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NUTRITION

OCTOBER 2022

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Sánchez Macarro M, Martínez Rodríguez JP, Bernal Morell E, Pérez-Piñero S, Victoria-Montesinos D, García-Muñoz AM, Cánovas García F, Castillo Sánchez J, López-Román FJ. Effect of a Combination of Citrus Flavones and Flavanones and Olive Polyphenols for the Reduction of Cardiovascular Disease Risk: An Exploratory Randomized, Double-Blind, Placebo-Controlled Study in Healthy Subjects. Nutrients. 2020 May 19;12(5):1475.

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

The potential for (poly)phenol compounds to be beneficial for cardiovascular health [see ***Geek Box**, below, for further detail] goes all the way back to the Seven Countries Study, in which flavonoids were independently associated with lower risk of coronary heart disease over 25-years [(1)].

(Poly)phenols are a family of compounds with diverse chemical structures, and flavonoids constitute the major source of (poly)phenols in the human diet, primarily from fruits and fruit juices, teas, red wine, and vegetables [(2)]. As you can see from the **figure** below, flavonoids may have multiple different configurations, each identified as a different subclass within the family of flavonoids [(3)].

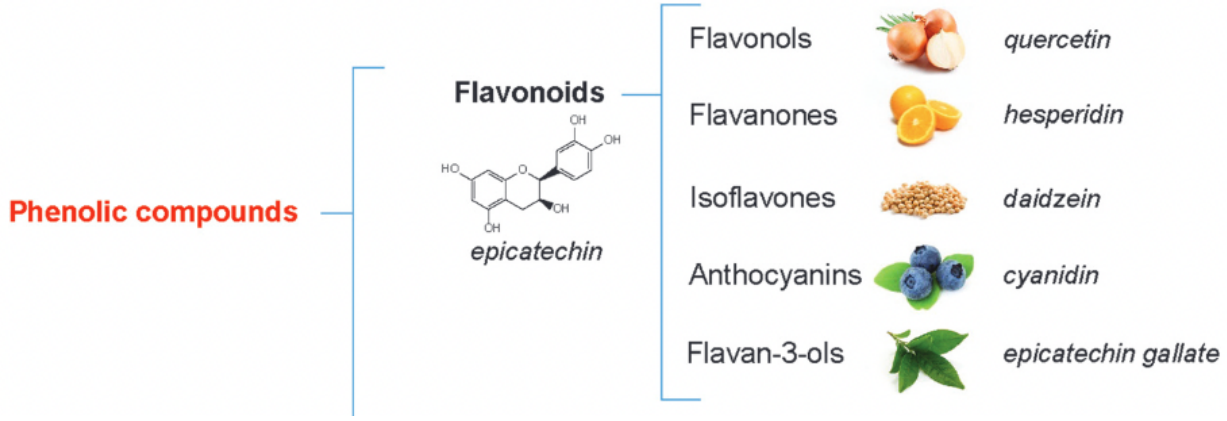

***Figure** from Fraga et al. [(3)] illustrating the structural family tree of flavonoids. Flavonoids belong to a group of compounds known as (poly)phenols, which are compounds found in plants that have a particular chemical structure, known as a phenolic ring. A compound with a single ring is a "phenol"; multiple phenolic rings is called a 'polyphenol'. Flavonoids are (poly)phenols, and constitute the major source of these compounds 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!*

While our mechanistic understanding of the bioavailability and bioactivity of these compounds continues to develop [3,4], human intervention trials are also demonstrating improvements in cardiovascular disease [CVD] risk factors [3,5,6]. In a previous Deepdive, we covered the COSMOS trial, which showed a 10% lower risk of all CVD events and 27% lower risk of CVD death from a cocoa flavanol supplement.

Thus, there may be some promise for flavonoids and CVD risk. But what of the potential for other flavonoid compounds? The present study investigated the effects of a supplement containing citrus flavanones and flavones, and olive oil (poly)phenols.

