*Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.*

# Apoaequorin

**Evidence Summary**

Serious doubts about apoaequorin are raised by a chemical structure that is most likely broken down in the gut before reaching the brain.

**Neuroprotective Benefit:** The manufacturer ran a randomized controlled trial claiming that apoaequorin improves cognitive function but no differences were shown from controls. Strong evidence suggests apoaequorin is digested in the stomach before it reaches the brain.

**Aging and related health concerns:** No data on lifespan or age-related disease exist. Insufficient data exists on whether it slows progression of amyotrophic lateral sclerosis in humans. Protection from ischemic cell death is seen, though only when applied directly onto rat brain slices.

**Safety:** Widespread consumer use and a 90-day randomized controlled trial in older adults suggest that apoaequorin is well-tolerated. High dose or long-term use has not been studied in humans.

**What is it?** Apoaequorin, the active ingredient in Prevagen®, is a calcium-binding protein isolated from jellyfish *Aequorea victoria* and has been used for many years as a calcium indicator. Because calcium dysregulation is associated with aging, disruption of synaptic functions, and cell death (Brini et al., 2014), calcium-binding proteins have been pursued as a possible target for maintaining healthy calcium levels.

Apoaequorin is a protein composed of 196 amino acids. While originally extracted from jellyfish, it is now produced as a recombinant protein exclusively by Quincy Bioscience (Madison, WI, USA). It is produced and extracted from *E. coli*, purified, concentrated, dried, and made into pill form.

**Neuroprotective Benefit:** The manufacturer ran a randomized controlled trial claiming that apoaequorin improves cognitive function but no differences were shown from controls. Strong evidence suggests apoaequorin is digested in the stomach before it reaches the brain.

*Types of evidence:*

- 0 meta-analyses or systematic reviews
- 1 clinical trial with 218 older adults, carried out by the manufacturer (Quincy Bioscience)
- 1 open-label sleep quality study with 55 healthy adults, carried out by the manufacturer
- 0 observational studies
- 0 laboratory studies

*Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?* In a randomized controlled trial (RCT) carried out by the manufacturer (Moran et al., 2016; all authors affiliated with Quincy Bioscience), 218 community-dwelling older adults (40-91 yo) with self-reported memory concerns took either 10 mg apoaequorin or placebo, daily for 90 days. The apoaequorin group showed a statistically significant 10-16% improvement ($p<0.001$) 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, i.e., failing to show that apoaquorin worked better than placebo.

*Human research to suggest benefits to patients with dementia.* None available.
*Mechanisms of action for neuroprotection identified from laboratory and clinical research.* The cognitive improvement claimed from the RCT above is baffling because in a separate study examining allergenicity, 90% of apoaequorin is broken down in a simulated digestive tract (acidic pH and pepsin)

in less than 30 seconds (the maximum time examined). 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 to enter the brain.

No data is available for neuroprotection *per se*. An open-label sleep quality study with 55 healthy adults was carried out by the manufacturer. 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 there is rationale for better cognition with improved sleep, it is unclear how much of these changes were due to placebo effects.

*APOE4 interactions:* Unknown.

**Aging and related health concerns:** No data on lifespan or age-related disease exist. Insufficient data exists on whether it slows progression of amyotrophic lateral sclerosis in humans. Protection from ischemic cell death is seen, though only when applied directly onto rat brain slices.

*Types of evidence:*

- 1 review by ALSUntangled Group that discusses a small open-label study in ALS with a treatment that was a concoction of apoaequorin, CoQ10, noni juice, etc.
- 0 observational studies
- 1 laboratory study in brain slices

No human studies on general aging exist. Because apoaequorin taken orally is almost entirely broken down in the gut, it is very unlikely to have meaningful effects for aging and related conditions. There is no data that it could be acting through mechanisms in the gut.

One open-label study involving 4 sporadic ALS patients was carried out, but apoaequorin (20 mg every 2-3 waking hours plus 20-40 mg before bedtime) was administered in conjunction with CoQ10, noni juice, turmeric extract, deprenyl, i.v. glutathione, lithium, and a diet rich in medium chain triglycerides (reviewed by ALSUntangled). All 4 patients experienced disease progression, but the author (physician) noted that the degree of progression is "decidedly less". The ALSUntangled contacted the author about details of the study outcome, but was told that "all these detailed data were lost". Thus, ALSUntangled concluded there is "insufficient information available" to determine whether apoaequorin slows ALS progression. It is also impossible to parse out the effects of apoaequorin from the other compounds administered.

