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.

L-Ergothioneine

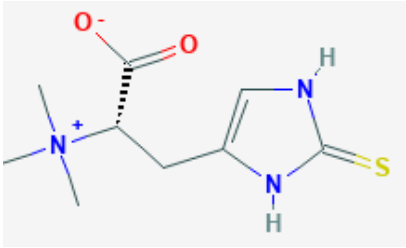
Evidence Summary

Protects against oxidative stress damage in preclinical studies, but very limited human data. Provided minor improvements in kidney function and diabetes associated anemia, and has strong safety profile.

Neuroprotective Benefit: No human studies on cognitive function have been performed. Preclinical models and decreased serum levels in patients with neurodegenerative disease suggest potential neuroprotection against oxidative stress damage.

Aging and related health concerns: Few human studies, which were not well-powered or well-designed, have been performed. Most potential is in combinatorial therapy for prevention against oxidative damage and in slowing progression in kidney disease.

Safety: Safe with no reported toxicity at very high doses far exceeding those obtained through food or supplements. Possible concern with drug interactions, especially metformin, gabapentin, and chemotherapeutics.

Availability: OTC and in diet (mushrooms).	**Dose:** 5-30 mg daily based on supplement supplier claims. Therapeutic dose not established.	**Chemical formula:** $C_9H_{15}N_3O_2S$ **MW:** 229.298 g/mol  Source: Pubchem
Half-life: Whole-body half-life 1 month in rat. Stored in cells/tissues. Tissue concentration dependent on transporter expression. Minimal metabolism, excreted as free sulfate and hercynine.	**BBB:** Not membrane permeable. Enters cells through OCTN1 transporter. Cells in brain (including neurons) express OCTN1 and take up l-ergothioneine.	
Clinical trials: 1 planned for AD (anticipated completion 2021)	**Observational studies:** 6 studies of l-ergothioneine levels in serum.	

What is it? L-ergothioneine is a sulfur containing (metabolite of histidine) diet derived amino acid. It is synthesized in Actinomycetales bacteria and non-yeast fungi. The highest concentrations of l-ergothioneine are found in mushrooms, particularly Boletus edulis (porcini mushroom, 528.14 mg/kg) and Pleurotus ostreatus (oyster mushroom, 118.91 mg/kg) [1]. The l-ergothioneine derived from mushrooms has been shown to be bioavailable (taken up by red blood cells) within one hour of consumption. It is also found in relatively high concentration in liver (chicken, 10.78 mg/kg), black turtle beans (13.49 mg/kg), red kidney beans (4.52 mg/kg), and oat bran (4.41 mg/kg).

L-ergothioneine is rapidly cleared from circulation and retained in cells/tissues [2]. It is minimally metabolized, possibly by an oxidative degradation mechanism into hercynine and free sulfate before being excreted [3]. L-ergothioneine is taken up by cells that express the organic cation / carnitine transporter 1 (OCTN1), expressed by the *SLC22A4* gene [4]. It is membrane impermeable to cells that lack the transporter, consequently, ergothioneine tissue levels correspond well with OCTN1 expression levels. The highest expression is in: erythrocytes, monocytes, lung, intestine, trachea, kidney, and brain [4; 5; 6]. Since the tissue expression pattern of OCTN1 is species specific, some animal studies may not be translatable to humans. While OCTN1 preferentially transports l-ergothioneine, it does not exclusively transport l-ergothioneine. Therefore, the phenotypes from OCTN1 knockout studies cannot be conclusively attributed to the loss of l-ergothioneine uptake.

L-ergothioneine is currently available as an OTC supplement, usually in combination with other vitamins/nutraceuticals, particularly Vitamin D (Entia Biosciences). It is also in several skincare and haircare products to prevent skin aging and promote hair growth, respectively. It is currently being developed for use in edibles (cakes, cookies, pastries, coffee, tea, fruit drinks, soft drinks, candy at 5 mg/serving) by Tetrahedron (France), and Blue California (USA).

