



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.

Klotho (Target)

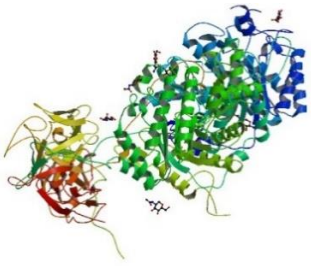
Evidence Summary

Preventing the normal decline in Klotho during aging may protect against age-related diseases through the mitigation of oxidative stress and inflammation. No human-validated therapies are currently available.

Neuroprotective Benefit: The CNS has its own source of Klotho, and higher Klotho levels are associated with higher cognitive reserve and a lower rate of dementia. Beneficial effects may be mediated by its anti-oxidant and anti-inflammatory activities.

Aging and related health concerns: Klotho levels decline with age. Maintenance of high Klotho may extend lifespan, and protect against age related diseases such as kidney disease, cardiovascular disease, inflammation, and cancer.

Safety: As an endogenous protein, Klotho itself is safe, but the methods used to induce chronically high Klotho levels need to be tested for safety in humans.

<p>Availability: Recombinant protein and viral expression vectors for research use.</p>	<p>Dose: Not yet determined</p>	<p>Chemical formula: Protein MW:130kDa (full-length) 70kDa (cleaved/secreted)</p>
<p>Half-life: 7.5 hours (estimated)</p>	<p>BBB: Not penetrant</p>	 <p>Source: Pubchem</p>
<p>Clinical trials: Serum Klotho levels are currently being used a biomarker for kidney function, and the ability of exercise to induce Klotho is being investigated. No trials testing Klotho therapies in progress.</p>	<p>Observational studies: Many studies show that Klotho levels decline with age and are associated with kidney function. Associations have also been found with lifespan, cognitive decline, hypertension, and cancer.</p>	

What is it? Klotho (α -Klotho) is located on Chromosome 13, and has been described as an anti-aging gene. It has two major isoforms, a 130kDa transmembrane protein that serves as an obligatory co-receptor for FGF23, and a 70kDa secreted form. The transmembrane form is primarily expressed in the kidneys, and can be cleaved by the metalloproteinases ADAM10 and ADAM17 to produce a soluble form that is detectable in the serum [1]. The secreted form is most highly expressed in the brain, and is produced primarily by the choroid plexus [2]. The soluble form of Klotho includes both the cleaved (majority) and secreted (minority) forms and acts as a hormone with pleiotropic functions. While several candidate interacting partners have been identified, the receptor(s) for the soluble form has not yet been definitively identified [3].

Klotho expression is induced by aerobic exercise and declines with age. Variants in the Klotho gene have been associated with lifespan extension and risk for cardiovascular disease. A measure of the circulating levels of Klotho has been proposed as a biomarker of kidney function. Supplementation of Klotho in the form of gene therapy or recombinant protein has shown promise in preclinical models and is currently being developed to target age-related diseases.



Neuroprotective Benefit: The CNS has its own source of Klotho, and higher Klotho levels are associated with higher cognitive reserve and a lower rate of dementia. Beneficial effects may be mediated by its anti-oxidant and anti-inflammatory activities.

Types of evidence:

- 16 observational studies
- Numerous laboratory studies

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

Prevention of cognitive decline: Potential benefit

While there have been no studies examining the effects of Klotho supplementation in humans, some observational studies indicate that **higher levels of Klotho circulating in the plasma or cerebrospinal fluid (CSF) are associated with higher cognitive function and a lower incidence of dementia** during the aging process [4; 5]. However, most of the studies regarding the relationship between Klotho and human cognition examine people with different genetic variants of the α -Klotho gene, and it is not clear how most of these polymorphisms alter Klotho expression or regulation in the brain.

Relationship between Klotho levels and cognitive decline

CSF: In an observational study (n=70) of older adults (average age 76) with and without Alzheimer's disease (AD) and younger adults (average age 30), CSF levels of Klotho were found to be affected by age, sex, and cognitive impairment (as measured by the Mini-Mental State Exam (MMSE) [4]. CSF Klotho was higher in men [mean 899 pg/mL, 95% Confidence Interval (CI) (8144-983) compared to women [716 pg/mL, 95% CI (632-801) (P = 0.002)]. It was higher in young adults [992 pg/mL, 95% CI (884-1100)] than older adults [766 pg/mL, 95% CI (658-874) (P=0.005)], and higher in older adults without AD [776 pg/mL 95% CI (705-828)] than those with AD [664 pg/mL 95% CI (603-725) (P = 0.02)]. Overall, **CSF Klotho levels were found to decrease with age** (Spearman correlation of age and Klotho: -0.37 (P = 0.003) **and during cognitive decline** (Spearman correlation of MMSE score and Klotho: 0.30 (P = 0.02)).

