis: Acute toxicity, genotoxicity, distribution and repeat-dose toxicity studies in gestation mice” (2020) https://doi.org/10.1016/j.yrtph.2020.104695

[W] “Pharmacokinetics, Safety, and Tolerability of Orally Administered Ursodeoxycholic Acid in Patients With Parkinson’s Disease—A Pilot Study” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7245554/

Neuroplasticity restoration

[1] "The citrus flavonoids hesperetin and naringenin block the lipolytic actions of TNF-a in mouse adipocytes" (2010) DOI:10.1016/j.bbrc.2010.03.060
[2] "Effect of Diosmin Administration in Patients with Chronic Venous Disorders on Selected Factors Affecting Angiogenesis" (2019) https://www.mdpi.com/1420-3049/24/18/3316/htm
[3] "7,8-Dihydroxyflavone improves neuropathological changes in the brain of Tg26 mice, a model for HIV-associated neurocognitive disorder" (2021) https://www.nature.com/articles/s41598-021-97220-8
[4] "7,8-dihydroxyflavone ameliorates cognitive and motor deficits in a Huntington's disease mouse model through specific activation of the PLCγ1 pathway" (2017) https://academic.oup.com/hmg/article/26/16/3144/3852118
[5] "Immunomodulatory Activities of a Fungal Protein Extracted from Hericium erinaceus through Regulating the Gut Microbiota" (2017) https://www.frontiersin.org/articles/10.3389/fimmu.2017.00666/full
[6] "Naringenin Promotes Thermogenic Gene Expression in Human White Adipose Tissue" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6309263/
[7] "Flavonoids Induce the Synthesis and Secretion of Neurotrophic Factors in Cultured Rat Astrocytes: A Signaling Response Mediated by Estrogen Receptor" (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3708423/
[8] "Naringin treatment improves functional recovery by increasing BDNF and VEGF expression, inhibiting neuronal apoptosis after spinal cord injury" (2012) https://pubmed.ncbi.nlm.nih.gov/22453521/
[9] "Antidepressant-like behavioral, neurochemical and neuroendocrine effects of naringenin in the mouse repeated tail suspension test." (2012) https://doi.org/10.1016/j.pnpbp.2012.06.009
[10] "Anti-depressant effect of hesperidin in diabetic rats" (2014) https://doi.org/10.1139/cjpp-2014-0281
[11] "Hesperidin exerts antidepressant-like effects in acute and chronic treatments in mice: possible role of L-arginine-NO-cGMP pathway and BDNF levels" (2014) https://doi.org/10.1016/j.brainresbull.2014.03.004
[12] "ERK-dependent brain-derived neurotrophic factor regulation by hesperidin in mice exposed to chronic mild stress" (2016) https://doi.org/10.1016/j.brainresbull.2016.03.016
[13] "BDNF signaling is necessary for the antidepressant-like effect of naringenin" (2014) https://doi.org/10.1016/j.pnpbp.2013.10.002
[14] "Antidepressant-like behavioral, neurochemical and neuroendocrine effects of naringenin in the mouse repeated tail suspension test" (2012) https://doi.org/10.1016/j.pnpbp.2012.06.009
[15] "Evidence for the involvement of the serotonergic 5-HT1A receptors in the antidepressant-like effect caused by hesperidin in mice" (2013) https://doi.org/10.1016/j.pnpbp.2012.09.003
[16] "Flavonoid Hesperidin Induces Synapse Formation and Improves Memory Performance through the Astrocytic TGF-β1" (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5468382/
[17] "Diosmetin Mitigates Cognitive and Memory Impairment Provoked by Chronic Unpredictable Mild Stress in Mice" (2020) https://www.hindawi.com/journals/ecam/2020/5725361/
[18] "Diosmin improved cognitive deficit and amplified brain electrical activity in the rat model of traumatic brain injury" (2021) https://pubmed.ncbi.nlm.nih.gov/28738538/
[19] "Diosmin is neuroprotective in a rat model of scopolamine-induced cognitive impairment" (2018) https://doi.org/10.1016/j.biopha.2018.09.127
[20] "Structures, biological activities, and industrial applications of the polysaccharides from Hericium erinaceus (Lion's Mane) mushroom: A review" (2017) https://doi.org/10.1016/j.ijbiomac.2017.01.040
[21] "Effect of anexo-polysaccharide from the culture broth of Hericium erinaceus on enhancement of growth and differentiation of rat adrenal nerve cells" (2002) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3449638/
[22] "Restoration of sensory dysfunction following peripheral nerve injury by the polysaccharide from culinary and medicinal mushroom, Hericium erinaceus (Bull.: Fr.) Pers.through its neuroregenerative action" (2015) https://www.scielo.br/j/cta/a/7Lqm37PzpCjxKn6VcQ5WbrF/?lang=en
[23] "Compounds for dementia from Hericium erinaceum" (2015) DOI:10.1358/DOF.2008.033.02.1173290
[24] "Hericium erinaceus Improves Recognition Memory and Induces Hippocampal and Cerebellar Neurogenesis in Frail Mice during Aging" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6521003/
[25] "Erinacine A-Enriched Hericium erinaceus Mycelium Produces Antidepressant-Like Effects through Modulating BDNF/PI3K/Akt/GSK-3β Signaling in Mice" (2018) https://www.mdpi.com/1422-0067/19/2/341/htm
[26] "Improving effects of the mushroom Yamabushitake (Hericium erinaceus) on mild cognitive impairment: a double-blind placebo-controlled clinical trial" (2008) https://doi.org/10.1002/ptr.2634

[27] "Anti-aging effects of Ribes meyeri anthocyanins on neural stem cells and aging mice" (2020) https://www.aging-us.com/article/103955/text
[28] "Hericerin derivatives from Hericium erinaceus exert BDNF-like neurotrophic activity in central hippocampal neurons and enhance memory" (2020) https://www.biorxiv.org/content/10.1101/2020.08.28.271676v1.full
[29] "Naringin Treatment Improves Functional Recovery by Increasing BDNF and VEGF Expression, Inhibiting Neuronal Apoptosis After Spinal Cord Injury" (2012) https://link.springer.com/article/10.1007%2Fs11064-012-0756-7 I.
[30] "The Citrus Flavanone Naringenin Protects Myocardial Cells against Age-Associated Damage" (2017) https://www.hindawi.com/journals/omcl/2017/9536148/
[31] "Endocrine disrupting activities of the flavonoid nutraceuticals luteolin and quercetin" (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3851288/
[32] "A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone" (2010) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2823863/
[33] "Biochemical and biophysical investigation of the brain-derived neurotrophic factor mimetic 7,8-dihydroxyflavone in the binding and activation of the TrkB receptor" (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4183797/
[34] "Optimized integration of fluoxetine and 7, 8-dihydroxyflavone as an efficient therapy for reversing depressive-like behavior in mice during the perimenopausal period" (2020) https://doi.org/10.1016/j.pnpbp.2020.109939
[35] "Crosstalk between the muscular estrogen receptor α and BDNF/TrkB signaling alleviates metabolic syndrome via 7,8-dihydroxyflavone in female mice" (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7811170/
[36] "7,8-Dihydroxyflavone, a Small Molecule TrkB Agonist, Improves Spatial Memory and Increases Thin Spine Density in a Mouse Model of Alzheimer Disease-Like Neuronal Loss" (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3948846/
[37] "7,8-Dihydroxyflavone improves neuropathological changes in the brain of Tg26 mice, a model for HIV-associated neurocognitive disorder" (2021) https://www.nature.com/articles/s41598-021-97220-8
[38] "The prodrug of 7,8-dihydroxyflavone development and therapeutic efficacy for treating Alzheimer's disease" (2018) https://www.pnas.org/content/115/3/578
[39] "Hesperidin promotes programmed cell death by downregulation of nongenomic estrogen receptor signalling pathway in endometrial cancer cells" (2018) https://doi.org/10.1016/j.biopha.2018.04.020
[40] "Hesperidin, a Citrus Flavonoid, Inhibits Bone Loss and Decreases Serum and Hepatic Lipids in Ovariectomized Mice" (2003) https://academic.oup.com/jn/article/133/6/1892/4688064
[41] "Diosmetin Protects Against Obesity and Metabolic Dysfunctions Through Activation of Adipose Estrogen Receptors in Mice" (2021) https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202100070
[42] "Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume" (2010) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3069644/
[43] "Exercise-Mediated Neurogenesis in the Hippocampus via BDNF" (2018) https://www.frontiersin.org/articles/10.3389/fnins.2018.00052/full
[44] "Luteolin Inhibits Microglia and Alters Hippocampal-Dependent Spatial Working Memory in Aged Mice" (2010) https://academic.oup.com/jn/article/140/10/1892/4600364
[45] "Calming the Storm: Natural Immunosuppressants as Adjuvants to Target the Cytokine Storm in COVID-19" (2021) https://www.frontiersin.org/articles/10.3389/fphar.2020.583777/full
[46] "Bioactivity of diosmetin glycosides isolated from the epicarp of date fruits, Phoenix dactylifera, on the biochemical profile of alloxan diabetic male rats" (2013) https://onlinelibrary.wiley.com/doi/10.1002/ptr.4777
[47] "Effects of Hericium erinaceus on amyloid β(25-35) peptide-induced learning and memory deficits in mice" (2015) https://www.jstage.jst.go.jp/article/biomedres/32/1/32_1_67/_pdf/-char/en
[48] "Protective effect of naringin, a citrus flavonoid, against colchicine-induced cognitive dysfunction and oxidative damage in rats" (2010) https://www.liebertpub.com/doi/10.1089/jmf.2009.1251
[49] "Naringin Dihydrochalcone Ameliorates Cognitive Deficits and Neuropathology in APP/PS1 Transgenic Mice" (2018) https://www.frontiersin.org/articles/10.3389/fnagi.2018.00169/full
[50] "Naringin ameliorates memory deficits and exerts neuroprotective effects in a mouse model of Alzheimer's disease by regulating multiple metabolic pathway" (2021) https://www.spandidos-publications.com/10.3892/mmr.2021.11971
[51] "Hesperidin ameliorates behavioral impairments and neuropathology of transgenic APP/PS1 mice" (2015) https://doi.org/10.1016/j.bbr.2014.12.012
[52] "Luteolin induces hippocampal neurogenesis in the Ts65Dn mouse model of Down syndrome" (2019) https://www.nrronline.org/article.asp?issn=1673-5374;year=2019;volume=14;issue=4;spage=613;epage=620;aulast=Zhou

