Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

S-equol

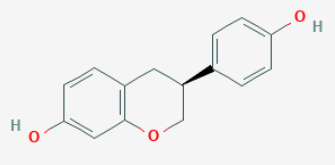
Evidence Summary

Higher S-equol levels correlate with lower mortality and better cognition. S-equol treatment shows benefits for menopause and bone health but mixed results for cognition, CVD, and metabolic health.

Neuroprotective Benefit: So far, small clinical trials of S-equol have not shown cognitive benefits. However, in observational studies, S-equol producers appear to be protected from cognitive decline.

Aging and related health concerns: S-equol decreases menopausal symptoms and bone resorption, but effects on cardiovascular and metabolic biomarkers are less consistent. Higher S-equol levels correlate with lower mortality and lower cancer risk, but this association may be limited to Asian populations.

Safety: Most clinical trials observed no adverse events, but one study reported treatment-emergent adverse events including abdominal distension and endometrial hypertrophy. S-equol does not alter serum sex and thyroid hormone levels.

Availability: not available on its own in the US besides research grade products; a supplement containing 60% equol is available in Japan	**Dose**: Most clinical studies have used a dose of 10 mg of S-equol, orally, per day.	**Chemical formula:** $C_{15}H_{14}O_3$ **MW**: 242.27  Source: PubChem
Half-life: plasma elimination half-life is 8 hours	**BBB**: penetrant in preclinical models	
Clinical trials: Numerous clinical trials have tested the effects of S-equol, mostly enrolling about 100 subjects with menopausal symptoms.	**Observational studies**: There have been numerous observational studies examining the association between soy isoflavone biomarkers and disease. One meta-analysis included 40 observational studies that examined mortality, cancer, cardiovascular disease, and metabolic syndrome.	

What is it? The whole soybean contains approximately equal amounts of genistein and daidzein, with smaller amounts of glycitein (Nakamura and Tonogai, 2000). S-equol is a major metabolite produced in the gastrointestinal tract by bacterial metabolism of the soy isoflavone daidzein. This metabolism of daidzein is dependent on gut microflora and many Asians (50-70%) and a smaller percentage of non-Asians (under 20%) possess these bacterial strains (e.g., *Lactococcus garvieae, Eggerthella sp. strain YY7918*)(Yokoyama et al., 2011; Chen et al., 2019). People who possess the intestinal bacteria to metabolize daidzein into S-equol are often referred to as 'equol producers'. Several studies have tested whether supplementation of pre- or probiotics along with soy isoflavones would induce S-equol production, but these interventions have mostly failed to induce S-equol production (reviewed in Sekikawa et al., 2019).

S-equol has been studied most extensively for its potential to alleviate menopausal symptoms due to its affinity for the estrogen receptor β (ERβ), and not ERα, which is associated with the classical effects of estrogen, including promoting breast and reproductive organ cancers. In addition, molecular docking studies have shown that isoflavones including S-equol bind directly to the G protein-coupled estrogen receptor (GPER) at the same position as 17β-estradiol, suggesting that some of the effects of S-equol

may be exerted in part by GPER signal transduction pathways(Ariyani et al., 2020). S-equol has also been studied for its chemoprotective effects in endocrine-related cancers (e.g., breast and prostate cancers)(Hod et al., 2021), anti-atherogenic properties in heart disease (Sekikawa et al., 2019), and antioxidant effects in various conditions like cardiovascular and metabolic diseases.

It is thought that S-equol may have greater cognitive benefits than dietary sources of soy isoflavones because S-equol has higher antioxidant properties, greater affinity to ERβ, longer bioavailability, and better ability to increase mitochondrial activities (Sekikawa et al., 2019).

Neuroprotective Benefit: So far, small clinical trials of S-equol have not shown cognitive benefits. However, in observational studies, S-equol producers appear to be protected from cognitive decline.

Types of evidence:

- 3 randomized clinical trials, of which 1 tested S-equol alone and 2 tested soy isoflavone formulations that included S-equol
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:
In a cross-sectional study of 152 older Japanese people (median age of 69.2 years old), equol producers (40% of the cohort) had higher scores on a cognitive test (measured by the Touch Panel-Type Dementia Assessment Scale; 14.7 ± 0.7 in equol producers vs 14.3 ± 0.8 in nonproducers, p=0.02) and lower prevalence of mild cognitive impairment (8% vs 21%, p=0.04) compared to equol non-producers (Igase et al., 2017).

No studies have tested the efficacy of S-equol alone for prevention of dementia or cognitive decline. But several clinical studies have investigated interventions with PhytoSERM (selective estrogen receptor modulators), which consist of soy isoflavones genistein, daidzein, and S-equol. The advantage of the PhytoSERM formulation is that S-equol is included, allowing people who are not able to metabolize daidzein into S-equol to also benefit from the effects of S-equol.

