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**Miller MG, Thangthaeng N, Rutledge GA, Scott TM, Shukitt-Hale B. Dietary strawberry improves cognition in a randomised, double-blind, placebo-controlled trial in older adults. Br J Nutr. 2021 Jan 20:1-11.**

## What We Know, Think We Know, or Are Starting to Know

In the literature on diet and brain health, polyphenols have emerged as a potentially important group of compounds. Flavonoids, a family of polyphenolic compounds, are the primary dietary source of polyphenols in humans, and contain six major subclasses: anthocyanidins, flavan-3-ols, flavonols, flavanones, flavones, and isoflavones.

The totality of evidence to date - both human and animal - suggests three main mechanisms* through which flavonoids may act on the brain:

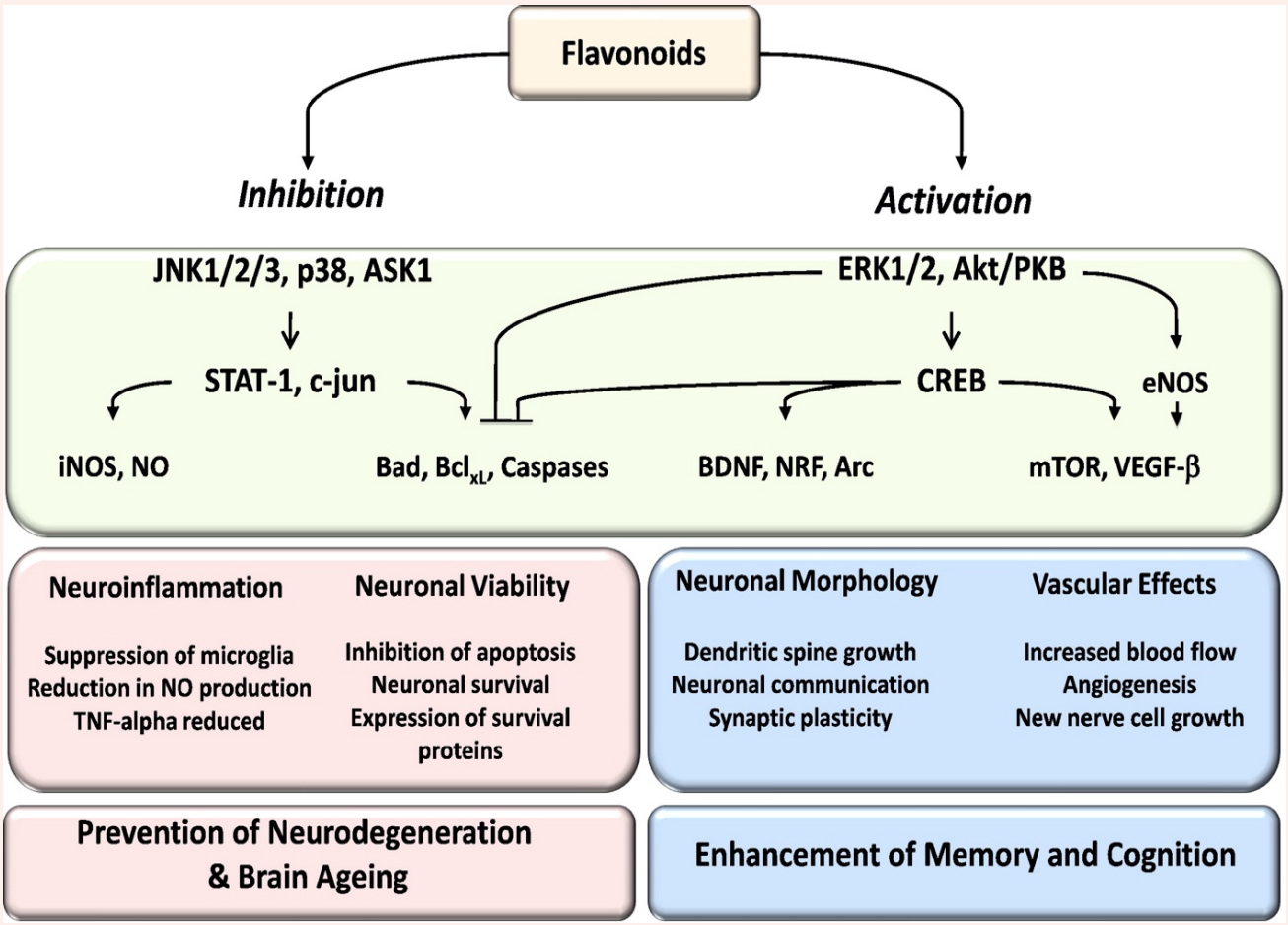
- Interaction with brain pathways that induce the growth, survival, and repair of brain cells, and synaptic plasticity [the ability of synapses, the junctions between brain cells that allow them to communicate, to strengthen in response to a task];
- Increased cerebrovascular blood flow [CBF] and angiogenesis [the formation of new blood vessels];
- Protection from inflammation and other processes, e.g., excess nitric oxide production [1].