[53] “Antihyperalgesic Effect of Hesperidin Improves with Diosmin in Experimental Neuropathic Pain” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5031820/
[54] "Activation of TrkB by 7,8-dihydroxyflavone prevents fear memory defects and facilitates amygdalar synaptic plasticity in aging" (2012) https://pubmed.ncbi.nlm.nih.gov/22710915/
[55] “7,8-dihydroxyflavone rescues spatial memory and synaptic plasticity in cognitively impaired aged rats” (2012) https://onlinelibrary.wiley.com/doi/10.1111/j.1471-4159.2012.07830.x
[56] “Hericium erinaceus Inhibits TNF-α-Induced Angiogenesis and ROS Generation through Suppression of MMP-9/NF-κB Signaling and Activation of Nrf2-Mediated Antioxidant Genes in Human EA.hy926 Endothelial Cells” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4707368/
[57] “Hesperidin Potentiates Ghrelin Signaling” (2014) DOI: 10.2174/2212798406666140825120623
[58] “Naringenin is a Senotherapeutic that Enhances Neurogenesis in Mice” (2020) https://www.fightaging.org/archives/2020/09/naringenin-is-a-senotherapeutic-that-enhances-neurogenesis-in-mice/
[59] “Hesperidin improves insulin resistance via down-regulation of inflammatory responses: Biochemical analysis and in silico validation” (2020) https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0227637
[60] “Hesperidin Benefits Cutaneous Functions” (2019) https://www.hindawi.com/journals/ecam/2019/2676307/
[61] “Effects of the flavonoid hesperidin in cerebral cortical progenitors in vitro: indirect action through astrocytes” (2012) https://doi.org/10.1016/j.ijdevneu.2012.01.008
[62] (Review) “Cognitive and Brain Plasticity in Old Age” (2016) https://link.springer.com/referenceworkentry/10.1007%2F978-981-287-082-7_146
[63] “TrkB activation by 7, 8-dihydroxyflavone increases synapse AMPA subunits and ameliorates spatial memory deficits in a mouse model of Alzheimer's disease” (2015) https://onlinelibrary.wiley.com/doi/10.1111/jnc.13432
[64] “7, 8-Dihydroxyflavone induces synapse expression of AMPA GluA1 and ameliorates cognitive and spine abnormalities in a mouse model of fragile X syndrome” (2015) https://doi.org/10.1016/j.neuropharm.2014.09.006
[65] “Exercise-induced enhancement of synaptic function triggered by the inverse BAR protein, Mtss1L” (2019) https://elifesciences.org/articles/45920
[66] “Diosmin reduces cerebral Aβ levels, tau hyperphosphorylation, neuroinflammation, and cognitive impairment in the 3xTg-AD Mice” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5074695/
[67] “7,8-Dihydroxyflavone Prevents Synaptic Loss and Memory Deficits in a Mouse Model of Alzheimer's Disease” (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3895241/
[68] “Short bouts of mild-intensity physical exercise improve spatial learning and memory in aging rats: Involvement of hippocampal plasticity via AKT, CREB and BDNF signaling” (2012) https://doi.org/10.1016/j.mad.2011.09.005
[69] “Hericium erinaceus enhances neurotrophic factors and prevents cochlear cell apoptosis in senescence accelerated mice” (2020) https://doi.org/10.1016/j.jff.2020.103832
[70] “Naringin Enhances CaMKII Activity and Improves Long-Term Memory in a Mouse Model of Alzheimer’s Disease” (2013) https://doi.org/10.3390/ijms14035576
[71] “Naringin Improves Neuronal Insulin Signaling, Brain Mitochondrial Function, and Cognitive Function in High-Fat Diet-Induced Obese Mice” (2015) DOI:10.1007/s10571-015-0201-y
[72] “Neuroprotective Metabolites of Hericium erinaceus Promote Neuro-Healthy Aging” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8232141/
[73] “New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory?” (2010) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2886712/
[74] “About NSI-189, a New Experimental Nootropic” https://selfhacked.com/blog/nsi-189-uses/
[75] “The neurogenic compound, NSI-189 phosphate: a novel multi-domain treatment capable of pro-cognitive and antidepressant effects” (2017) https://www.ncbi.nlm.nih.gov/pubmed/28460574
[76] “NSI-189, a small molecule with neurogenic properties, exerts behavioral, and neurostructural benefits in stroke rats” (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5518191/
[77] “Enhancement of synaptic plasticity and reversal of impairments in motor and cognitive functions in a mouse model of Angelman Syndrome by a small neurogenic molecule, NSI-189” (2019) https://doi.org/10.1016/j.neuropharm.2018.10.038
[78] “A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan” (2015) http://www.cell.com/cell-metabolism/fulltext/S1550-4131(15)00224-7

[79] “H. erinaceus provided a partial recovery of intellectual function of patients with a mild cognitive impairment or against other forms of neurodegenerative diseases, including dementia and Alzheimer’s” (2016) https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-016-0266-z
[80] “7,8-Dihydroxyflavone improves memory consolidation processes in rats and mice” (2013) https://pubmed.ncbi.nlm.nih.gov/24070857/
[81] “Naringenin Ameliorates Acute Inflammation by Regulating Intracellular Cytokine Degradation” (2017) https://www.jimmunol.org/content/199/10/3466
[82] “Naringin Decreases TNF-α and HMGB1 Release from LPS-Stimulated Macrophages and Improves Survival in a CLP-Induced Sepsis Mice” (2016) https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0164186
[83] “Luteolin Induces microRNA-132 Expression and Modulates Neurite Outgrowth in PC12 Cells” (2012) https://doi.org/10.1371/journal.pone.0043304
[84] “Luteolin Could Improve Cognitive Dysfunction by Inhibiting Neuroinflammation” (2018) https://doi.org/10.1007/s11064-018-2482-2
[85] “O-Methylated Metabolite of 7,8-Dihydroxyflavone Activates TrkB Receptor and Displays Antidepressant Activity” (2013) https://www.karger.com/Article/FullText/346920
[86] “7,8-dihydroxyflavone, a small molecular TrkB agonist, is useful for treating various BDNF-implicated human disorders” (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4702337/
[87] “Inhibition of autophagy via activation of PI3K/Akt/mTOR pathway contributes to the protection of hesperidin against myocardial ischemia/reperfusion injury” (2018) https://www.spandidos-publications.com/10.3892/ijmm.2018.3794
[88] “Hesperidin induces apoptosis and triggers autophagic markers through inhibition of Aurora-A mediated phosphoinositide-3-kinase/Akt/mammalian target of rapamycin and glycogen synthase kinase-3 beta signalling cascades in experimental colon carcinogenesis” (2014) https://pubmed.ncbi.nlm.nih.gov/25047426/
[89] “Autophagic activation may be involved in the mechanism of hesperidin's therapeutic effects on cognitive impairment” (2015) https://www.jns-journal.com/article/S0022-510X(15)00103-3/fulltext
[90] “Citrus Polyphenol Hesperidin Stimulates Production of Nitric Oxide in Endothelial Cells while Improving Endothelial Function and Reducing Inflammatory Markers in Patients with Metabolic Syndrome” (2012) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3085197/.
[91] “Reverse Underlying Cause of Degenerative Aging” (2018) https://www.lifeextension.com/magazine/2018/2/reverse-major-factor-in-degenerative-aging
[92] “Hesperidin and Chlorogenic Acid Synergistically Inhibit the Growth of Breast Cancer Cells via Estrogen Receptor/Mitochondrial Pathway” (2021) https://www.mdpi.com/2075-1729/11/9/950
[93] “Naringin Induces Lysosomal Permeabilization and Autophagy Cell Death in AGS Gastric Cancer Cells” (2020) DOI: 10.1142/S0192415X20500342
[94] “Naringin induces autophagy-mediated growth inhibition by downregulating the PI3K/Akt/mTOR cascade via activation of MAPK pathways in AGS cancer cells” (2015) DOI: 10.3892/ijo.2015.3095
[95] “Naringenin promotes cell autophagy to improve high-fat-diet-induced atherosclerosis in ApoE-/- mice” (2021) https://www.scielo.br/j/bjmbr/a/djBS7BwjPwrN673GLJjPWTv/?lang=en
[96] “Naringenin Attenuates Non-Alcoholic Fatty Liver Disease by Enhancing Energy Expenditure and Regulating Autophagy via AMPK” (2021) https://www.frontiersin.org/articles/10.3389/fphar.2021.687095/full
[97] “Protection by Naringin and Some Other Flavonoids of Hepatocytic Autophagy and Endocytosis against Inhibition by Okadaic Acid (*)” (1995) https://www.jbc.org/article/S0021-9258(18)81432-6/fulltext
[98] “Naringin induces autophagy-mediated growth inhibition by downregulating the PI3K/Akt/mTOR cascade via activation of MAPK pathways in AGS cancer cells” (2015) https://doi.org/10.3892/ijo.2015.3095
[99] “Diosmetin alleviates hypoxia-induced myocardial apoptosis by inducing autophagy through AMPK activation” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7339627/
[100] “Diosmetin inhibits apoptosis and activates AMPK-induced autophagy in myocardial damage under hypoxia environment” (2021) https://onlinelibrary.wiley.com/doi/10.1002/kjm2.12462
[101] “ Autophagy regulation using luteolin: new insight into its anti-tumor activity” (2020) https://cancerci.biomedcentral.com/articles/10.1186/s12935-020-01634-9
[102] “Luteolin Promotes Cell Apoptosis by Inducing Autophagy in Hepatocellular Carcinoma” (2017) https://www.karger.com/Article/FullText/484066

