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

The main compounds of interest in soy foods, namely isoflavones, are a subclass within the flavonoid class of (poly)phenols, and as such exert their biological effects differently to nutrients [(1)]. Specifically, the primary soy isoflavones, known as genistein, daidzein, and glycitin, may be characterised as “phytoestrogens” or plant-derived oestrogens, due to their structural similarity to hormonal oestrogens, albeit exerting much milder oestrogenic effect [(1)].

These properties have led to interest in the potential effects of soy isoflavones for women's health, particularly during the menopausal period, which is characterised by decreases in oestrogen production and associated increases in symptoms associated with menopause [(2)].

While soy is generally recognised for the phytoestrogen activity of its main isoflavones, it is the metabolism of daidzein by gut bacteria into a compound known as “equol” that may be of particular interest for the reported health effects of soy foods [(3)]. Equol is a non-steroidal oestrogen, which can be drugs [e.g., tamoxifen] or naturally occurring compounds that may exert oestrogenic activity.

In this regard, equol has been shown to have greater affinity for binding to oestrogen receptors than both genistein and daidzein, and exerts greater antioxidant activity compared to the precursor isoflavones [(3)]. The evidence also suggests that the capacity of gut bacteria to produce equol may be an important determinant of the effects of this soy isoflavone metabolite [see ***Geek Box** below for further detail] [(3)].

Equal is exclusively a product of bacterial metabolism of daidzein, and does not appear in urinary excretion [its elimination pathway] unless soy foods are consumed in the diet. Or, as the present study tested, as a dietary supplement.

*Geek Box: Equol Producing Capacity

Before they can be fully absorbed, soy isoflavones are metabolised by gut bacteria; this is a step that is now recognised as a critical stage in metabolism and ultimate bioactivity of all (poly) phenolic compounds. Due to the modifying effect of the gut microbiota, the concept of an "equol producer" has been identified, although the definition appears to be relatively arbitrary [based on levels of serum or urinary equol]. Equol-producing capacity may be a combination of genetics and the background diet; East Asian populations exhibit greater equol-production capacity compared to Western populations. Research also shows that increases in serum levels of equol may only be observed in "equol producers" compared to "equol non-producers". It has been suggested that equol provides more of a plausible mechanistic explanation for the association between soy foods consumption and lower risk of chronic diseases, compared to the precursor soy isoflavones. If this is the case, the distinction between "equol producers" and "nonproducers" may provide an explanation for certain of the inconsistencies in the literature. However, as we covered in a previous Deepdive, some health outcomes that appear to benefit from soy foods may not be dependent on changes in equol levels, i.e., on equol producing capacity. Like many areas related to the interaction between dietary compounds and the microbiota, there are a lot of unknowns with this area of research and it is worth keeping an open tab on the potential modifying effect of equol producing status on the health effects of soy isoflavones.

The Study

This study was a randomised controlled trial in 57 postmenopausal women in Japan. Women randomised to the equol supplement intervention group consumed 10mg of equol per day [extracted from fermented soybeans]. The total duration of the intervention was 12-weeks.

Prior to the study, equol producing status was quantified using urinary samples. Women were then classified into four groups:

1. Both intrinsic [i.e., natural equol producer] and extrinsic equol [i.e., also taking the supplement]
2. Exclusively extrinsic equol exposure [a non-producer taking the supplement]
3. Exclusively intrinsic equol exposure [an equol producer not taking the supplement]
4. No equol exposure [neither an equol producer nor taking the supplement]

Before and after testing was conducted for the main outcomes of interest. The primary outcome measures included blood lipid levels, visceral fat, and advanced glycation end products [AGEs; these are formed when proteins and lipids are exposed to sugars and become "glycated", forming a sticky compound which damages the vasculature and contributes to atherosclerosis. AGEs may be measured by assessing external damage to skin as AGEs accumulate in skin collagen, using a measure known as skin autofluorescence].

An analysis was also conducted with these four categories to examine their effects on visceral fat and AGEs.