Different foods possess different flavonoid compounds. For example, citrus fruits contain flav*onols*, while cocoa contains flav*anols*, and both of these compounds have some evidence for improving aspects of cognitive function [2-5]. Of the flavonoid subclasses, however, anthocyanins have received the lions share of attention to date, and a number of small interventions have suggested that anthocyanin-rich concord grapes and blueberries may improve cognition across a number of stages in the life cycle [7-13].

The first observational study to identify specific berry foods was the US Nurses Health Study, which found that consumption of blueberries >1/week and strawberries >2/week and high total flavonoid intake was associated with a delay in cognitive ageing equivalent to 2.5yrs on cognitive scoring [i.e., 75yo average scores were equivalent to a 73yo] [14]. The first study to look at specific flavonoid subclasses associated with these foods was the Chicago Health and Ageing Project, which found that the highest quartile of pelargonidin, the primary anthocyanidin contained in strawberries, had a 35% reduced risk of AD compared to the lowest quartile of intake [15].

These observational findings have generated interest in the potential effects of berry supplementation on cognitive function and overall brain health. The present study investigated the effects of a strawberry supplement on cognition and motor function in healthy elderly participants.

## *Geek Box: Getting Very Mechanistic About Flavonoids




*This figure is taken from Williams and Spencer [(1)], highlighting the various pathways through which flavonoids may act to protect the brain and improve cognition. Pay attention to this illustration as we go through this to stay with the detail, and by the end you'll have a good in-depth understanding of how these non-nutritive, bioactive food components exert biological activity in the brain and nervous system. The first thing to note is that antioxidant activity, traditionally considered the mechanism underlying the cognitive benefits of flavonoids, is not considered to account for their effect due to their low physiological brain concentrations: there is little evidence that flavonoids cross the BBB in significant quantities. Thus, the ability of flavonoids to exert an effect is due to sufficient quantities of flavonoids and, crucially, their metabolites, reaching the brain at low physiological concentrations and interacting with signalling pathways that regulate neuronal morphology [the shape of brain cells], viability [the proportion of live healthy cells], inflammation and vascular effects. Both anthocyanins and flavanones increase and maintain levels of brain-derived neurotropic factor [BDNF], a critical molecule in learning and memory. Enhanced vascular function may also increase BDNF ['Activation' side of the figure, above], and flavonoids may increase BDNF through enhancement of endothelial nitric oxide synthase [eNOS] and increased cerebrovascular blood flow [CBF]. Both neurogenesis [the formation of new neurons] and angiogenesis [the formation of new blood vessels] are strongly correlated in the hippocampus, and increased CBF may facilitate neurogenesis through activation of eNOS, which regulates angiogenesis and vasodilation.*

## *Geek Box: Getting Very Mechanistic About Flavonoids continued...

*Flavonoid activation of the Akt/PKB and mTOR pathway ['Activation' side of the figure, above], a process also mediated through eNOS activation, may also influence the relationship between neurogenesis and angiogenesis. The ERK1/CREB ['Activation' side of the figure, above] pathway is believed to be a pathway associated with long-term effects, and sustained activation of this pathway from flavonoids may increase synaptic plasticity. On the 'Inhibition' side of the figure, above, flavonoids may also exert protective mechanisms by influencing protein signalling pathways, including PI3, MAPK [p38] and NF-kB, that regulate inflammatory processes. This may underlie the suppression of neuro-inflammation by flavonoids. Uncontrolled activation of glial cells produces excess nitric oxide [NO], induced by increased nitric oxide synthase [iNOS] and TNF-α, stimulating neuro-inflammation through pathways which are inhibited by flavonoids. Flavonoids may also be directly neuroprotective, by inhibiting JNK/c-jun/caspase-3 pathways to prevent cell death caused by oxidized LDL-cholesterol. The inhibition of the caspase-3 pathway also serves to inhibit the action of pro-apoptotic proteins [i.e., inappropriate induction of cell death].*