[103] “REM sleep selectively prunes and maintains new synapses in development and learning” (2017) https://doi.org/10.1038/nn.4479
[104] (Review) “Cellular Senescence in Brain Aging” (2021) https://www.frontiersin.org/articles/10.3389/fnagi.2021.646924/full
[105] “Cellular Senescence Is Induced by the Environmental Neurotoxin Paraquat and Contributes to Neuropathology Linked to Parkinson’s Disease” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5806534/
[106] “Senolytic therapy alleviates Aβ-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6605052/
[107] “Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6206507/
[108] “Association of Daily Step Count and Step Intensity With Mortality Among US Adults” (2020) https://jamanetwork.com/journals/jama/article-abstract/2763292
[109] “BDNF mediates improvements in executive function following a 1-year exercise intervention” (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4263078/
[110] “Amelioration of Both Central and Peripheral Neuropathy in Mouse Models of Type 1 and Type 2 Diabetes by the Neurogenic Molecule NSI-189” (2019) https://diabetes.diabetesjournals.org/content/diabetes/68/11/2143.full.pdf
[111] “Ameliorating effect of luteolin on memory impairment in an Alzheimer's disease model" (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4838167/
[112] “Luteolin protects HUVECs from TNF-α-induced oxidative stress and inflammation via its effects on the Nox4/ROS-NF-κB and MAPK pathways” (2014) https://doi.org/10.5551/jat.23697
[113] “Do Small Molecules Activate the TrkB Receptor in the Same Manner as BDNF? Limitations of Published TrkB Low Molecular Agonists and Screening for Novel TrkB Orthosteric Agonists” (2021) https://www.mdpi.com/1424-8247/14/8/704/htm
[114] “Anti-inflammatory effect of hesperidin enhances chondrogenesis of human mesenchymal stem cells for cartilage tissue repair” (2020) https://journal-inflammation.biomedcentral.com/articles/10.1186/s12950-018-0190-y
[115] “Influence of Hesperidin on Systemic Immunity of Rats Following an Intensive Training and Exhausting Exercise” (2020) https://pdfs.semanticscholar.org/79cc/b5ff6c82c39b3727a4ef09d57707d879f648.pdf
[116] “7,8-Dihydroxyflavone Ameliorates Cognitive Impairment by Inhibiting Expression of Tau Pathology in ApoE-Knockout Mice” (2016) https://www.frontiersin.org/articles/10.3389/fnagi.2016.00287/full
[117] “7,8-dihydroxyflavone protects PC12 cells against 6-hydroxydopamine-induced cell death through modulating PI3K/Akt and JNK pathways” (2014) https://doi.org/10.1016/j.neulet.2014.08.016
[118] “Erinacine A-enriched Hericium erinaceus mycelium ameliorates Alzheimer’s disease-related pathologies in APPswe/PS1dE9 transgenic mice” (2016) https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-016-0266-z
[119] “Effects of 7,8-Dihydroxyflavone on Lipid Isoprenoid and Rho Protein Levels in Brains of Aged C57BL/6 Mice” (2020) https://link.springer.com/article/10.1007/s12017-020-08640-0
[120] “The TrkB agonist 7,8-dihydroxyflavone changes the structural dynamics of 2 neocortical pyramidal neurons and improves object recognition in mice” [2018] DOI: 10.1007/s00429-018-1637-x http://repositori.uji.es/xmlui/bitstream/handle/10234/176560/59851.pdf?sequence=1&isAllowed=y
[121] “Hericium erinaceus mycelium and its small bioactive compounds promote oligodendrocyte maturation with an increase in myelin basic protein” (2021) https://www.nature.com/articles/s41598-021-85972-2
[122] "Muscle-generated BDNF (brain derived neurotrophic factor) maintains mitochondrial quality control in female mice" (2021) https://doi.org/10.1080/15548627.2021.1985257
[123] “Neuroprotective potential of fisetin in an experimental model of spinal cord injury: via modulation of NF-κB/IκBα pathway” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7886366/
[124] “Fisetin provides antidepressant effects by activating the tropomyosin receptor kinase B signal pathway in mice” (2017) https://onlinelibrary.wiley.com/doi/full/10.1111/jnc.14226
[125] “Fisetin is a senotherapeutic that extends health and lifespan” (2018) https://www.sciencedirect.com/science/article/pii/S2352396418303736
[126] “New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463” (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5391241/
[127] “Fisetin Alleviated Bleomycin-Induced Pulmonary Fibrosis Partly by Rescuing Alveolar Epithelial Cells From Senescence” (2020) https://www.frontiersin.org/articles/10.3389/fphar.2020.553690/full
[128] “Modulation of p25 and inflammatory pathways by fisetin maintains cognitive function in Alzheimer’s disease transgenic mice” (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3954948/

[129] “The antidepressant-like effect of fisetin involves the serotonergic and noradrenergic system” (2012) DOI: 10.1016/j.bbr.2011.12.017
[130] “Flavonoid fisetin promotes ERK-dependent long-term potentiation and enhances memory” (2006) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1637622/
[131] “Fisetin inhibits TNF-α-induced inflammatory action and hydrogen peroxide-induced oxidative damage in human keratinocyte HaCaT cells through PI3K/AKT/Nrf-2-mediated heme oxygenase-1 expression” (2015) https://doi.org/10.1016/j.intimp.2015.11.014
[132] “Neuroprotective potential of fisetin in an experimental model of spinal cord injury: via modulation of NF-κB/IκBα pathway” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7886366/
[133] “Fisetin inhibits inflammation and induces autophagy by mediating PI3K/AKT/mTOR signaling in LPS-induced RAW264.7 cells” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8009086/
[134] “Fisetin induces autophagy in pancreatic cancer cells via endoplasmic reticulum stress- and mitochondrial stress-dependent pathways” (2019) https://www.nature.com/articles/s41419-019-1366-y
[135] “Effect of Hesperidin on Cardiovascular Disease Risk Factors: The Role of Intestinal Microbiota on Hesperidin Bioavailability” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7284956/
[136] “Cardiovascular event rates and mortality according to achieved systolic and diastolic blood pressure in patients with stable coronary artery disease: an international cohort study” (2016) DOI:https://doi.org/10.1016/S0140-6736(16)31326-5
[137] “Neurogenic-dependent changes in hippocampal circuitry underlie the procognitive effect of exercise in aging mice” (2021) https://www.cell.com/iscience/fulltext/S2589-0042(21)01421-8
[138] “Whole-body senescent cell clearance alleviates age-related brain inflammation and cognitive impairment in mice” (2021) https://onlinelibrary.wiley.com/doi/full/10.1111/acel.13296
[139] “Fisetin Rescues the Mice Brains Against D-Galactose-Induced Oxidative Stress, Neuroinflammation and Memory Impairment” (2021) https://www.frontiersin.org/articles/10.3389/fphar.2021.612078/full
[140] “Diosmetin attenuates metabolic syndrome and left ventricular alterations via the suppression of angiotensin II/AT 1 receptor/gp 91 phox/p-NF-κB protein expression in high-fat diet fed rats” (2021) https://doi.org/10.1039/d0fo02744h
[141] “Neuroprotective effect of hesperetin and nano-hesperetin on recognition memory impairment and the elevated oxygen stress in rat model of Alzheimer’s disease” (2018) https://doi.org/10.1016/j.biopha.2017.11.047
[142] “Restoration of hippocampal neural precursor function by ablation of senescent cells in the aging stem cell niche” (2021) https://doi.org/10.1016/j.stemcr.2021.12.010
- Removing senescent brain cells increased hippocampal neurogenesis by 30% in old mice.
- “Old Neurons Can Block Neurogenesis in Mice” (2022) https://neurosciencenews.com/hippocampal-neurogenesis-19944/
- “Senolytic Navitoclax Rescues Neurogenesis and Memory in Mice” (2022) https://www.lifespan.io/news/senolytic-navitoclax-rescues-neurogenesis-and-memory-in-mice/