In a double-blind randomized controlled phase 1b/2a trial, treatment with PhytoSERM (50 mg/day or 100 mg/day containing equal amounts of genistein, daidzein, and S-equol) was tested in 71 non-cognitively impaired perimenopausal women with at least 1 cognitive complaint and 1 vasomotor-

related symptom (Schneider et al., 2019). There were no statistically significant differences between the PhytoSERM and placebo groups on either vasomotor or cognitive scores (measured by the neuropsychological composite) after 4 or 12 weeks of treatment. However, this study was a safety and feasibility study and not powered to allow detection of cognitive effects. The authors noted that a rationally-designed phase 2b efficacy trial is warranted in order to further assess PhytoSERM for menopause-associated vasomotor and cognitive symptoms.

A retrospective analysis of the above study (the phase 1b/2a) testing PhytoSERM reported that the therapeutic effect of PhytoSERM was stratified by 2 genetic risk modulators for Alzheimer's disease: the mitochondrial haplogroup and the APOE genotype (Wang et al., 2020). No effect of PhytoSERM was observed on verbal fluency, episodic memory or global cognition throughout the trial, with or without stratification by genotype groups. Also 50 mg or 100 mg of PhytoSERM per day failed to improve immediate recall, delayed recall, or recognition, and genetic variation (by mitochondrial haplotype H vs non-H, and by APOE4 genotype) did not modulate any of these outcomes. However, subjects with the mitochondrial haplogroup H on placebo had significantly decreased "learning over trials" during the clinical study compared to non-H haplogroups (p=0.007), whereas haplotype H subjects receiving 50 mg of PhytoSERM per day successfully prevented the decline. No such preventative effect was observed in non-H haplogroups. Overall, PhytoSERM treatment did not result in significant improvement in executive function (as measured by Trails making B) compared to the placebo. However, the PhytoSERM 50 mg group exhibited significantly enhanced executive function compared to their own baseline, while no enhancement was observed in either placebo or the PhytoSERM 100 mg group. This measure of executive function was not modulated by mitochondrial genetic variances or APOE genotype.

Human research to suggest benefits to patients with dementia:
In a very small, single-arm pilot trial of 15 women with very mild or mild Alzheimer's disease (CDR score of 0.5 or 1), S-equol treatment (10 mg twice daily) for 4 weeks did not result in significant changes in cognitive scores (measured by MoCA) after 2 or 4 weeks of treatment (Wilkins et al., 2017). Given the small size of this study and the lack of a placebo arm, the absence of cognitive benefits is inconclusive.

A randomized controlled trial of 59 Alzheimer's disease patients reported that soy isoflavone treatment (Novasoy brand; 100 mg/day, 85% of which was daidzin and genistin as glycosides) for 6 months did not significantly improve cognitive function over placebo, despite increased plasma levels of isoflavones (Gleason et al., 2015). However, total plasma equol levels (but not of other soy isoflavones) were significantly correlated with performance on a verbal fluency test (Phonemic Fluency: $r = 0.29$, $p = 0.04$) and a measure of speeded dexterity (Grooved Pegboard, non-dominant hand: $r = -0.33$, $p = 0.02$;

dominant hand r = −0.29, p = 0.05). It is worth noting that the sample size was small with multiple outcomes, and these results must be interpreted as inconclusive and exploratory.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:
In a rat model of neurocognitive impairment (ovariectomized female HIV-1 transgenic rats), chronic S-equol treatment at postnatal day 28 prevented the development of neurocognitive impairments in all subjects (McLaurin et al., 2020).

In middle-aged ovariectomized female rats, treatment with S-equol (0.97 or 1.94 mg per day, orally) did not produce significant benefits on working memory (delayed spatial alternation task) and place learning (Neese et al., 2014). This is in contrast to previous studies from the same laboratory that showed that treatment with estradiol or the soy isoflavone genistein improves working memory and place learning.

In a mouse model of depression (induced by LPS, an inflammatory agent), treatment with S-equol (10, 20, and 40 mg/kg/day, orally) for 19 days partially reversed the depressive-type behavior while decreasing pro-inflammatory cytokines (TNF, IL-6, IL-10, IL-1β), up-regulating the expression of synaptic plasticity-related proteins (p-synapsin, synapsin, PSD-95), downregulating the TLR4/NFkB signaling pathway, increasing the levels of 5-hydroxytryptamine and norepinephrine, and normalizing the release of tryptophan and kynurenine in the hippocampi (Lu et al., 2021). Together, these findings suggest that S-equol may alleviate depressive-like behavior by inhibiting neuroinflammation via the TLR4/NF-κB signaling pathway, normalization of monoamine neurotransmitter levels, reversal of tryptophan metabolism dysfunction, and enhancement of synaptic plasticity.

In ovariectomized mice, treatment with S-equol-containing PhytoSERM (10 mg/kg/day) or racemic R/S-equol-containing PhytoSERM (10 mg/kg/day) increased expression of key proteins involved in energy production, restored the ovariectomy-induced decrease in key bioenergetic enzymes, and reduced the ovariectomy-induced increase in lipid peroxidation (Yao et al., 2013). Both the S-equol and the R/S-equol PhytoSERM treatments regulated mitochondrial function, with the S-equol version eliciting a greater response in mitochondrial potentiation. Both combinations regulated genes involved in glucose metabolism, energy sensing, lipid metabolism, cholesterol trafficking, redox homeostasis, and β-amyloid production and clearance.

