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Whyte AR, Cheng N, Butler LT, Lamport DJ, Williams CM. Flavonoid-Rich Mixed Berries Maintain and Improve Cognitive Function Over a 6 h Period in Young Healthy Adults. Nutrients. 2019;11(11):2685.

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

Nutrition is more than nutrients. While that may sound like a waxy wellness cliche, in fact we know that it is a biological reality. When we talk of ' nutrition' , we naturally think of vitamins and minerals, and this makes sense: they are required for life, and we must obtain them through the diet or we will suffer deficiency diseases.

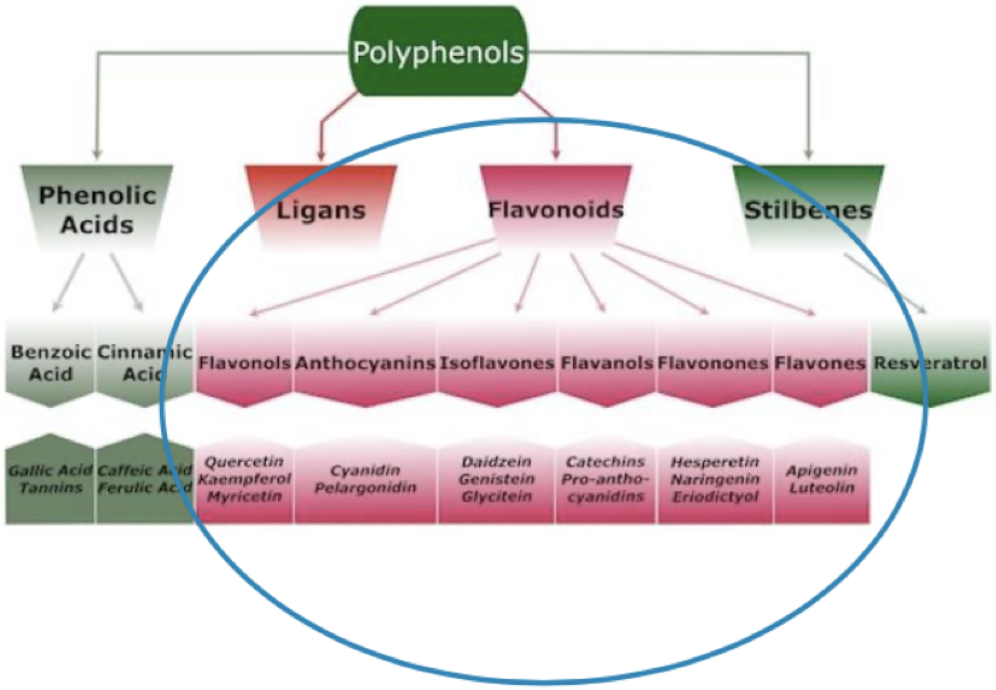
However, within the plant kingdom there is a chemical constellation of compounds, broadly known as 'phytochemicals' , which develop to protect the plant for environmental stressors - like heat and sunlight - and predators [(1)]. Research in these compounds was historically quite a niche area, as they were initially believed to be biologically inert [(1)]. We know now that they are far from inert, and phytochemicals have been proposed as "lifespan essentials", i.e., their presence in the diet may provide significant health enhancement over the lifespan, protecting against chronic lifestyle disease [(1,2)].

Their biological activity may be due to the fact that the body handles these compounds as xenobiotics, i.e., a chemical compounds not produced in the body, and which the body recognises as foreign [(1)]. Therefore, the body treats phytochemicals in much the same way as drugs, metabolising them through Phase I and Phase II detoxification enzymes in the intestines and liver, and converting them from the parent compound into secondary metabolites [(3)].

This is crucial: it is these metabolites that exert biological activity [(3)]. These metabolites act a low concentrations* through different signalling pathways, and it is important to note that antioxidant activity is now not considered to explain their effects [(3)].

The major classes of phytochemicals in the human diet are known as polyphenols [(4)]. Compounds known as flavonoids constitute the major human dietary source of polyphenols, with the primary food sources including vegetables, fruits and fruit juices, tea, red wine, coffee, and chocolate [(4)].

