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Kaplan A, Zelicha H, Yaskolka Meir A, et al. The effect of a high-polyphenol Mediterranean diet (Green-MED) combined with physical activity on age-related brain atrophy: the Dietary Intervention Randomized Controlled Trial Polyphenols Unprocessed Study (DIRECT PLUS). *American Journal of Clinical Nutrition*. 2022;115(5):1270-1281.

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

Given the paucity of available pharmacotherapy for neurodegenerative disease [1], the emphasis for interventions falls to non-pharmacological interventions, of which numerous modifiable risk factors have been identified: smoking, high cholesterol, hypertension physical inactivity, depression, and diet [2].

Of these factors, diet – predictably – falls under the *"more evidence needed"* qualifier [2], and this is predictable insofar as it reflects common methodological challenges for nutrition research: apparent inconsistency between epidemiology and intervention studies, challenges for intervention trials in terms of relevant treatment, dose, duration, etc., and relevant outcome measures that provide biological plausibility across lines of evidence.

At Alinea Nutrition, we love our (poly)phenols. And the nutrition world generally loves the Mediterranean diet, which happens to be rich in said (poly)phenols. We also know that many of the intervention trials investigating the effects of (poly)phenol compounds have been conducted over 12-weeks [3–5], while some of the berry anthocyanin interventions have tested cognition acutely over a 6-hour period [6,7] [see this Deepdive].

In a prior Deepdive we looked at the EPIC-Spain Dementia Cohort Study, which showed a 20% lower risk of dementia with higher Mediterranean diet scores. However, longer-term intervention trials of a Mediterranean diet and brain outcomes are rare; the present study is one such trial.

The Study

The DIRECT-PLUS trial was conducted in Israel on the effects of a low-carbohydrate Mediterranean diet enriched with (poly)phenols over 18-months in adults over 30yrs of age with metabolic syndrome. The primary outcomes of the study were changes in body composition, however, secondary outcomes included changes in brain anatomy and cognitive testing between baseline and the end of the 18-month intervention.

Participants were randomised to one of three groups; one active control group and two interventions:

- **Active Control aka 'HDG'**: Healthy Dietary Guidelines
- **Med Diet + Walnuts aka 'MED'**: Med diet with 28g/d walnuts [total of ~440mg/d (poly) phenols added]
- **Med Diet + Greens aka 'Green-MED'**: Med diet with the addition of 3–4 cups per day green tea and 100g of "duckweed", aka, Mankai, to be consumed in the evening replacing animal meat sources at dinner [total of ~1240mg/d (poly)phenols added]

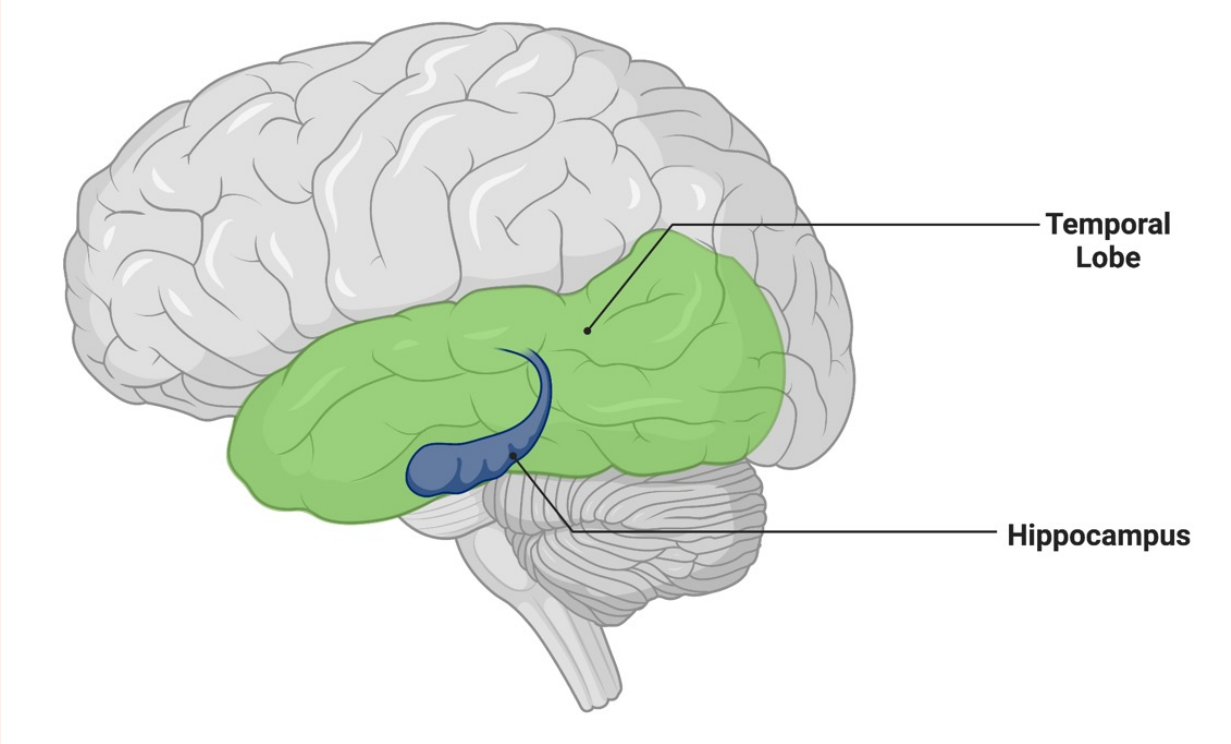
Both med diets were energy-restricted [1500–1800kcal/d men; 1200–1500kcal/d women] and carbohydrate-restricted [40g/d for 2-months then 80g/d thereafter].