In *C. elegans* exposed to the neurotoxin MPP+, S-equol exposure increased their survival from 72 to 108 hours (Johnson et al., 2020).

Cell culture studies have shown that S-equol treatment exerts cytoprotective effects (Johnson et al., 2020), promotes neuroprotection in the presence of neurotoxins (6-OHDA, MPP+)(Johnson et al., 2020), protects against the toxicity of Aβ(25-35) via ERα-mediated pathways (Tsai et al., 2019), and promotes dendritic/neurite growth (Ariyani et al., 2019).

In astrocyte-enriched culture, S-equol exposure (and other isoflavones) increased glial cell migration by direct binding to G protein-coupled estrogen receptor (GPER) and subsequent activation of the PI3K/FAK/Akt/RhoGTPase signaling pathway, which induces F-actin formation (Ariyani et al., 2020). In astrocytic culture, S-equol may also mitigate neuroinflammation and exert glioprotective effects by attenuating nitric oxide production (induced by LPS) through nongenomic pathways (Moriyama et al., 2018).

APOE4 interactions: In a randomized controlled trial of 59 people with Alzheimer's disease, APOE4 genotype (31 were E4 carriers) did not affect the response to soy isoflavone treatment (Gleason et al., 2015). However, it is unknown whether treatment with S-equol itself interacts with the APOE4 genotype.

Aging and related health concerns: S-equol decreases menopausal symptoms and bone resorption, but effects on cardiovascular and metabolic biomarkers are less consistent. Higher S-equol levels correlate with lower mortality and lower cancer risk, but this association may be limited to Asian populations.

Types of evidence:

- 6 meta-analyses or systematic reviews, mostly of observational studies evaluating soy isoflavone intake and/or plasma levels
- 8 clinical trials, most testing S-equol specifically while others testing a formulation that included S-equol
- 2 observational studies
- Numerous laboratory studies

Lifespan: HIGHER S-EQUOL LEVELS CORRELATED WITH LOWER MORTALITY IN JAPANESE PEOPLE
No studies have tested whether S-equol can decrease mortality or extend lifespan in humans. However, in a case-control observational study of Japanese people over 70 years old (from Tsurugaya, Sendai, Japan), of whom 165 had died and 177 were alive, higher serum equol levels (but not any other soy isoflavones) were inversely associated with the composite endpoint of disability and death (Hozawa et

al., 2013). The risk of disability or death among equol producers remained reduced after adjusting for age and sex (OR=0.55; 95% CI, 0.33 to 0.93). However, due to the observational nature of the study, these results do not mean that equol has preventive effects on disability or death. It is also not clear whether these findings would translate to non-Japanese/Asian populations.

Breast cancer: S-EQUOL LEVELS NOT CORRELATED WITH RISK / MIXED PRECLINICAL DATA
A 2017 meta-analysis of 40 observational studies examining soy isoflavone biomarkers (in plasma, serum, or urine) reported no significant associations with S-equol levels and breast cancer risk, though higher daidzein and genistein concentrations were associated with a 34% and 28% lower risk of breast cancer, respectively (Rienks et al., 2017).

In a systematic review of 23 studies (18 *in vitro*, 2 *in vivo*, 3 of both) studying the effects of S/R-equol on breast cancer models, 13 studies reported anticancer properties, 6 studies reported oncogenic properties, and 4 studies reported neither oncogenic nor anti-cancer properties (Hod et al., 2021). There was no specific pattern of dosage that conferred anticancer or oncogenic outcomes. In one of the cell culture studies using MCF-7 breast cancer cells, S-equol exerted an anti-breast cancer effect by upregulating miR-10a-5p and inhibiting the PI3K/AKT pathway (Zhang et al., 2019). A different breast cancer cell culture study (MCF-7 and MDA-MB-231 cells) reported that S-equol inhibited the activity of the breast cancer resistance protein (BCRP) and sensitized BCRP substrates (Rigalli et al., 2019). Also, in MDA-MB-231 human breast cancer cells, S-equol (and R-equol) treatment inhibited their invasive capacity by downregulating MMP-2 expression (Magee et al., 2014).

Prostate cancer: HIGHER S-EQUOL LEVELS CORRELATED WITH LOWER RISK IN ASIANS BUT NOT WESTERN POPULATIONS
No studies have tested S-equol interventions for prevention or treatment of prostate cancer in men.

A 2018 meta-analysis of 7 prospective studies (2 from Japan, 5 from Europe) that examined the relationship between circulating levels of isoflavones with prostate cancer risk reported that in men from Japan, those with high compared to low circulating equol concentrations had a lower risk of prostate cancer (highest quartile compared to lowest quartile; OR=0.61; 95% CI, 0.39-0.97), though a linear trend was not found (Perez-Cornago et al., 2018). Genistein and daidzein concentrations were not significantly associated with risk in Japanese men. In European men, circulating concentrations of genistein, daidzein and equol were not associated with prostate cancer risk.