Plasma: In the InCHIANTI Italian cohort study of adults \geq 55 years old (n=833), people with Klotho plasma concentrations greater than 669 pg/mL were, on average, younger, consumed fewer alcoholic drinks, had less depression, and were more likely to be female than participants with lower klotho concentrations (\leq 669 pg/mL), all P values <0.05 [5]. Individuals with higher Klotho levels had higher MMSE scores at the 3-year (P=0.002) and 6-year (P = 0.07) follow-up timepoints. Each additional natural



logarithm of Klotho (pg/mL) was associated with a 35% lower risk of meaningful decline in MMSE, defined as a decline exceeding three points.

In a study of older adults with different types of cognitive impairment (n=320), having the lowest level of plasma Klotho (1st tertile) was associated with older age, higher prevalence of coronary heart disease and stroke, and higher levels of creatinine, homocysteine, and high-sensitivity C-reactive protein [6]. The **risk for vascular dementia was highest for those with the lowest Klotho levels** (≤ 514.8 pg/mL, Odds ratio (OR): 3.54, 95% CI 1.05-11.93), and there was an increased risk for everyone with levels ≤ 680 pg/mL. Klotho levels were not found to be associated with the risk for AD (without vascular dementia) in this study.

Low plasma Klotho levels (≤ 400 pg/mL) have also been found to be associated with increased risk for Grade 2 (OR: 1.38) and Grade 3 (OR: 2.94) ($P < 0.05$) cerebral deep white matter lesions on MRI, and MMSE scores were correlated with α -Klotho levels ($P < 0.05$) in a study of community-dwelling people in Japan (n=280, average age 71)[7].

These studies suggest that systemic levels of circulating Klotho decrease with age, and that very low levels of Klotho are correlated with cognitive decline and vascular dysfunction. Notably, the sex difference showing higher CSF Klotho in men and higher plasma Klotho in women suggests that the two populations of Klotho are independently regulated.

Relationship between Klotho genetic variants and cognitive decline

The genetic and epigenetic regulation of Klotho is complex and varies with different ethnic groups. Overall, the basic trend appears to involve alterations that decrease Klotho expression being associated with a greater risk of cognitive impairment, although the particular mechanisms may vary. For example, in one ethnic Chinese population where the KL-VS variant is present in a similar frequency to Caucasians, the risk for mild cognitive impairment (MCI) was largely related to the presence or absence of the KL-VS associated SNPs [8]. Whereas in another ethnic Chinese population without this variant, MCI risk was better associated with the methylation status of the Klotho promoter [9]. Consequently, a personalized approach may be needed in order to effectively boost Klotho expression levels in a given person using 'Klotho enhancing therapy'.

KL-VS variant: KL-VS is the most well-studied genetic variant of Klotho. It is a haplotype containing 6 SNPs and is present in approximately 15% of Caucasians [10]. Two of the SNPs located in exon 2 lead to amino acid substitutions (F352V and C370S). *In vitro* studies suggest that the F352V substitution, which takes place in a highly conserved residue decreases transmembrane shedding and half-life, but is



compensated for by the C370S substitution [11]. Additionally, the KL-VS variant of Klotho has a lower propensity to homodimerize, and higher propensity to heterodimerize with FGF receptors, which may enhance signaling. There is a dosage effect to this genetic variant, in that homozygotes tend to have shorter lifespans, greater risk for cardiovascular disease, and greater rates of cognitive decline, whereas the opposite was found for heterozygote carriers [12]. However, there also appears to be an age-dependent effect, as the protective effects are seen during the middle age and early elderly period [13]. In contrast, the extreme elderly (≥ 90 yrs old) KL-VS carriers were found to have worse cognitive function (β : -0.59 , $P = 0.046$) and a faster rate of decline than noncarriers [14].

The early cognitive protection afforded to KL-VS carriers may stem from these individuals having both higher initial cognitive reserve and higher circulating levels of Klotho. KL-VS heterozygosity was found to be associated with larger volumes in the right dorsolateral prefrontal cortex (rDLPFC), which is an area important for executive function, in two independent cohorts of adults aged 55-85 in the US (Cohort 1: $P = 4.07 \times 10^{-5}$, $n=222$ and Cohort 2: $P = 0.02$, $n=200$) [15]. Heterozygotes were also found to have greater intrinsic connectivity within the rDLPFC, and this was associated with higher serum Klotho levels ($P_{\text{uncorr}} < 0.001$) [16]. Meanwhile, KL-VS homozygotes tend to have smaller rDLPFC volumes and lower serum Klotho levels [13; 15]. In the Lothian Birth Cohort 1921 study ($n=464$), people with the V/V (homozygotes) genotype (F352V) were found to have lower verbal reasoning ability at both age 11 and age 79, which was due to lower IQ [17]. The KL-VS haplotype does not prevent cognitive aging, rather, the slower rate of cognitive decline in the KL-VS heterozygotes is thought to be related to their higher baseline cognition. These studies suggest that the Klotho genotype may be more important for the development of cognitive reserve capacity than for cognitive aging per se.