All participants were counselled to target 3–4 exercise sessions per week of mixed aerobic and resistance training, and provided with a free gym membership. All participants underwent group sessions with a multidisciplinary team of dietitians, doctors, and fitness trainers, with a weekly frequency for the first month and then monthly frequency thereafter.

The main outcome was change in hippocampal occupancy score* [HOC], while changes in executive function were also investigated.

*Geek Box: Hippocampal Occupancy Score [HOC]

It is helpful for understanding the present study to have some grasp of what the main outcome measure was, and what it represents. The hippocampus is a key brain region for higher cognition, particularly learning and memory. It is located deep in the temporal lobe, one of the four major brain lobes, that is responsible for encoding sensory inputs, verbal reasoning, and visuo-spatial reasoning.



Atrophy of the hippocampus is one of the early stages associated with mild cognitive impairment [MCI], which itself is the beginning of neurodegenerative decline on the road to dementia and Alzheimer's Disease.

The HOC is an estimate of the atrophy in medial temporal lobes, and is calculated as the ratio of hippocampal volume to the sum of hippocampal and inferior lateral ventricle volume [LVV]. This is calculated for both left and right brain hemispheres, and expressed as an average for both hemispheres.

Thus, a reduction in HOC indicates brain atrophy in this region, while an expansion in LVV also represents loss of brain tissue in this region. Hippocampal volume is expressed in cubic centimetres [cm3]. One benefit of using HOC is that it can be standardised according to age and sex, because the average hippocampal volume at a given age, and between men and women, is known.

From the ages of 67 and 63 years in women and men, respectively, there is a decrease in hippocampal volume that exceeds the rate of loss of other brain grey matter [the major tissue component of the central nervous system]. This indicates that the hippocampus in particular may be vulnerable to age-related decline, and associated cognitive decline.

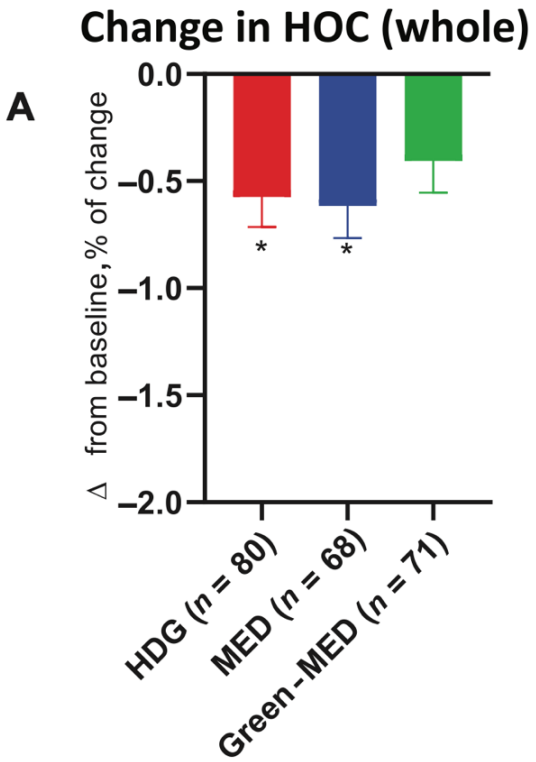
Results: 284 participants were randomised, of which 224 [79%] completed the intervention providing brain scans at both baseline and the end of the intervention. The number of dropouts was equal between groups. The average age of participants was 51yrs, with a range of 31yrs to 82yrs.

