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Klotho: The Protein of Faith

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Abstract

α-Klotho (Klotho) is a transmembrane protein that was initially thought to be expressed only in a limited number of tissues, most importantly the kidney, parathyroid gland and choroid plexus. Klotho is also known to be a co-receptor for Fibroblast Growth Factor-23. Recent data has now demonstrated that Klotho is more widely expressed in a variety of tissues. Klotho has been shown in various studies to have wide biological effects and be implicated in a variety of pathological diseases. However, its role in these conditions have yet to be elucidated, including neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer's, cardiovascular diseases, diabetes, cancer and obesity. In the present review, we evaluate the recent literature and provide evidence on tissue source of soluble Klotho, and how changes in the levels of Klotho may be associated in a variety of diseases.

Keywords: *Klotho; Protein; Diabetes; Cancer; obesity*

Introduction

The Greek Goddess Klotho was known for regulating the span of life [1]. α-Klotho (Klotho) is a 130 kDa type I membrane-bound protein consisting of two large extracellular domains with a short single-pass transmembrane and a short intracellular domain in its C terminus. Klotho is recently reported to promote longevity, regulate glucose, calcium and phosphate levels in the kidney and brain [1]. Studies also suggest Klotho influences pathways related to cancers and tumour suppressors in humans and in mice [1-5].

Klotho also influences the onset of several premature senescent phenotypes in humans and mice, including osteoporosis, stroke, cardiovascular disease and atherosclerosis [1,3,6]. Emerging evidence suggest Klotho has a role in musculoskeletal health and bone mineral density changes throughout life [7].

Klotho is found to be most abundant in areas of the brain and in particular at the apical plasma membrane of ependymal cells of the choroid plexus (See Figure 1A) [1,6,8]. It is believed to play an intrinsic role in re-myelination [2] and potentially in various diseased states associated with demyelination and neurodegeneration. As a result, the cerebrospinal fluid contains the protein Klotho which has a humoral factor, therefore Klotho can be transported by the circulatory system [6,8]. Klotho is also found in the pituitary gland, parathyroid gland, reproductive organs and especially localised in the distal renal tubules [6]. Blood serum Klotho levels has been described to be produced mainly from the kidneys [1,3,8]. Hormonal regulation is yet another function that Klotho has been found to have which further explains why mutations of Klotho gene results in a variety of diseased states as mentioned above [1,3,6].

The present article will present a systematic overview of recent literature regarding Klotho, the central dogma from gene to function and subsequent diseases in humans and in animals.

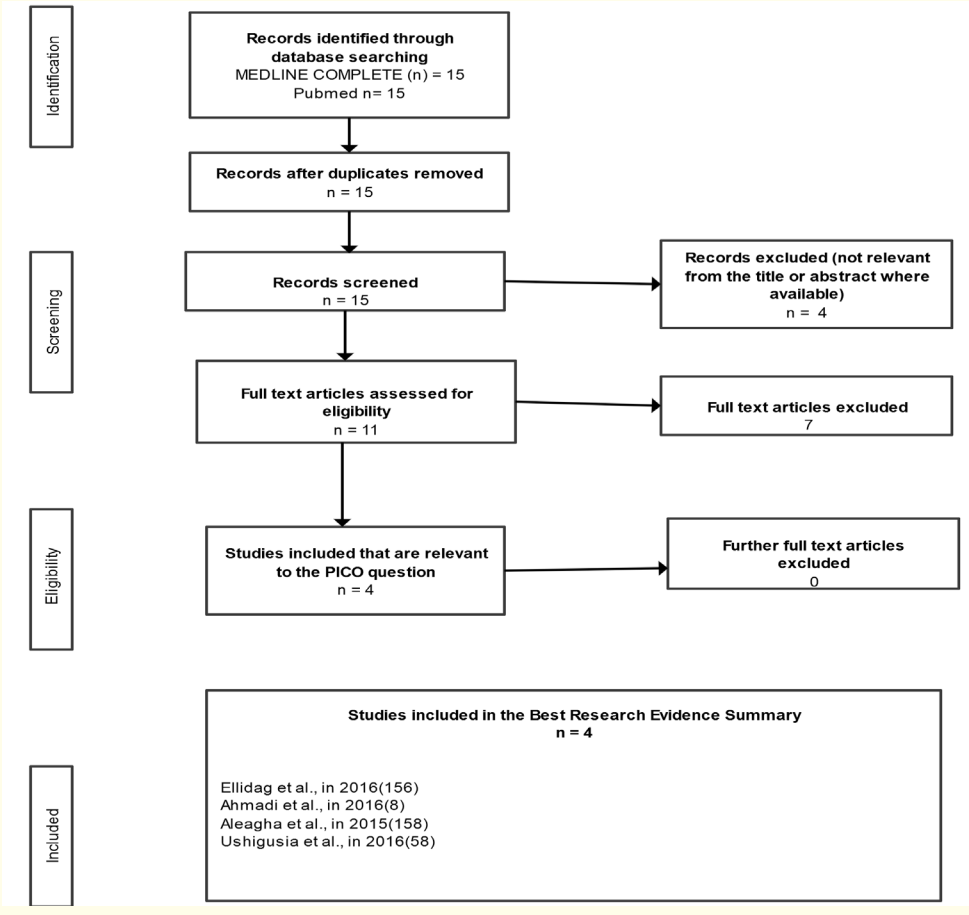
Methods

The eligibility criteria for studies to be included in the overview section:

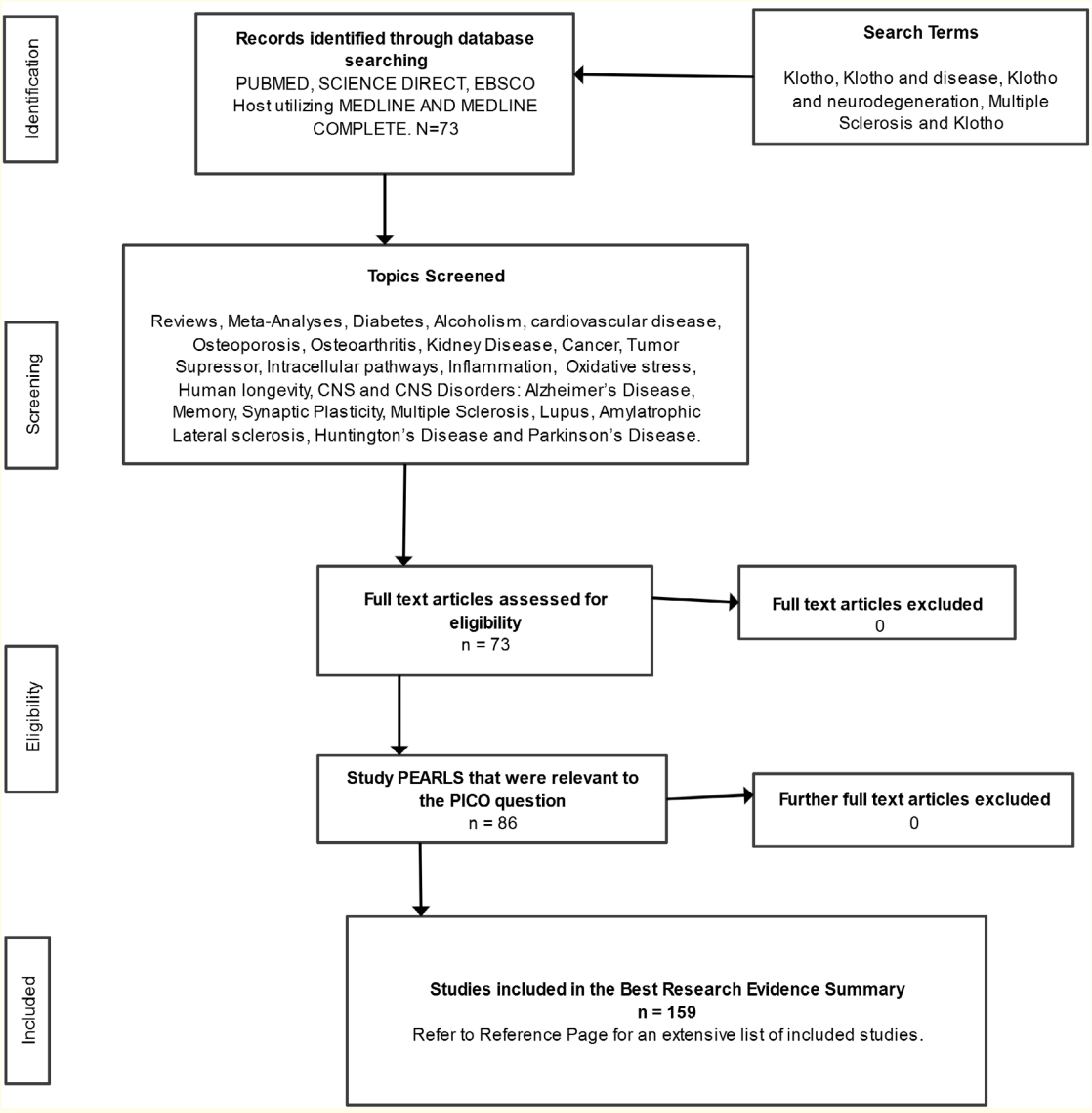
Any study type was included, with no restrictions to year or population. The primary inclusion criteria was Klotho protein (all types). Human and Animal studies were included as well as genome wide association studies. The only exclusion criteria was studies that were not published in English.

Search Strategy

In the initial stages of this review the leading authors conducted a search using several data bases as well as Grey Literature (e.g. Google, scholar and pearling). Klotho, Klotho and disease, Klotho and neurodegeneration, Multiple Sclerosis and Klotho (See Flow Diagram 1) were utilized. Databases such as PUBMED, SCIENCE DIRECT, EBSCO Host utilizing MEDLINE AND MEDLINE COMPLETE were specifically searched. All article titles were first scanned and if the abstract was related to Klotho the article was considered for inclusion. After the process, we end up with 73 related articles (See Flow Diagram 2). All articles were then further categorized into a relevant topic such as Klotho and cardiovascular diseases or neurodegenerative diseases or else. Review papers were kept as individual class of related articles. All topics were then assigned to each member to review the relevant literature that was found in order to synthesise the current evidence on that topic. In the case where articles were cited in a review these were further followed and scanned for relevance and incorporated if related to Klotho, resulting in 159 articles in total.



