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

When it comes to the broad category of bioactive plant food components that are *not* nutrients, we may be tempted to think of these so-called 'phytochemicals' as a relative recent. However, although our ability to characterise and analyse these compounds has certainly become more precise, our knowledge of their potential importance in the diet is not new.

So here is your fun nutrition history fact for the day: in the 25-year follow-up of the infamous Seven Countries Study [SCS], there were three variables that were independently associated with rates of coronary heart disease [CHD] mortality, namely saturated fat, smoking, and… *flavonoids* [1]. Yep, flavonoids. And neither vitamin E, vitamin C, or beta-carotene, were associated with CHD in the SCS.

Flavonoids had in fact been discovered in the 1930' s, however, their early interest was based on an assumption that their potential health benefits were due to their action as antioxidants [2]. Pay attention to the wording in the 25yr SCS follow-up and you' ll see they refer to them as *antioxidant flavonoids* [1]. The thinking was that you *ate* antioxidants, and those antioxidants present in foods did good things.

This view of flavonoids would come to define their assumed role in the diet until very recently, when it became clear that their action is much more complex [3,4]. In fact, it isn' t the antioxidant levels of the foods that matter; flavonoids mainly act through different signalling pathways that regulate processes like vascular function and inflammation [2].

Most of the previous Deepdives we have covered in relation to flavonoids have been investigating neurodegenerative diseases or cognitive function as outcomes: like this; and this; and this; and this. Today we take a dig into the potential cardiovascular benefits of flavonoids.

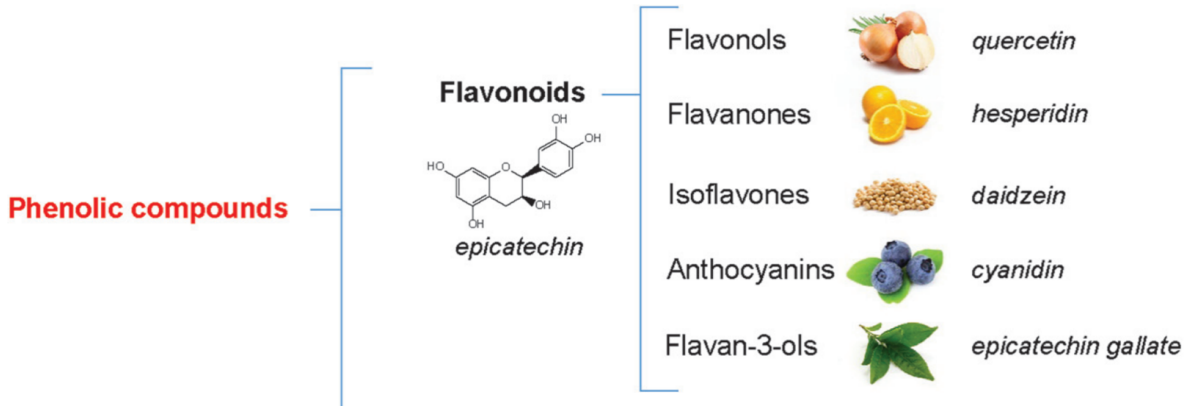


Figure *from Fraga et al.* [5] *illustrating the structural family tree of flavonoids. Flavonoids belong to a group of compounds known as* ***(poly)phenols****, which are compounds found in plants that have a particular chemical structure, known as a phenolic ring. A compound with multiple phenolic rings is called a 'polyphenol'. So, flavonoids are polyphenols, and flavonoids are the major source of polyphenols in the human diet. As you can see from the figure, flavonoids are the parent term for several subclasses, each structurally different to the other. The six major subclasses are anthocyanins, flavones, flavonols, flavan-3-ols, isoflavones, and flavanones. These compounds also contain their own specific compounds, for example quercetin, which is often studied in isolation, is a flavonol. There are >9,000 flavonoids!*

