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.

DHED

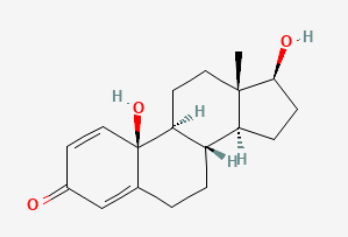
Evidence Summary

DHED, a prodrug that converts to 17β-estradiol exclusively in the brain, has therapeutic potential for cognitive decline, menopausal symptoms, and ischemia, but all evidence comes from preclinical studies.

Neuroprotective Benefit: DHED improved cognitive function in rodent models of aging/menopause and AD and showed neuroprotective benefits in models of PD and brain injury, but it has not been tested in people yet for any indication.

Aging and related health concerns: DHED has therapeutic potential for ischemia, hot flushes, ocular health, and depression, without concerns for breast, uterine, and ovarian cancers, but no studies have tested it in humans.

Safety: Because DHED does not increase circulating estradiol levels, it does not carry cancer (breast, uterine, ovarian) and cardiovascular risks, though no evidence is available yet in humans.

Availability: research grade only	**Dose**: not established	**Chemical formula:** $C_{18}H_{24}O_3$ **MW**: 288.4  Source: PubChem
Half-life: not documented	**BBB**: penetrant	
Clinical trials: none available	**Observational studies**: none available	

What is it? DHED (10β, 17β-dihydroxyestra-1,4-dien-3-one) is a small-molecule bioprecursor prodrug that converts to 17β-estradiol in the brain after systemic administration, but remains inert in the rest of the body (Prokai et al., 2015). This is because DHED is a substrate for an enzyme (SDR; short-chain NADPH-dependent dehydrogenase/reductase) that is selectively expressed in the brain. DHED is inactive as an estrogen because it has no measurable affinity to the classical estrogen receptors. Because female hormones have wide-ranging effects throughout the body, chronic use of estrogens to treat menopausal symptoms can have adverse effects on peripheral tissues, including cancers of breast and reproductive organs (Taylor and Manson, 2011). Because of the brain-specificity, DHED capitalizes on estrogen's neuroprotective effects without the harmful peripheral side effects. Thus, the translational potential of DHED is being evaluated in the hopes that it may more safely treat neurological and psychiatric symptoms in women undergoing early and surgical menopause.

Neuroprotective Benefit: DHED improved cognitive function in rodent models of aging/menopause and AD and showed neuroprotective benefits in models of PD and brain injury, but it has not been tested in people yet for any indication.

Types of evidence:

- 6 laboratory studies (2 in a mouse model of Alzheimer's disease, 2 in models of Parkinson's disease, 1 in a model of aging/menopause, and 1 in a rat model of brain injury)

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function: None available.

Human research to suggest benefits to patients with dementia: None available.

Mechanisms of action for neuroprotection identified from laboratory and clinical research: Systemically administered DHED is rapidly taken up by the brain and conversion to 17β-estradiol occurs in all brain areas, with the cortical areas showing the highest levels (Prokai et al., 2015).

In a mouse model of Alzheimer's disease (APPswe/PS1dE9 mice), DHED treatment (osmotic minipump, 2 µg/day) for 8 weeks reduced brain Aβ levels and improved cognitive performance (Tschiffely et al., 2016). Treatment started at 6 months of age when behavioral deficits first appear in this model. The magnitude of changes in Aβ levels were comparable to the effects seen with 17β-estradiol.

In the same Alzheimer's mouse model (APPswe/PS1dE9 mice), DHED treatment (2 µg/day, s.c. via osmotic minipump) for 2 months decreased amyloid precursor and amyloid-beta protein levels and enhanced performance on a cognitive task (radial arm water maze), while 17β-estradiol failed to reach statistical significance (Tschiffely et al., 2018).

In a mouse model of Parkinson's disease (alpha-synuclein tetramer-abrogating 3K mice), DHED treatment (100 µg/kg/day, s.c. in corn oil) for up to 90 days significantly increased brain estradiol levels, increased the tetramer-to-monomer ratio of alpha-synuclein, increased turnover by autophagy of aggregate-prone monomers, and improved neurite complexity of surviving dopaminergic and cortical neurons, while improving motor performance measured by the rotarod test (Rajsombath et al., 2019).

