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.

Blueberry

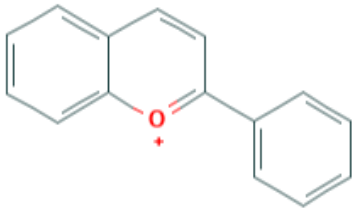
Evidence Summary

Blueberry consumption is probably a good addition to a healthy diet; however, it is not clear that taking blueberry supplements will confer any short- to medium-term cognitive or cardiovascular health benefits.

Neuroprotective Benefit: There are conflicting data from RCTs, but observational studies suggest long-term blueberry intake may be associated with a reduced risk of cognitive decline.

Aging and related health concerns: There are conflicting data from RCTs, but blueberries may improve vascular function and observational studies suggest that blueberries may be associated with improved cardiovascular (CVD) and metabolic outcomes.

Safety: Blueberry consumption is safe. There are some concerns of pesticides with high consumption of non-organic blueberries, and the content and safety of commercial supplements is not known.

Availability: Available from the supermarket or as a supplement	**Dose**: Clinical trials have typically tested 22-45g of freeze-dried blueberries (equivalent to 1-2 cups per day)	**Molecular Formula:** $C_{15}H_{11}O^+$ **Molecular weight:** 207.25g/mol  Source: PubChem (for base structure of anthocyanins)
Half-life: Depends on the metabolite; one study reported that >50% ^{13}C-labeled anthocyanin was detectable in the body 48 hours later (Kalt et al, 2019)	**BBB**: Possibly penetrant for certain anthocyanin metabolites in preclinical studies	
Clinical trials: Clinical trials have tested blueberry treatments in 654 non-AD individuals; 682 CVD patients; and 72 diabetes patients.	**Observational studies**: Large observational studies have included 16,010 women for cognitive aging; 129,617 for Parkinson's disease; 802,310 for CVD risk; 187,382 for diabetes risk; and 611,709 for colorectal cancer risk.	

What is it?

Blueberries contain many substances that may promote health including vitamin C, procyanidins, flavanols, phenolic acids, and derivatives of stilbenes. However, most benefits are ascribed to their anthocyanin content which can range from 25-495mg/100g. Anthocyanins give blueberries their dark color and are reported to be antioxidant, anti-inflammatory, antihypertensive, anti-atherosclerotic, antimicrobial, anticancer, and neuroprotective. However, these benefits may primarily come from anthocyanin metabolites as anthocyanin is extensively metabolized and only 2% of the parent compound reaches circulation. Much of it, in fact, is not even absorbed, with a reported 85% reaching the colon. One hypothesis is that blueberries increase beneficial gut microbes *Bifidobacterium* and *Lactobacillus* which may themselves provide health benefits.

There are different types of blueberries including "lowbush" and "highbush". "Highbush" blueberries are typically larger and grow in greater abundance, thus they are the most common commercial blueberries and the most commonly used in clinical trials. "Lowbush" blueberries are typically small, more intense in flavor, grow in harsher climates, and have around three times the amount of phenolic compounds (Hein et al, 2019; Kimble et al, 2019).

In clinical trials, blueberry supplementation is provided in various ways which may affect blinding of the study. Blueberries may be provided as blueberry juice or freeze-dried blueberry powder that is added to drinks or smoothies. In addition, most observational studies use food surveys to estimate the anthocyanin content of the diet. Anthocyanins are a class of flavonoid found in many colored foods including berries, cherries, black beans, purple corn, etc., so these studies may not be specific for blueberries, per se.

Neuroprotective benefit: There are conflicting data from RCTs, but observational studies suggest long-term blueberry intake may be associated with a reduced risk of cognitive decline.