G-395A variant: This SNP is located in the promoter region of the Klotho gene. It is thought to alter DNA-protein binding affinity, and has been predicted to be a missense variant [10]. Two observational studies indicate that there may be a slight age-dependent benefit to the SNP in terms of cognitive decline. A study in China ($n=706$) found that having the G/A SNP was associated with a lower risk of cognitive impairment (OR: 0.66; 95 % C.I 0.44 to 0.98) in those over age 90 [18]. In a Japanese study ($n=2234$), people aged 60-79 with the SNP had slightly higher IQ (102.6 ± 0.8 vs. 99.8 ± 0.5) and higher scores on the Japanese Wechsler Adult Intelligence Scale-Revised (JWAIS-R) – Information, Similarities, and Picture Completion task. However, there were no SNP dependent differences in individuals younger than age 60 [19].

CSF Klotho inducing therapy

In a small study of 8 geriatric patients (age 70.3 ± 8.65), electroconvulsive therapy (ECT) was shown to increase levels of CSF Klotho from 792.5 pg/mL to 991.3 pg/mL, ($P=0.0020$) [20]. The increase was



positively correlated with the number of ECT sessions ($F= 7.84$, $P=0.031$), however there was no correlation between CSF Klotho and depression-relief response to ECT. Notably, serum levels of Klotho were not affected by ECT, as they are derived from the kidney, whereas CSF levels are derived from the choroid plexus.

Human research to suggest benefits to patients with dementia:None

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

Neurogenerative diseases (pre-clinical models): Potential benefit

The secreted form of Klotho, generated through alternative splicing, is expressed at the highest level in the brain, particularly in the choroid plexus. The level of the secreted form is 10X higher in the brain than the kidney [2]. This expression decreases with age [21; 22], and decays more rapidly in animals with neurodegenerative disease [2]. In rodent models, Klotho supplementation or overexpression can prevent or reduce cognitive decline. Klotho's ability to regulate glutamate signaling, antioxidant activity, metabolism, neurogenesis, and inflammation may underlie its neuroprotective effects.

Alzheimer's disease: Klotho is a physiological target of APP, and is regulated by soluble APPs β [23]. It has been speculated that Klotho may play a role in protecting against A β -mediated toxicity. In hAPP transgenic mice, Klotho expression in the dentate gyrus of the hippocampus was 62% lower than in wildtype mice [24]. Transgenic overexpression of Klotho enhanced spatial learning and memory, increased the abundance of the GluN2B subunit of NMDA glutamate receptor at the synapse, and NMDA receptor-dependent synaptic plasticity [24]. A prior study found that transgenic Klotho overexpression was able to improve spatial memory task performance in 4-7 month-old and 10-12-month-old wildtype mice, and that this cognition enhancing effect was dependent on an increase in the expression of the GluN2B subunit of the NMDA receptor in the hippocampus [13].

Parkinson's disease: In the hSYN alpha-synuclein overexpression model of Parkinson's disease (PD) peripheral administration (10 $\mu\text{g}/\text{kg}$ i.p.) of a recombinant version of the cleaved (shed) form of Klotho (amino acids 35-982) improved motor learning and cognitive deficits [25]. It was also able to boost spatial and working memory in aged (18-month-old) wildtype mice. The effect appears to be related to altered glutamate receptor signaling, and increases in the cleaved form of the glutamate GluN2B NMDA receptor subunit. However, the mechanism underlying its protective effect is unclear, since it is not blood-brain-barrier (BBB) penetrant.

In the 6-OHD model of PD, pretreatment with recombinant Klotho (10 μg , 30 min prior to 6-OHD) via intracerebroventricular injection, mitigated drug-induced rotational behavior, and improved



performance on the narrow beam test [26]. It also reduced markers of oxidative stress and protected against neuronal degeneration in a PKA and CAMKII dependent manner.

Age-related cognitive decline: Exercise is a potent inducer of Klotho [27]. Continuous moderate exercise can slow the rate of cognitive decline in mice, but it cannot prevent or reverse cognitive decline [2]. In brain tissue (white matter) from rhesus monkeys, the level of Klotho promoter methylation increased with age [28]. These epigenetic modifications may decrease the ability to endogenously induce Klotho expression with age, and suggest that exogenous supplementation may be required to maintain high levels throughout life. Overexpression of the secreted form of Klotho via adenovirus (AVVrh10) delivery into the cerebral ventricles of 6 and 12-month-old mice, improved age-related motor declines and cognitive performance (Morris water maze $P=0.0018$), when tested 6 months later [29].