In a different mouse model of Parkinson's disease (MPTP-injected mice), DHED treatment (50 and 100 µg/kg/day, s.c.) for 4 weeks significantly mitigated behavioral impairments and dopaminergic neurodegeneration (Thadathil et al., 2021). DHED treatment also decreased oxidative stress and inflammation in the striatum compared to vehicle-treated mice.

In a rat model of aging and menopause (middle-aged and ovaries surgically removed), continuous administration of DHED (osmotic minipump, 4 µg/day) for 48 days significantly decreased working memory errors, as measured by the delay match-to-sample plus maze test, compared to controls (Prokai et al., 2015).

In a rat model of exercise-induced brain injury (from exhaustive swimming), intranasal DHED treatment (9 µg/mL in nasal drops, delivering a total of 20 µL each time, prepared in dimethyl sulfoxide) 15 minutes before exhaustive swimming reduced neuronal injury as measured by reduced expression of apoptosis-regulated proteins (cleaved caspase 3), the improvement of neural survival (NeuN), and the prevention of myelin loss (Peng et al., 2021). DHED treatment also attenuated mitochondrial fission, restoring dynamic equilibrium, and suppressed reactive gliosis and the release of inflammatory cytokines (IL-1β, TNF-α, IL-6) in the rat cerebral motor cortex. DHED treatment also increased the anti-apoptotic Bcl-2 levels compared to untreated rats undergoing exhaustive swimming.

DHED exerts its neuroprotective effects in manners similar to 17β-estradiol. DHED increased spine/synapse density in the hippocampal CA1 neurons, increased the number of cholinergic neurons in the medial septum and vertical diagonal band, and stimulated progesterone receptor expression in the hypothalamus (Prokai et al., 2015). The DHED effect on progesterone receptor expression was abolished when co-treated with an estrogen receptor antagonist, ICI 182780.

Roberta Diaz Brinton has proposed the "healthy cell bias" hypothesis of estrogen actions in the brain, which suggests that estrogens are protective in healthy cells but may be harmful in diseased states (Yao et al., 2012). For example, in cell culture, 17β-estradiol is protective when applied prior to or during Aβ42 insult. It is not protective if applied after—and exacerbates Aβ42-induced apoptosis. If this theory is true, DHED may only be protective at an early stage of AD (or before the appearance of neuropathology).

APOE4 interactions: Unknown.

Aging and related health concerns: DHED has therapeutic potential for ischemia, hot flushes, ocular health, and depression, without concerns for breast, uterine, and ovarian cancers, but no studies have tested it in humans.

Types of evidence:

- 6 rodent studies (2 on hot flushes, 2 on ocular health, 1 on stroke, and 1 on depression)

Ischemia: In a rat model of ischemic stroke (transient middle cerebral artery occlusion; tMCAO), DHED treatment reduced infarct volume in a dose-dependent manner, with concomitant attenuation of neurological deficits (ND score)(Prokai et al., 2015). The effective dose (ED_{50}) was about 15 µg/kg (s.c.). A

significantly higher (~10-fold) dose of systemic 17β-estradiol (200 µg/kg) was required to achieve the same level of protection. This is because DHED has better physicochemical properties for brain uptake from the circulation compared to 17β-estradiol (Prokai-Tatrai and Prokai, 2019). Furthermore, DHED exerted neuroprotective effects even after the stroke (up to 2 hours post-tMCAO).

Glaucoma and age-related macular degeneration: Retinal degeneration is a pathological feature of several blinding eye diseases, including glaucoma and age-related macular degeneration. Observational studies have found that early menopause, accompanied by lower circulating estradiol levels, is associated with a higher risk of open-angle glaucoma (Hulsman et al., 2001). In animal models of glaucoma (surgically-elevated intraocular pressure), 17β-estradiol eye drops showed therapeutic benefits, but systemic estradiol exposure could not be avoided with eye drops (Prokai-Tatrai et al., 2013). DHED treatment showed a greater preservation of the treated eye compared to 17β-estradiol, likely due to the greater diffusion of DHED through biological membranes, resulting in 75% and 50% preservation of contrast sensitivity with DHED and 17β-estradiol treatment, respectively, compared to ~20% in the untreated eye (Prokai-Tatrai and Prokai, 2019).

In a study in male rats and rabbits, DHED eye drops (10 µL eye drop once daily; 0.1% w/v) for 3 weeks resulted in a large concentration of 17β-estradiol levels in the retina without increasing circulating 17β-estradiol levels (Prokai-Tatrai et al., 2021).