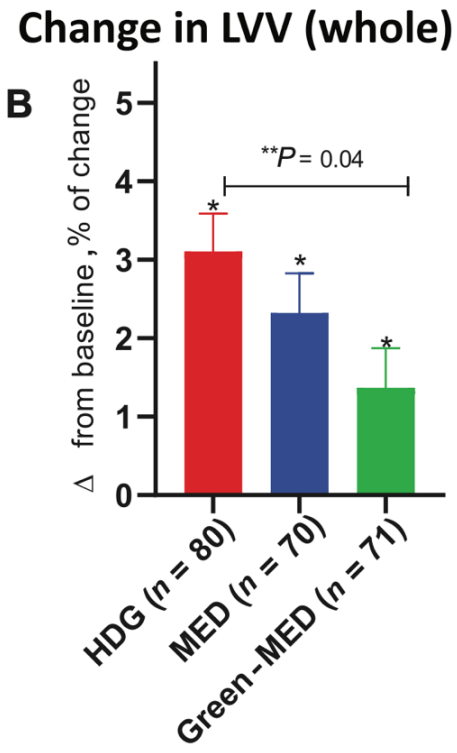
At baseline, age itself with correlated strongly with lower HOC, which was most evident in participants >50yrs. Several other risk factors were associated with HOC. Specifically, having lower waist circumference and lower systolic and diastolic blood pressure were associated with higher HOC, while higher blood total and LDL-cholesterol were associated with lower HOC.

Physical activity, measured using Metabolic Equivalents of Task [MET], increased by 5.2 units per week [light-moderate intensity, depending on baseline fitness].

- ***Effect of Age on HOC*:** Overall, 66% of all 244 participants who completed the intervention showed a decline in HOC after 18-months by 1.7%. However, in participants >50yrs of age the decline in HOC was 1%, while in those <50yrs it was 0.06%.
- ***Effect of Dietary Interventions on HOC*:** Without stratifying participants by age, in the total study group there was no significant effect of the dietary interventions on attenuating decline in HOC, albeit the magnitude of effect was lesser in the Green-MED group [**Figure A**, **left**, below].