Flow Diagram 1: *Depicting the research strategy for Article relating to Multiple Sclerosis.*



Flow Diagram 2: *Depicting the research strategy for Klotho.*

Results

Gene Structure and Function

The Klotho gene was discovered by Kuro-o., *et al*. in 1997 [3,9-12]. The gene was identified through observing a spontaneous mutation of the α-Klotho promoter region [9,10].

The Klotho gene is located on chromosome 13q12 in humans and chromosome 5 in mice and flanked by PDS5B and STARD13 (see Figure 1C) [3,9]. The human, rat and mice Klotho genes contain five exons and four introns (see Figure 1C) which transcribe 3036, 3042, and 3042 nucleotide mRNAs, respectively [9]. Interestingly, all aforementioned species show syntonic nature of the Klotho gene, i.e. the gene is the same in each species [9,13]. Up until now the genes encode three α-Klotho and 2 β-Klotho forms with known subsequent functions (See Alpha and Beta Klotho subsection).

The promotor region for the human Klotho gene does not have a TATA box, rather the 500-bp region located immediately upstream is the initiation site of transcription [9,14]. The lack of TATA box has been explained by being rich in Sp-1, and its cooperation with Oct-1 enhances the downstream Klotho expression [3,9,14]. Similarities have been reported in the mouse premotor region for Klotho, as it too is TATA-Box free and rich in Sp-1, but only 300bp in length. Experimentally it was shown to be 67% identical [9,15].

Studies [3,9,15,16] reveal that Klotho might enter into the circulation system through three pathways:

The secreted form of Klotho protein is produced by Klotho genes via alternative RNA splicing, which is then released into the extracellular space and then into the circulation [15,13,17,18].

The transmembrane form of Klotho protein could be cleaved and released into the circulatory system [15,19,20]. The extracellular domain of Klotho can be cleaved by members of the ADAM family (ADAM10 and ADAM17), to generate three circulating forms of α-Klotho: the α-cut product (~130 kDa), the β-cut product (~65 kDa), and secreted α-Klotho (~65 kDa) [3,9,19] as demonstrated in figure 1C.

Klotho is expressed in the cytoplasm of the kidney cells, mostly intersecting on the endoplasmic reticulum and Golgi apparatus, however, it is not present on the cell surface [15]. Nevertheless, Klotho can bind to Na^+, K^+-ATPase and is then recruited to the cell surface by the combination of a 'novel Klotho-dependent pathway' and 'the conventional pathway' in response to extracellular Ca^{2+}fluctuation [20]. Once the complex of Klotho and Na^+, K^+-ATPase arises to the cell surface, Klotho is cleaved and secreted into the extracellular space and hence the circulatory system [15,20].

α-Klotho and β-Klotho Gene

The genes encode three different forms with known subsequent functions. A short form of the human and mouse Klotho gene encodes secreted α-Klotho. Note, this form was found to be generated by alternative mRNA splicing [9,10,13]. The two transcripts were revealed by sequence analysis of isolated human Klotho complementary DNA clones [1,13]. One transcript encodes a singles-pass transmembrane protein whilst the other only a 50bp shorter transcript encodes a secreted form of the Klotho protein [1,13]. Interestingly the later form predominated in all tissues examined, leading to the conclusion that the secreted form (α-Klotho) is most abundant [1,13]. Consistent with other studies it has been reported that Klotho gene encodes two other forms, the β-Klotho and the Klotho-related protein (Klrp) [9,16,21]. Klrp is a transmembrane protein that binds to fibroblast growth factor receptor (FGFR) 1b, FGFR1c, and FGFR2c [9]. This complex's function remains unknown [9,23,23]. According to recent research Klotho family proteins share one or two glycoside hydrolase (GH) motifs homologous to GH family 1[24]. Though, the biological significance of GH motifs in Klotho family proteins remains indefinable [24]. It further states that KLrP is composed of a single GH motif and it is a cytosolic β-glucocerebrosidase (GCase, EC 3.2.1.145) [24].

In summary three forms of α-Klotho exist: a full-length transmembrane α-Klotho, a truncated soluble α-Klotho, and a secreted α-Klotho [7,9]; 2 forms exist for β-Klotho: a singles-pass transmembrane protein [9] and a Klotho Related Protein. Note α- and β-Klotho are from the same gene and they share unique and specific functions whilst being exactly the same [9].

α-Klotho and β-Klotho Function

α-Klotho

Several studies (see table 1) have shown that α-Klotho has been linked to several conditions (see section relating to diseased states) and it has several functions. Evidence has shown α-Klotho to be regulated by several physiological and pathological factors [24-28]. The main pathological states identified are, diabetes mellitus, chronical renal failure, acute inflammatory stress and high blood pressure (hypertension) [24-28]. α-Klotho may also act as a hormone, the receptor(s) are yet to be identified [9]. As stated previously, Klotho has an extensive function partly due to its secreted form.

β-Klotho

β-Klotho and its specific function are still being investigated, for details on β-Klotho see table 1.

A recent study by Xu and Sun., *et al*. in 2015 has described its primary function related to adipose tissue and its metabolic function e.g glucose uptake, fatty acid metabolism, and bile acid synthesis [9]. A secreted form of β-Klotho still remains elusive, hitherto only a transmembrane form has been identified [9].

Fibroblast Growth Factors (FGFs) family and relations to Klotho

Research about FGFs, their subfamilies and their relation to Klotho is extensive in the literature. It has been shown that FGFs play a key role in a variety of processes such as morphogenesis, proliferation, cell differentiation, and metabolism [29]. Conversely pathological conditions have been linked with FGF such as cancer and metabolic disease, and this has been substantiated in mouse models [29,30]. FGF15, FGF19, FG21, and FGF23 are unique in that they regulate in an endocrine fashion, energy metabolism, bile acid, glucose, lipid, phosphate, and Vitamin D metabolism [9,11,31].

- FGF15 acts through β-Klotho [9].
- Recent mouse genetic studies have linked FGF19 and its tissue specific bioactivity with Klotho, to bile acid homeostasis [9,11,30,31].
- FGF21 binds only to the C-terminus of β-Klotho, *in vitro* and is associated with lipid metabolism and glucose regulation [11,31-33].
- FGF23 and its tissue specific bioactivity with Klotho has been linked to mineral homeostasis regulation and substantiated in recent mouse genetic studies [1,9,11,30]. FGF23 binds only to the C-terminus of α-Klotho, *in vitro* [11,31-33].

It has been shown [7,33,34] that transmembrane Klotho functions as a receptor for FGF21 and FGF23 and is required for their metabolic activity. Moreover, these FGFs can leave the tissues of origin and serve as circulating hormones due to the lack of a heparin-binding domains [7,33,34].

FGF19

It was shown that FGF19 can bind to both A/β-Klotho and it attaches on the N-Terminus of α-Klotho and C-terminus of β-Klotho [11,30,31] FGF-19 induces hepatocyte proliferation [11]. One unique feature of FGF19 is its weak affinity to heparin sulfate proteoglycans of the pericellular space [11]. Both FGF-19 and FGF-21 transgenic mice are resistant to diet-induced obesity and have decreased body fat mass and improved insulin sensitivity, glucose disposal, and plasma lipid parameters [11].

FGF21

FGF21 is an endocrine hormone that is predominantly expressed in the pancreas and liver but also muscle and white adipose tissue [7]. FGF21 is a hormone induced by various metabolic stresses, including high-carbohydrate diets and ketogenic states, that regulates energy homeostasis [35].

Pavlatou MG., *et al*. in 2016 [1] and Bartali Benedetta., *et al*. in 2013 [7] have linked FGF21 as a lipid metabolism and glucose regulator. Bartali Benedetta., *et al*. reported FGF21 is a likely contributor to skeletal homeostasis in mice and humans. β-Klotho is required as the necessary co-receptor which permits FGF-21 to mediate activators *in vitro* and promotes FGF-21 action *in vivo* [2,12,29,33,36-39].

FGF21 crosses the blood-brain barrier and has effects on weight loss, growth, and female reproduction, by acting on its cognate receptor in the CNS [40-46]. Amongst its central actions, FGF21 induces corticotropin-releasing factor and suppresses arginine vasopressin expression in the hypothalamus [41,43-45].

FGF21 was associated with expression of β-Klotho and subsequent up regulation of glucose transport (GLUT) in 3T3-L1 type adipocytes [36]. However, it was absent in undifferentiated 3T3-L1 fibroblasts [36]. Therefore, Suzuki., *et al*. in 2008 [36] suggested the expression of β-Klotho plays an endocrine role and acts as a determinant of the FGF-21 specificity of the target cells.

Further studies have linked FGF-21 with glucose and lipid control in diabetic rodents and primates, as well as regulation of sweet and alcohol consumption [26,32,33,47]. Furthermore, pharmacologically, FGF21 improves insulin sensitivity and causes weight loss in obese

humans, mice and monkeys [48]. Chu., *et al.* in 2013 [49] and Tanaka., et al. in 2013 [50] stated that Single-nucleotide polymorphisms to the human FGF21 gene are associated with changes in macronutrient preference, including decreases in fat and protein intake and increases in carbohydrate consumption. Additionally, another study by Ding., et al. in 2012 [37] supported this statement where they used β-Klotho knockout mice that demonstrated decreased growth and metabolism associated with loss of FGF-21 function [37]. Again, showing the importance of β-Klotho and FGF-21 association [37].

FGF23

FGF23 is a hormone that is derived from the bone and acts on the kidney as a bone mineralization modulator by regulating phosphate excretion, and the synthesis of 1,25-dihydroxyvitamin D3 [1,25-$(OH)_2D_3$] and parathyroid hormone (PTH) [7,36,51]. One critical feature of FGF23 is that it requires a co-receptor, the single-pass transmembrane protein expressed Klotho in renal tubules in order to activate it [3,35].

It has been extensively reported in the literature that Klotho contributes to Calcium and phosphate homeostasis in mice. It has been reported that FGF23 also acts as an insulin-independent glucose transporter in adipocytes, and a regulator of body weight, as well as regulating ketogenesis and adaptive responses to starvation [36,52].