*Geek Box: Cardiovascular Effects of Flavonoids

In previous Deepdives where we have focused on flavonoids and the brain, we have discussed three main mechanisms: stimulation of pathways associated with learning and memory; resolving inflammation, and improving cerebrovascular blood flow. But in nutrition research there are strong common themes between what is good for the head and good for the heart. And so it is with flavonoids, as it turns out that the effects of flavonoids on vascular function and inflammation are also crucial to the cardiovascular effects of these compounds. Let's start with vascular function, and endothelial function in particular. The endothelium lines the insides of blood vessels, and endothelial cells release various signals to regulate blood vessel constriction/dilation, blood clotting, and immune responses. Flavonoids act by increasing expression of an enzyme called endothelial nitric oxide synthase [eNOS], which enhances vascular dilation and therefore lowers blood pressure. Flavonoids also inhibit inflammatory pathways that influence endothelial inflammation and immune responses. One notable effect of flavonoids in relation to the inflammatory-immune effects on the vascular system is to inhibit the adhesion of monocytes, a type of white blood cell, to the endothelium during inflammatory and immune responses. There is also some evidence that flavonoids may lower the susceptibility of LDL to oxidation, which is a critical step in the development of plaque in the arteries. However, in terms of strength of evidence it would be the vascular effects of flavonoids that are, at this point, likely to explain in part the cardiovascular benefits associated with these compounds, observed both in epidemiology and intervention studies.

The Study

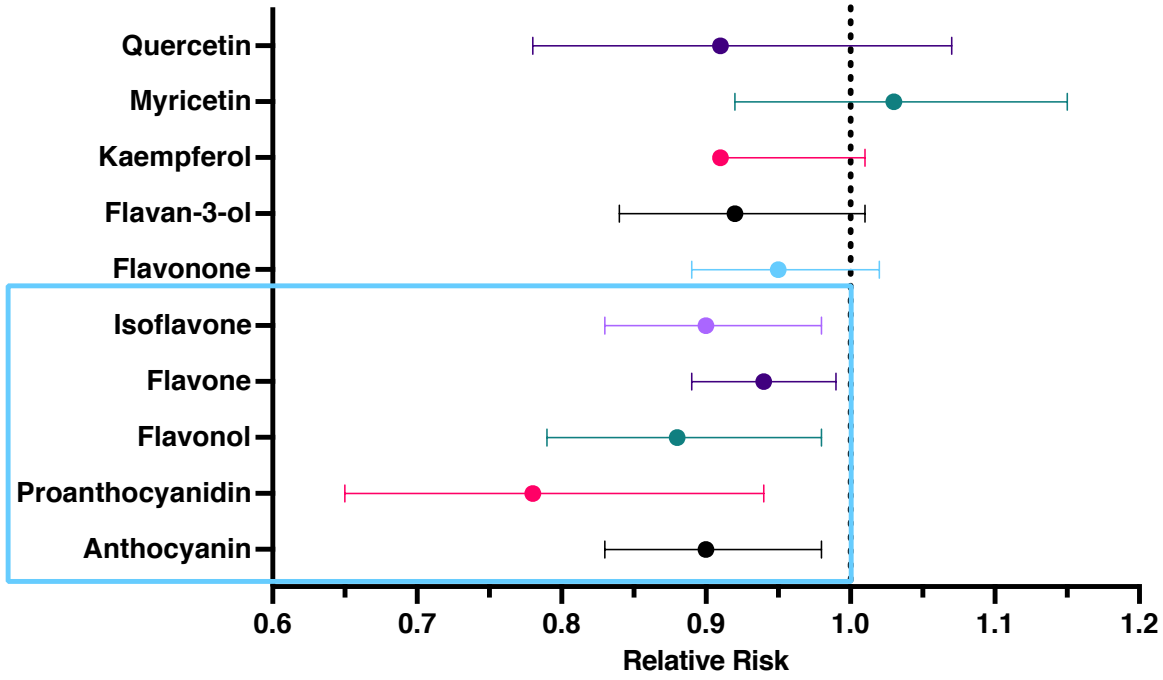
The study was a meta-analysis of observational research which have investigated associations between flavonoids and CHD risk. To be included, a study needed to meet the following criteria:

- Prospective cohort design;
- Assessed flavonoid subclasses as the exposure of interest;
- Assessed CHD incidence as the outcome of interest;
- Reported relative risks [RR] and accompanying 95% confidence intervals [CI].

The search for included studies encompassed a wider range of flavonoid subclasses, specifically anthocyanins, proanthocyanidins, flavones, flavonols, flavan-3-ols, isoflavones, flavanones, quercetin, kaempferol and myricetin. The included studies compared highest to lowest levels of intake of these flavonoids, so the findings of the meta-analysis reflect a high vs. low comparison.

Results: 19 prospective cohort studies were included in the final analysis, totalling 894,471 participants and 34,707 CHD events. The number of studies on each specific flavonoid subclass is included next to the subclass, below. The results laid out here are only those which were statistically significant.