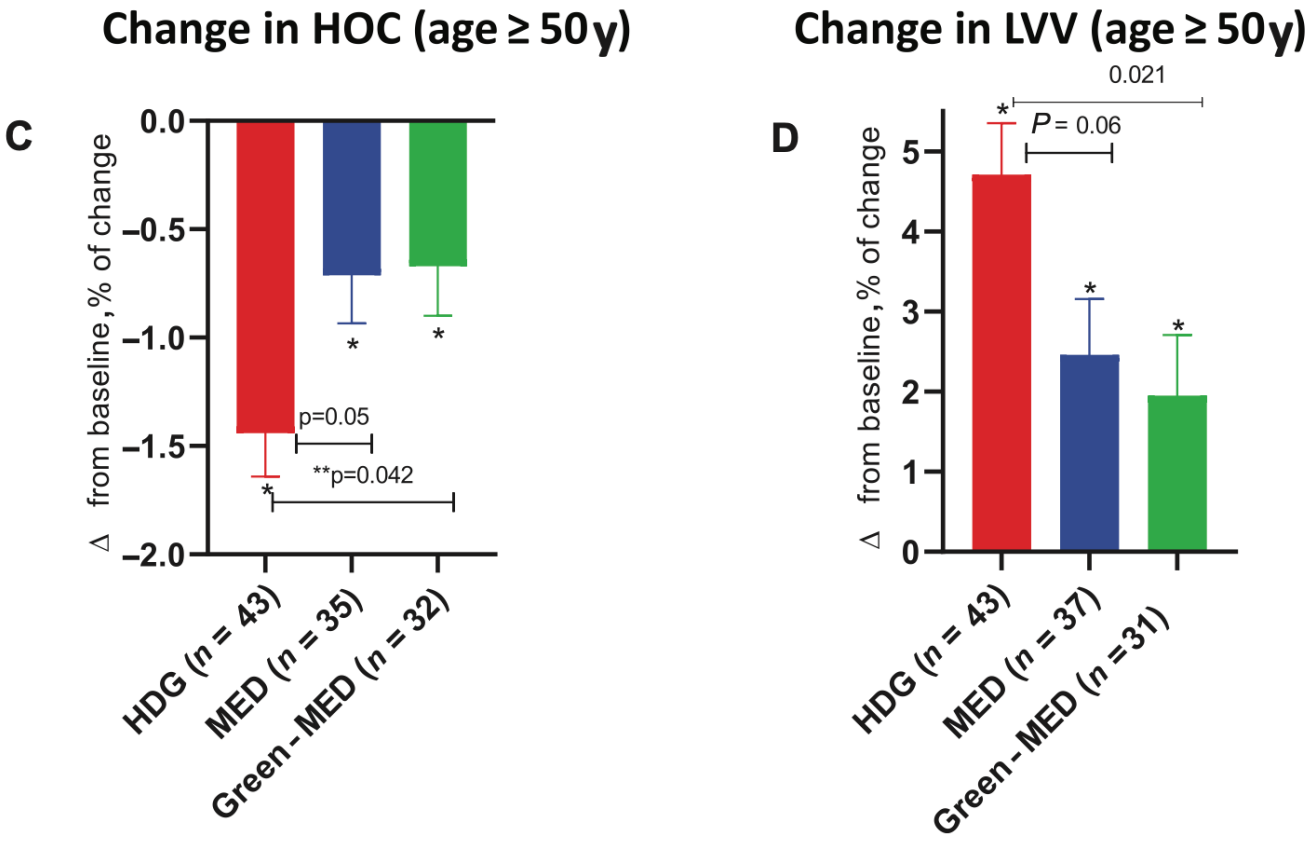
However, there was a significant difference in LVV, with an increase [remember: an increase in LVV represents brain atrophy] of 3.1% in the HDG group compared to 1.2% in the Green-MED group [**Figure B**, **right**, below].





However, when stratifying participants by age, in those >50yrs there was significantly less decline in HOC in both intervention groups [0.8% decline in both groups] compared to a 1.3% in the HDG diet group [**Figure C**, **left**, below].

LVV expansion was significantly attenuated by both intervention diets. Compared to the LVV expansion of 4.3% in the HDG, the MED and Green-MED diets resulted in 2.7% and 2.3% LVV expansion, respectively [**Figure D**, **right**, below].



- ***Effects of Cardio-metabolic Variables*:** In participants >50yrs, weight loss, lower triglyceride levels, and lower insulin resistance [HOMA-IR] all correlated with a lower decline in HOC. Including all these factors together, only HOMA-IR was independently associated with attenuated HOC decline.

The Critical Breakdown

Pros: The study is rare example of an intervention trial conducted over 1yr with brain scans before and after the intervention to sensitively quantify changes in the brain. The trial was pre-registered and there are no apparent deviations from the protocol. The research question and hypothesis were clearly stated. Randomisation method was appropriate [computer-generated] and was stratified by sex. Participants were well-matched at baseline. The study was conducted at an isolated centre, allowing for intensive delivery of the intervention, which was multidisciplinary. Importantly, each group was given the same intensity of intervention with the sessions. The study utilised urinary measures of (poly)phenol metabolites as a biomarker of intake [more under ***Key Characteristic***, below].

Cons: It is important to recall that these outcomes were secondary outcomes to the main trial. The power calculation was based off data on physical activity, although that likely reflects a lack of dietary data on the outcome, but the main outcome of hippocampal volume may have been underpowered for the two Med diet groups. Bizarrely for having physical activity in the title and intervention design, there is scant mention of it in the results, or the paper at all. Indeed, it does not appear to have been included as a covariate in the analysis of diet on HOC, so we cannot conclude that the outcome is independent of any effect of physical activity. No data is given on estimates of daily walnut intake, but is given for green tea and Mankai; while green tea showed compliance of an average of 3 cups per day, the average intake of Mankai shakes was 3 *per week*, indicating some compliance issues given it was intended to be daily. Maybe its hideous to drink, who knows. Also, let's be honest: this isn't *really* a Mediterranean diet; it's a low-carb diet with some Mediterranean foods washed down with Japanese tea and some obscure Thai aquatic plant. No biggie, I love both my green tea and Thai food, but if ever there was an example of "the Mediterranean diet is whatever you want it to be" in nutrition, this is it.