A 2017 meta-analysis of 40 observational studies examining isoflavone biomarkers (in plasma, serum, or urine) reported no associations between S-equol levels and prostate cancer risk, though a 19% lower risk of prostate cancer was observed with higher concentrations of daidzein (OR=0.81, 95% CI, 0.67-0.99) (Rienks et al., 2017).

A different 2017 meta-analysis of 23 observational studies examining serum or urinary phytoestrogen levels reported that S-equol levels (OR, 0.86; 95% CI: 0.66–1.14) were not significantly associated with prostate cancer risk, while daidzein (OR, 0.85; 95% CI: 0.75–0.96), genistein (OR, 0.87; 95% CI: 0.78–0.98), and glycitein (OR, 0.89; 95% CI: 0.81–0.98) were associated with a reduced risk for prostate cancer (Zhang et al., 2017). However, in a subgroup analysis stratified by study population, S-equol levels (as well as levels of total isoflavones, daidzein, and genistein) are associated with lower prostate cancer risk in the Asian population subgroup, but not in the Western populations.

In preclinical studies, S-equol has been shown to have anti-androgenic (through sequestration of 5alpha-dihydrotestosterone) and anti-proliferative properties in the prostate (Lund et al., 2004), though its role in humans remains unclear.

Cardiovascular diseases: DECREASES LDL, ARTERIAL STIFFNESS, AND POSSIBLY CHD
In a double-blind randomized controlled crossover trial of 54 overweight or obese Japanese people, S-equol treatment (10 mg daily, orally, Otsuka Pharmaceutical Co., Ltd) for 12 weeks led to a significant decrease in serum LDL-cholesterol levels and arterial stiffness (measured by the cardio-ankle vascular index)(Usui et al., 2013).

In a double-blind randomized controlled crossover trial of 28 men at moderate risk for cardiovascular disease, a single treatment with S-equol (40 mg of SE5-OH, Otsuka Pharmaceutical Co., Ltd) produced no effects on arterial stiffness (measured by carotid-femoral pulse-wave velocity), blood pressure, or endothelial function in non-equol-producers (Hazim et al., 2016). However, soy isoflavone treatment (80 mg) improved carotid-femoral pulse-wave velocity in equol producers (but not in non-producers) after 24 hours. Because this trial tested an acute dose of S-equol, it is possible that longer treatments could produce different outcomes.

A Chinese case-control study nested within 2 prospective cohort studies reported that urinary equol excretion (but not total urinary isoflavonoids) showed a significant inverse association with coronary heart disease in women (Zhang et al., 2003). The adjusted odds ratios for coronary heart disease from lowest to highest quartiles of equol levels in women were 1.0 (reference), 0.61, 0.51, and 0.46.

A review evaluating the effects of S-equol on vascular function noted that S-equol has numerous beneficial effects including anti-apoptosis, antioxidation, anti-atherosclerosis, production of nitric oxide in endothelial cells, and promotion of vascular smooth muscle cell relaxation (Matsumoto et al., 2020). For example, S-equol (and other phytoestrogens) may confer protective effects on vascular smooth muscle cells by inhibiting vascular remodeling and neointima formation (Dubey et al., 1999). In endothelial cells, S-equol suppresses oxidized LDL-induced apoptosis by decreasing reactive oxygen species (Kamiyama et al., 2009). S-equol also promotes relaxation of vascular tone in various arteries (reviewed in Matsumoto et al., 2020).

In a rat model of hypertension (deoxycorticosterone acetate salt-induced), S-equol treatment (10 or 20 mg/kg, orally) for 4 weeks dose-dependently decreased systolic blood pressure by inhibiting angiotensin-converting enzyme activity and increasing nitric oxide production (Liu and Tsai, 2016).

In another review studying the effects of S-equol and soy isoflavones on cardiovascular diseases, S-equol is noted as the soy isoflavone possessing the highest anti-atherogenic and antioxidant properties (Sekikawa et al., 2019). In ApoE knockout mice fed a high-fat diet, S-equol treatment (0.05-0.1%) for 12-14 weeks produced anti-atherosclerotic effects by inhibiting endoplasmic reticulum stress through the activation of the antioxidant transcription factor Nrf2 (Zhang et al., 2016).

Obesity, metabolic disease: DECREASES HbA1c / PRECLINICAL DATA ARE MIXED
In a double-blind randomized controlled crossover trial of 54 overweight or obese Japanese people, S-equol treatment (10 mg daily, orally, Otsuka Pharmaceutical Co., Ltd) for 12 weeks led to a significant decrease in HbA1c (measure used for diagnosis of diabetes)(Usui et al., 2013). In this study, 67.9% of the overweight and obese subjects were equol non-producers, a rate that is higher than previously reported percentages of equol non-producers in Japan (~50%).