Types of evidence:

- One systematic review of blueberry supplements in RCTs for cognition (and one counterargument)
- One additional RCT of blueberry supplementation in cognitively healthy patients
- One RCT for uric acid and lipid levels in patients with MCI
- On small open label study in patients with subjective cognitive impairment
- One observational study of blueberry consumption risk of cognitive decline
- One observational study of anthocyanin intake and risk of Parkinson's
- Multiple preclinical studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

Cognition – clinical trials

A recent systematic review of clinical trials testing the effect of blueberry supplementation on cognitive function in healthy elderly and individuals with MCI suggested that blueberries may improve some measures of cognition (Travica et al, 2020). However, this conclusion was countered in a subsequent letter to the editor (Brydges and Gaeta, 2020). One of the main concerns is that some studies suggest

improvements in only certain cognitive domains, but the results are inconsistent and most cognitive measures were insignificant. Below are some of the studies included in the systematic review along with additional studies.

Miller et al (2018) tested the effect of blueberry supplementation (12g twice per day of freeze-dried *Vaccinium ashei* blueberries from the US Highbush Blueberry Council, Folsom, CA; equivalent to 1 cup of fresh blueberries or 460mg of anthocyanins daily) in a double-blind RCT in elderly adults (n = 37, average age 67) over three months. Cognition was assessed with six cognitive tests in different domains (i.e., executive function, memory, attention, and reaction time). Although there was some evidence of an improvement in executive function in the blueberry group, there were no significant effects in other cognitive domains between groups.

In a double-blind RCT, Bowtell et al (2017) treated 26 healthy elderly individuals (average age 68) with 30mL/day of blueberry concentrate (provided by CherryActive, Ltd., Sunbury, UK; containing 387mg of anthocyanidins) over 12 weeks. They reported a significant increase in task-related brain activation in several brain regions. However, most measures of cognition (i.e., psychomotor function, visual processing, executive function, verbal and spatial memory, and working memory) were unaffected. The only exception was a significant improvement on a 2-back memory test after blueberry supplementation.

One hundred and twenty-two elderly individuals (average age 71) were treated with one of four treatments (wild blueberry powder, 500mg/day; wild blueberry powder, 1000mg/day; a proprietary blueberry extract, Thinkblue, developed by Naturex Inc, South Hackensack, NJ; 111mg/day; or placebo) over six months. Individuals taking the Thinkblue supplement had improved memory performance at three months (but not six months). Five memory and/or attention tasks were measured and only two memory tasks showed significant improvement at three months. There is no indication that data was corrected for multiple measures, and the study was funded by the company selling the supplement (Whyte et al, 2018).

Schrager et al (2014) randomized 20 elderly individuals to either two cups of prepackaged flash-frozen highbush (*Vaccinium corymbosum*) blueberries or a carrot juice drink control to be consumed evenly over the course of the day for six weeks. They assessed several measures of motor and psychomotor function (grip strength, simple reaction time, adaptive gait, and executive function). Between groups, there were no significant effects for blueberry supplementation with the exception of an improvement

on one difficult adaptive gait test (participants had to walk along a narrow path while reciting the days of the week backwards).

Benslam et al (2017) conducted a six-month RCT in 215 cognitively healthy elderly individuals with a proprietary polyphenol-rich extract from grape and blueberry (PEGB, a mixture of *Vitis vinifera L.* and *Vaccinium angustifolium Aiton* provided by Nutra Canada and Activ'Inside, containing 258mg of flavonoids per day). PEGB had no effect on the primary outcome (a paired associative learning task) or a working memory test but did improve performance on a verbal episodic and recognition memory test.

McNamara et al (2017) conducted an RCT in 76 patients with subjective cognitive impairment (but not diagnosed with MCI). Patients were treated with fish oil, blueberry powder (a blend of *Vaccinium ashei, Vaccinium corymbosum,* and *Vaccinium angustifolium* provided by the US Highbush Blueberry Council, Folsom, CA, and the Wild Blueberry Association of North America, Old Town, ME; equivalent to 1 cup of whole blueberries per day), a combination of the two, or placebo over 24 weeks. Compared to placebo, the blueberry group had a better score on perceived cognitive inefficiency (i.e., they felt that they were more cognitively effective). However, there were no effects on executive function or memory. There were also no significant effects on any measure for the group taking fish oil plus blueberry.

