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.

Licorice Flavonoids

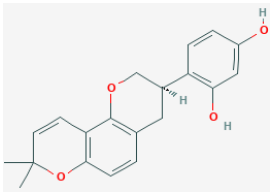
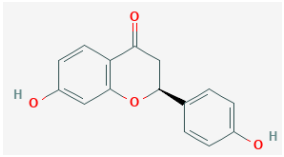
Evidence Summary

Antioxidant rich preparation with a more favorable therapeutic profile than licorice root. Glabridin-rich extracts may have anti-atherogenic and anti-inflammatory properties.

Neuroprotective Benefit: Has antioxidant and anti-inflammatory properties in preclinical models. Liquiritigenin may act as a neuro-selective estrogen agonist. The therapeutic potential of glabridin is limited by low brain levels.

Aging and related health concerns: Glabridin shows anti-atherogenic and cardiovascular protective properties. Liquiritigenin may reduce menopausal symptoms and osteoporosis by acting as a phytoestrogen.

Safety: More favorable safety profile relative to whole licorice root due to the lack of glycyrrhizin, but the safety of chronic long-term use has not been studied. May have drug interactions by inhibiting cytochrome P450 enzymes.

Availability: OTC	**Dose**: Not clinically established	Glabridin **Chemical formula:** $C_{20}H_{20}O_4$ **MW**: 324.4 g/mol  Source: PubChem Liquiritigenin **Chemical formula:** $C_{15}H_{12}O_4$ **MW:** 256.25 g/mol  Source: PubChem
Half-life: glabridin 10 hours liquiritigenin <1 hour	**BBB**: Penetrant	
Clinical trials: Metabolism and hypercholesterolemia for glabridin containing licorice flavonoid oils (range n=12 to n=103); Menopausal symptoms for liquiritigenin containing botanical extract (n=217)	**Observational studies**: None	

What is it?

Licorice root contains approximately 300 bioactive compounds, most of which fall into the chemical classes of triterpenoids and flavonoids [1]. Glycyrrhizin is the major bioactive triterpenoid and is associated with the mineralocorticoid effects of licorice. Therefore, there have been efforts to develop deglycyrrhizinated licorice extracts that are enriched in flavonoids. Clinical studies using these extracts have shown potential metabolic benefits. The **flavonoids primarily act as antioxidant and anti-inflammatory agents.** Although there are hundreds of bioactive flavonoids in licorice, there are only a few that have been well characterized [2].

Glabridin is the best studied licorice flavonoid, and is specific to the licorice species *Glycyrrhiza glabra*. It is an isoflavone located in the cork layer and decayed part of the roots. Glabridin's stability is influenced by temperature, pH, light, and humidity [3]. Isolated glabridin is best stored in a dry, dark area, with low oxygen, and neutral pH. It has different biological effects in different cell types, but it has been most extensively studied with respect to its anti-atherogenic and metabolic regulating activities. There is also

interest in glabridin for skin care products due to its ability to inhibit melanin synthesis by inhibiting the enzyme tyrosinase, thereby acting as a potential skin lightening agent. However, its activity *in vivo* is limited by its low oral bioavailability of approximately 7.5%, and brain levels are impacted by its ability to act as a substrate for the PgP transporter, which promotes its transport out of the brain [4].

Liquiritigenin and isoliquiritigenin are the major components of licorice extracts and considered to be the main bioactive phenols in licorice root [5]. Isoliquiritigenin is an isomeric precursor of liquiritigenin, and the two chemical species are interchangeable by pH and temperature. They are the most brain penetrant of the licorice root flavonoids that have been characterized, and can act as **phytoestrogens** by activating the estrogen receptor (ERβ).

Licochalcones (A/B) are chalconoids that have primarily been studied for their anticancer and anti-inflammatory properties [2]. They have the same caffeic acid scaffold as curcumin.

Neuroprotective Benefit: Has antioxidant and anti-inflammatory properties in preclinical models. Liquiritigenin may act as a neuro-selective estrogen agonist. The therapeutic potential of glabridin is limited by low brain levels.

Types of evidence:

- Several laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function? None

Human research to suggest benefits to patients with dementia: None

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

The neuroprotective effects of licorice flavonoids are primarily attributed to their antioxidant and anti-inflammatory properties [2]. Some of the compounds have additional properties that may enhance cognitive function and/or protect against decline, including anticholinesterase activity, estrogenic activity, and GABAergic activity.