In contrast, in mice fed a high-fat diet, S-equol supplementation (10 mg/kg body weight) for 4 weeks exacerbated the high-fat diet-induced metabolic disease as measured by decreased physical activity, reduced energy expenditure, and increased fasting serum glucose (Bax et al., 2019). In male mice, hyperinsulinemia and hypoleptinemia were also observed with S-equol treatment. Unlike humans, all mice are natural S-equol producers.

In ovariectomized and intact female rats, a phytoestrogen-rich soy diet (590 mg/kg; genistein and daidzein) for 28 weeks rescued metabolic dysfunction and inflammation while significantly altering gut microbiota (Vieira-Potter et al., 2018). Higher S-equol levels were correlated with better metabolic

indicators (reduced adipose tissue weight and hyperinsulinemia) and reduced visceral white adipose tissue inflammation, suggesting that S-equol may be a key mediator of soy diet effects on metabolism and inflammation.

In male mice, S-equol treatment (20 mg/kg, twice daily, oral gavage) for 7 days increased β-cell proliferation (Ki67-positive cells) by 27%, increased β-cell mass by 104%, and showed resistance to hyperglycemia after streptozotocin treatment (Horiuchi et al., 2017). In mouse pancreatic islets, S-equol treatment enhanced glucose-stimulated insulin secretion by 41%.

Menopausal symptoms: REDUCES HOT FLASHES, IMPROVES MUSCLE/JOINT PAIN/STIFFNESS
A review of 29 systematic reviews and meta-analyses in menopausal women reported that S-equol (along with acupuncture, hypnosis, paced respiration, cognitive behavioral therapy, genistein, soy isoflavones, etc.) significantly reduced vasomotor symptoms, based on systematic reviews and meta-analyses with moderate to high methodological quality (Guo et al., 2019).

A systematic review of 68 articles on the effects of soy isoflavones in menopausal women reported that the ability of women to produce equol may be the major determinant of whether or not soy isoflavones can effectively reduce vasomotor symptoms (Chen et al., 2019).

In a 2019 meta-analysis of 5 randomized controlled trials (728 subjects total) testing soy isoflavones or S-equol in postmenopausal women, a significant benefit of equol was observed for lowering hot flash scores (Daily et al., 2019). Three randomized controlled trials that tested an oral 10-mg daily dose of equol (SE5-OH, Otsuka Pharmaceutical Co. Ltd; each tablet containing 5.0 mg S-equol, 1.0 mg daidzein, 1.1 mg genistein) were included and the mean difference between 10 mg equol treatment (n=118) and placebo (n=119) groups was −0.30 (95% CI, −0.50 to −0.10; p=0.003) in the fixed-effect model. This meta-analysis also found that supplementing equol to equol nonproducers significantly lowered the incidence and/or severity of hot flashes in menopausal women. At least 2 of the randomized controlled trials that tested the SE5-OH supplement manufactured by Otsuka Pharmaceutical had received financial support along with the supplement to carry out the trials (Aso et al., 2012; Jenks et al., 2012). The authors of the meta-analysis suggested that women who are equol producers may obtain little additional benefit from equol supplementation if they already eat a diet rich in isoflavones.

In a double-blind randomized controlled trial of 102 menopausal women, treatment with S-equol (10, 20, or 40 mg daily; SE5-OH, Otsuka Pharmaceutical Co., Ltd) was compared with soy isoflavones (24 mg daidzein, 22 mg genistein, 2.0 mg glycitein; SoySelect S.p.A., Milan, Italy) and placebo (lactose)(Jenks et

al., 2012). Reductions in hot flash frequency after 8 weeks of treatment were similar for all treatment groups. However, cumulative analysis showed that after 8 weeks of treatment, 40 mg/day S-equol group experienced a greater reduction of hot flash frequency compared to the isoflavones group (p=0.021). A subgroup analysis showed that for subjects with 8 or more hot flashes per day at baseline, 20 and 40 mg daily S-equol treatments were superior to soy isoflavones in reducing hot flash frequency (p=0.045 and p=0.001, respectively). Also, S-equol treatment (10 and 20 mg/day dose) improved muscle and joint pain score compared with isoflavones (p=0.003 and p=0.005, respectively). In a different double-blind randomized controlled trial of 126 postmenopausal Japanese women, S-equol treatment for 12 weeks (10 mg/day, SE5-OH, Otsuka Pharmaceutical Co., Ltd) reduced neck or shoulder muscle stiffness (along with hot flashes) compared with the placebo group (p=0.012 and p=0.004, respectively)(Aso et al., 2012).

A retrospective analysis of a double-blind randomized controlled phase 1b/2a trial testing PhytoSERM (50 mg/day or 100 mg/day containing equal amounts of genistein, daidzein, and S-equol) in non-cognitively impaired perimenopausal women reported that 12 weeks of PhytoSERM treatment significantly decreased hot flash frequency in the 50 mg dose group compared to baseline, though this effect was not see in the 100 mg dose group (or placebo)(Wang et al., 2020). When stratified by APOE genotype, APOE4 non-carriers in the 50 mg dose group had significantly greater reduction in hot flash frequency compared to those in the placebo group (p=0.04). The sample size for APOE4 carriers was limited and a non-significant trend towards reduced hot flash frequency was observed in the 50 mg dose group. Participants receiving a dose of 100 mg daily did not experience significant improvements in hot flashes regardless of APOE genotype.