- ***Anthocyanins*:** Based on 8 studies, there was a 10% lower (RR 0.90, 95% CI 0.83 – 0.98) risk of CHD events. Each 50mg/d increase in anthocyanins was associated with a 5% (1–8%) lower CHD risk.
- ***Proanthocyanidin*:** Based on 4 studies, there was a 22% lower (RR 0.78, 95% CI 0.65 – 0.94) risk of CHD events. Each 100mg/d increase in proanthoycanidins was associated with a 5% (1–7%) lower CHD risk.
- ***Flavonol*:** Based on 8 studies, there was a 12% lower (RR 0.88, 95% CI 0.79 – 0.98) risk of CHD events. Each 25mg/d increase in flavonols was associated with a 5% (3–5%) lower CHD risk.
- ***Flavone*:** Based on 7 studies, there was a 6% lower (RR 0.94, 95% CI 0.89 – 0.99) risk of CHD events. Each 5mg/d increase in flavones was associated with a 5% (3–7%) lower CHD risk.
- ***Isoflavone*:** Based on 7 studies, there was a 10% lower (RR 0.90, 95% CI 0.83 – 0.98) risk of CHD events. Each 0.5mg/d increase in isoflavones was associated with a 5% (3–6%) lower CHD risk.



Graph *of the meta-analysis results for each flavonoid subclass investigated in the present study. Recall that the 'null' for calculating relative risks [or hazard ratios, etc.] is always 1.0; anything higher than that indicates an increase in risk, while lower indicates a reduction in risk. The statistically significant findings are highlighted in the blue rectangle, and each of those flavonoid subclasses was associated with a reduction in risk for CHD events. The circle represents the point estimate; the arms either side represent the 95% CI. While proanthocyanidins exhibited the greatest risk reduction, you can see the confidence intervals are quite wide, so it is not a more imprecise finding. You can also see that for the other significant findings, the point estimates are ~0.9 higher and the upper bound of the confidence intervals for the other significant findings are quite close to the 'null' – the 1.0 line. This means we want to have some caution as the effect size is modest.*

The Critical Breakdown

Pros: The exposure and outcomes were clearly defined, and the study was a first in deliberately assessing specific flavonoid subclasses as the exposure of interest. The study had a large sample size overall and large number of CHD events, and most flavonoid subclasses had a minimum of 6 studies on each. Relevant databases were searched, up to March 2021. Several subgroup analyses were conducted based on age, region of the study, and duration of follow-up. The Newcastle-Ottawa Scale was used to assess the quality of the included studies.

Cons: Of the 19 included studies, only 2 were outside North America and Europe [and 1 of those was in Australia]. For compounds like isoflavones, which are primarily found in soy and generally consumed in higher amounts in Asian populations, more data from Asian cohorts would be useful in future. The major limitation of this study is not necessarily it' s fault [more under Key Characteristic, below], but is the fact that there are some potential holes still in assessing flavonoid intake in epidemiology. One concern with the present study would be independence of effects of flavonoids; 7/19 studies did not adjust for total energy intake [a big no-no in nutritional epidemiology, because less/more energy = less/more nutrients]; 8/19 did adjust for total energy, but not fruit and vegetable intake [i.e., other compounds in veg/fruit may be at play!]. Annoyingly, it doesn' t present detail about what was 'high' or 'low' flavonoid intakes in the included studies.

Key Characteristic

With any epidemiological research in nutrition, well-conducted dietary assessment is the most important aspect of the study. And one caveat we must bear in mind is that the quality of assessing flavonoids in the diet is still highly variable in observational research [(6)]. This is particularly relevant given food sources of flavonoids may be influenced by seasonality, and region of the world may be an important variable [(7)]. Bear in mind that 18/19 studies in the present meta-analysis were from North America, Europe, and Australia; yet an analysis showed that in these populations, estimates of flavonoid intake ranged from 209mg/d to 1017mg/d [(6)]. This is a limitation of the present study in not presenting what the levels of intake were in the studies included in the meta-analysis.

13/19 studies assessed flavonoid subclass intake using a food-frequency questionnaire [FFQ], and this is generally a positive because FFQ capture average intake over time, while other measures like 24 h recalls only capture a snapshot of diet [(8)]. However, most FFQ in older cohort studies were not validated to assess flavonoids, and most analyses of flavonoid subclass intake are based on retrospectively calculating estimates of flavonoid intake from the FFQ used in that particular study [(7)].