[143] “7,8-Dihydroxyflavone Attenuates Alcohol-Related Behavior in Rat Models of Alcohol Consumption via TrkB in the Ventral Tegmental Area” (2020) https://doi.org/10.3389/fnins.2020.00467
[144] “7,8-Dihydroxyflavone Enhances Cue-Conditioned Alcohol Reinstatement in Rats” (2020) https://www.mdpi.com/2076-3425/10/5/270
[145] “7,8-Dihydroxyflavone alleviated the high-fat diet and alcohol-induced memory impairment: behavioral, biochemical and molecular evidence” (2020) https://link.springer.com/article/10.1007%2Fs00213-020-05502-2
[146] “Combination of dasatinib and quercetin improves cognitive abilities in aged male Wistar rats, alleviates inflammation and changes hippocampal synaptic plasticity and histone H3 methylation profile” (2022) https://www.aging-us.com/article/203835/text
[147] “Late-life physical activity relates to brain tissue synaptic integrity markers in older adults” (2022) https://doi.org/10.1002/alz.12530
- “Physical exercise during ageing protects brain health by increasing synaptic density” (2022) https://www.ehu.eus/en/web/campusa-magazine/-/physical-exercise-during-ageing-protects-brain-health

[148] “The Seattle Longitudinal Study: Relationship Between Personality and Cognition” (2004) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1474018/
[149] “Effect of Hesperetin against Oxidative Stress via ER- and TrkA-Mediated Actions in PC12 Cells” (2011) https://doi.org/10.1021/jf104632a
[150] “A single session of moderate intensity walking increases brain-derived neurotrophic factor (BDNF) in the chronic post-stroke patients” (2017) https://doi.org/10.1080/10749357.2017.1373500

[151] “7,8-Dihydroxyflavone reverses the depressive symptoms in mouse chronic mild stress” (2016) https://doi.org/10.1016/j.neulet.2016.10.035
[152] "Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation" (2011) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3155927/
[153] “Glycine and N-acetylcysteine (GlyNAC) supplementation in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, insulin resistance, endothelial dysfunction, genotoxicity, muscle strength, and cognition: Results of a pilot clinical trial” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8002905/
[154] “GlyNAC (Glycine and N-Acetylcysteine) Supplementation in Mice Increases Length of Life by Correcting Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Abnormalities in Mitophagy and Nutrient Sensing, and Genomic Damage” (2022) https://www.mdpi.com/2072-6643/14/5/1114/htm
[155] “Adjunctive N-acetylcysteine in depression: exploration of interleukin-6, C-reactive protein and brain-derived neurotrophic factor” (2017) DOI: https://doi.org/10.1017/neu.2017.2
[156] “Oxidative stress and ageing: is ageing a cysteine deficiency syndrome?” (2005) https://doi.org/10.1098/rstb.2005.1770
[157] “The Cyanthin Diterpenoid and Sesterterpene Constituents of Hericium erinaceus Mycelium Ameliorate Alzheimer’s Disease-Related Pathologies in APP/PS1 Transgenic Mice” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5855820/
[158] “Two New Cyathane Diterpenoids from Mycelial Cultures of the Medicinal Mushroom Hericium erinaceus and the Rare Species, Hericium flagellum” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5877601/
[159] “Physical Exercise Increased Brain-Derived Neurotrophic Factor in Elderly Population with Depression” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6698103/
[160] “Age-Dependent Accumulation of 8-Oxoguanine in the DNA and RNA in Various Rat Tissues” (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3657452/
[161] “Towards a comprehensive view of 8-oxo-7,8-dihydro-2’-deoxyguanosine: Highlighting the intertwined roles of DNA damage and epigenetics in genomic instability” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7926032/
[162] “Neurogenesis-dependent antidepressant-like activity of Hericium erinaceus in an animal model of depression” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8650354/
[163] “Hesperidin alleviates insulin resistance by improving HG-induced oxidative stress and mitochondrial dysfunction by restoring miR-149” (2021) https://dmsjournal.biomedcentral.com/articles/10.1186/s13098-021-00664-1
[164] “Oral administration of fisetin promotes the induction of hippocampal long-term potentiation in vivo” (2017) http://doi.org/10.1016/j.jphs.2017.12.00
[165] “Hesperidin Supplementation Alleviates Oxidative DNA Damage and Lipid Peroxidation in Type 2 Diabetes: A Randomized Double-Blind PlaceboControlled Clinical Trial” (2017) https://doi.org/10.1002/ptr.5881
[166] “Blood pressure lowering and anti-inflammatory effects of hesperidin in type 2 diabetes; a randomized double-blind controlled clinical trial” (2018) https://doi.org/10.1002/ptr.6046
{167] “Improvement of Executive Function after Short-Term Administration of an Antioxidants Mix Containing Bacopa, Lycopene, Astaxanthin and Vitamin B12: The BLAtwelve Study” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7824614/
- Bacopa monniera showed some improvement in memory but slower Trail Making Test scores. "The chronic effects of an extract of Bacopa monniera (Brahmi) on cognitive function in healthy human subjects" (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3800605/
- Vitamin B12 levels were found to be unrelated to Trail Making Test scores. “Trail Making Test performance in a community-based sample of healthy very old adults: effects of age on completion time, but not on accuracy” (1996) https://doi.org/10.1016/0167-4943(95)00681-8
- Low levels of lycopene (a carotenoid similar to astaxanthin) were significantly associated with worse Trail Making Test scores. “Lycopene and cognitive function” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6558668/

[168] “Astaxanthin alleviates brain aging in rats by attenuating oxidative stress and increasing BDNF levels” (2014) https://pubs.rsc.org/en/content/articlehtml/2014/fo/c3fo60400d
[169] “The effects of astaxanthin treatment on a rat model of Alzheimer’s disease” (2021) https://doi.org/10.1016/j.brainresbull.2021.04.020
[170] “Astaxanthin improves cognitive performance in mice following mild traumatic brain injury” (2017) https://doi.org/10.1016/j.brainres.2016.12.031
[171] “Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans” (2010) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2845588/