Bone health: DECREASED BONE RESORPTION, RENTENTION OF BONE MINERAL DENSITY
In a double-blind randomized controlled trial of 93 non-equol-producing menopausal Japanese women, treatment with S-equol (10 mg per day, Otsuka Pharmaceutical) for 1 year resulted in decreased levels of a biomarker of bone resorption (urinary deoxypridinoline; -23.94% with S-equol vs -2.87% in placebo; p=0.020) and slightly attenuated whole-body bone mineral density (1.040 g/cm^2 in the S-equol group, 0.994 g/cm^2 in the placebo group; p=0.015) (Tousen et al., 2011). The percent change in whole-body bone mineral density in people receiving the 10 mg dose of S-equol was −1.10%, which was significantly different from the −1.88% in the placebo group (p=0.027). However, other markers of bone resorption (NTx) or bone formation (OC and B-ALP) were unaffected by the equol intervention (2, 6, or 10 mg daily S-equol). Also, no significant differences in bone mineral density and percent changes in the bone mineral density of the lumbar spine, total hip, femoral neck, trochanter, Ward's triangle, and

intertrochanter were observed among the S-equol-treated groups (2, 6, and 10 mg daily) and the placebo group.

Safety: Most clinical trials observed no adverse events, but one study reported treatment-emergent adverse events including abdominal distension and endometrial hypertrophy. S-equol does not alter serum sex and thyroid hormone levels.

Types of evidence:

- 1 meta-analysis of randomized controlled trials testing soy isoflavones and equol
- 5 double-blind randomized controlled trials testing S-equol
- 2 double-blind randomized controlled trials testing soy isoflavones including S-equol
- 2 observational studies
- Several laboratory studies

Clinical studies with S-equol: In a double-blind randomized controlled trial of 126 postmenopausal Japanese women, S-equol treatment for 12 weeks (10 mg/day, SE5-OH, Otsuka Pharmaceutical Co., Ltd) resulted in no treatment-related adverse effects in any of the participants and there were no changes in any of the clinical laboratory test measures during and after the intervention period (Aso et al., 2012). SE5-OH showed no serious treatment-related adverse effects on the hormonal milieu, including sex hormones, gonadal hormones, and thyroid hormones.

In a double-blind randomized controlled trial of 102 menopausal women, treatment with S-equol (10, 20, or 40 mg daily; SE5-OH, Otsuka Pharmaceutical Co., Ltd) was compared with soy isoflavones (24 mg daidzein, 22 mg genistein, 2.0 mg glycitein; SoySelect S.p.A., Milan, Italy) and placebo (lactose)(Jenks et al., 2012). In contrast to the study above, 47% of subjects (48 out of 102) in this study reported at least one treatment-emergent adverse event (TEAE). Nineteen subjects experienced at least 1 TEAE that was considered to be potentially supplement-related: 8 (33%) with 10 mg S-equol, 5 (19%) with 20 mg S-equol, 4 (16%) with 40 mg S-equol, and 2 (8%) with soy isoflavones. The most common TEAEs that occurred in over 10% of subjects in S-equol treatment groups were abdominal distention (15% in the 20-mg dose and 13% in the 10-mg dose) and endometrial hypertrophy (13% in the 10-mg dose). TEAEs that related to the reproductive system or breast tissue, as detected by transvaginal ultrasonography or mammography, occurred in a total of 4 subjects, of whom 1 subject in the 20-mg S-equol group developed breast cancer. This was considered by the investigator to be unrelated to treatment. Eight subjects withdrew from the study because of TEAEs: 2 were in the 10-mg S-equol group (1 due to

endometrial hypertrophy, 7 mm in thickness with no other abnormal endometrial findings and 1 due to abdominal pain); 4 in the 20-mg S-equol group (1 due to breast cancer, 1 due to constipation, 1 due to urinary tract infection, 1 due to autonomic nervous system imbalance); 1 in the 40-mg S-equol group (due to depression); and 1 in the isoflavone group (due to positional vertigo). There were no notable or consistent changes in hematology, serum chemistry, urinalysis, hormone levels (FSH, TSH, SHBG), physical examinations, vital signs, or ECGs with any of the treatment groups.

In a double-blind randomized controlled trial of 134 pre-, peri-, or post-menopausal women in Japan, 1 subject receiving S-equol (10 mg) 3 times daily for 12 weeks developed a systemic rash, but all other subjects (receiving the same treatment or 10 mg of S-equol once daily or placebo) reported no adverse effects (Ishiwata et al., 2009).

In a double-blind randomized controlled trial of 93 non-equol-producing menopausal Japanese women, treatment with S-equol (10 mg per day, Otsuka Pharmaceutical) for 1 year did not alter serum levels of sex and thyroid hormones (estradiol, FSH, progesterone, and testosterone; free T3, free T4, and TSH) (Tousen et al., 2011).