An RCT in 16 patients with subjective memory impairment reported that 16 weeks of blueberry powder (a 50/50 blend of *Vaccinium ashei* and *Vaccinium corymbosum* provided by the US Highbush Blueberry Council; equivalent to about 148g of blueberries per day), versus a blueberry-flavored placebo powder had no effects on cognition but increased fMRI BOLD signals (indicative of brain usage) in a many brain regions (Boespflug et al, 2017). In an open label study in 9 patients with subjective memory impairment, 6-9ml/kg per day of wild blueberry juice (containing *Vaccinium angustifolium* blueberries provided by Van Dyk's Health Juice Products, Ltd., Caledonia, Nova Scotia, Canada, and the Wild Blueberry Association of North America, Old Town, ME) over 12 weeks slightly improved cognition from baseline (Krikorian et al, 2010). Finally, in 133 patients with MCI, 35g of blueberry powder (provided by the Wild Blueberry Association of North America, Old Town, ME, and Futureceuticals, Momence, IL; equivalent to 2 cups of fresh blueberries) each day over 6 months did not change uric acid levels after 6-12 months, but in women it decreased triglycerides, total cholesterol and HDL-c (Cheatham et al, 2016).

Cognition – Observational study

In an observational study of 16,010 women over the age of 70 from the Nurses' Health Study, the highest intake of blueberries was associated with less cognitive decline over four years on a global score of cognition (an average of six cognitive tests, $p = 0.014$), a verbal memory test ($p = 0.022$), and a

telephone interview on cognitive status (p = 0.022). These results were also seen with other sources of anthocyanins, such as strawberries, but not with other sources of flavonoids, such as tea, apples, onions, and oranges. They estimated that increased blueberry intake may delay cognitive aging by up to 2.5 years (Devore et al, 2012).

Parkinson's disease – observational study
In an observational study with 129,617 individuals from the Health Professional Follow-up Study and the Nurses' Health Study, Gao et al (2012) reported that flavonoid intake was not associated with a reduced risk of Parkinson's disease. However, when looking at individual flavonoids and foods, only anthocyanins (**HR = 0.76; 95%CI 0.61-0.96**) and berries (strawberries and blueberries) (**HR = 0.77; 95%CI 0.62-0.97**) were associated with a reduced risk.

Human research to suggest benefits to patients with dementia:
None reported

Mechanisms of action for neuroprotection identified from laboratory and clinical research
In aged rats, 8 weeks of blueberry supplementation was reported to improve cognition and motor performance, increase neurogenesis, increase IGF-1 and IGF-1R, increase ERK1/2, increase dopamine release from brain slices, and increase levels of HSP90 (a chaperone protein). Blueberry supplementation increased levels of anthocyanins throughout the brain (Joseph et al, 1999; Andreas-Lacueva et al, 2005; Shukitt-Hale et al, 2015; Casadesus et al, 2004; Shukitt-Hale et al, 2005).

In an Alzheimer's mouse model (APP/PS1), 8 weeks of blueberry supplementation improved cognition and increased ERK signaling in the hippocampus but did not change levels of amyloid beta (Joseph et al, 2003). Other studies in aged mice or in mouse models of accelerated aging suggest that blueberry supplementation may improve cognition, reduce lipid peroxidation, increase glutathione levels, increase SOD levels (another antioxidant protein), and decrease acetylcholinesterase activity (Papandreou et al, 2009; Tan et al, 2014; Beracochea et al, 2016). In vitro studies suggest that blueberry may be anti-inflammatory and neuroprotective (Zhu et al, 2008; Brewer et al, 2010).

APOE4 Interactions:
None reported

Aging and related health concerns: There are conflicting data from RCTs, but blueberries may improve vascular function and observational studies suggest that blueberries may improve cardiovascular (CVD) and metabolic outcomes.