Within the classification of flavonoid is a diverse chemical subgroup of compounds, including anthocyanins, flavonols, flavones, isoflavones, flavan-3-ols, flavanones, anthocyanidins and proanthocyanidins [(4)]. Anthocyanins are responsible for the pigmentation of fruits and vegetables, in particular purples, blues, and reds [albeit not beets, because fun-fact, beets are given their colour from betanins, not anthocyanins, and these compounds cannot be present together in the same food - a food contains one or the other]. The diverse chemical structures of the class of phytochemical its metabolites are associated with a wide array of biological affects, which may influence health and disease processes.



*Geek Box: Hormesis

'Hormesis' is a principle from toxicology, and describes how exposure of an organism to a physical or chemical agent may result in different physiological responses relative to the concentration dose of the agent. Hormesis is characterised by beneficial stimulatory effects exerted at low concentrations, but toxic effects at high concentrations. This biphasic dose-response is a fundamental mechanism of hormesis; a low dose exposure stimulates low levels of stress which the organism is capable of tolerating, and which induces beneficial adaptations as a compensatory response. The biphasic dose-response is quantitatively demonstrated by a "J-shaped curve", which illustrates the magnitude of response relative to the concentration dose. Hormesis can be initiated by extrinsic factors, like physical exercise, heat, toxins, or phytochemicals, and intrinsic factors, like energy availability, neurotransmitters, and hormones. The cellular stress induced by the initiating hormetic factor activates adaptive stress response pathways, which protect the cell from more severe stress. Intrinsic initiation of hormesis indicates that it is an integral aspect to normal physiological function, reflecting an evolutionary adaptation to survive harsh environmental conditions. Hormesis sounds complicated, but you already know of plenty of examples. Take exercise: too little is known to be bad for health, but too much may lead to negative adaptations (overtraining, decreased reproductive hormones, etc). In the middle ground, it takes time to build fitness; the initial stressor must be adapted to, so that initial stressor becomes insufficient - i.e., easier! - over time. In relation to phytochemicals, hormesis provides an explanation for why antioxidant supplement trials largely failed, and in the case of the famous Alpha-Tocopherol, Beta Carotene Cancer Prevention Study, increased risk for cancer. In effect: too much of a good thing is not necessarily a good thing, and hormesis dictates that we only require relatively low concentrations of polyphenol metabolites to benefit from their biological activity.

The Study

This study was a randomised, placebo-controlled, single-blind, parallel group intervention investigating the efficacy of a mixed-berry, flavanoid-rich preparation on acute cognitive measures. The testing was conducted over 6-hours.

40 participants in total were included; 20 in the intervention group and 20 in the control group. The mean age of participants was 22.8yrs, and all participants were healthy. Participants were instructed to follow a low-flavonoid diet for 24 h prior to the test day, and a 12 h fast preceding the baseline test battery, which was conducted at 09.00 h before the breakfast intervention smoothie, consumed at 09.30 h. The same cognitive test battery was then repeated at 1.5-2hrs, 3.5-4hrs, and 5.5-6hrs, post breakfast. Each battery of tests lasted 30mins.

The intervention consisted of a 400ml smoothie consisting of 75g strawberries, 75g blueberries, 75g blackberries, 75g raspberries, blended with water. Total polyphenol content 14.3g. Total flavonoid content was 569.7mg, comprised of the following compounds

- Anthocyanidins: 254.65mg
- Flavan-3-ol: 44.73mg
- Flavanone: 0.23mg
- Flavone: 0.15mg
- Flavonol: 13.55mg
- Proanthocyanidin: 256.4mg

Both groups also consumed 2 butter croissants and 35g light cream cheese. A standardised low-flavonoid lunch of chicken/ham sandwich with 5g spread, 25g pack ready-salted crisps, and one banana, was consumed between 2nd and 3rd battery of cognitive tests by all participants.

Cognitive tasks* consisted of the following tests in order:

- Modified Attention Network Task [MANT]: *designed to measure attention during different levels of demand. Participants have to recognise and correctly select the direction an arrow is pointing. The centre arrow is presented in either alone [easy level], or in a row of arrows, which may be facing in the same or opposite directions, presenting various levels of difficulty.*

- Task Switch Task [TST]: *designed to measure mental flexibility. Participants have to identify from displayed numbers displayed in segments whether a number is odd or even, before the task switches to having to identify whether the number is above or below 5; each number is displayed for 3 seconds, with 0.5 seconds between displays. The fist task switch is consider the most demanding, with slower response times and more errors.*

Primary outcome measures were accuracy [right answers] and response time in relation to both tests. In the MANT, the higher difficulty level included ‘incongruent tasks’ , where arrows were facing in different directions; incongruent tasks were also analysed separately for effects on accuracy and response time.