Alzheimer's disease: Potential benefit (preclinical models)

Glabridin pretreatment (4 mg/kg) was able to prevent memory impairments in the mouse model of scopolamine induced cognitive impairment, based on performance on the elevated plus maze and passive avoidance task [6]. The protective effect may stem from glabridin's anticholinesterase activity, as it was able to reduce brain cholinesterase activity to a comparable level as the irreversible cholinesterase inhibitor metrifonate (19% vs 21%).

However, the potential therapeutic efficacy for glabridin in the CNS is limited by its **poor brain distribution** following systemic administration. Glabridin has been found to be a substrate for P-glycoprotein (PgP), which is an ABC drug transporter located in the BBB which transports drugs out of the CNS into the blood [4]. In rats, the brain was the organ system with the lowest glabridin level following oral administration, with max brain levels only reaching up to 27% of plasma levels. Co-administration with a PgP inhibitor (i.e. verapamil) could increase glabridin brain levels between 33 and 143%.

Liquiritigenin and isoliquiritigenin are considered to be phytoestrogens which can act as **neuro-selective estrogen receptor agonists** (neuroSERMs) due to their ability to preferentially activate estrogen receptor beta (ERβ) [5]. Consequently, they are projected to help protect against the decline in estrogen-associated neuroprotection in women that takes place as a result of menopause. In ovariectomized female rats, an isoliquiritigenin supplemented (0.75%) diet was protective against a decline in performance on hippocampus sensitive cognitive tasks similar to estradiol, but had no effect on striatal or frontal cortex associated tasks [7; 8]. The lack of effect was notable, since in this model, supplementation of estradiol is associated with impaired performance on frontal cortex related tasks.

Liquiritigenin (30 mg/kg for 90 days) was also found to be protective in an Alzheimer's disease (AD)mouse model (Tg2576) via anti-inflammatory and anti-amyloid effects [9]. Liquiritigenin treatment reduced oligomeric Aβ by 65%, astrogliosis by 74% and improved performance on learning and memory tasks (Morris water maze, active avoidance task). In rat cortical neurons, isoliquiritigenin had similar efficacy to licorice root extract in protecting against Aβ induced reactive oxygen species (ROS) generation and cell death [10].

Isoliquiritigenin and liquiritigenin are expected to be the primary mediators of the neuroprotective effects associated with licorice root extracts, since they had the **highest BBB permeability rates of all the major compounds found in licorice root**. In an *in vitro* BBB permeability assay, the permeability

rates of isoliquiritigenin and liquiritigenin were 25.9 ± 2.9% and 33.4 ± 0.5%, respectively. The permeabilities of all the other tested licorice-derived compounds were less than 20%.

Licochalcone B derived from *Glycyrrhiza inflata* was shown to inhibit Aβ self-aggregation and metal ion-induced Aβ aggregation in cell culture (SY5Y cells). It was also protective against oxidative stress (H_2O_2) induced cell death via its antioxidant activity [11].

Excitotoxicity: Potential benefit (preclinical models)

Glabridin has been found to potentiate the $GABA_A$ receptor, resulting in an increase in inhibitory neurotransmission [12]. One study found that glabridin had the strongest effect on $GABA_A$ receptors with the α1β2γ2 subunit combination, which is the most abundant form in the CNS [13]. The promotion of inhibitory GABAergic neurotransmission may contribute to the protective effects of flavonoid-rich extracts of *G. glabra* in mouse models of epilepsy [14; 15]. The licorice extract associated mitigation of excitotoxicity has also been attributed to a decline in oxidative stress mediated damage.

Liquiritigenin was able to protect against glutamate mediated neurotoxicity in mouse hippocampal neurons by reducing the production of ROS and pro-apoptotic factors [16].

APOE4 interactions: Unknown

Aging and related health concerns: Glabridin shows anti-atherogenic and cardiovascular protective properties. Liquiritigenin may reduce menopausal symptoms and osteoporosis by acting as a phytoestrogen.