## The Study

Participants aged 60-75yrs were enrolled in a randomised, double-blind, placebo-controlled, parallel-group [both intervention and control run at the same time], study investigating the effects of strawberry supplementation over 90-days. Participants attended at the university clinical research centre on four separate occasions, as follows:

- Pre-baseline [Visit 1]
- Baseline [Visit 2 - Day 0]
- Midpoint (Visit 3 - Day 45]
- Endpoint [Visit 4 - Day 90]

Participants randomised to the intervention group received a powdered supplement of strawberries [SB], and were instructed to consume 12g in the morning with breakfast and 12g in the evening with dinner, for a total daily dose of 24g. This is equivalent to 2 cups [US measure] per day of strawberries.

The placebo group consumed a colour matched, strawberry flavoured placebo [PL] powder that was matched for calories with the intervention drink, and consumed the drink with the same instructions as the intervention group [12g with breakfast and dinner, respectively].

All participants were instructed to restrict intakes of berries and other flavonoid-rich foods from Visit 1 onwards. At Visit 1, participants completed abbreviated versions of the cognitive tests [to minimise practice effects]. On Visit 2 [Baseline], participants consumed a low-polyphenol standardised breakfast [50% carbohydrate, 31% fat, 19% protein] before undergoing the battery of scheduled tests. This was repeated on Visit 3 [Midpoint], except that participants consumed the SB or PL drink with the breakfast meal before tests. Visit 4 [Endpoint] was a repeat of Visit 3.

The primary endpoint was cognitive function, assessed by tests of short-term memory, long-term memory, executive function, and spatial cognition. The secondary endpoint was mobility, as assessed by stance and gait challenges.

**Results:** 37 participants completed the study [19 in the PL group, 18 in the SB group], and were well matched for baseline characteristics, including diet and physical activity levels. Compliance was assessed at >97% with no difference between groups.

- ***California Verbal Learning Test:*** This test requires participants to learn a 16-word list, which is compared in recall over time and against a distractor list of other words. Participants in both the SB and PL groups significantly improved number of words correctly recalled, and better recall after a 20min delay. These results suggest a practice effect of familiarity with the test over the study. In the SB group, there was a statistically significant increase in the number of correct words identified between Visit 2 Baseline and the Endpoint.