*Geek Box: Effects of Flavonoids on the Cardiovascular System

In previous Deepdives where we have focused on flavonoids and the brain, we have discussed three main mechanisms of action identified for flavonoids: 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 present study was conducted using a randomized, parallel, double-blind, placebo-controlled design. The intervention group were given a mixed (poly)phenol supplement derived from combinations of grapefruit extracts, bitter orange immature fruits extracts, and olive leaf extracts. The placebo consisted of maltodextrin [a sugar]. The supplement consisted of the following:

- 250–300 mg of citrus flavanones
- 175–200 mg of citrus flavones
- 85–90 mg of olive polyphenols

The duration of the study was 8-weeks; capsules were taken in the morning and again 12 h later in the evening. Measures were taken at baseline and again after 8-weeks, and changes after 8-weeks were compared to baseline in each group ("within-group differences"), and the magnitude of change after 8-weeks compared between groups ("between-group differences").

Compliance was assessed by counting returned supplement and placebo capsules from the respective containers provided to participants.

The primary outcome was flow-mediated vasodilation [FMD], as a measure of endothelial function [more under ***Key Characteristic***, below]. Secondary outcomes included blood cholesterol, blood pressure, oxidative stress biomarkers, and inflammatory biomarkers.

Results: Of 110 participants that were randomized [55 in each group], 96 completed the study. 51 participants completed the supplement intervention [27 male, 24 female; average age 47.3yrs], and 45 participants completed the placebo group [26 men, 19 women; average age 51.3yrs].

Primary Outcome – FMD

- **Supplement Before-After**: Increased from 6.73% to 9.77%.
- **Placebo Before-After**: Increased by 0.5%.
- **Between-Group Difference:** Compared to the placebo group, FMD differed significantly by 2.51% in the intervention group.

Secondary Outcome – Systolic Blood Pressure [SBP] and Diastolic Blood Pressure [DBP]

- **Supplement Before-After**: Decreased by 4.89mmHg and 2.49mmHg, for SBP and DBP, respectively.
- **Placebo Before-After**: SBP decreased by 1.98mmHg and DBP increased by 1.12mmHg.
- **Between-Group Difference**: Compared to the placebo group, SBP and DBP overall differed significantly by 2.91mmHg and 3.61mmHg, respectively, in the intervention group.

Secondary Outcome – Blood Cholesterol

- **Supplement Before-After**: Total and LDL-cholesterol decreased by 13.57mg/dL and 8.80mg/dL, respectively.
- **Placebo Before-After**: Total and LDL-cholesterol increased by 6.38mg/dL and 1.11mg/dL, respectively.
- **Between-Group Difference**: Compared to the placebo group, total and LDL-cholesterol differed significantly by 19.95mg/dL and 9.91mg/dL, respectively, in the intervention group.

Secondary Outcome – Inflammatory Markers

- **Supplement Before-After**: Interleukin-6 [IL-6] decreased by 0.57pg/mL.
- **Placebo Before-After**: IL-6 increased by 0.14pg/mL.
- **Between-Group Difference**: Compared to the placebo group, IL-6 overall differed significantly by 0.71pg/mL in the intervention group.