Key Characteristic

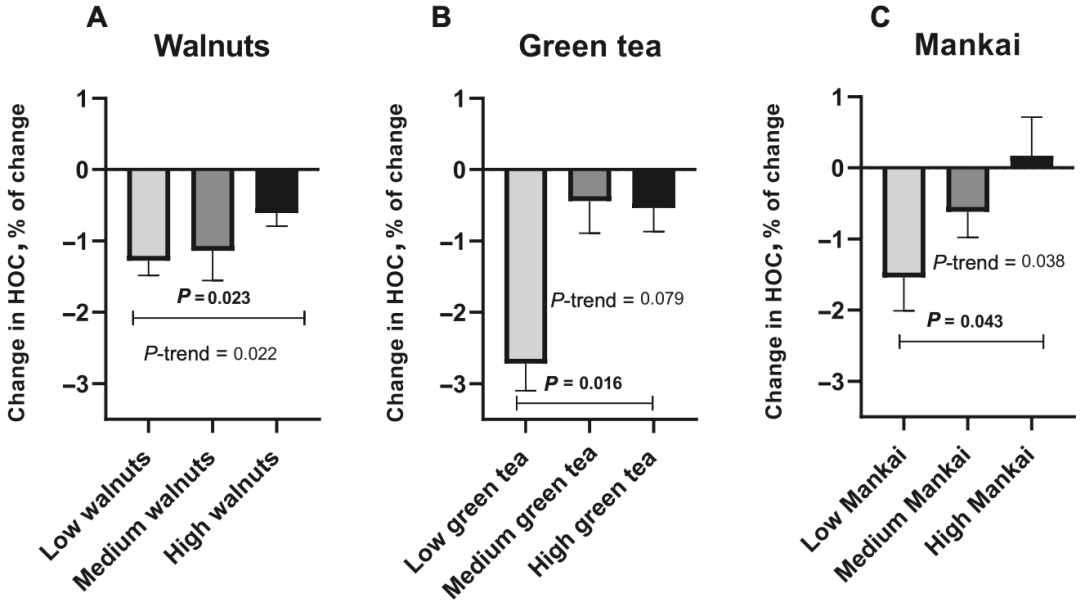
The assessment of (poly)phenols using urinary biomarkers is crucial. One characteristic of (poly)phenols is the fact that they undergo extensive metabolism by the gut microbiota, producing metabolites that are absorbed and exert biological activity [(8)]. This is considered crucial to their mechanism of action and allows for correlations between biomarkers and outcomes to be assessed. In the present study, increased urinary (poly)phenol biomarkers corroborated the adherence to the intervention, although this did differ relative to the food/beverage [i.e., 3/d cups green tea intake was achieved, but 3/week servings of the mankai smoothie].

More interestingly, however, two urinary (poly)phenol biomarkers – urolithin A and tyrosol – were both associated with significantly less decline in HOC in all participants, including those >50yrs of age. Thus, measuring urinary biomarkers allows for a stronger conclusion to be derived on the effects of the intervention foods, by linking effects the (poly)phenols between biomarkers and foods. For example, urolithin A is produced by gut bacteria in response to ellagic acid intake, which is found in high quantities in walnuts [(9)]. However, it appears around 40% of people possess the necessary microbiota to produce urolithin A, so there may be some regional factors in terms of background diet and/or genetics that may be relevant to this finding, but the exact species responsible for production of urolithins remain to be discovered [(9)].

Interesting Finding

The study also investigated the associations of the specific dietary components that comprised the additional (poly)phenol intake in the intervention groups, specifically in the participants >50yrs of age. Each food – walnuts, green tea, and mankai – was stratified into tertiles according to levels of achieved intake. For walnuts, this corresponded to 28g servings per week of <2, 3–4, or >5. For green tea, cups per day was <1, 2, or >3. And for mankai, smoothies per week was <3, 4–6, or 7 [i.e., daily].