Mechanisms of neuroprotection:

Antioxidant activity: Lentivirus mediated expression of full-length Klotho into the cerebral ventricles of 7-month-old senescence-accelerated prone (SAMP8) mice led to increases in levels of both transmembrane and soluble Klotho three months later, and reduced memory deficits, neuronal loss, and synaptic damage [30]. These effects may have been mediated by its **induction of an antioxidant response** by regulating the phosphorylation of Akt and FoxO1. The FoxO class of transcription factors are involved in the regulation of oxidative stress and are negatively regulated by the P13K/Akt pathway.

In cell culture with hippocampal neurons, pre-treatment with recombinant Klotho (0.4 $\mu\text{g}/\text{mL}$) reduced glutamate mediated cytotoxicity from 65% to 40%, and protected neurons against $\text{A}\beta$ -mediated toxicity [31]. This neuroprotective effect was partially mediated by the induction of the thioredoxin/peroxiredoxin redox control system.

Neurogenesis: Local downregulation of Klotho in the hippocampus reduces adult hippocampal neurogenesis in mice, and leads to decreased performance on hippocampal-dependent memory tasks [32; 33]. Transgenic Klotho overexpression [32] or supplementation [33] with shed klotho protein can increase the proliferation rate of neural progenitors and rescue these effects. Recombinant Klotho (0.4 $\mu\text{g}/\text{mL}$) can also increase the maturation of rat oligodendrocyte precursor cells (OPCs) in cell culture, and involves the Akt and ERK1/2 signaling pathways [34].

Glutamate signaling: In addition to affecting levels of glutamate NMDA receptor subunits in the hippocampus, Klotho also affects the expression of glutamate reuptake transporters (EAAT transporters) [35]. Treatment with recombinant Klotho increases EAAT3 and EAAT4 expression (by modulating PI3K signaling), which could help prevent glutamate-mediated excitotoxicity.



Metabolism: Klotho expression in neurons is modulated by glutamatergic and insulin signaling, and may play a role in the metabolic coupling between neurons and astrocytes. Soluble Klotho was shown to enhance astrocytic aerobic glycolysis and the release of lactate to be taken up and used as fuel by nearby neurons [36].

Inflammation: The age-related reduction of Klotho expression and secretion from the choroid plexus may be a key driver of neuroinflammation. The expression of major histocompatibility complex (MHC) Class II, which is involved in antigen presentation, was found to be 5.6-fold higher in 22-23-month-old mice, compared to 2-3-month-old mice [22]. There was also an increase in adhesion molecules, such as ICAM-1, which could promote the infiltration of immune cells into the CNS through the blood-CSF barrier. Indeed, lower Klotho levels promote macrophage infiltration and microglia activation. The anti-inflammatory effects of Klotho appear to be partially mediated through the inhibition of the NLRP3 inflammasome in a Klotho-FGF23 signaling dependent manner.

APOE4 interactions:

Klotho affects the risk for cognitive impairment independently from ApoE4 status. In the Aberdeen birth cohort of 1936 longitudinal study, the KL-VS variant's associations with white matter volume, rDLPFC volume, and rate of cognitive decline were not affected by ApoE4 status [37]. Klotho may also potentially act as a resilience factor in ApoE4 carriers. In the Wisconsin Registry for AD prevention (n=325), late middle-age KL-VS heterozygotes had less ApoE4-associated cortical thinning [38].

Ageing and related health concerns: Klotho levels decline with age. Maintenance of high Klotho may extend lifespan, and protect against age related diseases such as kidney disease, cardiovascular disease, inflammation, and cancer.

Types of evidence:

- 3 meta-analyses (longevity n=4 studies, Cardiovascular/kidney n=9 studies, Cancer n=18)
- 13 observational studies
- Numerous laboratory studies

Lifespan: Potential benefit

Circulating serum levels of Klotho decrease with age in humans and other long-lived primates [5; 39]. Animal studies suggest that the maintenance of high Klotho levels could slow the aging process and



extend lifespan. Rodent models initially identified the relationship between Klotho levels and lifespan, and studies of people with different genetic variants of Klotho suggest that this association may extend to humans.

Mice: Klotho knockout mice have a premature aging phenotype which includes atherosclerosis, decreased bone density, reduced skin thickness, abnormal lipid metabolism, and a shortened lifespan averaging only 60.7 days [40]. In contrast, transgenic overexpression of Klotho can extend lifespan in mice in a sex-dependent manner. In two different Klotho overexpression lines the lifespan of male mice was extended by 20% and 30.8%, while female lifespan was extended by 18.8% and 19%, respectively [41].