Neuroprotective Benefit: No human studies on cognitive function have been performed. Preclinical models and decreased serum levels in patients with neurodegenerative disease suggest potential neuroprotection against oxidative stress damage.

Types of evidence:

- 2 observational studies (l-ergothioneine serum levels)
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

There have been two observational studies examining blood serum levels of l-ergothioneine in individuals with neurodegenerative diseases [7; 8]. Both studies found that in comparison to healthy age-matched controls, patients with neurodegenerative diseases had lower levels of l-ergothioneine.

Mild Cognitive Impairment (MCI): Decreased levels. Individuals with MCI (n=25) were compared to age-matched individuals without signs of cognitive impairment (n=25) [7]. Individuals with MCI had lower whole blood levels of l-ergothioneine. This difference was not related to differences in mushroom consumption or differences in OCTN1 transporter expression. Decreased/altered OCTN1 transporter activity in the MCI cohort was offered as a possible explanation.

Parkinson's Disease (PD): Decreased levels. Patients with idiopathic PD without dementia (n=35, 49% female) were compared to age-matched healthy controls (n=15). PD patients had lower serum levels of l-ergothioneine [8]. Notably, other altered metabolites include: tryptophan, caffeine, bilrubin, and biliverdin. This suggests there may be a disruption in the balance of redox regulators, which could contribute to an increased susceptibility to oxidative damage.

Human research to suggest benefits to patients with dementia:

Currently, there is no evidence to suggest that l-ergothioneine treatment benefits patients with dementia. A planned clinical trial (NCT03641404) is aimed at addressing this question [9].

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

Ergothioneine is an antioxidant, however, it does not act like a conventional reactive oxygen species (ROS) scavenger or alkylthiol, and the molecular mechanism(s) by which it exerts its antioxidant activity is not fully understood.

Protection against oxidative stress:

In vitro assays using physiologically relevant concentrations of ergothioneine (based on tissue concentration of 1-2 mM) indicate that ergothioneine can inhibit the formation of free radicals (oxidative damage) following the interaction of transition metal ions (Copper and Iron) with peroxide [10]. Ergothioneine was shown to exhibit dose-dependent protection against DNA and protein oxidation by stabilizing copper in the Cu(I) state and forming a redox inactive ET-Cu(I) complex [11]. The strongest protection occurred in relation to heme protein associated oxidative damage, which is consistent with its high expression in erythrocytes [10].

Rat PC12 cells were protected against $A\beta_{25\text{-}35}$ induced apoptosis by treatment with l-ergothioneine (0.5 or 1mM ET for 36 hours) [12]. The protective effect was attributed to the suppression of peroxynitrite formation and nitration of tyrosine residues. It is expected to be most effective when used in combination with other types of antioxidants (i.e. conventional ROS scavengers).

The promoter for the ergothioneine transporter, OCTN1, was shown to be driven by the inflammatory mediators IL-1β, TNFα, and NF-κB in luciferase and gel shift assays [13]. OCTN1 expression is also independently regulated by the transcription factor RUNX1, which is involved in hematopoietic stem cell differentiation [13]. Both OCTN1 expression and ergothioneine tissue levels increased in a Guinea pig model of liver disease in response to rising cholesterol and iron levels/tissue damage [14]. Knockdown (RNAi) of OCTN1 in HeLa cell reduced ergothioneine uptake, increased H_2O_2 mediated toxicity, protein carbonylation, and lipid peroxidation [15]. Pretreatment of control cells with ergothioneine (1mM for 24 hours) rescued H_2O_2 induced toxicity. Ergothioneine preferentially protected water-soluble proteins from oxidative damage. Additionally, OCTN1 knockout mice showed lower tolerance to oxidative stress, as indicated by decreased survival in an intestinal ischemic injury model [6]. This work suggests that OCTN1 is induced in response to inflammation/cellular damage to increase transport of a cytoprotective agent(s). Ergothioneine may be the cytoprotective agent, but there is no conclusive evidence to support this hypothesis.