In a study of rats, rabbits, and pigs, DHED eye drops (0.1% w/v in 20% w/v HPβCD in saline) increased transcorneal flux and bioactivation to 17β-estradiol in the retina but not in the cornea (Prokai-Tatrai et al., 2020). Because circulating 17β-estradiol levels were not increased, there were no changes in uterine and anterior pituitary weights.

Hot flushes: In a rat hot flush model, oral DHED treatment (30 and 100 µg/kg) blunted the tail-skin temperature rise (Merchenthaler et al., 2016). The dose used to achieve this effect for ethinyl-estradiol (EE, orally bioavailable estradiol) was much higher (200 µg/kg).

Although hot flushes are best known as a symptom for menopause in women, men who undergo castration due to androgen-sensitive prostate cancer also experience hot flushes. While estrogens alleviate hot flushes, they cause unwanted side effects. In male orchidectomized (testicles removed) rats, DHED treatment (100 µg/kg, orally) alleviated androgen deprivation-associated hot flushes without peripheral side effects (Merchenthaler et al., 2020). Levels of 17β-estradiol were increased in the hypothalamus, but serum estrogen was unchanged after oral DHED treatment. In a pharmacological

model of hot flushes (morphine-dependent orchidectomized male rats), DHED treatment (300 µg/kg, twice daily, orally) for 13 days blunted the increase in hot flushes (measured by tail skin temperature).

Depression: Rats treated with DHED (50 µg/kg, s.c.) once daily for 5 days expressed less anxiety-like behavior on a forced swim test (Prokai et al., 2015). DHED treatment had greater antidepressant effects compared to 17β-estradiol given at the same dose.

Safety: Because DHED does not increase circulating estradiol levels, it does not carry cancer (breast, uterine, ovarian) and cardiovascular risks, though no evidence is available yet in humans.

Types of evidence:

- Several laboratory studies

Systemic administration of DHED in rats does not trigger or inhibit endogenous estradiol formation in the brain and does not produce estradiol in the circulation and peripheral tissues (Prokai et al., 2015). DHED is orally bioavailable, in contrast to 17β-estradiol (Prokai-Tatrai and Prokai, 2019).

Uterus: UNAFFECTED. DHED does not increase uterine weight, an effect seen with systemic estrogen treatment, regardless of the route of administration (i.v., s.c., or oral) or the length of treatment (acute or continuous long-term, up to 48 days)(Prokai et al., 2015; Tschiffely et al., 2016). There were also no changes in the expression of a wide panel of estrogen-regulated uterine proteins with DHED treatment.

Breast cancer: UNAFFECTED. DHED treatment does not stimulate the growth of breast cancer cells in a mouse model of breast cancer (Prokai et al., 2015).

Liver: UNAFFECTED. In contrast to systemic 17β-estradiol treatment, which causes estrogen receptor activation in the liver, DHED treatment, even at high oral doses (>200 µg/kg), does not cause hepatic side effects (Prokai et al., 2015).

Sources and dosing: DHED has only been tested in animal studies, not humans. In a mouse model of AD (APPswe/PS1dE9 mice), 2 µg/day (s.c., osmotic minipump delivery) of DHED was therapeutic (Tschiffely et al., 2016). In normal mice, doses ranging from 10-50 µg/kg (s.c.) showed anti-depressant effects (Prokai et al., 2015). In rats, doses ranging from 50-200 µg/kg (i.v.) showed neuroprotection in an ischemia model (Prokai et al., 2015). DHED has also been studied via the oral route (100 µg/kg),

intranasal route (9 µg/mL, total of 20 µL), and with eye drops (0.1% w/v)(Prokai-Tatrai and Prokai, 2019; Merchenthaler et al., 2020; Peng et al., 2021; Prokai-Tatrai et al., 2021).

Research underway: No clinical trials are underway based on ClinicalTrials.gov. Translational animal studies are ongoing to test DHED for menopausal hot flushes, funded by the NIH (R01AG070072).

Patents: Prokai-Tatrai, K. and Prokai, L. are authors of many papers investigating DHED and are inventors in the patents covering the use of DHED and related para-quinols as CNS-selective bioprecursor prodrugs for estrogens (e.g., Patent Numbers 7300926, 7026306).

Search terms:
Pubmed, Google: DHED, Prokai (author), brain-selective prodrug