KL-VS genetic variant: In a cohort of Italian adults aged 19-109 (n=1089), the proportion of individuals with the KL-VS heterozygous genotypes (who tend to have higher circulating Klotho levels) was significantly higher in the elderly (age 66-88) compared to younger adults (OR: 1.564 95% CI (1.126–2.174); P=0.008), but there was no difference in genotype frequency between centenarians (age >88) and younger adults [42]. A meta-analysis of 4 cohort studies (populations include: Bohemian Czech, US Caucasians, African Americans, Italians, and Indians) found that **KL-VS heterozygotes have slightly higher longevity** than non-carriers (OR:1.14, 95% CI (1.00–1.30); P=0.05) using a random-effects model [12]. KL-VS homozygotes show a non-statistically significant disadvantage during aging. Similar to what is seen with respect to cognitive function, KL-VS heterozygotes have a survival advantage that weakens over the course of the lifespan. The reason for this age-effect is unknown, but may be related to genetic-variant associated changes in Klotho expression and/or epigenetic regulation over time.

Insulin/IGF-1 -mediated mechanism: The effect of Klotho on lifespan extension is mediated through its ability to inhibit insulin and IGF-1 signaling [43]. Klotho overexpression in rodents is associated with insulin resistance primarily in males and IGF-1 resistance in both sexes. It has been proposed that the ability of Klotho to extend lifespan may be related to its regulation of lipid homeostasis by preventing intracellular lipid overload and associated lipotoxicity [44].

Frailty: Potential benefit

In the Italian InCHIANTI longitudinal cohort study of adults ≥ 65 years old, frailty status (n=744) and handgrip strength (n=804) were associated with plasma Klotho levels. Handgrip strength is an indicator of total body muscle strength and is a predictor of poor outcomes in older adults. Each increase in the natural logarithm of Klotho (pg/mL) was associated with lower odds of frailty versus robustness after adjustment for covariates (OR: 0.46, 95% C.I. (0.21- 0.98); P = 0.045) [45]. **Grip strength was positively correlated with plasma Klotho** at a threshold of <681 pg/mL. In adults with plasma Klotho <681 pg/mL,



each standard deviation increase in plasma Klotho was associated with increased grip strength ($\beta=1.20$, standard error = 0.35, $P = 0.0009$), after adjustment [46].

Kidney Disease: Potential benefit

The kidney has the highest Klotho expression. While the promoter is 30-40% methylated (silenced) in low or non-Klotho expressing cells, it is largely unmethylated in renal cells, which drives high expression in the kidney [47]. **The majority of the soluble fraction of Klotho circulating in the peripheral blood originates from the cleavage (shedding) of the transmembrane form in the kidney.** Unlike the CNS, the secreted form of Klotho does not appear to significantly contribute to this pool. In the kidney, the transcript for the alternatively spliced (secreted) form is a target for nonsense mediated mRNA decay [48]. Dysregulation of Klotho transcript splicing may contribute to the loss of Klotho protein that occurs in the context of kidney injury.

Kidney disease biomarker: A meta-analysis of 9 studies including 1457 patients with chronic kidney disease found that soluble α -Klotho levels were associated with the estimated glomerular filtration rate (eGFR), ($r=0.35$, 95%CI (0.23 to 0.46); $P<0.05$), and inversely correlated with FGF-23 ($r=-0.10$, 95% CI (-0.19- to -0.01); $P<0.05$) [49]. The eGFR is considered one of the more reliable measures of kidney function. Transmembrane Klotho is an obligate co-receptor for FGF-23, and the level of circulating FGF-23 itself is associated with chronic kidney disease progression [50]. FGF-23 is important for the regulation of phosphate and Vitamin D homeostasis by promoting the degradation of Vitamin D and inhibiting phosphate absorption [51]. High levels of FGF-23 can lead to toxicity, which can be prevented by the presence of the soluble (cleaved) form of Klotho [52]. This suggests that serum Klotho levels could serve as a biomarker for kidney function. Some clinical trials are already incorporating serum Klotho levels into their outcome measures, however, its use in this capacity may be premature. A standardized assay that can reliably measure soluble Klotho has not yet been validated.

Kidney disease treatment: In addition to regulating phosphate homeostasis through FGF-23, Klotho also helps regulate calcium homeostasis in the kidney through regulation of the calcium channel TRPV5. Klotho increases cell surface expression of TRPV5 in the renal epithelium by modifying sugar residues (removal of sialic acids) [53]. It is anticipated that the maintenance or restoration of Klotho levels could help restore mineral homeostasis and improve kidney function in the context of kidney disease. Animal studies provide proof-of-principle evidence to support this hypothesis.