In phase I double-blind randomized controlled trials of single- (SAD) and multiple-ascending dose (MAD) studies, 40 healthy volunteers were given S-equol (10-320 mg for SAD, 10-160 mg twice daily for MAD; synthesized by Girindus Solvay Organics, Kunsebeck, Germany; capsules prepared by Patheon, Toronto, Canada)(Jackson et al., 2011). S-equol was well tolerated and there were no significant drug-related adverse events, even at the highest dose tested of 320 mg. There were also no meaningful changes in routine serum chemistry values, complete blood count, electrocardiogram, or systolic or diastolic blood pressure in both studies. In both studies, all adverse events were considered mild or moderate (none were severe), and none required any action or led to study drug discontinuation.

In a very small, single-arm pilot trial of 15 women with very mild or mild Alzheimer's disease (CDR score of 0.5 or 1), S-equol treatment (10 mg twice daily) for 4 weeks did not result in any drug-related adverse events during the course of the study (Wilkins et al., 2017). Mean compliance was 96.4%.

Pharmacokinetics of S-equol: In phase I double-blind randomized controlled trials (SAD and MAD studies), 40 healthy volunteers were given S-equol (10-320 mg for SAD, 10-160 mg twice daily for MAD; synthesized by Girindus Solvay Organics, Kunsebeck, Germany; capsules prepared by Patheon, Toronto, Canada), and it was rapidly absorbed with time of peak plasma concentration (Tmax) ranging from 1.5 to 3 hours after a single dose (Jackson et al., 2011). Plasma area under the curve (AUC) and maximum

concentration (Cmax) increased linearly with dose. At the 20 mg single dose, a crossover study showed that food intake significantly decreased Cmax but not AUC for total S-equol.

In a double-blind randomized controlled trial of 93 non-equol-producing menopausal Japanese women, treatment with S-equol (2 mg, 6 mg, or 10 mg per day, Otsuka Pharmaceutical) for 1 year increased serum and urine concentrations of S-equol in a dose-dependent manner (Tousen et al., 2011).

In an open-label randomized 2-period crossover study of 12 healthy postmenopausal women, a single bolus oral dose of S-equol (10 or 30 mg, SE5-OH tablets, Otsuka Pharmaceutical) was rapidly absorbed and attained high plasma concentrations, with a plasma elimination half-life of 8 hours (Setchell et al., 2009). Three participants were equol-producers (25%) and equol producer status had no effect on S-equol pharmacokinetics.

In a pharmacokinetic safety study in 18 postmenopausal women, treatment with 50 or 100 mg of PhytoSERM (16.7 or 33.3 mg each of genistein, daidzein, and S-equol) resulted in a plasma concentrations of genistein, daidzein, and S-equol peaking at 9, 6, and 4 hours, respectively for the 50 mg dose and 6, 6, and 5 hours, respectively for the 100 mg dose (Hernandez et al., 2017).

Preclinical studies of S-equol: In a toxicokinetic study of S-equol in Sprague-Dawley rats and cynomolgus monkeys, orally-administered S-equol for 28 days did not alter uterine weight or morphology, even at the highest doses tested (1,000 mg/kg for monkeys, 500 mg/kg for rats)(Schwen et al., 2012). However, in ovariectomized rats treated with equol (400 mg/kg in chow) for 3 months resulted in mammotropic effects as measured by a significantly higher number of terminal ducts and type II lobules (Rachon et al., 2008). S-equol weakly induced liver cytochrome P450s *in vivo*, but did not inhibit the major human cytochrome P450s *in vitro*.

Clinical studies with PhytoSERM: In a double-blind randomized controlled phase 1b/2a trial, treatment with PhytoSERM (50 mg/day or 100 mg/day containing equal amounts of genistein, daidzein, and S-equol) was tested in 71 non-cognitively impaired perimenopausal women with at least 1 cognitive complaint and 1 vasomotor-related symptom (Schneider et al., 2019). The PhytoSERM formulation was overall well tolerated at 50 and 100 mg daily doses. Adverse events occurred in 16.7% (n=4) of the people in the placebo group, 39.1% (n=9) in the 50 mg dose group, and 29.2% (n=7) in the 100 mg dose group; 85% were mild and none were severe. Vaginal bleeding occurred in 1 subject (4.3%) in the 50 mg dose group, 3 subjects (12.5%) in the 100 mg dose group, and 0 in the placebo group. Overall, 93% of participants completed the 12-week trial (7% discontinued; 3 in 50 mg group, 2 in 100 mg group).

Reasons for dropout were loss to follow-up (n=4) and withdrawal of consent (out of concern about becoming dependent on the treatment and not being able to obtain it after the trial; n=1). No participant withdrew due to an adverse event. Based on these findings, a daily dose of 50 mg was considered preferable for a phase 2 efficacy trial.

In a safety/pharmacokinetic study of PhytoSERM in 18 postmenopausal women, a single treatment with PhytoSERM (50 mg or 100 mg containing equal amounts of genistein, daidzein, and S-equol) resulted in no adverse events (Hernandez et al., 2017).