Types of evidence:

- 1 meta-analysis of 26 trials for licorice flavonoid oil products and metabolic effects
- 7 clinical trials (3 clinical trials for deglycyrrhizinated licorice extract on cholesterol n=12, n=22, n=94; 2 trials for licorice flavonoid oil in diabetes and healthy controls n=11, n=17; 3 trials for deglycyrrhizinated licorice extract for ulcer n=47, n=68, n=90; 1 trial for licorice flavonoid containing Menerba n=217)
- Numerous laboratory studies

Metabolism: Glabridin rich extracts potentially reduce body fat mass

A meta-analysis of 26 clinical trials (n=985 participants) testing licorice containing products, primarily licorice flavonoid oil capsules, found that licorice product consumption was **significantly associated with a reduction in body weight** (Weighted mean difference WMD: −0.433 kg, 95% CI −0.683 to −0.183; p = 0.001) and body mass index (BMI) (WMD: −0.150 kg/m^2, 95% CI −0.241 to −0.058; p = 0.001), and the effects were dose related [17].

Most studies have used the Glavonoid® capsules (Kaneka, Japan) which contain a 30% ethanol extract of *G. glabra* with 3% w/w glabridin and 70% medium chain triglycerides (C8:C10 = 99:1) [18]. This preparation enriches for the hydrophobic licorice flavonoids, and glabridin is the major bioactive compound in this preparation. In the majority of these small studies, there was either a statistically significant difference or trend toward decreases in body mass, which were driven by decreases in fat mass [19; 20; 21; 22]. Some of the discrepancies in significance may be dose related, since the studies that saw the best effects were generally those that used levels two or three times higher than the manufacturer recommended dose of 300 mg/day. The participant population may also be a factor. Most studies tested healthy volunteers, and the results were more consistently positive in this population. The protective effects were less consistent in groups with metabolic disturbances, such as those with obesity or diabetes [19; 20; 22; 23]. It is unclear whether higher doses are required to have the same effect size in these groups.

Preclinical studies have found that glabridin's metabolic effects stem from the activation of the kinase AMPK, which stimulates fatty acid oxidation. Mice treated with glabridin containing licorice flavonoid oil showed increased muscle glucose transport, due to the AMPK mediated translocation of the glucose transporter GLUT4 [18]. A small study (n=50) showed an increase in trunk muscle mass in people with knee osteoarthritis following 16 weeks of Glavonoid® supplementation (+0.17±1.1 vs placebo -0.57±1.0 kg) [21]. A separate study examining the effect of Glavonoid® on metabolism in the context of light exercise (30 to 40% VO_2 max) found that supplementation was associated with higher fat oxidation, and the effect was dose dependent [24]. Although the effect sizes were small, these studies support the notion that this mechanism may be relevant to its effects in humans.

Cholesterol: Glabridin rich extracts reduce oxidized LDL

Preparations of deglycyrrhizinated licorice root extract (from *G. glabra*) were found to reduce LDL-cholesterol in three clinical trials. In healthy adults (n=22), treatment with extract (containing 60 mg glabridin) for six months led to a 10% **reduction in LDL-c, which was associated with a reduction in**

oxidative stress (TBARS) [25]. The oxidation level of LDL was reduced by 15% (95% CI 5 to 25%, p=0.008) within 3 months, and decreased by an additional 10% within 6 months, but the effects were not sustained following cessation of treatment. Deglycyrrhizinated licorice root extract was also beneficial in people with hypercholesterolemia (220 to 260 mg/dL). In a pilot study (n=12) licorice extract (0.1 g/day) increased the resistance of plasma LDL against oxidation by 55%, improved retention of chondroitin sulfate binding ability by 25%, reduced plasma LDL by 9%, and reduced systolic blood pressure by 10% [26]. The effects on cholesterol were not sustained, but the decrease in blood pressure was maintained for at least one month following cessation. In a double-blind, placebo controlled RCT (n=94), treatment with deglycyrrhizinated licorice root extract (containing 4 mg glabridin) for 12 months significantly decreased LDL-c (from 183±8.5 mg/dl to 174±9.1 mg/dl), carotid intima-media thickness (from 0.92±0.25 mm to 0.84±0.21 mm), systolic blood pressure (from 138±12 mmHg to 125±13 mmHg) and diastolic blood pressure (from 92±9 mmHg to 84±10 mmHg), while these measures either increased or did not change with placebo [27].