Klotho gene therapy: Treatment of nephrotomized mice (starting 1 week after injury) with a vector encoding the secreted form of Klotho, via hydrodynamic based gene therapy injury, prevented induction of the blood pressure regulating renin-angiotensin system (RAS), normalized blood pressure, and



ameliorated renal fibrotic lesions at 6 weeks post-injury [54]. In the Streptozotocin-induced diabetes rat model, an intravenous injection of adenovirus containing full-length Klotho was found to prevent progression of renal hypertrophy and fibrosis in for at least 12 weeks [55]. In a mouse model of chronic kidney disease, retro-orbital injection of adenovirus containing the soluble cleaved form of Klotho reduced hyperphosphatemia and prevented vascular calcification [56]. These gene therapy approaches allowed for the maintenance of Klotho levels under conditions where they would normally decrease.

Recombinant Klotho: In a sepsis-induced acute kidney injury model, i.p. injection of recombinant Klotho (0.01 mg/kg, 1-hour post-injury) attenuated renal dysfunction ($P < 0.05$) and partially restored endogenous renal Klotho expression ($P < 0.05$), but did not impact apoptosis or autophagy [57]. In an ischemia-induced acute kidney injury model, i.p. injection of recombinant Klotho (0.01 mg/kg/day for 4 days starting 24 hours post-injury), prevented the progression to chronic kidney disease, prevented cardiac remodeling, and restored Klotho levels long after the cessation of the therapy [58]. In a chronic kidney disease model, 0.3 mg/kg/day was delivered to mice via an osmotic pump starting 4-12 weeks after injury. When used as a treatment, rather than a preventative measure, Klotho was only partially effective in restoring renal function and reducing cardiac remodeling [58].

These studies suggest that in order to be most beneficial, Klotho therapy would need to be used either prophylactically, or very early in the course of disease/injury. The early benefits likely stem from Klotho's antioxidant activity, which could help to mitigate the induction of inflammatory and oxidative stress damage.

Cardiovascular disease: Potential benefit

Klotho can help protect against cardiovascular disease by regulating endothelial function, lipid homeostasis, ion transport, oxidative stress, inflammation, and protecting against kidney damage. Klotho can be induced as a protective mechanism in the context of inflammation, thus individuals with lower Klotho induction capacity are more likely to experience inflammation-associated adverse events. The risk for cardiovascular problems may be related to a decline in vascular expression of Klotho.

Serum Klotho and Cardiovascular disease risk: Several observational studies have found an association between low Klotho levels and higher risk for cardiovascular disease. Because serum Klotho levels are most closely associated with kidney function, the associations between Klotho levels and cardiovascular disease are strongest in people with impaired kidney function, such as those with diabetes or kidney disease. In the ARNOGENE cohort study of dialysis patients with kidney disease ($n=238$), individuals with levels ≥ 280 ng/L had a significantly reduced occurrence of cardiovascular events and cardiovascular death (OR: 0.39, 95% CI (0.19-0.78); $P = 0.008$) compared to patients with Klotho < 280 ng/L [59]. In type



2 diabetic patients in China (n=168), a high Klotho level was associated with a reduced risk of developing coronary artery disease and cerebrovascular accidents (adjusted OR: 0.397, 95% CI, 0.227–0.696; P = 0.001) [60]. Notably, diabetic patients taking statins were more likely to have higher Klotho levels, and those taking fibrins were more likely to have lower Klotho. Meanwhile, in the LURIC Cardiovascular Healthy Study (n=2948), serum Klotho levels did not add predictive power to cardiovascular and mortality risk assessment in patients with normal renal function [61]. After adjustment for cardiovascular risk factors the hazard ratios (HR) in the fourth quartile compared to the first quartile of s-klotho were HR: 1.14 (95%CI, 0.94–1.38; P= 0.187) for all-cause mortality and HR: 1.03 (95%CI, 0.80–1.31; P= 0.845) for cardiovascular mortality.

Mechanism: In mice, Klotho-associated cardioprotection is mediated by the down regulation of TRPC6 cation channels in the heart, via inhibition of IGF-1 and PI3K [62].

Atherosclerosis: Klotho levels may impact risk for atherosclerosis through the modulation of lipid metabolism and inflammation. KL-VS variant SNPs were found to be associated with serum levels of hemoglobin, albumin, and high-density lipoprotein cholesterol (HDL-C), fasting insulin, and fasting glucose [63]. In the InCHIANTI Italian cohort study (n=1023), plasma klotho was correlated with HDL cholesterol (r = 0.11, P =0.0004), and inversely correlated with C-reactive protein (r = -0.10, P = 0.0008) [64]. In a small study, patients with atherosclerosis (n=27) had lower Klotho serum concentrations than healthy controls (n=11) (413 pg/mL, 95%CI (317–479) vs. 1481 pg/mL (95% CI 1227–1889); P<0.0001) [65]. Vascular (arterial) Klotho expression was also 1.725 (95% CI 0.537–3.104) vs. 4.647 (95% CI 2.255–7.002); P<0.0001. **Higher vascular Klotho was associated with an anti-inflammatory profile** (high IL-10, low TNF α , and low LDL). Klotho expression was also found to be reduced in the cardiomyocytes of patients with atherosclerotic cardiovascular disease [66]. Therefore, vascular Klotho expression is likely to be a more reliable marker of cardiovascular disease risk than serum Klotho, and maintenance of high Klotho levels may be protective against the development of atherosclerosis.