Types of evidence:

- One meta-analysis of RCTs for blueberry supplementation for CVD outcomes or biomarkers
- Seven RCTs of blueberry supplementation on CVD outcomes or biomarkers
- Two RCTs for blueberry supplementation and metabolic outcomes in patients with type 2 diabetes
- Two meta-analyses of observational studies of anthocyanin intake for CVD outcomes (e.g., CVD mortality, CHD, hypertension, etc.)
- One observational study for intake of blueberries and risk of diabetes
- One observational study for intake of anthocyanins and risk of colorectal cancer
- Multiple preclinical studies for longevity

Longevity

Blueberry polyphenols increased mean and max lifespan of worms at standard (but not lower) temperatures by 28% and 14%, respectively. They also improved measures of healthspan (pharynx contraction) and reduced cellular damage (Wilson et al, 2006). Blueberry extract also increased lifespan in *C. elegans* by up to 44% (Wang et al, 2018). It decreased lipid peroxidation and cataract morbidity in a rat model of accelerated aging (Kolosova et al, 2004). It extended mean lifespan (10%), but not max, in flies and increased antioxidant proteins (Peng et al, 2012). Blueberry extract had no effect on lifespan in male mice when started at 12 months (Spindler et al, 2013).

Cardiovascular disease (CVD) – clinical trials: **Mixed results**

Miraghajani et al (2020) conducted a systematic review and meta-analysis of 11 RCTs (n = 500 healthy or unhealthy participants between the ages of 18-59). The interventions included blueberry powder, freeze-dried blueberry, blueberry fruit, juice, milkshake, or smoothie. One study was ranked as good, four as fair, and the others as poor quality (based on allocation concealment, blinding, incomplete data, etc.). Trial durations ranged from 4-16 weeks, and the outcomes measured included inflammatory mediators, lipids, glycemic indices, weight, BMI, and waist circumference. They reported that there were no significant effects on any outcome measure regardless of study design or length with the exception for a small insignificant trend for the reduction of triglycerides (p=0.06) and a subgroup analysis showing that blueberries may reduce body weight when treatment was longer than six weeks.

Some of the results (and studies) included in the above meta-analysis are presented below.

Basu et al (2010) reported that in a single-blinded study the consumption of a freeze-dried highbush blueberry beverage (with 50g freeze-dried blueberries per day with a blend of *Vaccinium ashei* and *Vaccinium corymbosum* provided by the US Highbush Blueberry Council, Folsom CA; equivalent to ~350g of fresh blueberries) over eight weeks in 48 obese individuals with metabolic syndrome (avg. age 50) improved blood pressure and decreased oxidized LDL and serum malondialdehyde (MDA) more than placebo. There were no changes on markers of metabolic health (HbA1c, insulin resistance, glucose), lipid levels, or on markers of inflammation (e.g., CRP, IL-6).

Stull et al (2015) randomized 46 individuals (avg. age 57) to a blueberry smoothie (with 45g of freeze-dried blueberry powder with a 50/50 blend of *Vaccinium ashei* and *Vaccinium corymbosum* provided by the US Highbush Blueberry Council, Folsom, CA; equivalent to 2 cups of blueberries) or a color- and taste-matching placebo twice per day over six weeks. There was no change in blood pressure or insulin sensitivity. However, endothelial function improved in the blueberry group.

To assess the acute effect of blueberry consumption on vascular function, Rodriguez-Mateos et al (2013) randomized 21 healthy men to 319, 637, 766, 1278, or 1791mg of blueberry polyphenols in a drink (blueberries provided by the Wild Blueberry Association of North America, Old Town, ME) or placebo and measured brachial flow-mediated dilation (FMD – a marker of endothelial function) over six hours. In the 766mg and higher groups, they reported a biphasic response where FMD improved over the first two hours and then again at six hours.

McAnulty et al (2014) randomized 25 men and postmenopausal women (avg. age 46) to blueberry powder (a 50/50 blend of *Vaccinium ashei* and *Vaccinium corymbosum* provided by Mercer Foods, Modesto, CA; equivalent to 250g blueberries per day) or placebo for six weeks. They reported improvements in arterial stiffness and aortic systolic blood pressure between groups. A reduction in blood pressure (SBP reduction ~3mmHg) and an increase in the number of natural killer cells were significant from baseline in the blueberry group but not between groups.