FGF-23 negatively regulates Parathyroid Hormone, vitamin D and blood phosphate levels [1]. Furthermore FGF-23 maintains the Calcium concentration within strictly narrow ranges, thus plays a central role in calcium homeostasis in both the periphery and the CSF [1].

Klotho Type	Function	Body location	Possible related Conditions
α-Klotho	**Hormone**: Potential maintenance of normal renal function (7,9,53). Regulates phosphate (Pi) absorption and 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] activity (8,9). May suppress the insulin and Wnt Signaling pathways, inhibit oxidative stress, also regulates phosphate and calcium absorption (7,9,53). Angiogenesis (4,54); Anti-ageing effects (54,56); α-Klotho is a potential biomarker (57); Potassium metabolism (58); Antioxidant effects (59); Calcium metabolism (60,61,62); FGF signalling (47,50,63,64); Insulin signalling (56).	DCT epithelial cells - kidney (7,9,53) Kidney (9,11) Brain Choroid Plexus (3,35,55)	Ageing (54,72); BP control (73-76); Osteoporosis(74,77-81); Diabetes(82); Osteoarthritis(83); Mortality in patients on haemodialysis(84,85); Kidney stones (86); Rickets(87); Tumoral Calcinosis(88)
1.full-length trans-membrane α-Klotho	FGF signalling (47,50,63,64); Phosphate metabolism (65,66); Potassium metabolism (58); Calcium metabolism (60,61,62).	-	-
2.truncated soluble α-Klotho	FGF signalling (7,9,47,50,63,64).	-	-
3.Secreted α-Klotho.	* May have direct actions on tissues or cells that do not express Klotho (eg, vascular endothelial cells and smooth muscle cells) (9). *lowering intracellular oxidative stress and regulation of ion channel and transport (7,67). FGF signalling (47,50,63,64); Insulin signalling (56).	Blood circulation Cerebral Spinal Fluid (8,54)	Atherosclerosis (76)

β-Klotho	Metabolic regulation, glucose uptake, bile acid synthesis, and fatty acid metabolism, which are independent of α-Klotho. No Secreted form has been found yet. (meaning transmembrane type) (9,53); Regulates alcohol drinking (47); Adipogenesis (68,69); Glucose metabolism (70,71)	Highly localised to cell membrane. Liver and white adipose tissue. The Brain (54)	Glucose metabolism (70,71)
Klotho Variant **KL-VS**	(Unknown)	-	Breast Cancer (89); HDL, LDL, Cholesterol and uric acid Regulation (90,91); Coronary Artery Disease (92,93,94); Longevity (72,90,94); Ischemic Stroke (95-96); Sickle Cells Anemia (97); Type 2 Diabetes (98); Valvular and vascular(99) coronary calcification (92); Telomere length in Leukocytes(100).

Table 1: *Demonstrating different types of Klotho, their function and different body locations.*

	Mode of Action of Klotho [101]:
1.	Klotho acts as a glucuronidase
2.	Humoral factor (Secreted form. Binds to a Klotho receptor -but not yet identified) e.g as with cAMP pathways
3.	Co-receptor and cofactor for other proteins e.g. FGF-23
4.	Physical interaction with Na^+/K^+/ATPase in parathyroid cells and regulates the Ca^+ -stimulated PTH secretion.

Table 2: *Klotho Mode of Action.*

The role of Klotho in Cardiovascular disease

Lower plasma Klotho levels have been recently associated with atherosclerosis, oxidative stress, endothelial dysfunction and with an increased risk of cardiovascular disease [1,102]. Klotho levels in plasma were also negatively associated with C-reactive protein and age and positively associated with high-density lipoprotein cholesterol levels [1,102]. Genetic variants of Klotho have also been related with stroke [90,96,102], coronary artery disease [93,94,102,103] and longevity [72,90,102] in humans.

Subsequent studies [72,90,94] in humans have identified a functional variant of Klotho, labelled KL-VS (Table 1), that has been associated with early-onset occult coronary artery disease and with longevity. This common KL-VS allele harbours three mutations in the coding region which alter Klotho metabolism, of which two code for missense mutations F352V and C370S and one is silent [90].

Numerous other human Klotho gene polymorphisms have been related with changes in cardiovascular function and increased vulnerability to coronary artery disease [1]. Klotho single-nucleotide polymorphisms (SNP) G395 was found to be an independent risk factor for atherosclerotic coronary artery diseases and associated with systolic blood pressure in healthy Japanese women [103,104]. The Klotho SNP rs650439 was significantly associated with carotid atherosclerosis in hypertensive subjects and mean carotid artery intima-media thickness, suggesting that it may influence the progression of atherosclerosis in hypertensive patients [76]. Lower plasma nitric oxide

(NO) levels were found in individuals older than 40 years with the Klotho gene variant C1818T, suggesting an age-specific effect of Klotho C1818T variant, which becomes prominent with age [149].

Mechanism of Action of Klotho in Cardiovascular Disease

The Klotho-deficient mouse shows decreased NO biosynthesis and impaired vasodilation [106]. Subsequently, diminished bioavailability of NO contributes to vascular endothelial dysfunction, which leads to atherosclerosis [107]. Endothelial dysfunction occurs when endothelial cells lose their capability to defend against thrombosis and inflammation in response to several environmental and metabolic stressors [108]. Klotho has a positive role in endothelium physiology reducing endothelial dysfunction through multiple pathways and possibly protects the cardiovascular system through endothelium-derived NO production [90,109]. Klotho in the systemic circulation maintain endothelial integrity by regulating transient receptor potential canonical-1-mediated calcium entry in endothelial cells [1]. *In vitro*, Klotho stimulates NO production in human umbilical vein endothelial cells via activation of c-AMP-PKA pathway [1,110]. The activation of c-AMP pathway by Klotho also stimulates a two-fold increase in manganese superoxide dismutase (Mn-SOD) expression, either directly or through increased NO production [1,110]. Klotho may also buffer against endothelial inflammation. Thus, Klotho inhibited downstream effects of TNF-α [1,111].

A study by Saito, Yuichiro., *et al*. in 2000 [112] used various animal models with conditions including: atherosclerotic disease with multiple atherogenic risk factors e.g obesity, hypertension, dyslipidemia and severe hyperglycemia, were induced with adenovirus-mediated Klotho gene. This improved vascular endothelial function, increased NO production, reduced blood pressure, prevented perivascular fibrosis and medial hypertrophy [112].

Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) is a protein that in humans is encoded by the HCN4 gene [27]. In mammals, the HCN channel family comprises four members (HCN1-HCN4) [27]. HCN4 is prominently expressed in the pacemaker region of the mammalian heart [28]. Individuals with Sick sinus syndrome and bradycardia have been shown to have mutations in their HCN4 gene and the role of HCN channels in autonomic control of heart rate is currently under investigation [113,114].

A recent study by Qiu., *et al*. (2016) investigated whether Klotho is involved in the therapeutic effects of Astragaloside-IV (an anti-inflammatory herb) on bradycardia, as well as evaluated the effect of Klotho on HCN4 [25]. The results indicated that Klotho is involved in the treatment mechanism of ASG-IV increasing heart rate. Additionally, the expression of Klotho was also up-regulated ($P < 0.05$) and i*n vitro*, after incubation with LPS for 24h, HCN4 expression was significantly decreased in neonatal rat myocardial cells (0.6 ± 0.07 vs. 1.0, $P < 0.01$) and If was significantly declined [25].

Obesity and Diabetes

In today's society diabetes represents one of the most costly conditions worldwide [82]. There are 2 known forms of diabetes. Type-1 is associated with gene defects and subsequent immune response and with damage of Beta pancreatic cells, and the type 2 diabetes is known as the acquired type. In a novel study by Keles, Nursen., *et al*. in 2016(84) (n = 76) where an attempt to connect the known atherosclerotic formation relationship and Klotho with type 1 diabetes was undertaken. The epicardial fat thicknesses (EFTs) values of the study population were inversely correlated with serum Klotho levels ($r = -0.411$, $p = 0.013$).

In this study patients with type 1 DM had lower Klotho levels than the normal population and lower serum Klotho levels in patients with type 1 DM were related to higher, carotid artery intima-media thickness (CIMT) and Epicardial fat thicknesses (EFT) values, but lower left ventricle longitudinal global strain (LVLGS) and flow-mediated dilation (FMD) percentages. Since these parameters are associated with endothelial dysfunction and increased atherosclerosis risk, it can be concluded that serum Klotho may have protective effects against atherosclerosis and endothelial dysfunction in patients with type 1 DM.

Recent *in vitro* and *in vivo* studies demonstrated Klotho in remodelling of adipose tissue relevant in human pathophysiology [54]. These studies showed that Klotho may act as an adipocyte maturation factor vital for adipocytes turnover. Moreover, knockout mice models showed a phenotype of leanness i.e. not able to become and lacking steatosis [54].

In a recent study by Wu., *et al*. in 2012 [11] where an FGF-23 was modified to become FGF23-21c it was observed to have a similar function on glucose receptors as were previously seen with FGF-21. FGF-23-21c induced glucose uptake into adipocyte and improved glucose metabolism *in vivo* [11]. Klotho is relevant as FGF's require Klotho to interact with FGFR's and subsequent glucose uptake by the adipocytes. Administration of recombinant FGF-19 or FGF-21 in diabetic mice results in decreased serum glucose and insulin levels, improved glucose tolerance and reduced liver steatosis and body weight [11]. This study reports significant plasma glucose concentration decrease with injection of FGF-21 and FGF-23-21c, into diabetic mice, consistent with previous findings with adipocytes uptake of glucose [11]. Whilst the author states the obvious potential of this massive effect, further investigation into the pharmacological benefit of FGF-19/21 should be investigated as the mechanism of action is not yet fully understood. Similar findings have been additionally found in humans and monkeys [26].

Likewise, further studies by Chu., *et al*. in 2013 [49] and Tanaka., *et al*. in 2013 [50] reported that Single-nucleotide polymorphisms SNPs to the human FGF21 gene are associated with changes in macronutrient preference, including decreases in fat and protein intake and increases in carbohydrate consumption. In mice, these effects need the FGF21 co-receptor β-Klotho in the central nervous system and associate with decreases in dopamine concentrations in the nucleus accumbens [26]. Since analogues of FGF21 are currently undergoing clinical evaluation for the treatment of obesity and type 2 diabetes, these findings endorse the probability that FGF21 administration could affect nutrient preference and other reward behaviours in humans [26].