Menopausal symptoms: Potential benefit for liquiritigenin rich extracts

Phytoestrogens isoliquiritigenin and liquiritigenin are hypothesized to be protective against menopausal symptoms stemming from changes in estrogen levels, such as hot flashes or night sweats [28]. The botanical extract Menerba (MF101) is comprised of 22 herbs and acts as a selective estrogen receptor modulator [29]. Liquiritigenin was identified as the most active estrogenic compound in the extract, and exerted its effects by acting as an agonist at ERβ. It had been in clinical development for this indication, but development has been discontinued due to the bankruptcy of the company sponsoring the Phase 3 trial (Bionovo). In the positive Phase 2 RCT (n=217), the proportion of women treated with the highest dose (10 g/day) who had a 50% reduction in hot flashes was 16% higher than the placebo group, while there were no significant effects in the low dose group (5 g/day) [30].

Osteoporosis/Osteoarthritis: Potential benefit for (iso)liquiritigenin (preclinical models)

Preclinical studies provide evidence for a potential protective role for isoliquiritigenin and liquiritigenin in the context of inflammation related bone loss. In cell culture, they have been found to **inhibit the differentiation of osteoclasts**, which reabsorb bone tissue, and promote the differentiation of osteoblasts, which promote bone formation [31]. Mechanistically, isoliquiritigenin and liquiritigenin inhibit NF-kB activation and signaling, which in turn, reduces expression of the transcription factor NFATc1, a master regulator of osteoclast differentiation [31; 32]. Isoliquiritigenin was protective in an LPS model of inflammatory bone loss in mice by inhibiting osteoclasts [32].

Isoliquiritigenin (40 mg/kg) was also protective in a mouse model of osteoarthritis by inhibiting inflammation driven osteoclastogenesis and inhibiting aberrant bone angiogenesis [33]. These processes led to a reduction in aberrant subchondral bone remodeling, cartilage calcification, and osteoarthritis progression. The aberrant remodeling of the underlying subchondral bone is thought to trigger the calcification of the cartilage in the joint and drive osteoarthritis.

Glabridin has also been identified as a phytoestrogen and was found to prevent the loss of bone producing osteoblasts in the context of inflammation *in vitro* [34]. However, glabridin (5 mg/kg) failed to show benefit in ovariectomized female rats [35], suggesting that the effects may not be relevant *in vivo* at the concentrations attainable through systemic administration, perhaps due to low bioavailability.

Cancer: Unclear benefit

Numerous studies have found that all of the major licorice flavonoids (licochalcones, isoliquiritigenin/liquiritigenin, glabridin) show anti-cancer activity in cell culture models using cancer cell lines [2]. The anti-proliferative effects are related to the ability of these flavonoids to affect cell cycle progression, the expression of anti- and pro-apoptotic factors, and oxidative stress [36]. Benefits have also been shown in mouse xenograft cancer models, however, due to the low translatability of these preclinical models, it is unclear whether these effects would be clinically meaningful in humans. There is currently no epidemiological or clinical evidence that licorice flavonoid consumption is protective against cancer.

Ulcer: No benefit

Carbenoxolone, a synthetic derivative of glycyrrhizin, is used for the treatment of duodenal and gastric ulcers, suggesting that potential therapeutic benefits of licorice for ulcers is related to its glycyrrhizin content. Multiple double-blind, placebo-controlled clinical trials have found that deglycyrrhizinated licorice extract showed no clinical benefits for duodenal ulcers [37; 38; 39].

Anti-microbial/Anti-viral: Potential benefit (*in vitro* models)

Although much of the anti-microbial/anti-viral activity of licorice has been attributed to glycyrrhizin, some *in vitro* studies suggest that the licorice flavonoids may also contribute to these effects. Clinical trials have found that licorice root extract containing products can reduce levels of *Streptococcus mucans*, a bacterium associated with dental cavities [40; 41]. Deglycyrrhizinated licorice root extract (derived from *G. uralensis*) was shown to be able to inhibit *Streptococcus mucans* biofilm formation [42]. This effect is likely mediated by liquiritigenin and isoliquiritigenin as their anti-bacterial activity has been

replicated in other studies, and *in vitro* studies suggest that they can enhance the efficacy of β-lactam antibiotics [2].

The licorice flavonoids may also drive the anti-viral effect of licorice root extract toward the influenza virus [43]. Neuroaminidase inhibitors are the first line drugs used for influenza, since neuroaminidase A is an important enzyme in viral replication. Virtual screening identified multiple flavonoids in licorice root (*G. glabra*) with neuroaminidase inhibitor activity [44].

Safety: More favorable safety profile relative to whole licorice root due to the lack of glycyrrhizin, but the safety of chronic long-term use has not been studied. May have drug interactions by inhibiting cytochrome P450 enzymes.