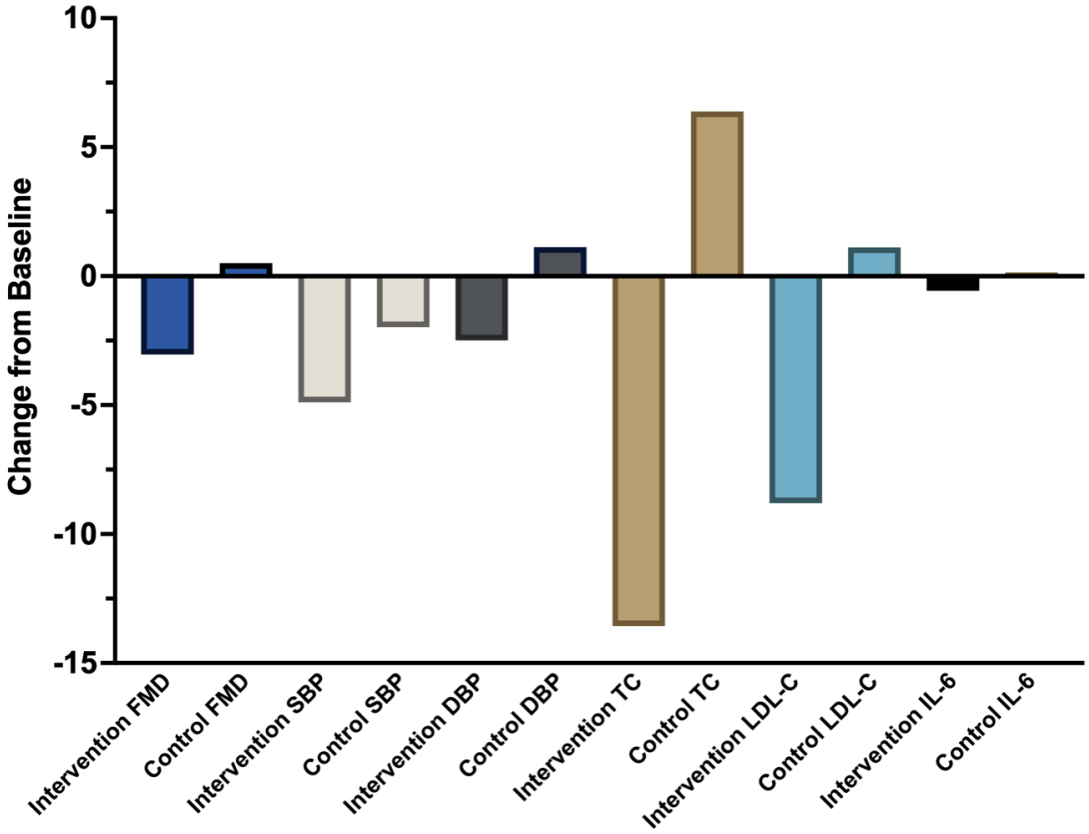

Figure *illustrating the change from baseline in the intervention and control groups, respectively, over 8-weeks. The magnitude of between-group differences are detailed in writing under the* ***Results*** *section, above. FMD = flow-mediated vasodilation; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; IL-6 = interleukin-6. It is important to note that in some cases, the magnitude of difference between groups was driven by decreases in a measure in the intervention group and concurrent increases in that measure in the control group.*

The Critical Breakdown

Pros: The study had a strong design as a randomized, parallel, double-blind, placebo-controlled trial. Randomisation was computer-generated and conducted by a researcher not involved in the study. The trial preregistered at ClinicalTrials.gov, and there do not appear to be any major deviations from the protocol as preregistered, although study was not actually registered until 2018 and the trial had begun in 2016, which could leave a few open questions. The study group was overall well balanced for sex, and the sample size was decent an exploratory trial of this type, albeit still small overall.

Cons: No power calculation appears to have been conducted, although the authors state the intervention was an exploratory trial, so this can be forgiven [i.e., the data from this trial can be used for a power calculation for future, larger studies]. With 14 drop-outs, and an imbalance of 51 to 45 participants between intervention and control groups, the statistical analysis should have used the intention-to-treat [ITT] method, where data from drop-outs is included in the analysis using their last data point from the study. Instead, the data was analysed using a "per-protocol" approach, which only includes the data of participants who completed the study according to which intervention they were originally allocated to, and may introduce bias. FMD shows variation over the course of the menstrual cycle [7], and cycle phase of the female participants in this study was not considered, potentially introducing some variability into the before-after results for the primary outcome. There was no assessment of diet, which also may have influenced the outcomes.

Key Characteristic

The key characteristic of the present study is the selection of FMD as the primary outcome, when this is often a secondary outcome in nutrition trials on CVD risk factors [which commonly focus on blood cholesterol and blood pressure as the main outcomes of interest]. So, what is FMD and why is it relevant?

FMD is a non-invasive method of assessing the reaction of the brachial artery to blood flow restriction; ultrasound is used to determine changes in the diameter of the brachial artery after a blood pressure cuff is inflated on the arm to restrict blood flow [8].

The ability of the brachial artery to vasodilate is dependent on the vascular endothelium releasing nitric oxide to expand the artery [8]. In this way, FMD represents endothelial function, and impaired endothelial function is a critical factor in the processes of atherosclerosis [8].