The secondary outcome was the Climacteric Scale, a composite scale of menopausal symptoms, which were administered every month for the intervention.

Results: Of the 57 women in the study, 13 [22.8%] were classified as equol producers; 4 in the intervention group and 9 in the control group. Average age of the participants was 56yrs, and BMI of 21.4kg/m2.

- ***Blood Lipids, Visceral Fat, and AGEs*:** There was no significant difference in blood cholesterol, triglycerides, visceral fat levels, or skin autofluorescence [as a marker of AGEs], between groups.
- ***Effects of the Equol Categories*:** More women in the "no equol exposure" category showed a worsening of visceral fat levels, and less improvement in skin autofluorescence [as a marker of AGEs], compared to the other categories [more under ***Key Characteristic***, below].
- ***Effects on Menopausal Symptoms***: There was a significant difference between groups in the Climacteric Scale scores, which decreased from 16.4 to 11.3 in the equol supplement group and were unchanged from 14.7 to 14.7 in the control group over 12-weeks, respectively [between-group difference of 3.4] [more under ***Interesting Finding***, below].

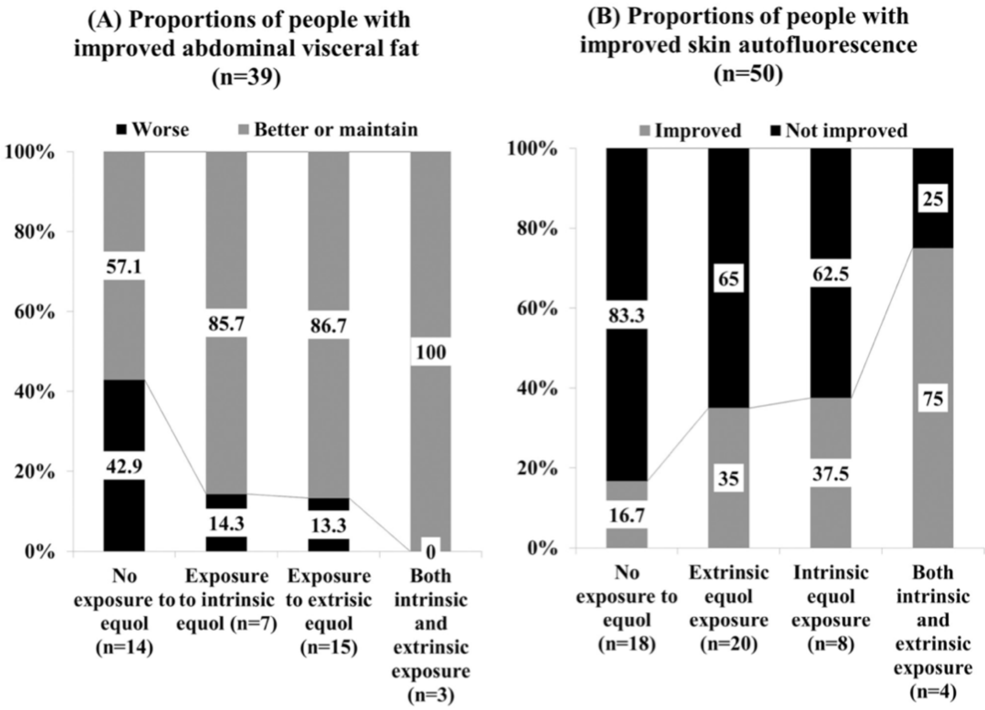
The Critical Breakdown

Pros: The study was the first to investigate the effects of an equol supplement on visceral fat and AGEs. Equol producing status was quantified, which allowed for the analysis to consider the effects of equol producing status on the outcomes. Yep, that' s all the positives this one has.