Hypertension: Klotho appears to be beneficial for **preventing and reducing high blood pressure** through regulation of endothelial function and inflammation. Klotho may be important for the vascular production of nitric oxide (NO). An observational study (n=109) looking at the relationship between serum Klotho and hypertension found that older patients (average age 64) in China with hypertension had lower levels of Klotho protein (0.303 ± 0.096 vs. 0.489 ± 0.216 , P<0.01) and NO (43.95 ± 21.85 $\mu\text{mol/L}$ vs. 62.63 ± 21.26 $\mu\text{mol/L}$, P<0.01) than age-matched people without hypertension [67]. Another observational study of hypertensive patients in Egypt (n=80) found that patients treated with anti-hypertensives (angiotensin converting enzyme (ACE) inhibitors) had higher levels of Klotho and NO and



lower levels of carotid thickening and the oxidative stress marker malondialdehyde (MDA) [68]. The study found a positive correlation between Klotho and NO levels.

Mechanism: Mice with reduced levels of Klotho (Klotho heterozygotes) show evidence of endothelial dysfunction with an attenuated dilation response and reduced production of NO [69]. These mice develop spontaneous hypertension and are highly sensitive to salt-induced hypertension and an associated (CCR2-dependent) infiltration of macrophages and T-cells in the kidney [70]. Klotho can also prevent angiotensin-II mediated proliferation, migration and inflammatory signaling responses in vascular smooth muscle cells [71].

Klotho gene therapy: In a strain of rats that develop spontaneous hypertension, a single dose of adenoviral vector mediated full length (mouse) Klotho (2×10^8 particles per rat via tail vein i.v.) prevented the further increase in blood pressure past the baseline level for at least 12 weeks [72]. The Klotho treatment also induced an anti-inflammatory (IL-10) and antioxidant response (decreased Nox2, NADPH oxidase, and superoxide production) in the aorta and kidney. In senescence accelerated (SAMP1) mice, treatment with adenovirus mediated secreted-form of Klotho inhibited inflammation (macrophage infiltration) and attenuated fibrosis in the aorta [73]. In a rat model of atherosclerosis, there is a reduction in endogenous levels of Klotho. Adenovirus mediated Klotho expression improved the NO mediated endothelial relaxation response to acetylcholine (from $62 \pm 3\%$ to $82 \pm 5\%$), reduced blood pressure to control levels, and reduced the perivascular fibrosis area (from 10995 ± 1303 mm to 6448 ± 986 mm, $P < 0.05$) [74].

Klotho-inducing small molecule therapy: Treatment of aged mice (24-27 months old) with a small molecule called Compound H (15 mg/kg, i.p. daily for 2 weeks) that induces Klotho expression by promoting its demethylation [75], attenuated age-related increases in arterial stiffness (pulse wave velocity) and blood pressure [76]. It reduced levels of fibrosis-associated molecules (MMP2, MMP9, TGF- β) and rescued the age-related downregulation of Sirt1 deacetylase.

Cancer: Potential benefit

In a meta-analysis of 18 studies (17 in Asians, 1 in Caucasians) regarding the relationship between cancer and Klotho expression, tissue Klotho protein expression was found to be significantly lower in overall malignancies [77]. Furthermore, **higher expression of tissue Klotho was associated with a good prognosis** (Overall OR: 0.482 95% CI (0.379–0.613); $P < 10^{-4}$). The loss of Klotho expression in various cancers has been shown to be associated with extensive promoter hypermethylation (gene silencing) [78; 79; 80; 81]. These studies suggest that Klotho may act as a tumor suppressor by inhibiting multiple



cancer-associated signaling pathways, such as the insulin/IGF-1 pathway, FGF pathway, Wnt signaling pathway, and transforming growth factor- β 1 pathway [3].

Safety: As an endogenous protein Klotho itself is safe, but the methods used to induce chronically high Klotho levels need to be tested for safety in humans.

Types of evidence:

- Several laboratory studies

Thus far, methods to enhance or induce Klotho expression via small molecules, gene therapy, or recombinant proteins have only been tested in cell and animal models. At this point, the safety data is extremely limited, since most of the studies perform a one-time intervention and do not provide any information about potential toxicity. In the context of the Streptozotocin-induced diabetes model, adenovirus mediated expression of Klotho did not affect the blood glucose or body weight of the rats [55], and recombinant Klotho did not either exacerbate or ameliorate hyperglycemia in the diabetic mice [82]. Mice with kidney disease that were chronically administered recombinant Klotho for 20 weeks did not show any evidence of overt toxicity [58]. Long-term effects are unknown.