Alcoholism

Extreme alcohol consumption can lead to addiction and further dramatic consequences on public health [28,47]. Drinking habits may be inherited. However, few genes have been identified that are strongly associated to alcohol drinking [47]. A recent study by Schumann., *et al*. in 2016 conducted the largest genome-wide association meta-analysis and replication study to date, among > 105,000 individuals of European ancestry, and identified β-Klotho as a locus associated with alcohol consumption [47]. Additionally, using pharmacologic administration of FGF21 and mouse models, the study demonstrated that the hormone FGF21 inhibits alcohol drinking by acting on the brain [47]. Whereas brain-specific β-Klotho knockout mice have an increased alcohol preference. These results suggest that a liver–brain endocrine axis acts as an alcohol intake regulating mechanism and may provide a distinctive pharmacological target for decreasing alcohol consumption [47].

Likewise, another recent study by Talkdar., *et al*. in 2016 examined the effect of FGF21 on sweet and alcohol preference in mice and monkeys and reported that FGF21 administration evidently decreases sweet and alcohol preference in mice, and sweet preference in Cynomolgus monkeys [26].

Intracellular Signaling Pathways and Klotho

1. IGF-1 Signaling pathway
2. P53/p21 signaling pathway
3. cAMP signaling
4. PKC signaling
5. Wnt signaling

IGF-1 Signaling pathway

A recent study by Xu, Yuechi, and Zhongjie Sun in 2015 [9] reports that α-Klotho supresses downstream signalling pathway of insulin receptor substrate (IRS) and IGF-1 receptor, without directly acting on these receptors, the mechanism of which is not yet clear. However, it has been reported [3,9] to involve the forkhead box proteins and subsequent phosphorylation and decreased gene expression.

Circulating Klotho shows increased resistance to oxidative stress through its ability to inhibit IGF-1 signalling, this also occurs via fork head box proteins, subsequent upregulation of SOD2 promoter resulting in gene expression and removal of reactive oxygen species [1].

DNA damage was shown due to Mice over expressing Klotho, this was proved by a lower level of urinary 8OHdG - a biomarker of DNA damage [3].

Reasons for IGF-1 inhibition [3]:

1. Klotho negative mice are hypoglycaemic and extremely sensitive to insulin
2. Mice with overexpression of Klotho are resistant to insulin (in males) and to IGF-1(in females) although they maintain normal fasting blood glucose levels.

A study by Wang, Yuhong, and Zhongjie Sun in 2009 has substantiated that Klotho circulates as a hormone and by binding to the IGF-1 receptor it extends lifespan [3]. Again, a complete picture of its mechanistic action remains unknown.

p53/p21

All cells have a finite proliferative ability and they share phenotypic changes associated with cell death e.g cell swelling before arrest [3]. The role of p53 in cellular senescence is well understood in that it regulates gene transcription in cell cycle arrest and apoptosis during the transition from G1-S phase of cell replication. This process is mediated by the transcriptional activation of the cyclin dependent kinase (CDK) inhibitors e.g. p16 and p21 [3]. The author reported after DNA damage, Klotho can reduce the cellular senescence phenotype in MRC-5 primary human fibroblast cells and human umbilical vascular endothelial cells (HUVECs) through the p53/p21 pathway. In the same cell line loss of Klotho activity by RNAi, resulted in cell senescence. Thus, Klotho reduces senescence by the p53/p21 dependent pathway [3].

cAMP signaling pathway:

Cyclic adenosine monophosphate (cAMP) is a nucleotide generated from ATP through the action of the enzyme adenylate cyclase [3].

Protein Kinase A can become activated by increased cAMP. This results in activation or deactivation of many other cellular enzymes. Wang, Yuhong, and Zhongjie Sun in 2009, described how circulating hormone Klotho can cause an upregulation of cAMP, in particular in endothelial cells [3]. Results suggest that Klotho protein acts as a humoral factor to increase Adenosine-1-converting enzyme (ACE) activity in human umbilical vascular endothelial cells (HUVECs) via a cAMP–PKA-dependent pathway [3]. The author concluded Klotho protein may improve endothelial dysfunction by regulating reactive oxygen and antioxidant agents, that are supported by numerous studies [3].

PKC signal pathway

Protein kinase C (PKC) transduces the cellular signals that promote lipid hydrolysis. Additionally, PKC pathways are essential in controlling cell growth and differentiation. Klotho suppression of 25-hydroxyvitamin D3 1α-hydroxylase by is independent of the cAMP or PKC pathway [3]. This is the case as Klotho was shown to upregulate the PKC pathway in the kidney and testis where the Klotho gene is most abundantly expressed.

Wnt signal pathway

It has also been reported that α-Klotho may suppress Wnt Signaling [9]. Wnt3 signaling pathway prolongs the cell cycle by arresting the cell at G2/M phase and up regulating fibrogenic cytokines. However, experimentally it is shown α-Klotho treated cells bypass this phase and decrease cytokine production. Furthermore, under hypoxic conditions it was noted Klotho may be a natural antagonist of endogenous Wnt/Beta-catenin. Furthermore, loss of Klotho may contribute to kidney injury by causing pathogenic Wnt/Beta-catenin signaling. The *in vivo* expression of Klotho decreases the activation of renal β-catenin and improves renal fibrosis in chronic kidney disease [9].

Wnt signalling pathway is integral in stem cell differentiation, whilst over expression is associated with cellular senescence [3,4]. Wang, Yuhong, and Zhongjie Sun in 2009 reports that Klotho inhibits Wnt signal pathway by its extracellular domain binding [3]. The authors also describe how Klotho deficient mice showed aging phenotype of the skin [3]. This was explained to be caused by the role Klotho has on maintaining the balance required for normal cell proliferation rather than over differentiation resulting in early senescence. This has also been verified by Wang and Zhou in 2015 [4]. This same effect was seen in relation to the bone mineral density in Klotho deficient mice. Therefore, Klotho plays an inhibitory effect on Wnt signalling, required for normal cell proliferation [3].

In an extensive and recent review by Wang & Zhou (2015) [4] it was stated that dysregulation of the Wnt/B-catenin signalling has central roles in tumorigenesis and angiogenesis. Wnt could cause cancer through various genetic defects, such as mutations of B-catenin and other transcription factors.

Klotho and kidney disease

Abnormal mineral metabolism can lead to advanced chronic kidney disease (CKD) [14]. Recent studies and reviews [1,35,115-118] have identified a unique bone-kidney-endocrine-axis (See Figure 1) that sustains phosphate homeostasis. In order to maintain phosphate balance, Bone secretes FGF23 when phosphate is in excess, were it acts on the kidneys to endorse phosphate excretion into urine and suppress vitamin D synthesis [1,35,115-118]. However, FGF receptors requires co-receptor Klotho to be activated [1,35].

Klotho is profusely expressed in the renal tubules where it regulates mineral metabolism [1]. Klotho inhibits phosphorous reabsorption by moderating Na-coupled Pi transporters via enzymatic glycan modification of the transporter proteins [1].

Defects in either Klotho or FGF23 are related with phosphate retention, various kidney conditions and to premature-aging syndrome [1,35,115]. In contrast, high serum FGF23 levels in several hereditary disorders are associated with impaired bone mineralization and phosphate wasting [35,115].

Systemic Klotho deficiency can lead to acute kidney injury and CKD since Klotho deficiency intensifies kidney injury and decreases markers of renal function. However increased Klotho expression acts as a Reno-protective factor, in animal models of both acute kidney injury and CKD [1,119,120]. Reno-protective effects of Klotho has been attributed to its anti-inflammatory, antioxidant, and anti-apoptotic properties [1,119].

Klotho deficiency is also linked to various conditions such as vascular calcification, cellular senescence, oxidative stress, accelerated ageing, renal fibrosis and to renal osteodystrophy which leads to bone alteration [1,106,111]. According to reviews by Kuro-o, Makoto in 2009 [35] and Olauson, Hannes, and Tobias E. Larsson in 2013 [115], phosphate retention accelerates aging since the aging-like phenotypes in FGF23 or Klotho-deficient mice can be saved by reducing hyperphosphatemia with genetic or dietary management. Moreover, severe complications of CKD is soft tissue calcification, and vascular calcification [121]. Thus the reason why CKD is the highest risk factor for cardiovascular disease [1]. Klotho deficiency leads to phosphorus overload which aggravates calcification [1,121].

Renal fibrosis is the hallmark of CKD and its progress are significantly affected by epigenetic modifications [122]. Rhein, a plant-derived anthraquinone, displays strong anti-fibrosis properties, but its protective mode of action remains unknown [122]. A recent study by Zhang, Qin., *et al*. in 2016 tested the hypothesis that Rhein beneficial regulation of Klotho contributes to its anti-fibrotic functions [122]. The stidies results indicate that Klotho hypermethylation and aberrant DNA methylation expression significantly contribute to

renal fibrosis. Zhang, Qin., *et al*. reported that Rhein is an impressive up-regulator of Klotho, and the Rhein- Klotho promoter demethylation reduces Klotho loss and prevents renal interstitial fibrosis. These results support the concept that Klotho-targeted strategies with demethylating agent such as Rhein have strong preventing and treatment possibilities for renal fibrosis-related kidney disorders [122].