Cons: The study stated its aims in the broadest terms, and did not clearly state any hypothesis; thus, with such an array of outcome measures, this leaves the authors free to emphasise any findings they like and we don't know what outcomes really matter for the research question being addressed. The study used a simple randomisation method, which randomised women in chronological order according to even or odd numbers. While simple to implement, the disadvantage of simple randomisation is that it may lead to imbalanced characteristics between group, which occurred in this study; equol producing capacity was not matched, neither was alcohol habits. No detail is provided regarding the control, which appears to just have been an inactive control, i.e., they were not provided with any placebo. The study doesn't specify which of the many outcomes listed as "primary" were in fact the primary outcome, and the results section randomly leads off with HDL-cholesterol, vitamin D, and parathyroid hormone, which do not appear related to any specific hypothesis [because none were actually stated]. The statistical analysis is bizarre, combining both analysis of continuous data – which is what you would expect with outcomes like blood cholesterol or blood pressure, for example – with analysis of proportions, which we would expect for categorical data [more under ***Key Characteristic***, below].

Key Characteristic

The approach to the statistical analysis in this study is a little strange. For example, in the quantitative analysis of both visceral fat and skin autofluorescence [as a marker of AGEs], there was no significant difference between groups [although the control group did show a small decrease in visceral fat levels, compared to no change in the equol supplementation group]. However, the paper then went on to analyse the effects of the four respective "equol exposure" categories using a categorical statistical test [see **figure**, below].



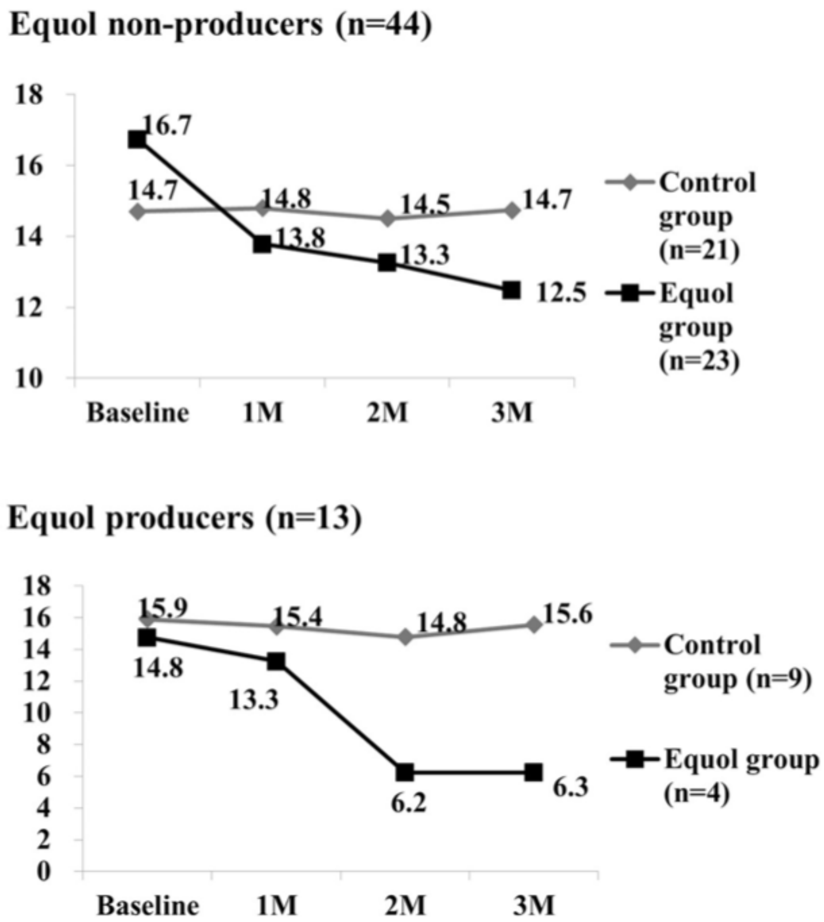
To do this, they had to dichotomise each outcome – which you can see in the figure legend above is "improved", but we are not told in the paper what constitutes "improved" for skin autofluorescence, and for visceral fat we can only assume "worse" meant an increase while "better" meant a decrease.