[172] “Effects of Lutein and Astaxanthin Intake on the Improvement of Cognitive Functions among Healthy Adults: A Systematic Review of Randomized Controlled Trials” (2020) https://doi.org/10.3390/nu12030617
[173] “Neuroprotective Effects of Hesperidin, a Plant Flavanone, on Rotenone-Induced Oxidative Stress and Apoptosis in a Cellular Model for Parkinson’s Disease” (2013) https://doi.org/10.1155/2013/102741
[174] “Astaxanthin delays brain aging in senescence-accelerated mouse prone 10: inducing autophagy as a potential mechanism” (2022) https://doi.org/10.1080/1028415X.2022.2055376
[175] “Lion’s Mane Mushroom, Hericium erinaceus (Bull.: Fr.) Pers. Suppresses H2O2-Induced Oxidative Damage” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6720269/
[176] “Erinacine A-Enriched Hericium erinaceus Mycelium Delays Progression of Age-Related Cognitive Decline in Senescence Accelerated Mouse Prone 8 (SAMP8) Mice and LPS-Induced Inflammation in HT22 Hippocampal Neurons and BV2 Microglia” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8537498/
[178] “Preventive Effect of 7,8-Dihydroxyflavone against Oxidative Stress Induced Genotoxicity” (2009) https://doi.org/10.1248/bpb.32.166
[179] “7,8-Dihydroxyflavone Suppresses Oxidative Stress-Induced Base Modification in DNA via Induction of the Repair Enzyme 8-Oxoguanine DNA Glycosylase-1” (2013) https://doi.org/10.1155/2013/863720
[180] “Flavones 7,8-DHF, Quercetin, and Apigenin Against Tau Toxicity via Activation of TRKB Signaling in ΔK280 TauRD-DsRed SH-SY5Y Cells” (2021) https://doi.org/10.3389/fnagi.2021.758895
[181] “Antioxidant action of 7,8-dihydroxyflavone protects PC12 cells against 6-hydroxydopamine-induced cytotoxicity” (2014) https://doi.org/10.1016/j.neuint.2013.10.018
[182] “Antioxidant activity of 7,8-dihydroxyflavone provides neuroprotection against glutamate-induced toxicity” (2011) https://doi.org/10.1016/j.neulet.2011.05.054
[183] “7,8-Dihydroxyflavone alleviated the high-fat diet and alcohol-induced memory impairment: behavioral, biochemical and molecular evidence” (2020) https://doi.org/10.1007/s00213-020-05502-2
[184] “Erinacines A, B and C, strong stimulators of nerve growth factor (NGF)-synthesis, from the mycelia of Hericium erinaceum” (1994) https://doi.org/10.1016/S0040-4039(00)76760-8
[185] “The effect of Hericium erinaceum on the prevention of chemically induced experimental colitis in rats” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8009150/
[186] “Hericium erinaceus potentially rescues behavioural motor deficits through ERK-CREB-PSD95 neuroprotective mechanisms in rat model of 3-acetylpyridine-induced cerebellar ataxia” (2020) https://www.nature.com/articles/s41598-020-71966-z
[187] “Protective Effects of Hericium erinaceus Mycelium and Its Isolated Erinacine A against Ischemia-Injury-Induced Neuronal Cell Death via the Inhibition of iNOS/p38 MAPK and Nitrotyrosine” (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4200813/
[188] “Astaxanthin inhibits cytokines production and inflammatory gene expression by suppressing IκB kinase-dependent nuclear factor κB activation in pre and postpartum Murrah buffaloes during different seasons” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6048090/
[189] “Protective effects of a dietary carotenoid, astaxanthin, against light-induced retinal damage” (2013) https://doi.org/10.1254/jphs.13066FP
[190] “Astaxanthin: A Mechanistic Review on its Biological Activities and Health benefits” (2018) https://doi.org/10.1016/j.phrs.2018.08.012
[191] “7,8-dihydroxyflavone, a small-molecule tropomyosin-related kinase B (TrkB) agonist, attenuates cerebral ischemia and reperfusion injury in rats” (2014) https://doi.org/10.1007/s10735-013-9539-y
[192] “Administration of the TrkB Receptor Agonist 7,8-Dihydroxyflavone Prevents Traumatic Stress-induced Spatial Memory Deficits and Changes in Synaptic Plasticity” (2016) https://doi.org/10.1002/hipo.22599
[193] “Neuroprotective Effects of 7,8-dihydroxyflavone on Midbrain Dopaminergic Neurons in MPP+-treated Monkeys” (2016) https://doi.org/10.1038/srep34339
[194] “The Neuroprotective Effects of Astaxanthin: Therapeutic Targets and Clinical Perspective” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6680436/
[195] “The Neuroprotective Effect of Astaxanthin on Pilocarpine-Induced Status Epilepticus in Rats” (2019) https://www.frontiersin.org/articles/10.3389/fncel.2019.00123/full
[196] “Astaxanthin is neuroprotective in an aged mouse model of Parkinson’s disease” (2018) https://www.oncotarget.com/article/23737/text/

[197] "Molecular Mechanisms of Astaxanthin as a Potential Neurotherapeutic Agent" (2021) https://www.mdpi.com/1660-3397/19/4/201/htm
[198] "Neuroprotective effects of astaxanthin against oxygen and glucose deprivation damage via the PI3K/Akt/GSK3β/Nrf2 signalling pathway in vitro" (2020) https://onlinelibrary.wiley.com/doi/full/10.1111/jcmm.15531
[199] "Protective Effects of Hesperidin (Citrus Flavonone) on High Glucose Induced Oxidative Stress and Apoptosis in a Cellular Model for Diabetic Retinopathy" (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5748762/
[200] "Brain-derived neurotrophic factor mimetic, 7,8-dihydroxyflavone, protects against myocardial ischemia by rebalancing optic atrophy 1 processing" (2019) https://doi.org/10.1016/j.freeradbiomed.2019.09.033
[201] "7,8-Dihydroxyflavone ameliorates mitochondrial impairment and motor dysfunction in the α-synuclein 1–103 transgenic mice" (2022) https://doi.org/10.1016/j.nbd.2022.105736
[202] "7,8-Dihydroxyflavone alleviates apoptosis and inflammation induced by retinal ischemia-reperfusion injury via activating TrkB/Akt/NF-kB signaling pathway" (2022) https://www.medsci.org/v19p0013.htm
[203] "Lion's Mane Mushroom, Hericium erinaceus (Bull.: Fr.) Pers. Suppresses H2O2-Induced Oxidative Damage and LPS-Induced Inflammation in HT22 Hippocampal Neurons and BV2 Microglia" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6720269/
[204] "Astaxanthin Ameliorated Parvalbumin-Positive Neuron Deficits and Alzheimer's Disease-Related Pathological Progression in the Hippocampus of AppNL-G-F/NL-G-F Mice" (2020) https://www.frontiersin.org/articles/10.3389/fphar.2020.00307/full
[205] "Astaxanthin supplementation modulates cognitive function and synaptic plasticity in young and aged mice" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6423184/
[206] "Neuroprotective mechanisms of astaxanthin: a potential therapeutic role in preserving cognitive function in age and neurodegeneration" (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5352583/
[207] "Astaxanthin Improves Stem Cell Potency via an Increase in the Proliferation of Neural Progenitor Cells" (2010) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3100832/
[208] "Astaxanthin improves cognitive deficits from oxidative stress, nitric oxide synthase and inflammation through upregulation of PI3K/Akt in diabetes rat" (2015) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4525820/
[209] "Astaxanthin modulates age-associated mitochondrial dysfunction in healthy dogs" (2013) https://doi.org/10.2527/jas.2012-5341
[210] "Effects of astaxanthin-rich Haematococcus pluvialis extract on cognitive function: a randomised, double-blind, placebo-controlled study" (2012) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3432818/
[211] "Hesperidin reverses cognitive and depressive disturbances induced by olfactory bulbectomy in mice by modulating hippocampal neurotrophins and cytokine levels and acetylcholinesterase activity" (2016) https://doi.org/10.1016/j.ejphar.2016.07.042
[212] "The Neuroprotective Properties of Hericium erinaceus in Glutamate-Damaged Differentiated PC12 Cells and an Alzheimer's Disease Mouse Model" (2016) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5133811/
[213] "Hesperidin Reduces Memory Impairment Associated with Adult Rat Hippocampal Neurogenesis Triggered by Valproic Acid" (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8708262/
[214] "Protective effects of 7,8-dihydroxyflavone on neuropathological and neurochemical changes in a mouse model of Alzheimer's disease" (2018) https://europepmc.org/article/med/29510124
[215] "Single-nucleus transcriptomic landscape of primate hippocampal aging" (2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8403220/
[216] "β-Amyloid burden in healthy aging" (2012) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3280058/
[217] "The Role of BDNF in Age-Dependent Changes of Excitatory and Inhibitory Synaptic Markers in the Human Prefrontal Cortex" (2016) https://www.nature.com/articles/npp2016126
[218] "Critical Issues in the Analysis of Dendritic Extent in Aging Humans, Primates, and Rodents" (1997) https://doi.org/10.1016/0197-4580(93)90058-J
[219] "Layer-specific dendritic regression of pyramidal cells with ageing in the human prefrontal cortex" (1998) doi: 10.1046/j.1460-9568.1998.00137.x
[220] "Increased Hippocampal ProBDNF Contributes to Memory Impairments in Aged Mice" (2017) https://doi.org/10.3389/fnagi.2017.00284
[221] "Age-related Dendritic and Spine Changes in Corticocortically Projecting Neurons in Macaque Monkeys" (2003) https://academic.oup.com/cercor/article/13/9/950/342633

[222] “Age-dependent and differential effects of Smad7ΔEx1 on neural progenitor cell proliferation and on neurogenesis” (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4162458/
[223] “Aging and Rejuvenation of Neural Stem Cells and Their Niches” (2020) https://doi.org/10.1016/j.stem.2020.07.002
[224] “Evidence for reduced neurogenesis in the aging human hippocampus despite stable stem cell markers” (2017) https://onlinelibrary.wiley.com/doi/full/10.1111/acel.12641
[225] “Dendritic spine changes associated with normal aging” (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3654095/
[226] "Pharmacological blockade of TNFα prevents sarcopenia and prolongs survival in aging mice" (2020) https://doi.org/10.18632/aging.202200
[227] “Aging triggers an upregulation of a multitude of cytokines in the male and especially the female rodent hippocampus but more discrete changes in other brain regions” (2021) https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-021-02252-6
[228] “Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo“ (2021) https://doi.org/10.1016/j.neuron.2021.06.008
[229] “A Single Dose of Psilocybin Increases Synaptic Density and Decreases 5-HT2A Receptor Density in the Pig Brain” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7830000/
- “Psychedelic spurs growth of neural connections lost in depression” (2021) https://www.eurekalert.org/news-releases/744231