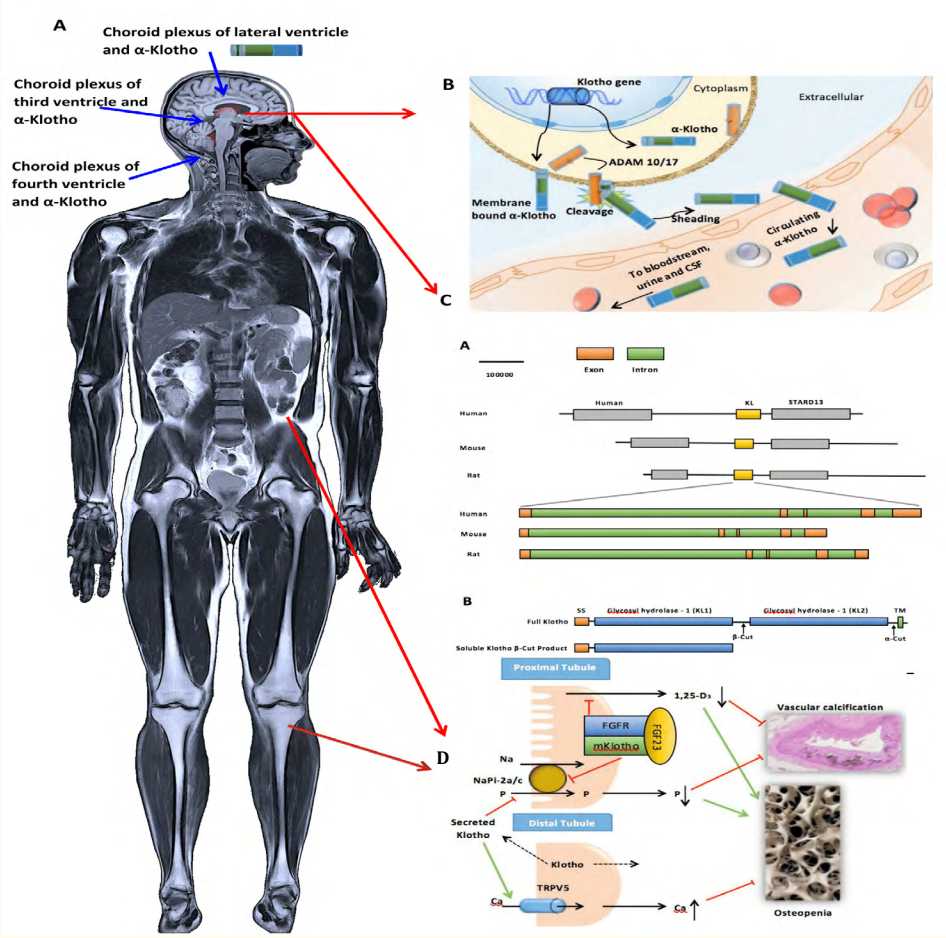


Figure 1: *(A) Site of Klotho production in Choroid Plexus of Ventricles in the brain [1]. (B) Post-translation process of Klotho protein exerting its potential humoral effects in the blood stream. This figure depicts the enzymes thought to be required for processing of the transmembrane form of Klotho, ADAM10 and 17 (α-secretases) and BACE1 (β-secretases). The cleaved ectodomain can proceed to be released into the circulation to exert its humoral effects [1]. (C) Depicts the architecture of the Klotho gene and protein in Human, Rat and Mouse models [9]. (D) Site of Klotho production in Distal Convoluted Tubules of the Kidney (Indicated by dotted line) [1]. Also depicted, the possible relationship between the effects of Klotho on Calcium and Phosphate Metabolism and subsequent effects on bone and vascularity. Binding of FGF23 to the membrane bound Klotho and FGFR Coreceptor inhibits the production of 1,25-Vitamin D3 and inhibition of Na+ dependent phosphate cotransporter (NaPi-2a/2c) in the proximal tubule. In Addition, secreted Klotho directly inhibits NaPi-2a/2c. The inhibition process of Klotho is protective for vascular calcification. However, over a prolonged period of time this causes bone demineralization 'osteoponeia' (Green arrow). Secreted Klotho stimulates renal Ca reabsorption which counteracts the effects of lower phosphates levels on bone. Additionally, Klotho exerts its effects on calcium metabolism through a bone-kidney-parathyroid axes (not shown here). In the parathyroid gland FGF23-Klotho bound supresses PTH production, which in turn leads to less production of 1,25 Vitamin D3. In comparison with the kidney-bone axis, FGF23-Klotho bound directly decreases expression of 1a-hydroxylase, the rate limiting enzyme in 1, 25 Vitamin D3 synthesis [1].*

A recent review by Olauson, Hannes, and Tobias E. Larsson in 2013 [115] stated that anti-FGF23 treatment using neutralising antibodies improved bone and biochemical issues. However, increased vascular calcification and mortality, whereas animal proof-of-concept studies have demonstrated positive effects of Klotho delivery in CKD. Thus, stressing the importance of developing treatment regimens that can maintain normal serum calcium and phosphate concentrations, reduce parathyroid hormone secretion, and correct any deficiencies [115].

Moreover, FGF23 and soluble α-Klotho are emerging potential biomarkers of phosphorus and vitamin D metabolism, which change in concentration in early CKD in order to maintain normal phosphorus levels [15,115,117,118]. Hu MC., *et al.* in 2010 [123] on healthy individuals and CKD patients of various stages, indicated that there is a gradual decrease in secreted Klotho protein in urine occurring during progression of the disease. Therefore, Klotho measurement in urine could assist as an early biomarker of CKD [123]. Likewise, a recent review by Dubal, Dena B., *et al*. in 2014 [15] reported that blood FGF23 and Klotho levels increase beginning at the early stages of CKD, stating that blood measurement could assist as an early biomarker of CKD. Tubular reabsorption of phosphate (TRP) has also been frequently used to evaluate renal tubular phosphate transport [117]. TRP is a simple and cost-saving marker for the assessment of altered mineral metabolism in CKD patients and can be used as a substitute to serum FGF23, particularly for mild to moderate renal insufficiency [117]. Clinical biomarkers may postulate novel therapeutic strategies to slow CKD progression and alleviate cardiovascular risks [115,118].

Osteoporosis

Blood phosphate levels are determined by:

1. Dietary phosphate,
2. Mobilization from bone (the major reservoir of phosphate and calcium),
3. Excretion from the kidney into urine

The body regulates the balance via various mechanisms of which Klotho is now part [35]. These processes are regulated by various endocrine factors such as vitamin D and PTH. The active form of Vit-D is synthesized in the kidney and acts on the intestines to increase the absorption of dietary calcium and phosphate [35]. It also acts on bone to promote mobilization of kidney and phosphate from the reservoir, thereby increasing blood levels respectively [35]. PTH acts on the kidney to promote both Vit-D synthesis and phosphaturia. Note PTH can selectively increase blood calcium levels without an increase in blood phosphate levels [35]. The bone-kidney endocrine axis mediated by FGF-23 and Klotho is described [35] as a major mediator of phosphate homeostasis.

A recent review by Pavlatou MG., *et al*. in 2016 [1] described evidence to support independent correlation of osteoporosis and spondylosis of the lumbar spine. Klotho represents a novel addition to the knowledge of pathogenesis of osteoporosis (See Figure 1). Originally where calcium metabolism consisted of PTH, calcium receptor, calcitonin and vitamin D. As mentioned many times previously Klotho's relationship with Calcium homeostasis of the body is integral with its function and thus represents a new piece of the puzzle of osteoporosis [1,78]. An example of the intermit relationship is that of hypocalcemia where Klotho responds by facilitating Na^+, K^+ - ATPase to the cell membrane of the parathyroid gland and distal convoluted tube of the kidney resulting in PTH secretion and calcium transport from the kidney [1,78]. TRPV5 (channel) is co-expressed in the kidney for the purposes of Ca^{2+} influx, Klotho is required to bind to TRPV5 prior to $Ca2^+$ influx [1]. Therefore, it becomes obvious what may occur in Klotho deficiency.

The net effect in Klotho deficient mice through allowing FGF-23 to inhibit Vitamin D levels results in increased plasma concentration of calcium and phosphorous [1,7,12,78]. When phosphate levels are too high FGF-23 released from the bone acts to reduce blood phosphate levels [12]. Osteoporosis results as seen with ageing. It was also stated by the authors citing recent studies [1,78] that Klotho deficient mice also have increased inflammatory markers related to osteoporosis.

A mini review by Bartali B., *et al*. in 2017 [7] sites a study that reported Klotho levels were associated with activities of daily living in elderly community dwelling adults. Also, the roles that Klotho has with FGF-23 and regulation of Vitamin D and serum phosphate levels. As extracellular phosphate is required for bone mineralization and intracellular for energy storage and production such as with muscle function this relates to one of the multi uses of Klotho described in the literature. Is Klotho linked to muscle mass and muscle function? Further studies have been reported on this matter [7].

A review by Torres, Pablo Ureña., *et al*. in 2009 discuss further studies looking at how Klotho deficient mice show radiological, histomorphometric osteopenia [101]. Interestingly osteoclastogenesis is upregulated in Klotho deficient mice [101], this suggests bone mineralization requires Klotho as a mediator and an underlying impairment. In humans SNPs have been associated with these bone changes, in particular in the Asian and Caucasian populations [101]. Pablo Ureña., *et al*. in 2009 goes on to describe the effects of Klotho on bone mineralization density as follows:

1. Vit D Metabolism: low serum 24,25-Dihydroxycholecalciferol, but elevated levels of 1, 25(OH)2D3, due to an increase in both renal 25-hydroxyvitaminD and CYP27b1 (1, 25(OH)2D3 - calcitriol).
2. PTH Metabolism: It has been suggested that mechanism is via physical interaction of Klotho with Na^+/K^+/ATPase. A high Na^+ gradient created after activation has been proposed to create the trans-epithelial calcium transport in the choroid plexus, kidney, and parathyroid gland [101].

Osteoarthritis

Osteoarthritis is a very common condition and often and mistakenly linked with ageing. In a study by Tsezou, Aspasia., *et al*. in 2008 [124] titled the 'Association of Klotho Gene with osteoarthritis in a Greek population', it was reported that recent evidence suggests the Klotho gene is linked to hand osteoarthritis in Caucasian people [124]. Furthermore, the study was designed to investigate the Klotho gene relation to Knee osteoarthritis in a Greek population. Of four SNPs genotyped, two were found to be linked with osteoarthritis, one in the promoter region and the other in exon 4, G395A and C2998T respectively. The case control study involving 752 (n = 369 patient group and n = 383 control group) Greeks from Thessalia. A significant difference for both SNPs of $P < 0.05$ was observed. More specifically in females for G395A patient's vs Control and only patient's vs controls for C2998T. However, there was no significant difference when stratified by sex [124]. It is postulated that cartilage metabolism is affected by Klotho levels and hence related to variations in the gene [124].