Neuroprotection:

The uptake of ergothioneine by mouse neural progenitor cells (NPCs) decreased mitochondrial activity (less ROS generation) and neurosphere area (decreased proliferation) [16]. It also promoted neuronal differentiation in an OCTN1 dependent manner. Mice on diets supplemented with mushroom extract (containing 1.2% ergothioneine) had increased doublecortin+ cells in the brain, indicating a possible effect on neuronal differentiation [17]. The authors hypothesize that ergothioneine could be neuroprotective by promoting the differentiation of NPCs in the context of neuronal damage.

In a rat NMDA-mediated excitotoxicity induced model of retina degeneration, i.p. injection (0.2 ml 70 mg/ml) of l-ergothioneine at the time of injury decreased retinal ganglion cell loss from 81% to 44% [18]. However, their sample size (n=3) is insufficient to account for the variability in this model and the neuroprotective effect may be overestimated.

ErgoD2 (4000 IV Vitamin D + 3mg ergothioneine per serving) was tested in a preclinical study for its ability to reduce indicators of PD activity [19]. The stated preliminary findings indicate improved grip strength of PD mice and reduced levels of alpha-synuclein in the midbrain, however, the results have not been published and the status of the study is unknown.

Cognitive function:

Ergothioneine pre and post treatment (0.5 mg/kg daily) was found to be protective in mice against $A\beta_{1-40}$ [20] or D-galactose [21] mediated neurotoxicity. Ergothioneine treatment decreased latency (improved performance) on avoidance and water maze tests, prevented Aβ accumulation, prevented brain lipid peroxidation, restored acetylcholine esterase activity, and maintained the glutathione ratio and SOD activity in the brain. In the D-galactose model, ergothioneine was also combined with melatonin (10 mg/kg) and found to have a synergistic effect [21]. This combinatorial effect is hypothesized to result from increased antioxidant activity, since ergothioneine and melatonin are different types of antioxidants.

APOE4 interactions: Unknown

Aging and related health concerns: Few human studies, which were not well-powered or well-designed, have been performed. Most potential is in combinatorial therapy for prevention against oxidative damage and in slowing progression in kidney disease.

Types of evidence:

- 4 clinical trials
- 4 observational studies examining l-ergothioneine blood levels
- Numerous laboratory studies

Human studies:

Aging: Decreased levels. This study involved community-dwelling adults aged 55-85 (n=439, 48% female) in Newcastle, Australia [22]. Participants were classified as healthy and unhealthy based on the presence of a chronic medical condition. Serum ergothioneine levels were inversely correlated with age. Levels were slightly, but not significantly, higher in males, and not significantly affected by health status, body mass index (BMI), L-cysteine-L-glycine (involved in thiol redox homeostasis) levels, or tau levels. An inverse relation between serum ergothioneine levels and age also found in a cohort of adults in Singapore [7].

Disease susceptibility: Potential increased risk with low levels. Expression/activity of OCTN1 can influence disease susceptibility. Polymorphisms in the OCTN1 gene (SLC22A4) (rs273909 (T→C)) are associated with susceptibility to ischemic stroke and chronic kidney disease progression (Japanese cohort) [23]. The L503F variant shows altered substrate specificity [24] and is associated with Crohn's disease in Caucasians [25]. Crohn's disease patients (Japanese cohort) have been shown to have decreased blood levels of ergothioneine [6]. Progression of chronic kidney disease (CKD) (Japanese cohort) also correlated with decreasing blood levels of ergothioneine, with levels being restored following kidney transplant [26].

Clinical/intervention studies

Exercise: Potential minor benefit/Unclear Healthy men in Poland (n=14) consumed a diet supplemented with Shitake mushroom extract (700 mg 2x/day, estimated ergothioneine intake 2.77 mg) for 10 days [27]. Following exercise designed to induce skeletal muscle damage, ergothioneine supplementation increased thiol redox status and nitric oxide (NO) concentration, but had no other measurable effects on inflammation or antioxidant activity.