[230] “Prolonged epigenomic and synaptic plasticity alterations following single exposure to a psychedelic in mice“ (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8582597/
[231] “Aging impairs dendrite morphogenesis of newborn neurons and is rescued by 7, 8-dihydroxyflavone” (2017) https://doi.org/10.1111/acel.12553
[232] “Neuroprotection of Fasting Mimicking Diet on MPTP-Induced Parkinson's Disease Mice via Gut Microbiota and Metabolites” (2019) https://pubmed.ncbi.nlm.nih.gov/30815845/
[233} “Effects of the flavonoid hesperidin in cerebral cortical progenitors in vitro: indirect action through astrocytes” (2012) DOI: 10.1016/j.ijdevneu.2012.01.008
[234] “Hesperidin Alleviates Cognitive Impairment, Mitochondrial Dysfunction and Oxidative Stress in a Mouse Model of Alzheimer’s Disease” (2014) https://doi.org/10.1007/s10571-014-0098-x
[235] “7,8-Dihydroxyflavone improves motor performance and enhances lower motor neuronal survival in a mouse model of amyotrophic lateral sclerosis” (2014) doi: 10.1016/j.neulet.2014.02.058
[236] “Chemical Constituents from Hericium erinaceus Promote Neuronal Survival and Potentiate Neurite Outgrowth via the TrkA/Erk1/2 Pathway” (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5578049/
[237] “Low Doses of LSD Acutely Increase BDNF Blood Plasma Levels in Healthy Volunteers” (2020) https://doi.org/10.1021/acsptsci.0c00099
[238] “Direct comparison of the acute effects of lysergic acid diethylamide and psilocybin in a double-blind placebo-controlled study in healthy subjects” (2022) https://www.nature.com/articles/s41386-022-01297-2#MOESM1
[239] “Age-Related Decline in Multiple Unit Action Potentials of CA3 Region of Rat Hippocampus: Correlation With Lipid Peroxidation and Lipofuscin Concentration and the Effect of Centrophenoxine” (1993) https://doi.org/10.1016/0197-4580(93)90117-T
[240] “Centrophenoxine improves chronic cerebral ischemia induced cognitive deficit and neuronal degeneration in rats” (2004) PMID: 15569402 https://www.researchgate.net/publication/8158903_Centrophenoxine_improves_chronic_cerebral_ischemia_induced_cognitive_deficit_and_neuronal_degeneration_in_rats
[241] “Gerontopsychological studies using NAI (' Ntirnberger Alters-Inventar') on patients with organic psychosyndrome (DSM III, Category 1) treated with centrophenoxine in a double blind, comparative, randomized clinical trial “ (1989) https://doi.org/10.1016/0531-5565(88)90003-4
[242] “Centrophenoxine: effects on aging mammalian brain” (1978) https://doi.org/10.1111/j.1532-5415.1978.tb02544.x
[243] “Effects of centrophenoxine on the lipofuscin pigments in the nervous system of old rats” (1974) https://doi.org/10.1016/0006-8993(74)90864-6
[244] “Transient Stimulation with Psychoplastogens Is Sufficient to Initiate Neuronal Growth” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8033616/
[245] “Key Mechanisms and Potential Implications of Hericium erinaceus in NLRP3 Inflammasome Activation by Reactive Oxygen Species during Alzheimer’s Disease” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8615045/

[246] “Cognitive Impairment and Endoplasmic Reticulum Stress Induced by Repeated Short-Term Sevoflurane Exposure in Early Life of Rats” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6083612/
[247] “Chemical Chaperones Reduce ER Stress and Restore Glucose Homeostasis in a Mouse Model of Type 2 Diabetes” (2006) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4741373/
[248] (Review) “Tauroursodeoxycholic acid: a potential therapeutic tool in neurodegenerative diseases” (2022) https://translationalneurodegeneration.biomedcentral.com/articles/10.1186/s40035-022-00307-z
[249] (Review) “Elimination of endoplasmic reticulum stress and cardiovascular, type 2 diabetic, and other metabolic diseases” (2013) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3581057/
[250] “Tauroursodeoxycholic acid suppresses amyloid β-induced synaptic toxicity in vitro and in APP/PS1 mice” (2012) https://doi.org/10.1016/j.neurobiolaging.2012.04.018
[251] “Tauroursodeoxycholic acid increases neural stem cell pool and neuronal conversion by regulating mitochondria-cell cycle retrograde signaling” (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4613652/
[252] “Protective effects of tauroursodeoxycholic acid on lipopolysaccharide-induced cognitive impairment and neurotoxicity in mice” (2017) https://doi.org/10.1016/j.intimp.2019.03.065
[252] “Mitochondria: Major Regulators of Neural Development” (2015) https://doi.org/10.1177/1073858415585472
[253] “Tauroursodeoxycholic Acid Enhances Mitochondrial Biogenesis, Neural Stem Cell Pool, and Early Neurogenesis in Adult Rats” (2018) https://www.ncbi.nlm.nih.gov/pubmed/28534273
[254] “Anti-inflammatory effect of Tauroursodeoxycholic acid in RAW 264.7 macrophages, Bone marrow-derived macrophages, BV2 microglial cells, and spinal cord injury” (2018) https://www.nature.com/articles/s41598-018-21621-5
[255] “TLR4 promoted endoplasmic reticulum stress induced inflammatory bowel disease via the activation of p38 MAPK pathway” (2022) https://doi.org/10.1042/BSR20220307
[256] “Tauroursodeoxycholic acid (TUDCA) supplementation prevents cognitive impairment and amyloid deposition in APP/PS1 mice” (2012) https://doi.org/10.1016/j.nbd.2012.09.003
[257]
[258] “Tauroursodeoxycholic Acid Protects Hepatocytes From Ethanol-Fed Rats Against Tumor Necrosis Factor–Induced Cell Death by Replenishing Mitochondrial Glutathione” (2001) DOI: 10.1053/jhep.2001.28510
[259] “Bile acid TUDCA improves insulin clearance by increasing the expression of insulin-degrading enzyme in the liver of obese mice” (2017) https://www.nature.com/articles/s41598-017-13974-0
[260] “Tauroursodeoxycholic Acid Alleviates H2O2-Induced Oxidative Stress and Apoptosis via Suppressing Endoplasmic Reticulum Stress in Neonatal Rat Cardiomyocytes” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6052504/
[261] “Tauroursodeoxycholic acid mediates endoplasmic reticulum stress and autophagy in adrenocortical carcinoma cells” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6888259/
[262] “Trehalose upregulates progranulin expression in human and mouse models of GRN haploinsufficiency: a novel therapeutic lead to treat frontotemporal dementia” (2016) https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-016-0114-3
[263] “EFFECT OF DIMETHYLAMINOETHYL p-CHLOROPHENOXYACETATE ON THE LIFE SPAN OF MALE SWISS WEBSTER ALBINO MICE” (1973) https://doi.org/10.1016/0531-5565(73)90024-7
[264] “Age-related change in the multiple unit activity of the rat brain parietal cortex and the effect of centrophenoxine” (1988) https://doi.org/10.1016/0531-5565(88)90003-4
[265] “Influences of Vitamin B12 Supplementation on Cognition and Homocysteine in Patients with Vitamin B12 Deficiency and Cognitive Impairment” (2022) https://doi.org/10.3390/nu14071494
[266] “Vitamin B12 in Relation to Oxidative Stress: A Systematic Review“ (2019) https://doi.org/10.3390/nu11020482
[267] “7,8-Dihydroxyflavone Attenuates Alcohol-Related Behavior in Rat Models of Alcohol Consumption via TrkB in the Ventral Tegmental Area” (2020) https://www.frontiersin.org/articles/10.3389/fnins.2020.00467/full
[268] “Amyloid-β pathology is attenuated by tauroursodeoxycholic acid treatment in APP/PS1 mice after disease onset” (2015) https://doi.org/10.1016/j.neurobiolaging.2014.08.034
[269] “Associations between moderate alcohol consumption, brain iron, and cognition in UK Biobank participants: Observational and mendelian randomization analyses” (2022) https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1004039#pmed-1004039-g007
[270] “Hesperetin promotes longevity and delays aging via activation of Cisd2 in naturally aged mice” (2022) https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-022-00838-7

- “A Modest Gain in Mouse Lifespan via Pharmacological Means of CISD2 Upregulation” (2022) https://www.fightaging.org/archives/2022/08/a-modest-gain-in-mouse-lifespan-via-pharmacological-means-of-cisd2-upregulation/

[271] (Review) “Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases” (2015) https://doi.org/10.1016/j.lfs.2014.12.030

[272] “Hesperidin metabolite hesperetin-7-O-glucuronide, but not hesperetin-30 -O-glucuronide, exerts hypotensive, vasodilatory, and anti-inflammatory activities” (2013) DOI: 10.1039/c3fo60030k

[273] “Hesperetin protects against inflammatory response and cardiac fibrosis in postmyocardial infarction mice by inhibiting nuclear factor κB signaling pathway” (2017) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5609142/

[274] “Upregulation of Cisd2 attenuates Alzheimer's-related neuronal loss in mice maintains Ca2+ homeostasis” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7065100/