Human Cancer Development

There is a growing body of evidence implicating Klotho as a tumor suppressor [4]. In particular cervical, colorectal, gastric and lung carcinoma, pancreatic, hepatocellular carcinoma and breast cancer amongst a few [1,30]. In general, Higher Klotho expression was associated with smaller tumor size and Soluble Klotho treatment slowed progression of cancer. The author states that Klotho deficient mice show proliferation specifically the inhibition of IGF-1/insulin pathway by bFGF [4].

In a study investigating Klotho's involvement in oligodendrocyte maturation simultaneous research of the expression of Klotho in brain cancers was performed using Oncoming database www.oncomine.org [2]. The results of their search revealed that Klotho is significantly downregulated in almost all cancer types including brain malignancies [2]. Two exceptions were noted, that being thyroid and B-cell cancers where no change was observed [2].

A recent review by Zhou, Xiangxiang, and Xin Wang in 2015 [4] with the primary aim of synthesizing the literature for Klotho's relationship with cancer concludes that a downregulation of Klotho was observed across the different cancer types. Surprisingly the role of Klotho in cancer i.e. as a tumor suppressor was also mentioned by Wolf I., *et al*. in 2009 [89], this shows how novel and recent Klotho is in regard to cancer.

Breast Cancer

In breast cancer Klotho levels were significantly lower compared with normal tissue [2,4]. Carriers of BRCA-1 mutation in Jewish

woman also showed association with Klotho functional variant. It was found previously that Klotho has a tumor suppressor role in Breast Cancer [4,54]. Extensive methylation and histone deacetylation (epigenetic mechanisms) have been identified in several breast cancer related genes. Zhou, Xiangxiang, and Xin Wang., in 2014 [4] and Razzaque, M. Shawkat., in 2012 [54] cited a recent study that showed a linkage disequilibrium between functional KL-VS and BRCA2 617delT mutation. Compared with wild type, Klotho KL-VS could reduce the secretion and growth inhibitory activity of Klotho in breast cancer, IGF-1/IGF1R was again possibly involved [4]. Furthermore, over-expression of Klotho resulted in enhanced activation of the FGF pathway in breast cancer cell lines [4].

Lung Cancer

Lung cancer is one of the leading causes of death worldwide and is notoriously known for its low survival rates. A study by Chen Bo., *et al.* in 2010 found a role of Klotho in the pathogenesis of human lung cancer [125]. Klotho expression was shown to reduce proliferation and increase apoptosis of A549 cells, the same cell line as lung cancer [4,125]. The potential for Klotho yet again is mysterious and widespread. The authors identify the pathway is yet again associated with IGF-1/insulin signaling inhibition and Bax/Bcl-2 expression, not just in this study but in other studies as well [4]. An even more interesting point worth noting is the dose dependent nature of Klotho on preventing proliferation and inducing apoptosis in lung cancer cells A549 cells. Like other studies previously mentioned (see Intracellular signaling pathways and Klotho section) Klotho was also shown to inhibit Wnt pathway when in excess.

In small lung cell carcinoma Klotho expression levels predict good survival in patients with limited disease who received surgery [4]. Additionally, patients who were Klotho Negative received a much lower overall survival than positive Klotho levels, in Large-cell neuroendocrine carcinoma [4].

In a most recent study by Chen TianJun., *et al*. in 2016 [126] investigated drug resistant lung cancer cell type, the results showed significant decreased levels of Klotho. Experimentally it was shown over expression significantly inhibited cancer cell lines and under expression significantly promoted lung cancer cell proliferation [126].

Colorectal

Klotho gene promoter methylation was found to be associated with colorectal and gastric cancers, i.e Klotho gene was suppressed. Furthermore, downregulation was associated with poorer prognosis [2].

Cervical

The same author mentions a different study reporting hypermethylation of Klotho was associated with cervical cancer, but that it only occurred in the later stages of the cancer, thus resulting in loss of Klotho as a Wnt antagonist [2].

Recent data published in the British Medical Journal in 2015 [57] reveals the tumor suppressor nature of Klotho. The data suggested it is an epigenetically silenced mechanism through which cervical cancer is caused. The study aimed to study the expression pattern of Klotho in squamous cell carcinoma (SQCC) and adenocarcinoma (ADC). In 7 out of 38 SQCC samples no Klotho expression was observed compared to high levels of Klotho in normal surrounding healthy tissue and 2 of 44 samples in ADC group showed no Klotho expression [57].

Hepatocellular carcinomas

Similarly, with other types of cancers, apoptosis and autophagy are intact with restoration with Klotho levels as described in the same review by Zhou, Xiangxiang, and Xin Wang in 2014 [4]. Uniquely β-Klotho was identified to be upregulated in hepatocellular carcinomas, as stated by the author this was unique in that it conflicted with other reports saying β-Klotho was downregulated in regard to HCC and normal healthy tissue. Finally, the conclusion presented was that β-Klotho overexpression resulted in G1 to S phase arrest, reductive phosphorylation of Akt and GSK-3Beta, and restraining cyclin D1, all implicating β-Klotho in the Akt/GSK-3beta/cyclin D1 pathway [4].

FGF-role in Cancer

Activation and overexpression of the FGF receptors plays a significant role in cancer cell survival and proliferation [4]. As mentioned previously Klotho has an intimate role with FGFR's and thus binding could be a significant part of the activation and subsequent tumorigenesis.

The reader of this review is encouraged to visit these references in order to read the individual studies and research papers presented in the review on Cancer and Klotho. The extensive work presented is a suggested starting point for further additional studies in order to add to the current review [4].

Human Longevity

The Klotho gene cleverly named after the Greek Goddess '*Clotho*' who spins the thread of life, with Lachesis, and Atropos who measures and cuts the thread of life, respectively [1,127]. The Klotho gene accidently identified in mice in 1997 was shown to cause a marked decrease in lifespan and display characteristics associated with ageing [127].

Ageing is broadly defined as the decline in physiological functions necessary for survival and fertility. Previously models of ageing indicate its multifactorial nature from genetic variability and environmental stress to excessive protein, lipid and nucleic acid modification [3]. Until recently the specific effects of ageing on myelin sheath in white matter of the brain has not been well understood and a study by Duce, James A., *et al*. in 2008 [128] reveals the effects on cell function including increased cell cycle inhibition and proteolysis, as well as decreased mitochondrial function, signal transduction and protein translation [128]. These specific effects are the cause for the resulting cognitive decline and function seen. Furthermore, other phenotypes typical of ageing include e.g atherosclerosis, vascular calcifications, soft tissue calcifications muscle atrophy etc. [3].

Klotho and its relation to longevity presents a novel element and has been extensively studied and verified in many models of which has made the understanding of the ageing process greatly improved [1,9,12,27,30,31,72,76,101,128-131]. Phenotypes seen in Klotho deficient mice closely resemble that of ageing e.g. shortened lifespan, muscle atrophy, osteopenia, and infertility, arteriosclerosis, gonadal dysplasia, skin atrophy, ataxia, hypoglycemia and severe hyperphospatemia [3,7,101,127,130,131]. Klotho deficient mice stop growing and hardly gain body weight and typically only live for 8 - 9 weeks [127]. Conversely, overexpression of Klotho increases life span in mice [7,34,127,131]. This has also been verified in the rhesus monkey [128] and epigenetic mechanisms such as methylation has shown to decrease gene expression (30.26). In Klotho, deficient mice who are able to stimulate Klotho expression by uptake of Zinc Sulfate in water, are able to produce Klotho in the gastrointestinal tract and reverse the ageing effects of Klotho deficiency [127]. In humans, a recent review on Klotho [1] supports that Bohemian Czechs with a variant allele of Klotho (KL-VS) showed an increased lifespan past that of 80 years of age.

Another recent study demonstrated that the lifespan-extending variant of the Klotho gene (KL-VS) is associated with increased Klotho levels in serum and enhanced cognition in heterozygous aging population [15]. Also across multiple age groups [15]. In a systematic review and a meta-analysis [15,34] it was shown that KL-VS variant Klotho carriers were associated with higher cognition vs non-carriers in human cohorts. Cognitive abilities of KL-VS carriers and non-carriers were examined using multiple neuropsychological tests and compared with global averages [15].

Mechanisms of Action

Klotho increases life span via several mechanisms, including: the reduction of calcitriol synthesis, serum calcium, and phosphorus levels, the induction of insulin resistance; and by increasing the resistance to oxidative stress and inflammation [101,131]. The link between Klotho and inflammation has been shown in a recent study [131]. Increased levels of phosphate observed seems to be the cause for premature death, and longevity is revived by reducing phosphate levels through diet or genetic mutation, as seen in mice [130].

Longevity in mice is attributed to a decreased insulin/IGF-1 signaling pathway, that occurs by suppressing tyrosine phosphorylation of insulin and IGF-1 receptors [1]. Another proposed mechanism is that a reduction in insulin-stimulated intracellular glucose availability potentially prevents intracellular lipid overload and lipotoxicity [1].

The author states that mutations observed in the KL-VS variant may alter Klotho levels or function contributing to the development and premature appearance of various diseases associated with increased morbidity and mortality.

According to recent reviews by Olauson, Hannes, and Tobias E. Larsson n 2013 [115] and Zeldich E., *et al*. in 2014 [130], phosphate retention accelerates aging since the aging-like phenotypes in FGF23 or Klotho-deficient mice can be saved by reducing hyperphosphatemia with genetic or dietary management. One of the occurrences of Klotho deficiency is hyperphosphatemia [132]. Therefore, it appears the change in phosphate metabolism is related to premature ageing. Both Klotho and FGF-23 are intimately required for phosphate metabolism and thus a deficiency in FGF-23 results in similar phenotype. Ultimately hyperphosphatemia occurs via increasing intestinal phosphate absorption and increasing renal phosphate reabsorption.

How does hyperphosphatemia cause premature ageing?

1. Causes intracellular insulin signaling and unfavourable cell metabolism
2. Increased mitochondrial membrane potential and oxidative stress
3. Promotes vascular calcification

Interestingly Huang, Chou-Long in 2010 cites how dietary restriction of phosphate reverses this happening [132].