Diabetes/Anemia: Potential minor benefit/Unclear In a clinical study sponsored by Entia Biosciences (manufacturer of ErgoD2), ErgoD2 Hemo (Vitamin D + l-ergothioneine) was tested for its ability to alleviate diabetes associated anemia. The company claims that ErgoD2 stimulated iron export from cells and increased red blood cell production, but results have not been published.

Joint pain: Potential minor benefit/Unclear In a clinical study sponsored by OXIS International, the manufacturer of ErgoFlex (glucosamine, hyaluronic acid, glucosaminoglycans, collagen, acai, cat's claw, white willow bark, and 500 ug ergothioneine), individuals with range of motion (ROM) limiting joint pain (n=12, 50% female) received 2 capsules ErgoFlex/day for 6 weeks [28]. Supplementation showed slightly significant improvements in joint range of motion (ROM) during the 6-12-week period, and decreased perceived pain in primary and secondary areas at use within 1 week and sustained for 12 weeks. All changes were relative to baseline and could be attributed to the placebo effect. Since the supplement contains multiple ingredients, these effects can also not be clearly attributed to ergothioneine.

Kidney disease: Potential benefit. In an ongoing clinical study sponsored by Entia Biosciences (manufacturer of Ergo4Health), patients with CKD (n=60) are being treated with 500mg capsules of Ergo4Health /Kidney (0.75mg l-ergothioneine +1250 IU Vitamin D2/capsule) 2x daily [29]. The reported results for 3 and 6 months (Bonaire site only) indicate moderate improvement in the estimated glomerular filtration rate (eGFR), decreased creatine and blood urea nitrogen levels at 3 months (not significant at 6 months), and improved quality of life (survey).

Animal/cell studies

Lifespan: Unknown. A *C. elegans* study showed that worms with mutant OCT-1 had decreased (mean and max) lifespan and decreased survival in response to an oxidative stress challenge [30]. However, these mutant worms were still able to uptake ergothioneine, suggesting OCT-1 is not the correct transporter homolog or the effects are due to another function of transporter. Therefore, this study does not provide meaningful information about the role of ergothioneine in longevity.

Skin/UV damage: Potential benefit (cell culture) OCTN1 was shown to be expressed in skin cells and capable of ergothioneine uptake. Ergothioneine pretreatment (10, 50, or 100 uM for 24 hours) protected cells against UV induced apoptosis, DNA fragmentation, ROS generation, lipid peroxidation. This study has been used as the basis for the use of l-ergothioneine in skincare products.

Ischemic injury: Potential benefit (rodents). In a rat model of ischemia and reperfusion, serum inflammatory and oxidative stress markers (TNFα, IL-1β, MDA, MPO) were reduced and Hsp70 levels increased by ergothioneine pretreatment (10 mg/kg for 15 days prior to injury)[31]. There was also less histological evidence of tissue damage at 4 hours after reperfusion suggesting possible cytoprotection for ischemic injury. Results are consistent with a connection between OCTN1 polymorphisms and ischemic stroke susceptibility.

Kidney Disease: Potential benefit (rodents). Ergothioneine (70 mg/kg 7 days pretreatment) protected against lipid peroxidation of some fatty acids (22:6, 20:3 n6, 20:4, 18:2, 18:1) in the kidney and liver, significantly reduced levels of conjugated dienes and conserved the concentrations of α-tocopherol and glutathione in the kidney and liver in rats with ferric-nitrilotriacetate induced oxidative damage [32].

In a mouse model of CKD, the intestinal uptake of ergothioneine by OCTN1 was impaired, leading to lower blood levels. OCTN1 knockout mice with CKD had worse kidney fibrosis and increased oxidative stress damage [26].

Diabetes: Potential benefit (cell culture) In a model of hyperglycemic cytotoxicity in PC12 cells, treatment with ergothioneine, the PKC inhibitor hispidin, or the combination prevented hyperglycemia induced cytotoxicity, and increased advanced glycation endproducts (AGE), the AGE receptor (RAGE) and NF-κB, which are involved in inducing pro-inflammatory genes [33]. There was a synergistic effect on antiglycation activity/inhibiting AGE formation, but not on antioxidant activity (ROS, protein carbonyl levels), due to possible inhibitory effect of ergothioneine on hispidin activity *in vitro*.