*Geek Box: Cognitive Tests in Research

Assessing cognitive effects is very challenging from a research methodology perspective! There are many different tests that could be used, and so a key considerations are validity [how well does it measure what it intends to measure], reliability [how consistent is it over repeated measures in the same person], and sensitivity [does it correctly show an actual effect]. Factors like baseline cognitive health status, age (both often related!), and neural correlations of the nutrient or compound's proposed mechanism of action, are all important, and may influence the outcomes. In particular, an important factor is the length of the intervention relative to the absorption and metabolism of the intervention food/bioactive compound, which could influence results based on the timing of the tests. A range of external factors may influence performance, including sleep quality, mood, motivation, and physical well-being. In addition, the role of wider diet may be important. If there are effects, they may also be in very small scales, for example, in this study response time was measured in nanoseconds. It is also important to provide a practice opportunity to participants before the testing; otherwise, if the difference in later tests appears greater, it may be because the first performances of the test were poorer due to lack of familiarity, potentially influencing the results. These factors all coalesce to make assessing the effects of dietary compounds on cognitive function quite difficult. This means that the onus is on us to really critically evaluate studies with 'null' findings, and to distinguish between immediate effects vs. long-term benefit.

Results: In response to the Modified Attention Network Task [MANT], participants in the berry group performed more accurately than the placebo group, which was statistically significant. The significance was driven by preservation of accuracy up to the 6 h Test in the berry group, while in the placebo accuracy declined significantly from 2 h Test to 6 h Test.

The magnitude of effect was greater between groups when comparing the most cognitively demanding levels, incongruent tests of the MANT. These effects were significantly different at 6 h Test time, while there was no significant difference at 2 h or 4 h Tests.

Response time was faster in the berry group vs. placebo, which was statistically significant at 2 h and 4 h Tests.

For Switching Task [TST] accuracy, there was a borderline significant difference evident, with reduced accuracy in the placebo group between 2 h and 6 h Tests. For ST reaction time, there was a significant decrease in response time in intervention group at 6h Test compared to the between 2 h and 4 h Tests.