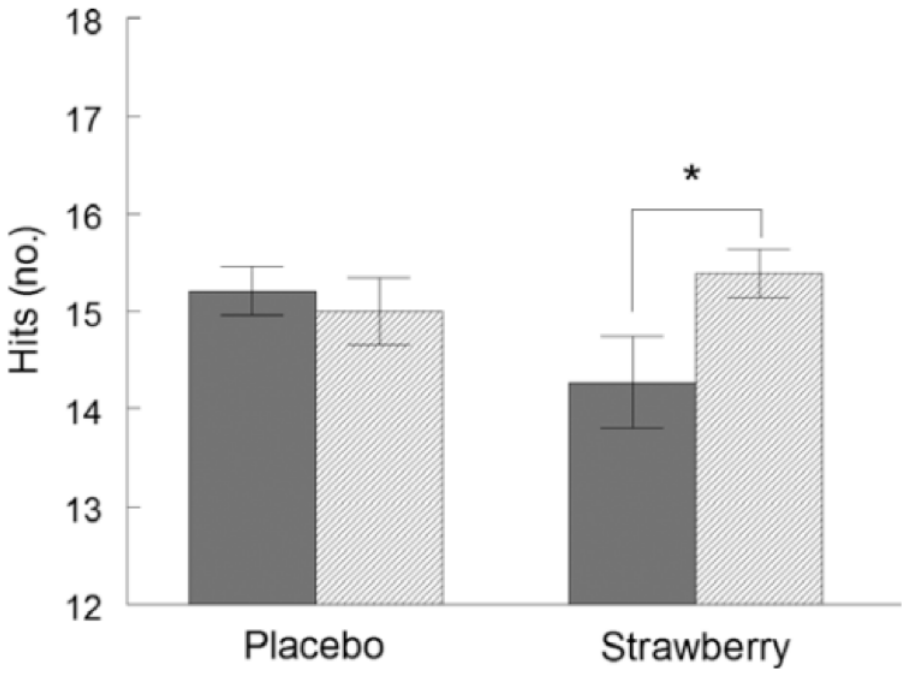



***Figure*** *from paper illustrating the difference between Visit 2 Baseline [dark grey bars] and Visit 4 Endpoint [light grey bars] in the PL group [left bars] and SB group [right bars]. The PL group did not change between visits, while there was a statistically significant increase between visits in the SB group. However, as the figure illustrates, this was largely a reflection of the lower baseline test score in the SB group.*

- ***Morris Water Maze (vMWM) Test:*** This test requires participants to use arrow keys on a computer to locate an invisible platform with a specific arena on the screen. The time to correctly identify the platform decreased significantly in both groups over the course of the study, suggesting a practice effect; there were no differences between groups. The SB group spent more time searching for the hidden platform in the correct zone compared to the PL group at Visit 3 Midpoint, which was statistically significant. However, there was no difference from baseline by Visit 4 Endpoint.

There was no statistically significant differences in other cognitive measures, or in mobility measures, between groups.

## The Critical Breakdown

**Pros:** Randomisation was appropriate and reported, and both researchers and participants were blinded to the treatments. All participants were instructed to restrict intakes of berries and other flavonoid-rich foods from Visit 1 onwards, which may have reduced the potential for a dilution effect of dietary intake. Well-validated cognitive tests were used. The exact flavonoid composition of the intervention drink was quantified. Compliance was assessed both by a participant-recorded calendar and by assessing any unused powder sachets, and was reportedly very high.

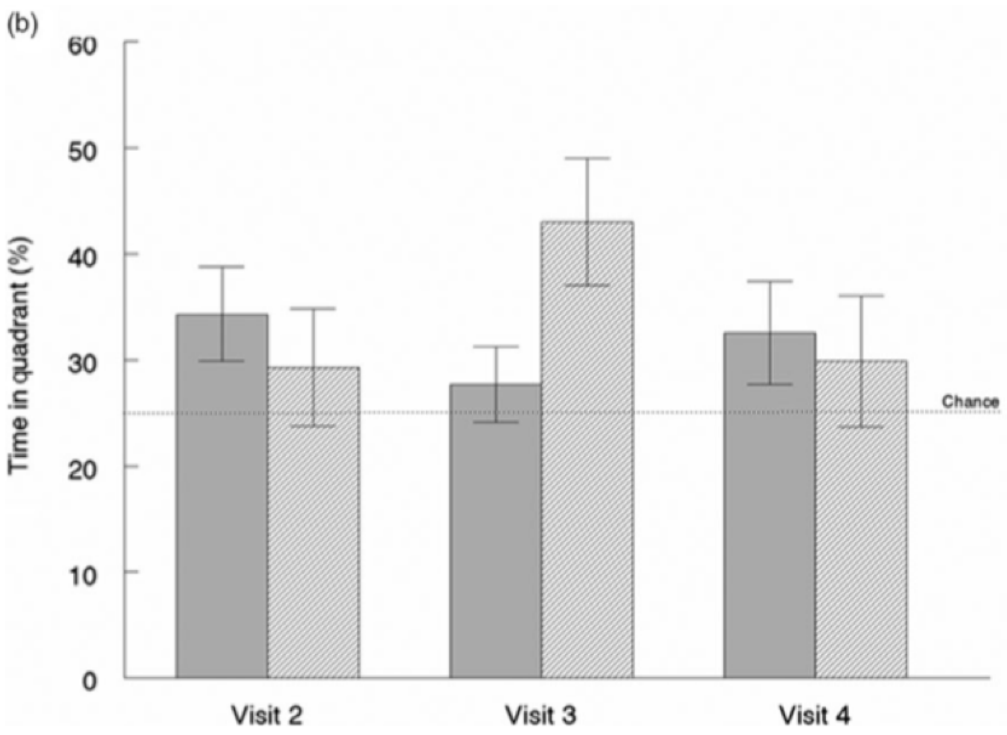
**Cons:** The study required 20 participants per group for statistical power, and although a statistically significant finding was reported for the outcome on which the power calculation was based, the study may have been underpowered. Nonetheless, the overall sample size was small [n = 37]. It is possible that the participants benefitted from practicing the tests over the study, with the implication that certain outcomes may reflect this practice effect and not a true effect of the intervention. Participants were allowed coffee with breakfast before testing, and this is a fairly unacceptable potential confounder to allow for given the noted relationship between caffeine and cognition. Other potential confounders include the sugar content of the PL vs. the SB drinks, and sex differences in the vMWM test [see ***Key Characteristic*** and ***Interesting Finding***, below, respectively]. Only a per-protocol analysis was conducted, and with 16 drop-outs it would have been appropriate to conduct an intention-to-treat analysis. Although funding is not a Con per se, it is always a factor to acknowledge, and this study was industry-funded from the California Strawberry Commission. BigBerry has a good ring to it.