Longevity occurs in part due to Klotho's ability to promote phosphate excretion via the kidneys. Conversely prolonged decreased phosphate levels cause bone disease [132]. Stimulation of TRPV5 and renal Ca^{2+} reabsorption by secreted Klotho promotes positive Ca^{+} balance and counteracts the effect of low phosphate balance on bone. The author presents the role of regulation of TRPV5 (in the kidney) by a working model of Klotho's effect on the proximal and distal convoluted tubule and subsequent effect on phosphate serum levels and calcium absorption and subsequent use for bone mineralization. Furthermore, the author describes how the stimulation of TRPV5 and renal Ca^{2+} reabsorption by secreted Klotho, as demonstrated in an animal study by Alexander RT., *et al*. in 2009 [133] will promote positive Ca^{2+} balance and counteract the effect of low phosphate balance on bone.

A recent review by Abraham, Carmela R., *et al*. in 2012 [131] reports a case of a 13-year old girl with a homozygous missense mutation of the Klotho gene with decreased levels of Klotho, this girl showed osteopenia and it was noted no neuropsychiatric symptoms were observed. The FGF-23-Vitamin-D- Phosphate Axis was implicated as the pathway affected [131].

Neurodegenerative diseases and Klotho

The most prevalent neurodegenerative diseases are Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD) and amyotrophic lateral sclerosis (ALS) [131]. In the last 30 years, in rare cases of these disorders, gene duplications, mutations, deletions or expanded triple repeats have been identified to cause either autosomal recessive or dominant inheritability [131]. HD is always dominantly inherited, whereas the majority of PD, ALS, and AD cases are sporadic or idiopathic [131].

Vitamin D deficiency are associated with a dysregulation in both redox and Ca^{2+} signalling and has been linked to many human diseases such as hypertension, cardiovascular disease, AD, PD and in MS as described before [131,134,135]. The ability of vitamin D to sustain healthy cells appears to depend on its ability to maintain the reactive oxygen species (ROS), the redox and Ca (2+) signalling systems [104,134,135]. The expression of the signalling components responsible for stabilising the low-resting state of these two signalling pathways are preserved by vitamin D [134,135]. This phenotypic stability function is enabled through the capability of vitamin D to increase the expression of both Klotho and Nrf2 (nuclear factor-erythroid-2-related factor 2) which are also major regulators of redox and Ca (2+) signalling [134,135]. Therefore, a decline in vitamin D will disrupt the stability of this regulatory signalling network and may lead to various disorders [104,134,135].

Klotho and Inflammation

Klotho is a novel β-glucoronidase transmembrane enzyme that is highly expressed in both hippocampus and choroid plexus where it produces antioxidative and anti-inflammatory effects, and also regulates phosphate and calcium homeostasis [131,135]. The anti-aging properties of Klotho are also related with increased resistance to inflammation and oxidative stress [131,136]. Secreted Klotho functions as a humoral factor, it regulates ion channel and transport, and lowers intracellular oxidative stress [7].

Oxidative stress occurs when there is an elevated intracellular level of ROS, causing damage to proteins, lipids and DNA [35]. Oxidative stress has been associated to several pathologies [35]. Inflammation has been characterised as the "secret killer" and is associated with many modern-day diseases such as heart, cancer, and neurodegeneration disease (9.1). Klotho's downregulation is associated with increased inflammation in kidney, rheumatoid arthritis and inflammatory bowel disease [119,131,137].

FGF21 has recently been demonstrated in playing a role in inhibiting the activation of the transcription factor nuclear factor-kappa B (NF-kB) [131,138], the main inflammation regulator that can be activated in skeletal muscle cells under inflammatory conditions [7,131]. NF-kB activation is strongly associated to elevated oxidative stress, which changes the balance between protein synthesis and degradation [7,138]. Thus, may disturb the rate of protein degradation in skeletal muscle [7,138]. Therefore, the cytoprotective properties of Klotho can defend against oxidative stress and decrease protein degradation and muscle loss [7]. The defensive influence of Klotho through the negative regulation of NF-κB signaling was also observed in the model of inflammation in injured and diabetic kidneys [132,138]. The protection mechanism comprised the inhibition of RelA Ser-536 phosphorylation as well as promoter DNA binding of this phosphorylated form of RelA [131,138].

The expression of manganese superoxide dismutase (MnSOD), an essential enzyme for mitochondrial antioxidant defences in mammalian cells, is up-regulated by FOXO3a [9]. FOXO3a functions as a negative regulator of mitochondrial ROS generation [9]. α-Klotho stimulates FOXO3a phosphorylation, indicating that α-Klotho could suppress ROS-related oxidative stress [9,35].

It was recently reported by Wang, Yuhong., *et al*. in 2012 [139] that the expression of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase 2 is down-regulate by α-Klotho. NADPH oxidase 2 is a catalytic subunit of NADPH oxidase that transfers electrons from NADPH to the outside of the membrane [139]. This study also revealed that α-Klotho decreases oxidative damage, AngII-induced superoxide production and apoptosis through the cAMP/PKA pathway [9,139].

A recent review by Xu, Yuechi, and Zhongjie Sun in 2015 [9] stated that α-Klotho overexpression can reduce mitochondrial DNA fragmentation, H_2O_2-induced apoptosis, β-galactosidase activity, lipid peroxidation, superoxide anion generation and Bax protein expression, a function that was reported to be related to the apoptosis signal-regulating kinase 1.

Alzheimer's

Alzheimer's is the most common form of dementia in the elderly [131]. It has been shown to have a strong genetic/epigenetic and environmental component and has been projected to affect more the 100 million people by 2050 worldwide [131]. The phenotype of which is a gradual decline in memory and cognitive ability affecting activities of daily living [131].

AD risk by early linkage studies [140-142] has shown that mutation effects the Amyloid Beta Precursor Protein (APP) pathway and causes subsequent toxicity and accumulation of plaques of beta-amyloid protein. APP has been suggested to support neurons and allow movement of neurons such as with neuroplasticity [140].

In one of the largest genome wide studies to date APOE gene on chromosome 19 was consistently observed in patients with AD phenotype [140,141]. In summary, there is confirmed genome wide association in AD patients, for the effects specific genes have on: 1. Immune response and inflammation, 2. APP processing, 3. Tau Pathology, 4. Cell migration, 5. Lipid Transport and endocytosis, 6. Hippocampal synaptic function and 7. Cytoskeletal function and active transport as well as 8 Regulation of gene expression, post-tran protein modification, microglial and myeloid cell function [140].

The precise role of Klotho in ageing type neurodegenerative disease remains unknown yet it is still implicated in such conditions like Alzheimer 's disease [131]. Its pleiotropic effects in ageing and neurodegeneration have shown that it enhances cognition and increases life span [34].

It is observed that Klotho levels are even lower in AD states in monkeys when compared to just the ageing type [131]. The white matter and myelin is significantly affected in the Klotho deficient mice, monkeys and rats but even more so in the Alzheimer's phenotype. Consistently there is a decreased level of Klotho in the CSF of the aforementioned mammalian models [34]. Ultrasounds and MRIs have shown that Klotho Knock-out-mice show significantly decreased myelin on the optic nerve [131]. If it has been shown in numerous studies [2,131,143] previously mentioned that Klotho enhances myelination by facilitating maturation of Oligodendrocyte Precursor Cells (OPCs), then loss of Klotho needless to say would not have such a desired effect.

As shown previously by Pavlatou MG., *et al*. in 2016 [1] that in a population of Bohemian Czechs with Klotho variant KL-VS gene and an increased survival rate, KL-VS actively causes an increase in circulating Klotho levels, larger prefrontal cortical regions and increased cognitive function [15,34].

These studies beg the question 'Can improving Klotho levels restore phenotype in AD patients?'

In a recent study by Dubal., *et al*. in 2015 [34] published in the Journal of Neuroscience, transgenic mice with an overexpression of Klotho were crossed with transgenic mice with Human Amyeloid Precursor Protein, carrying mutations causing AD (Specifically early onset subtype). These mice were considered to have the same phenotype as humans with AD in terms of cognition and other deficits. Interestingly it was shown that altering the Klotho reduced premature mortality and all other phenotypic characteristics of AD mice [34]. The author continues to explain that these effects are due to modulation of NMDA and receptor (NMDAR) function [34].

In an experimental study by Schafer, Marissa J., *et al*. in 2015 [144] calorie restriction was shown to enhance longevity, but the physiological response on the cells was not clear. In particular, the study focused on the effects of calorie restriction on areas of the brain specifically affected during AD, the hippocampus and CA1 region. This was the case as it has been shown before [144] this area of the brain is affected in learning and memory deficits and neurodegeneration. It was shown that CR caused a suppression of aging related genes and an upregulation of neuroprotective factors including Klotho and transthyretin. This study showed an increase in a functional transcriptional state of the aforementioned neuroprotective factors in CA1 of the hippocampus, consistent with a relative youthful state [144].

An interesting study by Zeldich., *et al*. in 2014 [130] sought to understand the mechanism through which the elixir protein Klotho seems to have its effects. In both rat and mice models, pre-treatment of the Hippocampal regions with Klotho protected them from glutamate and oligomeric amyloid Beta - induced cytotoxicity [130]. The resistance was even greater in transgenic overexpressing Klotho mice [130]. Klotho significantly enhances the expression of thioredoxin/peroxiredoxin (trx/Prx) system, and Klotho was also shown to repress PI3/Akt pathways important in apoptosis and ageing, and this was associated with sustained inhibition of phosphorylation of forkhead BOX O3a and was essential for the induction of Prx-2. Downregulation of Prx removed the ability of Klothos neuroprotective effect against glutamate showing the key role of Prx in neuroprotection [130]. This was one of the first studies this systematic review included that showed an underlying mechanism through which Klotho exerts its effects.

Learning and memory

Learning and memory involve different brain regions, including the cortex and hippocampus [15,145]. It also comprises coordinated activities of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptors (NMDARs and AMPARs) [34,116,145,146]. Excitatory neurotransmission mediated by NMDARs is necessary to the physiology of the mammalian central nervous system [15,145,146]. These are heteromeric ion channels receptors that require binding of glycine to the NR1 and glutamate to the NR2 subunits in order to be activated [145]. Once activated, influx of cations initiates signal transduction cascades that are essential for critical functions including learning and memory [145]. These NMDAR- and AMPAR-mediated functions are disrupted by aging and age-related neurodegenerative disease [15].