[275] “Hesperidin Preserves Cognitive Functions and Hippocampus Histological Architecture in Albino Wistar Rats Subjected to Stress Through Enhancement of Brain-Derived Neurotrophic Factor" (2021) https://doi-org.prox.lib.ncsu.edu/10.1007/s12640-021-00433-y

[276] “Supplementing Glycine and N-Acetylcysteine (GlyNAC) in Older Adults Improves Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Physical Function, and Aging Hallmarks: A Randomized Clinical Trial” (2022) https://doi.org/10.1093/gerona/glac135

[277] "Centrophenoxine improves chronic cerebral ischemia induced cognitive deficit and neuronal degeneration in rats" (2005) https://pubmed.ncbi.nlm.nih.gov/15569402/

[278] "Evidence for centrophenoxine as a protective drug in aluminum induced behavioral and biochemical alteration in rat brain" (2006) DOI: 10.1007/s11010-006-9125-7

[279] "Modulatory effects of centrophenoxine on different regions of ageing rat brain" (2005) DOI: 10.1016/j.exger.2005.06.016

[280] “CENTROPHENOXINE INCREASES THE RATES OF TOTAL AND mRNA SYNTHESIS IN THE BRAIN CORTEX OF OLD RATS: AN EXPLANATION OF ITS ACTION IN TERMS OF THE MEMBRANE HYPOTHESIS OF AGING” (1983) DOI: 10.1016/0531-5565(84)90035-4

[281] “The differential effects of meclofenoxate on memory loss in the elderly” (1977) https://doi.org/10.1093/ageing/6.2.123

[282] (Review) “The older adult brain is less modular, more integrated, and less efficient at rest: A systematic review of large-scale resting-state functional brain networks in aging” (2022) https://doi.org/10.1111/psyp.14159

- “The brain undergoes a great “rewiring” after age 40” (2022) https://bigthink.com/neuropsych/great-brain-rewiring-after-age-40/

[284] “Fasting-mimicking diet cycles reduce neuroinflammation to attenuate cognitive decline in Alzheimer’s models” (2022) https://www.cell.com/cell-reports/fulltext/S2211-1247(22)01258-X

[285] “Effects of Acetyl-L-Carnitine on the Brain Lipofuscin Content and Emotional Behavior in Aged Rats” (1988) https://doi.org/10.1254/jjp.48.365

[286] “Acetyl-L-carnitine treatment increases choline acetyltransferase activity and NGF levels in the CNS of adult rats following total fimbria-fornix transection” (1993) DOI: 10.1016/0006-8993(94)91524-5

[287] “Flavones 7,8-DHF, Quercetin, and Apigenin Against Tau Toxicity via Activation of TRKB Signaling in ΔK280 TauRD-DsRed SH-SY5Y Cells” (2021) https://www.frontiersin.org/articles/10.3389/fnagi.2021.758895/full

[288] "Reduced lipofuscin accumulation in senescent rat brain by long-term acetyl-L-carnitine treatment" (1989) https://doi.org/10.1016/0167-4943(89)90035-6

[289] “Acetyl-l-carnitine enhances Na+, K+-ATPase glutathione-s-transferase and multiple unit activity and reduces lipid peroxidation and lipofuscin concentration in aged rat brain regions” (2001) https://doi.org/10.1016/S0304-3940(01)01576-2

[290] “Neuronal mitochondrial amelioration by feeding acetyl-L-carnitine and lipoic acid to aged rats” (2009) https://doi.org/10.1111/j.1582-4934.2008.00324.x

[291] “Orally-active, clinically-translatable senolytics restore α-Klotho in mice and humans” (2022) https://doi.org/10.1016/j.ebiom.2022.103912

[292] "Acute Effects of Hesperidin in Oxidant/Antioxidant State Markers and Performance in Amateur Cyclists" (2019) https://doi.org/10.3390/nu11081898

[293] "High throughput screening of mitochondrial bioenergetics in human differentiated myotubes identifies novel enhancers of muscle performance in aged mice" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6010423/

[294] “Tauroursodeoxycholic Acid Protects Retinal Pigment Epithelial Cells from Oxidative Injury and Endoplasmic Reticulum Stress In Vitro” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7555559/

[295] “Tauroursodeoxycholic Acid Mitigates High Fat Diet-Induced Cardiomyocyte Contractile and Intracellular Ca2+ Anomalies” (2017) https://doi.org/10.1371/journal.pone.0154907
[296] “Tauroursodeoxycholate (TUDCA), chemical chaperone, enhances function of islets by reducing ER stress” (2010) https://doi.org/10.1016/j.bbrc.2010.06.022
[297] “ESCALATING ETHANOL INTAKE IS ASSOCIATED WITH ALTERED CORTICOSTRIATAL BDNF EXPRESSION” (2009) https://doi.org/10.1111/j.1471-4159.2009.06073.x
[298] "Estimating the Prevalence of Dementia and Mild Cognitive Impairment in the US. The 2016 Health and Retirement Study Harmonized Cognitive Assessment Protocol Project" (2022) doi:10.1001/jamaneurol.2022.3543
[299] “Trail Making Test A and B: Normative data stratified by age and education” (2004) https://doi.org/10.1016/S0887-6177(03)00039-8
[300] "Intermittent Fasting Attenuates Hallmark Vascular and Neuronal Pathologies in a Mouse Model of Vascular Cognitive Impairment" (2022) https://www.ijbs.com/v18p6052.htm
[301] “The bile acid TUDCA reduces age-related hyperinsulinemia in mice” (2022) https://doi.org/10.1038/s41598-022-26915-3

Additional review articles and key references

References related to restoring age-related decline in cognitive function

1. “7,8-dihydroxyflavone rescues memory and synaptic plasticity in cognitively impaired aged rats” (2012) https://onlinelibrary.wiley.com/doi/10.1111/j.1471-4159.2012.07830.x
2. “Hericerin derivatives from Hericium erinaceus exert BDNF-like neurotrophic activity in central hippocampal neurons and enhance memory” (2020) https://www.biorxiv.org/content/10.1101/2020.08.28.271676v1.full
3. “Hericium erinaceus Improves Recognition Memory and Induces Hippocampal and Cerebellar Neurogenesis in Frail Mice during Aging” (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6521003/
4. “Effects of Hericium erinaceus on amyloid β(25-35) peptide-induced learning and memory deficits in mice” (2015) https://www.jstage.jst.go.jp/article/biomedres/32/1/32_1_67/_pdf/-char/en
5. “The Citrus Flavanone Naringenin Protects Myocardial Cells against Age-Associated Damage” (2017) https://www.hindawi.com/journals/omcl/2017/9536148/
6. “Diosmetin Mitigates Cognitive and Memory Impairment Provoked by Chronic Unpredictable Mild Stress in Mice” (2020) https://www.hindawi.com/journals/ecam/2020/5725361/
7. “Enhancement of synaptic plasticity and reversal of impairments in motor and cognitive functions in a mouse model of Angelman Syndrome by a small neurogenic molecule, NSI-189” (2019) https://www.sciencedirect.com/science/article/pii/S0028390818305999?via%3Dihub
8. (Review) “Cellular Senescence in Brain Aging” (2021) https://www.frontiersin.org/articles/10.3389/fnagi.2021.646924/full
9. “7,8-dihydroxyflavone, a small molecular TrkB agonist, is useful for treating various BDNF-implicated human disorders” (2016) https://translationalneurodegeneration.biomedcentral.com/articles/10.1186/s40035-015-0048-7

References - Brain Plasticity & BDNF

1. “TNF-Mediated Homeostatic Synaptic Plasticity: From in vitro to in vivo Models” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7556297/
2. (Review) “Cognitive and Brain Plasticity in Old Age” (2016) https://link.springer.com/referenceworkentry/10.1007%2F978-981-287-082-7_146
3. “Exercise-Mediated Neurogenesis in the Hippocampus via BDNF” (2018) https://www.frontiersin.org/articles/10.3389/fnins.2018.00052/full
4. “BDNF mediates improvements in executive function following a 1-year exercise intervention” (2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4263078/
5. “TrkB Receptor Agonists” https://en.wikipedia.org/wiki/Category:TrkB_agonists
6. (Review) “Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain” (2019) https://www.frontiersin.org/articles/10.3389/fncel.2019.00363/full
7. “The Role of BDNF in Age-Dependent Changes of Excitatory and Inhibitory Synaptic Markers in the Human Prefrontal Cortex” (2016) https://www.nature.com/articles/npp2016126

8. "New insights into brain BDNF function in normal aging and Alzheimer disease" (2008) https://doi.org/10.1016/j.brainresrev.2008.07.007
9. (Review) "Involvement of BDNF in age-dependent alterations in the hippocampus" (2010) https://www.frontiersin.org/articles/10.3389/fnagi.2010.00036/full
10. (Review) "The Role of BDNF on Neural Plasticity in Depression" (2020) https://www.frontiersin.org/articles/10.3389/fncel.2020.00082/full