Preclinical studies of PhytoSERM: In ovariectomized mice, treatment with S-equol-containing PhytoSERM (10 mg/kg/day) or racemic R/S-equol-containing PhytoSERM (10 mg/kg/day) did not produce any uterotrophic effects (Yao et al., 2013).

Toxicity studies for SE5-OH: SE5-OH is a supplement manufactured by the Otsuka Pharmaceutical Co., Ltd and each tablet contains 5.0 mg of S-equol and smaller amounts of other soy isoflavones (see details under "Sources and dosing"). Several clinical trials testing S-equol have used this product (Aso et al., 2012; Tousen et al., 2011; Jenks et al., 2012). In an acute toxicity study in Sprague-Dawley rats, the oral LD50 for SE5-OH was over 4,000 mg/kg (oral gavage); in the 91-day subchronic study in Sprague-Dawley rats, the no-observed-adverse-effect-level (NOAEL) was 2,000 mg/kg/day (oral gavage), the highest dose tested (Yee et al., 2008). There were no deaths in either the control or S-equol-treated rats throughout the study and there were no remarkable pathologies observed following microscopic or macroscopic examination from any group of rats. No significant differences between groups were observed for water consumption, organ weights, or behavior.

SE5-OH was negative in *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) and in *Escherichia coli* tester strain (WP2uvrA)(Yee et al., 2008). SE5-OH was also negative for chromosome aberrations in Chinese hamster lung cells up to 3,000 µg/ml with and without metabolic activation. SE5-OH treatment at a dose up to 4,000 mg/kg twice daily for 2 consecutive days did not increase micronucleated polychromatic erythrocytes.

Drug interactions: Drug interactions with S-equol are not well-documented. S-equol binds to estrogen receptors so it is likely to interact with drugs that target the estrogen system.

Sources and dosing: S-equol on its own is not available as a supplement or drug in the US. A soy isoflavone product that includes S-equol as an ingredient (PhytoSERM) is under development by Dr.

Roberta Diaz-Brinton's laboratory for menopause symptoms and age-related memory decline (Hernandez et al., 2017). Clinical studies testing S-equol have typically used SE5-OH by Otsuka Pharmaceutical Co., Ltd, Japan, at a dose of 10 mg/day, taken orally (Jenks et al., 2012; Tousen et al., 2011; Aso et al., 2012). This product is manufactured by fermenting soy germ with Lactococcus 20-9217 using a patented and proprietary process (patent number JP3864317B, W02005000004). Each tablet contains 5.0 mg of S-equol, 0.8 mg of daidzein, 1.0 mg of genistein, 2.2 mg of glycitein, 298 mg of protein, 113 mg of fat, 375 mg of carbohydrate, 56 mg of ash, and 110 mg of fiber. In Japan, a supplement containing 60% S-equol is available by Otsuka Pharmaceutical (product name, Equelle).

For many Asian people (50-70%) and a smaller proportion of non-Asian people (20-30%), S-equol is produced in the gut microbiome as a metabolite of dietary soy isoflavone intake (Sekikawa et al., 2019). In an observational study of healthy adults in the US and Australia (89 and 70 subjects, respectively), equol production appeared to be associated with a greater intake of polyunsaturated fatty acids, maltose, and vitamins A and E (Setchell et al., 2013). While the determinant for equol production is the presence of intestinal bacteria capable of catalyzing the conversion from daidzein to S-equol, small differences in dietary micronutrient composition may be important as cofactors in stimulating bacterial activity.

Individuals with plasma levels of S-equol under 40 nM/L (10 µg/L) are classified as "non-equol-producer" while individuals with levels higher than 83 nM/L (20 µg/L) are classified as "equol producers" (Hod et al., 2021). Also, individuals with urine equol concentrations over 1,000 nM/L can also be classified as "equol producers".

In a study in 90 healthy infants, none of the breast-fed 6-month-old infants produced S-equol, while 3.8% and 6.0% of soy and cow milk formula-fed infants were equol producers (Brown et al., 2014). By 3 years old, 50% of the formula-fed infants were equol producers, compared with 25% of breast-fed infants. Thus, equol producer status is developmentally regulated and initially related to diet composition, with a trend for formula feeding favoring S-equol production.

Research underway: There is one double-blind randomized controlled trial testing the effects of S-equol on mitochondrial activity (measured by cytochrome oxidase/citrate synthase activity) in 40 patients with Alzheimer's disease (SEAD2 trial)(NCT03101085). This trial is scheduled to be completed in September 2021.

Search terms:

Pubmed, Google:

- S-equol, equol

Websites visited for S-equol:

- Clinicaltrials.gov
- NIH RePORTER
- Examine.com (soy isoflavones)
- DrugAge (0)
- Drugs.com (0)
- WebMD.com (0)
- PubChem
- DrugBank.ca
- Labdoor.com (0)
- ConsumerLab.com (0)
- Cafepharma (0)
- Pharmapro.com (0)