This analysis provided some interesting insights, as you can see from the **figure** from the paper, below. For example, walnuts only attenuated the decline in HOC with the highest of >5 servings per week. For green tea, it also appears that 2 cups per day was the minimum effective dose, and there was no additional benefit to higher daily intakes. And for mankai, we can see that that in participants >50yrs who achieved the highest intake with the target of a daily 100g intake with dinne, there were no changes in HOC.



Findings like this open up questions regarding the (poly)phenol content of the respective foods, and the relevance of 'dose'. For example, a separate analysis which investigated the (poly)phenol content of mankai showed that the plant contains over 200 (poly)phenol compounds, mostly flavonoids [10] Participants in the Green-MED group exhibited significantly elevated urinary levels of a compound called naringenin, which belongs to the flavanone subclass of flavonoids [11].

Previous research on the cognitive effects of citrus fruits, rich in flavanones, has attributed potential benefits to naringenin, particularly due to its anti-inflammatory effects and influence on lower nitric oxide levels in the brain [11,12]. Importantly, these effects have been shown at levels that are physiologically relevant for dietary intake [11,12].

Of course, this calls into question why this "Mediterranean diet" didn't use oranges or orange juice, and we have no qualitative assessment of what participants thought of the mankai smoothie. Nevertheless, this appears to be the first study to specifically use this newly discovered, (poly)phenol rich food, and it appears to have – with daily intake – exerted significant benefits on the brain.

Relevance

As we highlighted at the start, the evidence-base for dietary interventions and brain health outcomes from intervention trials is often short-term or even acute one-day studies. These studies have provided some degree of biological plausibility to explaining the beneficial effects of (poly)phenols observed in prospective cohort studies, in demonstrating effects on various aspects of cognitive function.

The main limitation of the present study is that the investigation of brain-related outcomes were secondary endpoints to the trials design and protocol. Nevertheless, as an 18-month trial with brain scans to determine changes in a highly relevant and predictive marker of cognitive decline in HOC [13], the findings are encouraging. Longer-term interventions designed with cognitive outcomes as primary endpoints are rare.

One such recently published study investigated the effects of eating 15% of energy from walnuts over 2-years, and overall found no effect in healthy older adults between two research sites in Loma Linda, California, and Barcelona, Spain [14]. However, subgroup analysis indicated a significant benefit in participants in Barcelona, who had a higher risk profile at baseline [14].

In my opinion studies like that, which aim to have that level of proportion of daily intake from a single food, are a waste of time, resources, and funding. Why hedge your bets on walnuts, specifically, when the diversity of (poly)phenols, and their related diversity in mechanisms of action, is the very reason they may be "lifespan essential"? As we covered in a previous Deepdive, anthocyanins appear to demonstrate a greater magnitude of benefit in lower dementia risk compared to other flavonoid subclasses, although they all exert benefit. Bear in mind that at 40-80g/d carbs, the present study possibly tied its hands behind its back with achieving high (poly)phenol intakes.

It is important to note that other interventions have also investigated brain atrophy as outcomes, not just cognitive testing. For example, the VITACOG trial, which investigated a combination vitamin B6/B9/B12 supplement in elderly adults with mild cognitive impairment, showed significantly lower rate of brain atrophy through the homocysteine-lowering effects of the B-vitamin intervention [15]. In an example of why the "15% walnuts" is a bugbear, this effect in VITACOG was mediated by higher levels of the omega-3 fatty acids EPA+DHA at baseline [16]. Interaction effects in nutrition matter.

Overall, however, one would think there are more encouraging signals than not for the effects of (poly)phenols on the brain, and more longer-term interventions will help to corroborate that interpretation.

Application to Practice

If you'd like to follow more of a Mediterranean diet, go ahead. And if you like green tea, as I do, fire away with your 2-3 cups a day. And you feel like trying the ol' mankai out, by all means have at it. Just remember how broad a category (poly)phenols are, and the diversity of pigmentation that denotes flavonoid subclasses also corresponds to the diversity of metabolites in circulation. Based on current evidence, it is difficult to justify emphasising single foods the way the present study did. What about citrus flavanones? Cocoa flavonols? Berry anthocyanins? Eat the lot.

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