But again, neither of these are evident in the continuous data analysis. Now, it is likely that because those data just analysed each full group – all 30 participants in the control vs. 27 in the intervention – that the quantitative test lacked sensitivity to test the differences between these equol exposure categories. However, there remain statistical tests for determining differences between groups according to different categories or levels of an exposure [such as an analysis of variance, aka ANOVA].

To be clear, this doesn't make this particular analysis invalid; the authors intention appears to have been to test for a linear trend between the different levels of equol exposure, from lowest [no equol exposure] to highest [both intrinsic equol production and extrinsic equol supplementation]. Nevertheless, this is a test of *association*, and an analysis using the continuous data would have shed more light on the magnitude of effect of these equol exposure categories on these outcomes, and potential differences between levels of equol exposure, if any. This "if any" is important because there is the potential that the statistical test was chosen to fit the data, not *vice versa*.

Interesting Finding

The analysis of the effect of the intervention on menopausal symptoms also stratified the analysis by equol producing status. In the **figure**, below, you can see that in that in equol non-producers the supplement was still effective, reducing the symptom score from 16.7 to 12.5, while in the equol producers the supplement reduced the symptom score from 14.8 to 6.3.



In the statistical analysis, there was no interaction between equol producing status and the outcome, which the authors interpret to mean that equol supplementation was effective regardless of equol producing status. And this interpretation is valid, however, the lack of "statistically significant" interaction could have been influenced by the very small numbers of equol producers that were taking the equol supplement [*n* = 4].

The absolute magnitude of change in the equol supplement group stratified by equol producing status was, from baseline to 3-months, 2.2 and 8.5 in the non-producers and equol producers, respectively. Thus, another interpretation is that while the equol supplement was beneficial overall irrespective of equol producing status, the magnitude of effect appeared greater in equol producers. But this finding is not robust, and should be considered hypothesis-generating for future, larger studies.

Relevance

This is quite a poor study overall, and one which in the screening process for Deepdives looked good in the abstract summary. Dammit. Nevertheless, all research serves a purpose, including to highlight poor research.

And there is always some wider context that we can consider. In relation to the menopausal scores, the scale used was a 21-item questionnaire with a 0-3 rating [3 = severe]; thus a score of 63 was the maximum overall score. With baseline scores of 16.4 and 14.7 in the intervention and control groups, respectively, the participants in this study were women with very mild menopausal symptoms. This could have an influence on the outcomes, as the effects of soy isoflavones may be more pronounced in women with lower oestrogen levels [(4)], although oestrogen was not measured in this study.

A more rigorously controlled RCT in Japan found improvements in certain menopausal symptoms, mostly mood-related, following 12-weeks of equol supplementation [(5)]. The evidence for benefits from equol supplementation specifically is also confined to Japanese peri-menopausal women [(6)], and therefore some caution is warranted in extrapolating the findings beyond this population group.

The Kupperman Index [KI] represents a more comprehensive grouping of 11 menopausal symptoms [hot flushes (vasomotor), paresthesia, insomnia, nervousness, melancholia, vertigo, weakness, arthralgia/myalgia, headache, etc.], and a recent meta-analysis suggests that soy phytoestrogens are not superior to placebo for menopausal symptoms assessed using the KI, however, they may reduce hot flushes [(7)]. Although even the finding in relation to hot flushes is not consistent, with the most recent meta-analysis on this question showing no effect of soy isoflavones on this outcome [(8)].

Application to Practice

Currently the data is too inconsistent to recommend either soy isoflavone supplementation, or equol supplementation specifically, for menopausal symptoms. This is consistent with the wider research on soy foods and isoflavones and different health outcomes, which despite the “halo effect” of this food group, the evidence is often characterised by weak effect sizes and inconsistencies [see this Deepdive, this Deepdive, and this Deepdive for further reading].

However, this may very well be a reflection of the research itself – the quality of the available evidence – and overall soy isoflavones exert a range of biological activity, similar to other flavonoids, that justify inclusion in the diet, but likely not as a supplement.

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