Johnson and colleagues tested the effect of 22g of freeze-dried blueberry powder per day (50/50 blend of *Vaccinium virgatum* and *Vaccinium corymbosum*, provided by the US Highbush Blueberry Council, Folsom, CA) for eight weeks in two RCTs in postmenopausal women with pre- and stage 1-hypertension (n = 40 and 48). Blueberry supplementation reduced systolic and diastolic blood pressure compared to baseline (SBP~7mmHg, DBP~5mmHg) and improved brachial-ankle pulse wave velocity. It also improved

a marker of oxidative DNA damage and increased nitric oxide. The treatment had no effect on several other outcome measures (anthropomorphic measures and blood biomarkers of inflammation and oxidative stress) (Johnson et al, 2015; Johnson et al, 2016).

Curtis et al (2019) conducted the largest and longest RCT to date. One hundred and fifteen overweight and obese participants (avg age 63) were randomized to freeze dried blueberry powder (provided by the US Highbush Blueberry Council, Folsom, CA; equivalent to either ½ or 1 cup of blueberries) or placebo per day over six months. Individuals taking the equivalent of 1 cup of blueberries had improved flow-mediated dilation and reduced systemic arterial stiffness. There was also a slight increase in HDL-c and ApoA1. No significant effects were reported for insulin resistance, pulse wave velocity, blood pressure, or nitric oxide. In addition, there were no significant effects reported for individuals taking the equivalent of ½ cup of blueberries per day.

Cardiovascular disease – Observational studies: **Potential benefit**
Kimble et al (2019) conducted a systematic review and meta-analysis on dietary intake of anthocyanins and risk of cardiovascular disease from 19 studies including a total of 602,054 participants. High anthocyanin consumption was associated with a reduced risk of chronic heart disease (CHD) (**RR = 0.91; 95%CI 0.83-0.99**) and CVD mortality (**RR = 0.92; 95%CI 0.87-0.97**). There were no significant effects for stroke, myocardial infarction, or total CVD. From a subgroup analysis, the reduced risk of CHD and CVD mortality was more pronounced in studies that considered total anthocyanin intake rather than those that determined anthocyanin intake from berries only.

A meta-analysis of five prospective cohort studies (n = 200,256 individuals) reported that flavonoid intake (highest vs. lowest) was not associated with a reduced risk of hypertension. However, when individual subclasses of flavonoids were considered, anthocyanin intake was associated with a reduced risk of hypertension (**RR = 0.92; 95%CI 0.88-0.97**).

Diabetes – clinical trials: **Mixed evidence**
Twenty women at risk for diabetes were treated with wild lowbush blueberry juice (240mL per day, from *Vaccinium angustifolium* blueberries harvested in Tignish, Prince Edward Island, Canada and provided by the Prince Edward Island Juice Works, Prince Edward Island, Canada) or placebo over seven days. There were no changes on outcome measures including metabolic parameters, inflammatory markers, or oxidative stress markers (Stole et al, 2017).

Stote et al (2020) conducted an RCT in 52 men who are US Veterans (avg age 67) with type 2 diabetes. Patients were given either 22g of freeze-dried blueberry powder (a 50/50 blend of *Vaccinium virgatum* and *Vaccinium corymobosum*, provided by Mercer Foods, Modesto, CA – equivalent to one cup of fresh blueberries) or placebo per day (divided over two meals) for eight weeks. Blueberry treatment improved HbA1c and reduced liver enzymes and triglycerides but had no effect on other lipid levels, fasting plasma glucose, serum insulin, CRP, or blood pressure.