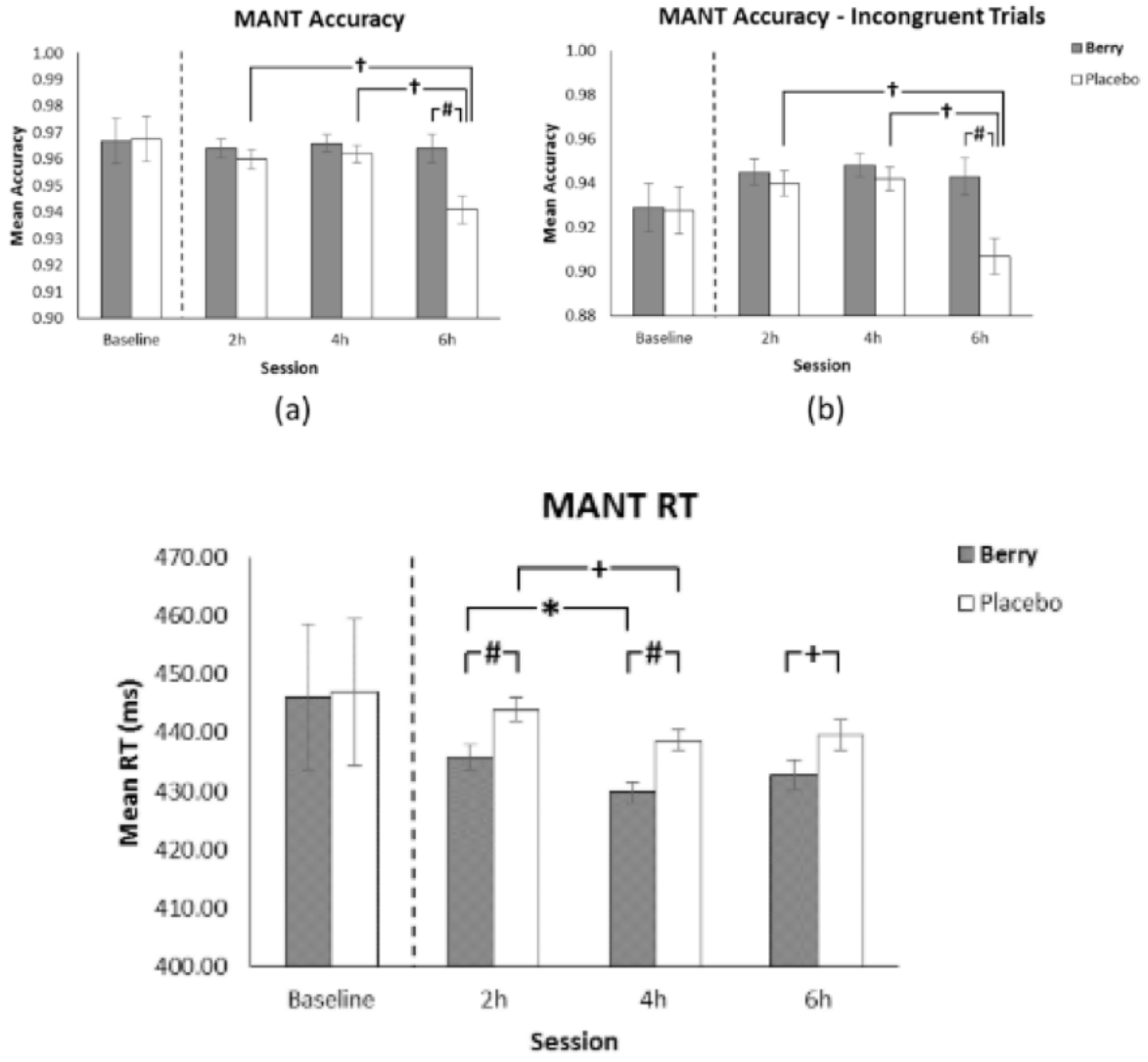


Figure *from the paper illustrating (**top graphs**) mean accuracy (as % of right answers) in overall test (**a**) and in the more cognitively demanding incongruent tests (**b**) in the MANT. The bottom graph illustrates response times in the MANT in milliseconds.*

The Critical Breakdown

Pros: The study had a strong design and participants were blinded to the intervention. Participants were young and otherwise healthy, in an area where much research has been conducted in the elderly with or without mild cognitive impairment. The intervention was food-based using commercially available berries, whereas other research uses concentrated supplemental powdered berry extracts. Both intervention drinks were matched for fructose, glucose, total energy, and vitamin C [more under *Key Characteristic*, below]. Both intervention and placebo drinks were consumed through opaque flasks with black straws; this is important because wider research has shown that sensory properties of foods/drinks may influence outcomes.

Cons: Although matched for nutrient contents [except flavonoids], the drinks were not matched for taste, which may have had some effect. The provision of additional foods at breakfast and at lunch means interactions cannot be ruled out. While the banana with lunch is a lower flavonoid fruit, bananas contain sugars, vitamin B6, choline, etc., and bioactive compounds, all of which may have cognitive effects at. It is not possible to draw conclusions in relation to individual berries used in the intervention, which may have differential effects given their respective flavonoid content. Finally, although this depends on the individual, the total amount of berries [300g] may be an amount that is impractical for one meal.

Key Characteristic

The placebo drink was matched with the berry intervention for both sugar [and total carbohydrate] content, and vitamin C. A methodological challenge inherent in these study designs is concocting an appropriate placebo.

Given fruits contain sugar, this is the most important variable to match given glucose serves as the brains primary fuel source, and glucose has been shown to directly link with improved mental effort, memory and attention responses to acute cognitive testing [(5)].

Previous research has failed to match compounds between flavonoid-rich interventions and placebos; for example, a trial using blueberry supplementation in older adults failed to match glycaemic load of the drinks, potentially influencing cognitive outcomes [(6)].

While there is also some evidence to suggest that flavonoids may slow sugar absorption, and thus may result in more prolonged glucose availability compared to a sugar-only control drink [(5)], this is not yet well-established. Thus, at this point it would appear that the main difference between diets in this acute one-day intervention was the flavonoid content.