## Key Characteristic

Cognitive testing presents a number of challenges for nutrition research. It is important that a selected test have validity [i.e., been shown to measure what it purports to measure], reliability [i.e., can reproduce results], and sensitivity [i.e., can detect small changes to determine whether a performance is valid or not]. The tests used in studies tend to have undergone these validation processes in prior research. The challenge is relating dietary exposures to any effects, and it is important to control as many variables as possible in this regard. In this study, while the PL may have have been isocaloric with the intervention SB, we do not know whether sugars were matched: the PL drink contained sucrose, dextrose, maltodextrin, and dextrose. Glucose has been shown to directly link with improved mental effort, memory and attention responses to acute cognitive testing [(16)]. And coffee was not controlled for between groups before testing, thus the particular cognition-boosting effects of caffeine may have influenced testing [(17)]. Thus, even if we were to accept that the the minor observed differences in the SB group were a true difference from baseline, we could not confidently conclude the the effects of testing in both groups were not confounded by these unmatched factors.

## Interesting Finding

The improvement observed in spatial learning was only observed after 45-days [Visit 3 Midpoint] of the intervention [see ***Figure***, below], and had returned to a similar score as baseline by the end of the intervention. Spatial learning is the process of acquiring a mental representation of an environment, and being able to recall that information. However, there are sex differences in performance on the vMWM, and performance has been shown to improve in men with age [18]. Specifically, older men are associated with higher percent time spent in the target region [18], the exact finding observed in this study. The SB intervention group contained 38% women and 62% men, with a mean age of 66yrs; it is possible that these factors influenced the result. It is possible that this result was a transient effect reflecting random variation in performance in the groups, rather than any true effect of the SB intervention in that timeframe.



## Relevance

There is still a long way to go with establishing the effects of flavonoids, and specific flavonoid-rich foods, on cognitive function. A number of questions remain to be fully elucidated, including:

- The effects of different flavonoid subclasses
- Whether particular flavonoid compositions in foods are important any observed effects
- Whether specific cognitive domains are influenced more than others
- The relationship between particular cognitive domains and specific flavonoids

The present study found no significant differences in healthy older adults in the majority of cognitive tests, and no difference in any mobility tests. The statistically significant findings were primarily observed in both groups, suggesting good overall cognitive function in these healthy elderly participants, and a practice effect of habituating to the tests. The two statistically significant differences are minor in magnitude, and subject to a number of limiting caveats discussed in the above sections.

A number of interventions using anthocyanin/flavonoid-rich drinks or foods have found improved cognitive function and/or memory performance, primarily from blueberries or concord grape [7-13]. Citrus flavonols, in particular hesperidin and narirutin, have been shown to result in increases in global cognitive function [2], including increases in assessments of executive function [3] that were not observed in trials using anthocyanins [6,7]. Cocoa flavanols have been shown to increase visual acuity and visual-spatial working memory [4]. Cocoa flavanols also led to reduced subjective mental fatigue across different time points, where cognitive test batteries were performed 2-minutes apart [5].

Taken together, there remains sufficient evidence of effects to warrant further research. The present study contains a number of important limitation that prevent any conclusions from being made at this juncture, as to whether strawberries' flavonoid composition and intake yields any benefit to cognitive function.

## Application to Practice

The overall weight of data - epidemiology, interventions, and mechanistic research - remains in favour of flavonoid consumption in the diet, with potential benefit beyond the brain for cardiovascular health. While the present study makes no case for eating 2-cups of strawberries a day, the wider research does point to the regular inclusion of dark-pigmented berries in the context of total fruit intake, as a source of flavonoids.

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