Ergothioneine pretreatment (0.01-1 mM for 12 hours) protected endothelial cells from high-glucose induced cytotoxicity and cell senescence, and reduced ROS production. Cytoprotective effect was mediated through the upregulation of SIRT1 and SIRT6 and downregulation of p66Shc and NF-κB [34].

Safety: Safe with no reported toxicity at very high doses far exceeding those obtained through food or supplements. Possible concern with drug interactions, especially metformin, gabapentin, and chemotherapeutics.

Types of evidence:

- 1 observational study
- Numerous laboratory studies

Mutagenesis: Bacterial mutagenesis assays [35] and cell culture genotoxicity assays [36] have indicated no mutagenic activity.

Toxicity: Mice treated with doses of l-ergothioneine up to 1500 mg/kg (Human equivalent dose (HED)= 122 mg/kg) showed no treatment-associated mortality, but did exhibit decreased activity at the highest dose (1500 mg/kg) [36]. No clinical signs of toxicity were demonstrated at lower doses (375, and 750 mg/kg).

Rats treated with oral l-ergothioneine daily for 90 days (400, 800, 1600 mg/kg) showed no associated mortality, changes in food consumption or body weight, or macroscopic changes at necropsy [37]. At high doses there was some intermittent alopecia and minor hematological changes considered non-adverse. Hematological changes were generally dose and gender specific. The no-observed-adverse-effect-level (NOAEL) of 800 mg/kg/day (HED= 129 mg/kg) used in safety assessments by the FDA and EFSA came from this study.

No reproductive toxicity was demonstrated in a rat study (diet contained with up to 0.9% l-ergothioneine). No effects were observed on mating, reproduction performance, lactation, duration of gestation, fertility, size of pups, litter size, cannibalization of pups, or litter sex-ratio [38].

One human safety study has been conducted. 25 healthy men (Chinese ethnicity n=45, aged 21-35) were treated with oral placebo, 5mg or 25 mg l-ergothioneine daily for 7 days [39]. Plasma ergothioneine levels varied widely, possibly due to polymorphisms in OCTN1. No adverse effects were reported.

FDA and EFSA guidelines:

L-ergothioneine from OXIS International was granted Generally Recognized as Safe (GRAS) status by the FDA in 2011 [40]. L-ergothioneine from Blue California (fermentation based product) was granted GRAS status by the FDA in 2018 for use in foods at 5 mg/serving [41]. Consumption of products based on company recommendations expected to produce total daily exposure to l-ergothioneine of 0.633 mg/kg/day for adults.

L-ergothioneine (Ergoneine) from Tetrahedron was determined to be safe by the European Commission EFSA panel in 2016 for use in food at 5 mg/serving and as supplements at 30 mg/day for adults and 20mg/day for children [42]. Total daily exposure based on company recommendations not expected to exceed 1.7 mg/kg/day. Margin of safety determined to be 470 mg/kg/day for adults and 216 mg/kg/day for children. Also considered safe for infants, toddlers, and pregnant women.

Ergothioneine is classified as likely safe by WebMD.

Drug interactions

The l-ergothioneine transporter, OCTN1, is also capable of transporting other drugs (tetraethylammonium (TEA), verapamil, gabapentin, oxaliplatin, donepezil, metformin and phenformin) and endogenous biological molecules (carnitine, acetylcholine, betaine), albeit at lower affinity [43]. High levels of l-ergothioneine could potentially affect the ability of the transporter to transport other drugs.

Biguanides: Polymorphisms in OCTN1 and/or OCTN1 cellular expression levels can also affect the transport of these molecules. The OCTN1 L503F variant (prevalent in Caucasians) increases transport of biguanides, such as metformin, while the I306T variant reduces transport of gabapentin [43].