Interesting Finding

Although the magnitude of effect in the tests was small, the effect of the berry intervention was to preserve, not necessarily improve cognitive function. But this is still an important finding, because the 6 h time period over which cognition was preserved in fact lends support to the potential mechanisms underpinning the observed effect.

There is little evidence that flavonoids cross the blood-brain barrier in significant quantities [(5)]. Their action is due to acting at low concentrations, and one factor in their persistent circulation is the factor that flavonoids pass to the large intestine and undergo bacterial degradation into phenolic metabolites, which are subsequently reabsorbed through enterohepatic circulation [(7)]. This prolonged colonic production and absorption provides one mechanism for the extended bioavailability* of phenolic metabolites up to 48-hours post-ingestion [(7)].

Significant acute improvements in memory with berry anthocyanins have been shown to occur at 1.5 h and 6 h post-ingestion [(8)], which corresponds to peak concentrations of anthocyanin bioavailability [(9)]. These peak bioavailability times and corresponding effects also correspond to increases in cerebrovascular blood flow, which is believed to be an important factor in the cognitive effects observed [(5)].

Thus, the acute cognitive benefits shown in the present study correspond with flavonoid pharmacokinetic activity from wider research.

*Geek Box: Bioavailability

Bioavailability is the amount of a compound that reaches circulation, generally expressed as a percentage of the total intake. In this regard, it is not quite the same as absorption, because a compound is only bioavailable after it has been not only absorbed, but passed through the liver. In nutrition, the bioavailability for macronutrients is generally very high, because there are specific transporters in the gut for fats, sugars, and amino acids. For micronutrients, it can differ, as often a number of minerals may share the same transporter, thus competing for absorption. In addition, homeostasis for minerals and trace elements is maintained at the level of the gut, which can upregulate or downregulate absorption based on levels in the body. The bioavailability of polyphenols was originally believed to be quite low overall, but also variable, with different compounds showing bioavailability of anywhere from 1 to 60%. However, it is now known that the bioactivity if polyphenols is from metabolites, ***not*** *the parent compounds, and metabolites may circulate in concentrations up to 100-fold greater to the parent compounds, and much greater bioavailability.*

Relevance

The associations between flavonoids and reduced risk of cognitive decline have been consistent. In the PAQUID cohort study in France, higher dietary flavonoid intake in subjects aged >65yrs was associated with better cognitive performance at baseline assessment, and reduced risk of dementia at 10-years follow-up [10]. In the Rotterdam Study, high flavonoid intake was associated with reduced risk for Alzheimer's Disease, while in the US Nurses Health Study, analysis in women >70yrs found that high anthocyanin intake was associated with 2.5-years delayed cognitive ageing [11]. These observations have suggested chronic effects; however early research found concentrations of the parent compounds peaked at 1.5 h post-ingestion, calling into question chronic health benefits. However, understanding the bioavailability of metabolites provides a better mechanistic explanation for both acute and chronic health benefits [7,9].

It is important to note that this study investigated two particular aspects of cognition: attention and motor control [i.e., reaction time]. It did not assess learning and memory, and it is particularly the latter which is of interest for the role of flavonoids in the prevention of dementia.

It is also important to consider that practically, both groups were scoring 96% accuracy on the MANT test at the 2 h Test mark; the effect of the berry intervention was to attenuate decline in performance compared to the placebo, but the difference was 2% overall, and 3.5% when comparing the higher difficulty test level. In the reaction time test, the difference in response time in the berry intervention group from baseline to 6 h was 0.015 seconds. So, we are ultimately talking about fractional effects. However, this is science: there is ***an effect***. And the million-dollar question thus becomes: what would be the cumulative effect of a small effect over 10, 20, 30, 40 years? This is the nature of diet-health outcomes: exposure is always a function of dose x duration.

Application to Practice

This study is acute, and the effects are small, but suggest potential ability to preserve cognition over the course of the day in response to demanding task. However, a study like this is not cause to down a 300g berry smoothie every morning [unless you want to, of course]. Best practice advice in nutrition includes fruit consumption, which is a ubiquitous characteristic of healthy diet patterns. However, a case is building that advice within the broad categorisation of 'fruit' could make specific reference to berries. It doesn't appear at this point to matter, although most research is in blueberries, but the take-home is this: if it is dark purple-to-red, and a berry, regular consumption may be beneficial over time.

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