Since Klotho is an endogenous protein, and high levels are positively associated with health in a variety of organ systems, the maintenance of chronically elevated Klotho is expected to be safe. However, safety still needs to be demonstrated for the methods used to deliver exogenous Klotho, such as the vector and method of gene delivery in any type of gene therapy. The optimal form of the Klotho protein (full length, soluble cleaved, secreted), method of delivery, and dosing still need to be established for recombinant protein therapy.

Sources and dosing:

Exercise: Aerobic exercise is the most potent natural inducer of Klotho, and may contribute to the life expectancy benefit of prolonged aerobic exercise [27]. Induction of soluble Klotho is related to fitness level, as those with higher aerobic capacity have greater induction. The induction of Klotho may occur as a mechanism to repair exercise associated skeletal muscle tissue damage, and is related to the level of exercise intensity [83]. In mice, Klotho is induced in muscle progenitor cells in response to muscle injury, but the induction is attenuated in older animals [84]. This regenerative effect on muscle involves the promotion of mitochondrial bioenergetics. In a study, younger women (age 36.0 ± 7.0) were able to boost their Klotho levels ($30.08 \pm 11.94\%$ vs $15.25 \pm 6.56\%$) in response to exercise to a higher degree

than older women (age 68.3 ± 3.0) [83]. This may be due to a combination of both lower Klotho induction capacity and lower exercise intensity capacity for the older women.

Klotho inducing compounds: Many different compounds, particularly those with antioxidant activity, have been shown to induce Klotho expression. This list includes (but is not limited to): Vitamin D, curcumin, ginsenoside Rg1, statins, resveratrol, testosterone, EGCG, glitazars, ligustilide, rhein, HDAC inhibitors, and molecular hydrogen. However, most of the evidence comes from animal studies, and it is not clear whether they can appreciably boost Klotho levels in humans over a prolonged period of time.

The method of induction is also likely to impact long-term efficacy. The age-related decline of Klotho appears to be related, at least in part, to epigenetic silencing in the form of promoter methylation and histone deacetylation, and is likely to vary slightly from person to person. Therefore, agents capable of altering these epigenetic marks would need to be used in combination with agents that drive Klotho expression at the mRNA and protein levels. However, currently available broad-spectrum demethylating agents and HDAC inhibitors have pleiotropic effects. Due to these complexities, exogenous Klotho replacement therapy is likely to be the most effective method for lifelong maintenance of Klotho.

Klotho replacement therapy: There are a couple companies working on developing Klotho replacement therapy.

Recombinant Protein: [Klotho Therapeutics](#) is a virtual biotechnology company that is working on developing recombinant Klotho for the treatment of kidney disease. They have provided proof-of-concept in preclinical models of acute kidney injury and chronic kidney disease [58] (see Kidney disease treatment section). Notably, this study showed that Klotho treatment was most effective at early stages of the disease, and may be better suited for the prevention of kidney disease than for treatment of late-stage disease. In 2017, the company announced that it had obtained Series A financing to develop its recombinant Klotho for use in clinical trials for acute kidney disease, but no further information is available.

Klotho Gene Therapy: [Klogene Therapeutics](#) is a Boston based biotechnology company that is working on developing small molecules to upregulate Klotho, but little progress has been made to date. In late 2017, Klogene announced that it was merging with [Kogenix](#) Therapeutics (Barcelona), which specializes in the development of gene therapy-based approaches to Klotho enhancement. They are developing adenovirus (AAV) based gene therapy using CRISPR/dCas9 to activate the secreted form of α -Klotho. In early 2018, they published a study in which they identified two guide RNAs (sgRNAs) that bind in to the Klotho promoter (-1 to -300 bp region) and enhance expression of the Klotho gene [85].

Research underway:

According to Clinicaltrials.gov, there are several trials using serum Klotho levels as a biomarker of kidney function and/or treatment response. There are also a couple of trials on the ability of exercise to induce Klotho in different populations. There are currently no registered trials testing Klotho replacement therapy.

Search terms:

Pubmed, Google: Klotho +

- dementia, Alzheimer's, neurodegeneration, cognitive, aging, lifespan, genetics, cardiovascular, hypertension, atherosclerosis, kidney, cancer, inflammation, meta-analysis, safety, gene therapy, recombinant, exercise, enhancers, insulin, ApoE4, biomarker, clinical trials, companies

Websites visited for Klotho:

- Clinicaltrials.gov
- PubChem

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