Consumption of a high Shitake mushroom (250 g 3x/day) diet at a level that elevated blood l-ergothioneine levels (425 ng/ml vs 172 ng/ml in controls) impacted renal clearance of gabapentin but did not affect other gabapentin pharmacokinetics [44].

Chemotherapeutic agents: OCTN1 is capable of transporting nucleoside analogs, such as cytarabine (chemotherapy agent). Low expression of the OCTN1 gene (SLC22A4) is associated with worse survival in acute myeloid leukemia (AML) following chemo, and is a predictor of treatment response [45]. OCTN1 also transports the chemotherapeutic agent oxaliplatin into neurons, which can lead to peripheral neuropathy in some patients. In a rat study, supplementation with ergothioneine (15 mg/kg) reduced oxaliplatin-associated neuropathy [46]. Consequently, ergothioneine supplementation could potentially impact responsiveness to some chemotherapy agents.

Anti-tuberculosis (or anti-fungal) drugs: L-ergothioneine plays a critical role in maintaining redox balance in Mycobacterium tuberculosis (TB) [47]. Strains that can't utilize l-ergothioneine have decreased virulence and are more susceptible to anti-TB drugs. L-ergothioneine is also required by some opportunistic fungal pathogens [48]. Therefore, supplementation with ergothioneine could impact (weaken) the efficacy of anti-TB or anti-fungal drugs.

Sources and dosing:

L-ergothioneine is currently available on the market in the US and Europe. Blue California (US) is developing its fermentation based l-ergothioneine and Tetrahedron (France) is developing its product Ergoneine for use in food and beverage products. The few clinical intervention studies performed have involved l-ergothioneine in combination.

ErgoD2 (Entia Biosciences) (4000 IU Vitamin D3 + 3 mg Ergothioneine per serving) is available from Total Nutraceutical Solutions (TNS), and is also available as part of GlucoSANO Diabetes Health Formula (ErgoD2 + blend of medicinal mushrooms).

Ergo4Health (Entia Biosciences) (0.75 mg l-ergothioneine +1250 IU Vitamin D2/capsule) 2 capsules/day. Ergo4Health has 3.4/5.0 star rating from Consumer Health Digest.

L-ergothioneine is also available in a variety of skincare products. Entia Biosciences has a line of products (Groh) that use the ErgoD2 formulation.

Timing:

The OCTN1 transporter (*SLC22A4*) is regulated in a circadian manner. Slc22a4 is a PPARα-regulated gene and in mice its intestinal expression exhibited circadian oscillations in a bile acid–dependent manner (protein levels peak before start of active/feeding phase) [49]. Consequently, there were dose timing dependent changes in OCTN1 substrate (gabapentin) intestinal uptake, with higher uptake at times with higher OCTN1 expression. This suggests that the time the l-ergothioneine is taken could affect its uptake/efficacy. This work also calls into question the reliability of studies looking at levels of serum l-ergothioneine in different populations if the samples were collected at different times of day in different patients/groups.

Research underway:

There is 1 clinical trial planned (anticipated completion by 2021, not yet recruiting) entitled 'Investigating the Efficacy of Ergothioneine to Delay Cognitive Decline' (NCT03641404) [9]. This study is sponsored by the National University Hospital, Singapore. It includes males and females aged 60-90 years of Chinese ethnicity in Singapore with MCI and no other severe conditions. Participants will take oral capsules containing 25mg l-ergothioneine or placebo (99% cellulose) 3X weekly (M, W, F) for 52 weeks. Primary outcomes are changes on cognitive assessment tests. Secondary outcomes include imaging and biomarker changes.

Search terms:

Pubmed, Google: L-ergothioneine +

aging, dementia, Alzheimer's, safety, tissue distribution, lifespan, oxidative stress, clinical trials, diabetes, kidney, human, mushrooms, transporter, cancer, diet, supplements, inflammation, mechanism

Websites visited for l-ergothioneine:

- Clinicaltrials.gov
- PubChem

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