FMD is often represented as the percentage change in artery diameter, i.e., the greater the percentage change, the better, representing greater endothelial nitric oxide reactiveness. Such percentage changes correlate with meaningful reductions in CVD risk; meta-analyses indicate that for each 1% increase in brachial FMD, the risk of CVD events may be up to 12-13% lower [9,10].

There is, however, one caveat to bear in mind; the use of percentages for FMD has been questioned based on potentially overestimating effects in small arteries, such as the brachial artery [11]. Thus, the magnitude of change in FMD of 3.04% in the intervention group may contain some measure of overestimation.

Interesting Finding

Sticking with a theme here, but the significant improvements in endothelial function, as assessed by FMD, warrant further comment as the main interesting finding of the present study. This is where adding pieces of the evidential puzzle together, i.e., multiple converging lines of evidence, is always important.

On a mechanistic level, we have evidence that flavonoids increase expression of the enzyme, endothelial nitric oxide synthase [3,5]. And, as we covered in a previous Deepdive of longer-term cohort studies, flavanones and flavones were associated with modest reductions in heart disease risk.

If we home in on the citrus flavanones and flavones that formed most of the flavonoids in the present study supplement, previous RCTs have shown increases in endothelial function and vasodilation from orange juice and its primary flavanone, hesperidin [12,13].

The fact that these effects were shown with isolated hesperidin suggests that the effect of this flavanone on endothelial function is direct and causal [12,13].

Thus, although there is the potential for the actual magnitude of change in FMD in the present study to be overestimated, the wider literature lends confidence to the finding that the citrus flavonoid supplement was in fact causative of the improvement in endothelial function observed in this study, particular considering that all the cardiovascular risk factor outcomes were observed independent of weight loss or significant changes in body composition.

Relevance

The present trial is a small, exploratory study and first and foremost, it is important to state the any interpretation of the findings should be hypothesis-generating, i.e., requiring confirmation in larger studies. With that caveat, what of the potential of the findings in the present study?

Even if we assume some overestimation in the 3.04% change in FMD observed with the intervention, the fact that meta-analysis indicate reductions in risk of CVD events for just a 1% increase in FMD suggests that the effect of the (poly)phenol supplement in this study could yield meaningful reductions in "hard" endpoints. The most robust intervention trial to date specifically using a flavonoid-enriched supplement was the COSMOS trial, which we covered in a previous Deepdive, but this trial focused specifically on cocoa flavanols.

In order for a similar strength of evidence to be produced for citrus flavonoids, it would be necessary to conduct a longer-term trial [COSMOS average follow-up was 3.6yrs] with CVD events and/or mortality as endpoints. This is, of course, where nutrition is often struggling to produce these drug-trial-esque intervention trials, however, it is easier to achieve with a supplement than with food-based interventions. Until such a trial is conducted, however, we are left with inferences from short-term changes in intermediate risk factors, for which we should be cautious against over-extrapolation.

Some of the other findings are also interesting, particularly the reduction in IL-6. Targeting reductions in IL-6 specifically was an aim of the CANTOS trial, which showed that even participants below the baseline average IL-6 level of 1.65pg/mL had a 32% lower risk of major CVD events [14]. Participants in CANTOS were recruited deliberately to have high inflammatory markers, so are not comparable to the healthy participants in the present study. Nevertheless, the reduction observed in the present study may be clinically relevant.

Overall, the study demonstrates improvements in a range of important CVD risk factors, including modest reductions in LDL-C, but as an exploratory study, the supplement used in the present trial will need to be shown to be as effective in a larger intervention, preferably with CVD events as a primary endpoint.

Application to Practice

It is frustrating when the authors of a supplement intervention don't at least attempt to put the doses used in their study into a food-based context. Either way, it would be difficult to attain the levels of (poly)phenol compounds in the present study through food alone.

For example, it would take ~100ml of extra virgin olive oil to obtain ~90mg olive oil polyphenols. And there is little chance of obtaining the relevant levels of citrus flavanones and flavones through food alone, although orange juices [~200ml] could provide some decent levels [~40mg].

However, a constant theme for these compounds is not necessarily that we need to whack the full dose in every day, but that a constant intake of a wide variety of (poly)phenol-rich foods is likely the best preventative bet for otherwise healthy people over time.

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