Types of evidence:

- 1 meta-analysis of 26 studies for licorice flavonoid products
- 1 clinical trial in healthy volunteers for safety and PK n=117
- Numerous laboratory studies

Licorice flavonoids have a superior safety profile relative to complete licorice root because of the absence of glycyrrhizin, which is the mediator of licorice's mineralocorticoid related side effects. In preclinical toxicology studies, licorice flavonoid oil did not show signs of genotoxicity in cell culture or in rats at doses up to 5000 mg/kg [45]. Based on a 90-day toxicology study in rats, the no-observed-adverse-effect-level (NOAEL) was determined to be 800 mg/kg/day for female rats and 400 mg/kg/day for male rat, due to a sex-related difference in anticoagulation effects [46]. In a single-blind, placebo-controlled safety study in healthy volunteers (n=117), the NOAEL was determined to be 1200 mg/day [47]. Plasma glabridin levels had a linear dose relationship, and reached a steady state within 2 weeks of daily dosing. At 2 weeks, there were significant changes in platelet count for the highest dose (1200 mg/day), but the changes were not considered clinically significant. Overall, there were no clinically relevant changes in hematological or biological parameters. Clinical trials examining the metabolic effects of licorice flavonoid oil reported good tolerability and a lack of serious adverse events.

Although the glycyrrhizin content of deglycyrrhizinated licorice extract containing products such as licorice flavonoid oil is generally very low (less than 0.005% in Glavonoid®), it may vary across supplements due to differences in the extraction process and a lack of regulatory standardization.

Therefore, a small risk for glycyrrhizin mediated mineralocorticoid effects remains a possibility with use of these products.

In *in vitro* systems, extracts from licorice root (*G. glabra*, *G. inflata*, *G. uralensis*) were found to inhibit cytochrome P450 enzymes, suggesting that they may interfere with drug metabolism [48]. Since the different flavonoids were found to differentially inhibit different CYP enzymes to varying degrees, different licorice flavonoid based products would be expected to have different drug interactions. It is not yet known if licorice flavonoid supplements inhibit CYP enzymes in a clinically meaningful manner.

Sources and dosing:

Deglycyrrhizinated licorice extract is sold as a supplement. Glavonoid® from Kaneka (Japan) has been the most clinically tested licorice flavonoid extract. It was granted generally recongnized as safe (GRAS) status by the FDA in 2008 and Novel Food Status by the European Commission in 2011. It is primarily available from Japanese and European suppliers. It is comprised of an ethanol extract of *G. glabra* containing 3% w/w glabridin and the formulation is a mixture of 30% extract with 70% medium chain triglycerides (MCTs) (C8:C10 = 99:1) [18]. The licorice flavonoid oil containing MCTs has higher dissolved flavonoid content than powdered licorice ethanolic extract. The manufacturer recommended dose is 300 mg/day, however, in clinical studies, potential benefits were more apparent at higher doses. There is currently no established clinically effective dose for any indication.

The phenolic content of the licorice varies depending on species, growing and harvesting conditions, and extraction methods. *G. uralensis* has the highest flavonoid content, and contains the highest levels of liquiritigenin and isoliquiritigenin [49]. While only *G. glabra* contains glabridin. *G. glabra* harvested in Spring (May) and late Fall (November) were found to have the most potent antioxidant activity, with Spring harvested licorice having high liquiritigenin content, whereas glabridin was highest in Fall harvested licorice [50]. Due to high solubility in alcohols, ethanolic based extracts have higher concentrations of licorice flavonoids than aqueous extracts.

Research underway:

There are currently no clinical trials for licorice flavonoids or deglycyrrhizinated licorice on Clinicaltrials.gov. There are various groups working on methods to enhance the solubility and bioavailability of the major licorice flavonoids, such as through nanoparticle encapsulation.

Search terms:

Pubmed, Google: Licorice flavonoids, deglycyrrhizinated licorice, glabridin, liquiritigenin, isoliquiritigenin, licochalone

- Alzheimer's disease, neurodegeneration, BBB, GABA, metabolism, atherosclerosis, cancer, estrogen, menopause, osteoporosis, ulcers, anti-microbial, clinical trials, safety

Websites visited for Licorice Flavonoids:

- Examine.com (Licorice)
- PubChem Glabridin, Liquiritigenin

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