Alzheimer's
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# Apoaequorin

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Apoaequorin is a protein isolated from the jellyfish *Aequorea victoria*. It is the active ingredient in some supplements marketed for memory and brain health (e.g., Prevagen®). Apoaequorin is a calcium-binding protein that has been used in research for many years as a calcium sensor. Because increased calcium levels in neurons are associated with aging, disruption of synaptic functions, and cell death [1; 2], agents that help maintain healthy calcium levels have been pursued as possible therapeutic targets. However, serious doubts about the efficacy of apoaequorin are raised because the chemical structure of apoaequorin is most likely broken down in the gut before reaching the brain [3; 4].

## EVIDENCE AND POTENTIAL BENEFIT FOR BRAIN HEALTH

### Rated 1/4 based on 1/4 evidence

Strong evidence suggests apoaequorin is digested in the stomach before it ever reaches the brain, and the only existing clinical trial (carried out by the manufacturer) claimed to show a difference from baseline but failed to show a memory-enhancing effect that was different from controls.

Randomized controlled trials: In a randomized controlled trial carried out by the manufacturer, 218 community-dwelling older adults (40–91 years old) with self-reported memory concerns took either 10 mg apoaequorin or placebo, daily for 90 days [5]. The apoaequorin group showed a statistically significant 10–16% improvement ($p<0.001$) from baseline in verbal learning and working memory at the end of the 90 days, while the control group showed a more modest (3–8%) change that was not significant ($p>0.2$). A noted weakness of this study is that the authors failed to directly compare the apoaequorin group with the control group (i.e., did not show that apoaequorin worked better than placebo).

Other human research: None available.

Biology: The cognitive improvement claimed by the manufacturers from the randomized controlled trial above, albeit with inadequate statistics, is baffling because a separate study examining allergenicity showed that 90% of apoaequorin is broken down in a simulated digestive tract (acidic pH and pepsin) in less than 30 seconds (the maximum time examined) [3]. Even in the unlikely event a small amount of the protein survives the digestive tract, it is highly doubtful that a 196 amino-acid protein would cross the blood-brain barrier and enter the brain [6].

No direct data is available for neuroprotection. An uncontrolled and unblinded sleep quality study with 55 healthy adults was carried out by the manufacturer [7]. After 90 days, subjects reported an average increase in sleep time by 40 minutes per night, fewer numbers of waking events (from 3.56 to 1.81 times), and an improvement in self-reported quality of sleep. While improved sleep might in turn improve cognition, the study lacked a control group and provides no evidence that apoaequorin supplements improve sleep more than placebo.

One open-label study involving four sporadic Amyotrophic Lateral Sclerosis (ALS) patients was conducted, but apoaequorin was administered in conjunction with other supplements (e.g., CoQ10, noni juice, turmeric extract). All four patients experienced disease progression, but the author (a physician) noted that the degree of progression was "decidedly less" [8]. Representatives from ALSUntangled contacted the author for details of the study outcome, but were told that "all these detailed data were lost". ALSUntangled concluded there is "insufficient information available" to determine whether this combination of supplements can slow ALS progression [9]. It is also impossible to parse out the effects of apoaequorin from the other compounds administered.

In a preclinical ischemia study, apoaequorin was protective when administered on rat brain slices prior to oxygen-glucose deprivation (i.e., a stroke model) [10]. The treated brain slices had significantly fewer dead/dying neurons, along with increased inflammatory markers, which were interpreted as a "protective preconditioning" before the insult. It is important to note that in this study the apoaequorin was administered directly onto the brain slices. One cannot expect the same protective effect in humans, given that little if any protein is likely to reach the brain.

## For Dementia Patients

Apoaequorin has not been evaluated as a treatment for dementia in randomized controlled trials. Given the evidence suggesting that apoaequorin taken orally does not reach the brain, it is unlikely to have positive effects in dementia patients.

Randomized controlled trials: None is available for apoaequorin in dementia patients.

Other human research: None available.

Biology: Mechanisms of action are questionable given that the chemical structure of apoaequorin makes it unlikely to reach the brain [3; 4].

## SAFETY

### Rated 3/4

Widespread consumer use and a 90-day randomized controlled trial in older adults suggest that apoaequorin supplements are well-tolerated [5]. Safety has not been carefully evaluated for higher doses, longer periods of treatment, or for vulnerable patients who have existing conditions, health risks, or use other medications and supplements. In an in vitro and bioinformatics study, authors affiliated with the manufacturer examined the allergenicity and gastric digestion of apoaequorin [3]. They showed that the apoaequorin protein met the criteria that it is not a known allergen and is not likely to cross-react with known allergens. Authors also demonstrated that apoaequorin was easily broken down in a simulated digestive tract, with 90% being digested in less than 30 seconds. In other words, it is unlikely to either harm or help because it is so rapidly broken down in the gut.

Within the PatientsLikeMe community, two multiple sclerosis patients who were taking apoaequorin reported serious adverse events; one described hypotension severe enough to cause a coma, and the other described depression with suicidal thoughts. The supplement manufacturer retained two physicians to complete a comprehensive review of the 2,281 adverse events reported by the consumers using Prevagen®, which are included in the Generally Recognized as Safe (GRAS) Notification (later withdrawn). The most common adverse event was headache (18.76%), followed by dizziness (7.54%), nausea (6.80%), and hypertension (3.68%). All serious adverse events were judged by the physicians to be associated with pre-existing chronic conditions or unrelated to supplement use.

In 2012, the FDA issued a warning letter to Quincy Bioscience, the supplement manufacturer. Although apoaequorin was originally extracted from jellyfish, the manufacturer now produces and extracts it from *E. coli*, and therefore FDA considers it a drug, though Quincy Bioscience maintains that it is a supplement. On September 2, 2014, Quincy Bioscience submitted a notice to the FDA that apoaequorin is GRAS (generally recognized as safe), but withdrew the notice on Oct 21, 2015, at which point, FDA ceased to evaluate the notice.

In a toxicity study in rats, authors affiliated with the manufacturer showed that the No Observed-Adverse-Effect (NOAEL) level for apoaequorin was 666.7 mg/kg per day, the highest dose tested [11]. The human equivalent dose is 106.67 mg/kg, which is ~640 times what an average person (weighing 60 kg) would take, assuming he/she takes 1 pill daily. In the rodent study, there were 1–2 (out of 40 treated rats) incidents each of blepharospasm (eye closure), immature lymphocytes, hypersegmented neutrophils, decreased prothrombin time (decreased blood clotting time), increased heart weight, oviduct cysts, mottled adrenal, red-brown stomach mass, misshapen spleen, ovarian cyst, coronary arteritis, cardiomyopathy, ulcers, thyroid inflammation, and others. However, these incidents were

"considered as incidental changes/biological variations and not treatment-related adverse events" by the authors.

## HOW TO USE

Apoaequorin is available as an over-the-counter supplement (e.g., Prevagen®). The dose used in the randomized clinical trial was 10 mg daily and deemed safe by the manufacturer that ran the trial. The ALS patients took 20 mg every 2–3 hours while they were awake (up to ~200 mg daily).

## WHAT'S THE FUTURE?

There are no studies or trials of apoaequorin underway.

- The Food and Drug Administration has detailed analyses of adverse events associated with an apoaequorin supplement.
- Details of a class-action lawsuit filed in January 2015 against an apoaequorin supplement manufacturer regarding the claims that its product improves memory function. As of June 2016, the results of this lawsuit aren't available online.

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