Diabetes – observational studies: **Potential benefit**
Muraki et al (2013) conducted an observational study in 151,209 women from the Nurses' Health Study I and II and 36,173 men from the Health Professionals Follow-up Study (a total of 187,382 individuals) to assess consumption of blueberries and the risk of type 2 diabetes. Compared to consumption of <1 serving per month, consuming 2-4 servings/week (**HR = 0.77; 95%CI 0.67-0.87**) and more than 5 servings per week (**HR = 0.74; 95%CI 0.66-0.83**) was associated with a reduced risk of diabetes. Consumption of even 1-3 servings/month or 1 serving per week was also associated with a reduced risk (**HR~0.90**).

Cancer – observational studies: **Mixed evidence**
A meta-analysis of seven observational studies (n = 611,709 individuals) reported that higher anthocyanin intake was associated with a reduced risk of colorectal cancer (**RR = 0.78; 95%CI 0.64-0.95**). However, this association was only seen with case-control studies and in males. None of the prospective cohort studies (n = 3) or results specifically in females in this meta-analysis reported significant results. They also reported that using the GRADE system, the quality of the meta-analysis was very low (there was significant heterogeneity between studies) (Wang et al, 2019).

Safety: Blueberries consumption is safe. There are some concerns of pesticides with high consumption of non-organic blueberries.

Types of evidence:

- Multiple RCTs of different types of blueberry supplements
- One preclinical toxicology study

Blueberry consumption is safe, and no side effects have been reported in clinical trials. There are some concerns that blueberries may contain a higher level of residual pesticide than many other fruits (see article from CNN); however, blueberries tested for pesticides by USDA were below tolerated levels established by the EPA. In addition, no published studies suggest that blueberry consumption is

associated with side effects. However, there is little data concerning the safety of blueberry supplements sold commercially. One preclinical toxicology study reported that purified blueberry polyphenols were safe at up to 1000mg/kg body weight (Cladis et al, 2020).

Drug interactions: There is some concern that blueberry supplementation may interact with anti-diabetic drugs that lower blood glucose levels. Glucose levels should be monitored when taking blood-glucose lowering drugs while consuming blueberries (webmd.com).

Sources and dosing: Most of the previous studies used the equivalent of 1-2 cups of blueberry/day (generally 22-45g of freeze-dried blueberry powder supplement per day, either in a pill form or reconstituted in water). Epidemiological studies generally use food questionnaires to estimate the anthocyanin content of an individual's diet. None of the studies reported effects with a specific supplement (other than Thinkblue) so it is not clear which blueberry supplement would be most effective. Notably, commercial blueberry supplements were not studied, and there is no evidence whether they contain their reputed contents.

Research underway: At least 15 blueberry clinical trials are currently underway (ClinicalTrials.gov). Select studies include one three-month RCT study examining blueberry powder + exercise or exercise alone in 60 participants over 12 weeks. They will examine arterial stiffness, cognition, and blood pressure in overweight adults (NCT04049162). Another study will examine the effects of blueberry powder in 60 healthy individuals for effects on endothelial function (flow-mediated dilation) and cognition after 12 weeks (NCT04084457). They will also look at changes in cerebral blood flow, blood pressure, and arterial stiffness.

Search terms:

Blueberry + cognition, lifespan, cardiovascular, aging, hypotension, observational, neuropathy, apoe4, pesticide, safety
anthocyanin ([title/abstract] + cardiovascular, alzheimer, cognition, neuropathy, aging, mortality, hypertension, apoe4, cancer

Websites visited:

- Clinicaltrials.gov
- Pubmed
- Drugs.com

***Disclaimer**: Cognitive Vitality Reports® do not provide, and should not be used for, medical advice, diagnosis, or treatment. You should consult with your healthcare providers when making decisions regarding your health. Your use of these reports constitutes your agreement to the **Terms & Conditions**.*

If you have suggestions for drugs, drugs-in-development, supplements, nutraceuticals, or food/drink with neuroprotective properties that warrant in-depth reviews by ADDF's Aging and Alzheimer's Prevention Program, please contact INFO@alzdiscovery.org. To view our official ratings, visit Cognitive Vitality's Rating page.