In an *in vitro* ischemia study, apoaequorin was protective when administered on rat hippocampal brain slices prior to oxygen-glucose deprivation (i.e. a stroke model). The treated brain slices had significantly fewer dead/dying neurons, along with increased inflammatory markers (cytokine and chemokine expression), 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 when Prevagen (apoaequorin) is taken orally, given that little if any protein will reach the brain.

**Safety:** Widespread consumer use and a 90-day randomized controlled trial in older adults suggest that apoaequorin is well-tolerated. High dose or long-term use has not been studied in humans.

*Types of evidence:*

- 1 review by ALSUntangled Group
- 1 clinical trial with 218 older adults, carried out by the manufacturer (Quincy Bioscience)
- 0 observational studies
- 2 laboratory studies, 1 in rodent and 1 using *in vitro* and bioinformatics
- FDA issued a warning letter to Quincy Bioscience for selling an "unapproved drug"
- Class-action lawsuit filed against Quincy Bioscience regarding efficacy (not safety)

In the RCT carried out by the manufacturer, active and placebo capsules were well-tolerated. Only 2 participants out of the 218, 1 from each group, experienced adverse events (irritability in the apoaequorin group and despondence/lethargy in the control) and both withdrew from the study.

Within the PatientsLikeMe community, 2 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. Quincy Bioscience retained 2 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 Prevagen® use.

There are some controversies surrounding Prevagen® and its manufacturer, Quincy Bioscience. In 2012, FDA issued a warning letter to Quincy Bioscience because of: 1) sale of an "unapproved drug", 2)

illegal claims on Prevagen®'s memory improving properties, 3) clinical trials lacking FDA approval, 4) failure to adequately report adverse reactions to Prevagen®, and 5) failure to comply with various Good Manufacturing Practices. Although apoaequorin was originally extracted from jellyfish, the manufacturer now produces and extracts it from *E. coli*, and therefore FDA considers it a drug, even though Quincy Bioscience claims it is a supplement. On Sept 2, 2014, Quincy Bioscience submitted a notice to 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 Jan 2015, a class-action lawsuit was filed against Quincy Bioscience LLC, because of questionable claims that the product improves memory.

In a toxicity study in rats (first author employed at Quincy Bioscience), Moran et al. showed that the No Observed-Adverse-Effect (NOAEL) level for apoaequorin was 666.7 mg/kg per day, the highest dose tested. 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.

In an *in vitro* and bioinformatics study, Moran et al. (first & last author employed at Quincy Bioscience) examined the allergenicity and gastric digestion of apoaequorin. They showed that the apoaequorin sequence met the criteria that the protein is not a known allergen and is not likely to cross-react with known allergens. Apoaequorin was also easily digested by pepsin, with 90% being digested in under 30 seconds. While comforting that the protein is not allergenic, these studies show that apoaequorin taken orally is unlikely to survive digestion or cross the blood-brain-barrier to enter the brain.

**Sources and dosing:** Apoaequorin is available commercially under the brand name Prevagen® and is sold at 30,000 retail stores including CVS, Duane Reade, Walgreens, Rite Aid, etc. Quincy Bioscience owns the patent on the compound. 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). A toxicity study (first author employed at Quincy Bioscience) in rats used much higher doses of up to 666.7 mg/kg. Other usage and safety information can be found here.

**Research underway:** There are no studies or trials underway.

**Search terms:**

Pubmed:

- Prevagen (only 1 hit)
- Apoaequorin (155 hits on PubMed but vast majority on its bioluminescence properties and scientific applications)

Google, Google Scholar, PatientsLikeMe, Natural Medicines, WebMD, Scopus

- Prevagen
- Apoaequorin

Clinicaltrials.gov, clinicaltrialsregister.eu, NIH Reporter

- Prevagen (0 hits)
- Apoaequorin (0 hits)