References - Phytosubstances, Brain Plasticity & Inflammation

11. (Review) "Antidepressant Flavonoids and Their Relationship with Oxidative Stress" (2017) https://www.hindawi.com/journals/omcl/2017/5762172/
12. (Review) "Citrus Polyphenols in Brain Health and Disease: Current Perspectives" (2021) https://www.frontiersin.org/articles/10.3389/fnins.2021.640648/full
    a. "Chronic consumption of flavanone-rich orange juice is associated with cognitive benefits: an 8-wk, randomized, double-blind, placebo-controlled trial in healthy older adults" (2015) https://academic.oup.com/ajcn/article/101/3/506/4569404?login=false
        i. Global cognitive function was significantly better after 8-wk consumption of flavanone-rich juice than after 8-wk consumption of the low-flavanone control.
13. (Review) "Hesperidin as a Neuroprotective Agent: A Review of Animal and Clinical Evidence" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6384806/
14. (Review) "Flavonoids as Therapeutic Agents in Alzheimer's and Parkinson's Diseases: A Systematic Review of Preclinical Evidences" (2018) https://www.hindawi.com/journals/omcl/2018/7043213/
15. (Review) "Functions of flavonoids in the central nervous system: Astrocytes as targets for natural compounds" (2016) https://www.sciencedirect.com/science/article/pii/S0197018616300092?via%3Dihub
16. (Review) "Effect of flavonoids on learning, memory and neurocognitive performance: relevance and potential implications for Alzheimer's disease Pathophysiology" (2013) https://doi.org/10.1002/jsfa.6473
17. (Review)"Advances in the Preclinical Study of Some Flavonoids as Potential Antidepressant Agents" (2018) https://doi.org/10.1155/2018/2963565
18. (Review) "Dietary Flavonoids Interaction with CREB-BDNF Pathway: An Unconventional Approach for Comprehensive Management of Epilepsy" (2019) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7057203/
19. (Review) "Polyphenols and brain health" (2017) https://www.ocl-journal.org/articles/ocl/full_html/2017/02/ocl170010/ocl170010.html
20. "High-flavonoid intake induces cognitive improvements linked to changes in serum brain-derived neurotrophic factor: Two randomised, controlled trials" (2016) https://content.iospress.com/articles/nutrition-and-healthy-aging/nha1615
21. (Review) "Flavonoids as a Natural Enhancer of Neuroplasticity—An Overview of the Mechanism of Neurorestorative Action" (2020) https://doi.org/10.3390/antiox9111035
22. (Review) "Phytochemistry and mechanisms of neuroprotection and cognitive enhancement" (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6857646/
23. (Review) "Effects of Citrus Fruit Juices and Their Bioactive Components on Inflammation and Immunity: A Narrative Review" (2021) https://www.frontiersin.org/articles/10.3389/fimmu.2021.712608/full
24. (Review) "Fisetin Acts on Multiple Pathways to Reduce the Impact of Age and Disease on CNS Function" (2015) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5527824/
25. (Review) "Effect of Hesperidin on Cardiovascular Disease Risk Factors: The Role of Intestinal Microbiota on Hesperidin Bioavailability" (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7284956/

References - Lion's Mane Mushroom & Its Neurological Activity

26. "Chemistry, Nutrition, and Health-Promoting Properties of Hericium erinaceus (Lion's Mane) Mushroom Fruiting Bodies and Mycelia and Their Bioactive Compounds" (2015) https://pubs.acs.org/doi/10.1021/acs.jafc.5b02914
27. (Review) "Structures, biological activities, and industrial applications of the polysaccharides from Hericium erinaceus (Lion's Mane) mushroom: A review" (2017) https://doi.org/10.1016/j.ijbiomac.2017.01.040
28. "Inflammation Part 7: Neurohormesis, neuroinflammatory diseases, and their treatment by mushroom substances (Section 1)" (2019)

https://www.anti-agingfirewalls.com/2019/10/09/inflammation-part-7-neurohormesis-neuroinflammatory-diseases-and-their-treatment-by-mushroom-substances-section-1/
29. (Review) “Neurological Activity of Lion’s Mane (Hericium erinaceus)” (2017) https://doi.org/10.14200/jrm.2017.6.0108
30. (Review) “Therapeutic Potential of Hericium erinaceus for Depressive Disorder” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6982118/
31. (Review) “Neurohealth Properties of Hericium erinaceus Mycelia Enriched with Erinacines” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5987239/
32. “The Cyanthin Diterpenoid and Sesterterpene Constituents of Hericium erinaceus Mycelium Ameliorate Alzheimer’s Disease-Related Pathologies in APP/PS1 Transgenic Mice” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5855820/
33. “Two New Cyathane Diterpenoids from Mycelial Cultures of the Medicinal Mushroom Hericium erinaceus and the Rare Species, Hericium flagellum” (2018) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5877601/
34. “Erinacines A, B and C, strong stimulators of nerve growth factor (NGF)-synthesis, from the mycelia of Hericium erinaceum” (1994) https://doi.org/10.1016/S0040-4039(00)76760-8
35. “Improvement of cognitive functions by oral intake of Hericium erinaceus" (2019) https://doi.org/10.2220/biomedres.40.125
36. “Hericium erinaceus potentially rescues behavioural motor deficits through ERK-CREB-PSD95 neuroprotective mechanisms in rat model of 3-acetylpyridine-induced cerebellar ataxia” (2020) https://www.nature.com/articles/s41598-020-71966-z

References - Glutathione

33. (Review) “Oxidative stress and ageing: is ageing a cysteine deficiency syndrome?” (2005) https://doi.org/10.1098/rstb.2005.1770
34. “L-cysteine efflux in erythrocytes as a function of human age: correlation with reduced glutathione and total antioxidant potential” (2012) doi/10.1089/rej.2012.1394
35. “Inhibition of Glutathione Synthesis via Decreased Glucose Metabolism in Stored RBCs” (2018) https://www.karger.com/Article/Fulltext/495864
36. "Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation" (2011) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3155927/
37. “Glycine and N-acetylcysteine (GlyNAC) supplementation in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, insulin resistance, endothelial dysfunction, genotoxicity, muscle strength, and cognition: Results of a pilot clinical trial” (2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8002905/
38. “Supplementing Glycine and N-acetylcysteine (GlyNAC) in Aging HIV Patients Improves Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Endothelial Dysfunction, Insulin Resistance, Genotoxicity, Strength, and Cognition: Results of an Open-Label Clinical Trial” (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7601820/
39. “GlyNAC (Glycine and N-Acetylcysteine) Supplementation in Mice Increases Length of Life by Correcting Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Abnormalities in Mitophagy and Nutrient Sensing, and Genomic Damage” (2022) https://doi.org/10.3390/nu14051114
40. “A Randomized Controlled Clinical Trial in Healthy Older Adults to Determine Efficacy of Glycine and N-Acetylcysteine Supplementation on Glutathione Redox Status and Oxidative Damage” (2022) https://www.frontiersin.org/articles/10.3389/fragi.2022.852569/full

Trail Making Tests Percentiles with Age

- Create a Trails Making Test form generator & percentile calculator.
    - “Computerizing Trail Making Test for long-term cognitive self-assessment” (2017) https://doi.org/10.1108/IJCS-12-2016-0002
- “Trail Making Test A and B: Normative data stratified by age and education” (2004) https://www.sciencedirect.com/science/article/pii/S0887617703000398

Trail Making Test A, Educated Cohorts. x=age in years		
Percentiles	**LMS Fit, 20 to 65 years old**	**LMS Fit, > 65 years old**

10%	y = 0.285x + 35.945	y = 22.031 exp(0.016 x)
20%	y = 0.2241x + 24.993	y = 1.6514x - 69.198
30%	y = 0.2642x + 19.84	y = 1.2629x - 46.25
40%	y = 0.3162x + 15.987	y = 1.0057x - 28.592
50%	y = 0.3073x + 14.69	y = 0.9314x - 26.225
60%	y = 0.2646x + 14.104	y = 0.9086x - 28.522
70%	y = 0.2432x + 13.245	y = 0.8114x - 24.118
80%	y = 0.2431x + 10.823	y = 0.6229x - 13.903
90%	y = 0.2183x + 9.1197	y = 0.5086x - 10.055

Trail Making Test B, Educated Cohorts. x=age in years		
Percentiles	**LMS Fit, 20 to 65 years old**	**LMS Fit, > 65 years old**
10%	y = 1.0501x + 40.382	y = 0.0736x + 151.67
20%	y = 0.3874x + 53.674	y = 5.3429x - 270.54
30%	y = 0.4455x + 45.536	y = 3.6914x - 163.68
40%	y = 0.6872x + 33.569	y = 3.46x - 144.42
50%	y = 0.6245x + 33.204	y = 3.2x - 134.6
60%	y = 0.5756x + 31.481	y = 2.54x - 96.98
70%	y = 0.5814x + 28.354	y = 1.6x - 34.2
80%	y = 0.5399x + 24.426	y = 1.553x - 38.82
90%	y = 0.485x + 21.699	y = 1.4x - 37.8