Efforts continue to be made to update relevant food databases with more refined detail regarding the flavonoid content of foods, and researchers are moving to make dietary assessment methods like FFQ or 24 h recalls validated for flavonoids [(6)]. However, it is a caveat to note to date. As we know that the effect of measurement error in nutritional epidemiology is generally to underestimate intakes, it is possible that the "true" effect of flavonoid subclasses may actually be stronger than the modest effects found in the present study.

Interesting Finding

It is always important to reconcile potentially divergent findings between epidemiological research and evidence from intervention studies. And one finding from the present study which stands out in this regard is that the associations between the flavon-3-ol subclass and CHD. Let' s start with the actual finding; an estimated 8% [RR 0.92] lower risk and a 95% CI of 0.84 – 1.02, i.e., although it was not statistically significant, the direction of effect was toward a lower CHD risk [< 1.0].

Why is this interesting? Well, because the best available current evidence for cardiovascular health and flavonoids from intervention studies is for the flavon-3-ol subclass, which are abundant in foods like cacao [9]. In a meta-analysis of 42 randomised controlled trials, cocoa flavan-3-ols were shown to significantly improve vascular function and lower blood pressure, effects which were noted both in acute studies and in longer-term trials up to 18-weeks [10].

Again, given the good evidence from intervention studies, and the challenges with more robust estimates of specific flavonoid subclasses in epidemiology, I would be inclined to think the direction of effect in the present study is one, echoing the ***Key Characteristic***, above, that could have a stronger "true" effect elucidated with more validated dietary assessment of flavonoid subclass intakes.

Relevance

The primary relevance of this study is its claim to being the first meta-analysis of specific flavonoid subclasses on CHD risk. As noted under ***What We Know***, above, the associations between flavonoids and CHD are not new, and go right back to the SCS. But as we also noted, those findings occurred at a time when flavonoids were believed to act as dietary antioxidants, which we now know is not their main mechanism of action [2,4,9].

What is interesting is that each of the flavonoid subclasses associated with a significant reduction in CHD risk in the present study have experimental evidence supporting a particular cardiovascular benefit; anthocyanins have been shown to decrease monocyte adhesion, flavonols and isoflavones to induce eNOS and vasodilation [4,9]. There is also the body of human intervention studies, which shows effects on intermediate risk factors like blood pressure, while also demonstrating some of the more specific vascular effects like platelet aggregation [adhesion of blood clotting cells] [4,9].

The present study illustrates several themes for how we think about evidence in nutrition. On the one hand, this is a synthesis of epidemiological research with modest effect sizes, and it is easy to hand-wave off the findings. On the other hand, those findings are congruent with both experimental evidence on potential underlying mechanisms *and* human interventions with risk factors as outcomes. This is an example of the type of research critics of nutrition science like to dismiss [epidemiology] without acknowledging that it is yet another example of where different lines of evidence are congruent with each other, and converge to the same conclusion.

Thus, while the limitations of the present study should not be ignored, neither should its consistency with the wider evidence for the effects of flavonoids overall, and specific flavonoid subclasses, on cardiovascular health.

Application to Practice

The most basic, incontrovertible fact in nutrition is that vegetables and fruits exert health benefits. Only an outlier motley crew of imbeciles deny this fact. The *why* of veg and fruit has traditionally been their micronutrient contributions to diet. But our understanding of non-nutritive phytochemicals is much further along in 2022, and the evidence-based for flavonoids is expanding.

Generic advice for consuming veg/fruit is often based on non-specific platitudes like *"eat the rainbow"*, which assumes that anyone can remember the colours of said rainbow and/or will not purchase Skittles instead. It was the late Professor Martha Clare Morris whom I first saw make a case for a more specific approach to recommendations for veg/fruit based on the compounds reflected in their pigmentation; dark-green leafy veg, red-purple pigmented berries, cherries, high-cacao dark chocolate.

I'm inclined to agree that, where possible, the benefits of flavonoids generally, and specific subclasses like (pro)anthocyanins, flavanones, and isoflavones, are something worth thinking about in the shopping aisle. Of note, many of the food-based interventions often use doses that are replicable in diet: 20-40g dark chocolate, ~150g berries, ~300ml orange juice. A positive of the flavonoid research, unlike some other areas of nutrition science, is exactly that: it can be implemented in the daily diet.

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