Aging is the primary risk factor for cognitive deterioration [116]. Klotho can regulate age when overexpressed and may extend lifespan [1,9,15,101,130,131]. In humans, a single variant allele of Klotho (KL-VS), increases secreted Klotho, enhances FGF23 signalling in cell culture, promotes longevity and diminishes age-related diseases [1,15,147]. A recent study by Dubal., *et al*. in 2014 [15], analysed transgenic mice with systemic overexpression of Klotho and reported that the mice performed better than controls in multiple tests of learning and memory. Additionally, increased Klotho also enhanced long-term potentiation and improved synaptic GluN2B, an NMDAR subunit with important functions in learning and memory [15]. Klotho mediated effects were abolished from GluN2B blocked [15]. Thus, Dubal., et al. concluded that enhancing Klotho may enhance cognition and counteract cognitive deficits at different life stages [15].

However, it is unknown whether Klotho elevation can counteract cognitive disorders, such as Alzheimer disease (AD) [34]. Another recent study by Dubal., *et al*. in 2015 [34] crossed Klotho transgenic mice that overexpress wild-type mouse Klotho throughout the brain and body with human amyloid precursor protein (hAPP) transgenic mice from line J20, which carry mutations that cause early onset AD in humans. As in humans with AD, singly transgenic hAPP-J20 mice have elevated levels of amyloid-β (Aβ) peptides in the brain as well as neuritic amyloid plaques, synaptic impairments, neural network dysfunction, deficits in cognition, behavioural abnormalities, and premature mortality [34]. Dubal., *et al*. in 2015 [34] revealed that elevating Klotho expression decreased synaptic and network dysfunction, premature mortality as well as behavioural and cognitive deficits in hAPP transgenic mice, without changing levels of soluble Aβ, plaque-associated neuritic dystrophy, Aβ deposition, β-CTFs, or tau. Additionally, the depletion of NMDAR subunits in the hAPP mice hippocampus was prevented with Klotho elevation, enhancing spatial learning and memory [34]. Klotho elevation also increased GluN2B subunit abundance in postsynaptic densities and NMDAR-dependent long-term potentiation (LTP), which is crucial for learning and memory [34] as seen in figure 2 [15]. TP provides a measure of synaptic plasticity and is a cellular substrate of learning and memory [34]. Therefore, Dubal., *et al*. in 2015 [34] concluded that increasing Klotho levels or activities improves cognitive and synaptic functions, and may be of therapeutic benefit in AD and other cognitive disorders.

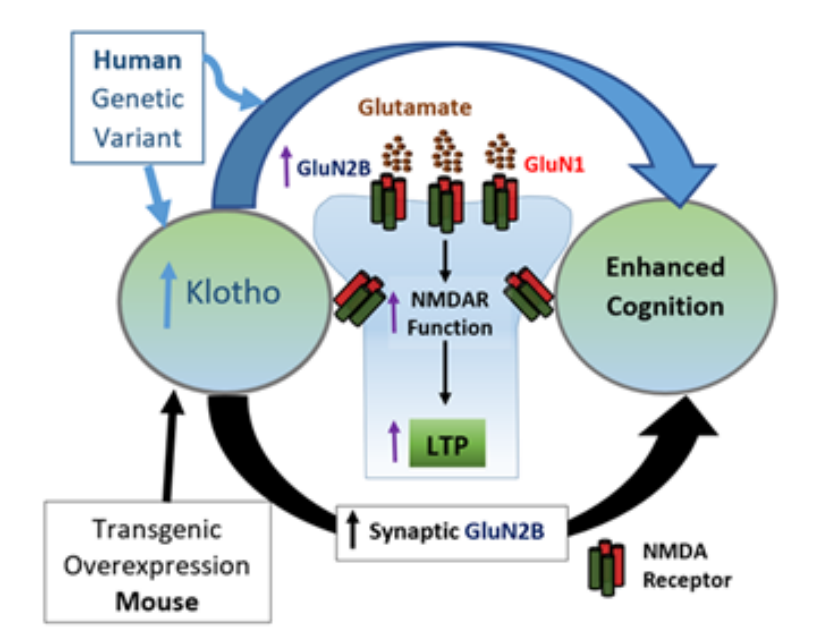


Figure 2: *Demonstrating an increase in GluN2B abundance in post synaptic neurons and NMDAR-dependent long-term potentiation in response to increased expression of Klotho, this is considered crucial for cognition and memory [15].*

CNS and CNS Disorders

This review primary aim is to synthesis the literature and provide an overview of the newly discovered protein Klotho. Klotho is primarily produced in two places, the choroid plexus and the kidneys [1]. Specifically, the ependymal cells of the choroid plexus which face the ventricles produce Klotho [1] (see Figure 1A).

A single study by Pavlatou MG., *et al*. in 2016 [1] of 804 subjects over 65 years of age, designed simply to compare lower and upper tertiles of Klotho levels, reported that those in the lowest textile had a significant increase in all-cause mortality and significantly greater cognitive impairment, as assessed by a Mini Mental State examination score of less than 24 [1].

The mechanism through which Klotho acts in the CNS is via a Na^{+}, K^{+}-ATPase channel, that possibly in harmony with Ca^{2+} binding proteins and calcium channels (TRPV4) promote calcium export and substantiated in mice models [1,60].

Unbalanced calcium homeostasis in neurons is associated with neuronal degeneration and death such as seen in Alzheimer's disease [1]. Several studies [114,148,149] have shown that Klotho deficiency was associated with a decreased number of large neurons, nerve terminals and degeneration within the cortex, hippocampus and purkinje cells as well as the anterior horn cells of the spinal cord [1,131]. A recent study by Clinton, Sarah M., *et al*. 2013 [150] compared knockout mice to wild type. The knockout exhibited a phenotype of memory loss, altered cholinergic function, increased apoptotic markers and elevated oxidative stress, decreased purkinje and dopaminergic cells, as well as showed insufficient transport and synaptic expression [150,151].

It has been recently shown that Klotho enhances the maturation of oligodendrocytes and in the absence of Klotho, Oligodendrocytes Precursor Cells (OPCs) do not maturate [2,131,148]. Additionally, Klotho enhances myelination of the central nervous system [131,148]. Therefore, the autocrine hormonal function of Klotho is evident. Neuropathologically Klotho deficient mice displayed characteristics of neurodegeneration concluding that Klotho had a major impact on health and function of neurons and oligodendrocytes [152]. Analysis of protein mRNA levels shows that Klotho also enhances expression of proteins involved in myelination by oligodendrocytes [143,148]. In OPCs, Abraham., *et al*. in 2016 [143] showed Klotho to upregulate major proteins required in myelination, proteolipid protein, myelin basic protein, and myelin-associated glycoprotein. Klotho deficient mice also show impairment in the nodes of Ranvier, as the paranodal region is diminished and nodal region is enlarged. Furthermore, there is a growing body of evidence that Klotho plays a significant role in enhancing myelination. This has been verified in both *vitro* and *vivo* animal studies [143,148]. In a microarray analysis comparing young and old monkey white matter, we discovered that Klotho is downregulated in the aged brain [131].

Several groups have established using MRIs observing the subsequent hyper-intensities in white matter of patients with AD and MS, the remyelination is incomplete [12,31,101,115,130]. As MS is an inflammatory demyelinating disease of the central nervous system a diminished number of OPCs has been observed which has led to the conclusion that a factor that would induce OPC maturation may be missing and desired [12,31,101,115,130].

Multiple Sclerosis

MS is the most common Disease of the central Nervous System [151]. MS is characterized by demyelination of neurons that is accompanied by loss of neural cells required for cell integrity for example oligodendrocytes [131,151]. Although not a typical neurodegenerative disease as neurons are not a direct target of destruction, rather the myelin sheaths begin to unravel and as a result normal neural condition is inhibited [131]. MS is considered an autoimmune disease where immune cells are targeted against self-cells and are able to cross the blood brain barrier into the central nervous system where they can act on the myelin in the brain and spinal cord [131].

Ellidag, Hamit Yasar., *et al*. in 2016 [151] and Abraham, Carmela R., *et al*. in 2012 [131] have shown that as the body begins to repair the damaged myelin, re-myelination is incomplete and oligodendrocyte precursor cells (OPCs) are found near the lesions. OPCs are immature oligodendrocytes that require Klotho for maturation. Abraham, Carmela R., *et al*. in 2012 [131] described evidence where Klotho

enhances the maturation of OPCs into mature oligodendrocytes. Therefore, can administering Klotho enhance the maturation of OPCs? Therapeutic research appears to be lacking and should be considered for the treatment of not only MS but also neurodegenerative diseases and other pathologies associated with the Klotho.

As stated previously the required role off FGF-23 is to decrease the expression of type 2 Na+/Phosphate co-transporters and increase phosphate secretion. Also, FGF-23 suppression of 1a-hydroxylase activity which decreases Vitamin-D levels, which in turn leads to the suppression of Phosphate absorption from the kidneys. The importance of Vitamin D in the progression and prevalence of MS has been demonstrated previously by Ellidag, Hamit Yasar., *et al*. 2016 [151]. Hu, Ellidag, Hamit Yasar., *et al*. explored the interactions of FGF-23, Klotho, P and Vit-D in MS patients. This is a huge focus in some countries where vitamin D levels are of concern. Decreased vitamin D levels as seen with children who have shown less exposure in childhood is a risk factor for MS [151].

In this study, a systematic literature search was done to identify relevant studies relating to MS and Klotho (see table 3 and flow diagram 1) for a summary of the included studies and methods.

Study	Design / Level of Evidence	No.	Participant type age	Intervention/ analysis method	Comparator(s) /Exposure	Outcome(s)/ Exposure	Main findings/ Statistics
Ellidag., *et a*l. in 2016 [151]	Level III-2	32	RRMS Males:12 Females: 19 (38.5yrs)	Serum Klotho, FGF-23 and Phosphate levels/ Serum PTH - Becker Cultman Kit; sKlt and FGF-23 - ELISA; Serum Ca, P, urea - assay kits	31 Control - No risk factors	Serum Concentration	All three comparators were higher in RM-MS pts compared to control (P,0.01, P < 0.01 and P = 0.02 respectively) Serum Vit-D and Calcium was lower in RM-MS pts (P < 0.01 and P = 0.04) Serum PTH was lower - n.s.d (p = 0.09)
Ahmadi., *et al*. in 2016 [8]	Retrospective observational case control Level III-2	45	15 New cases RRMS; 15Chronic cases RRMS	ELISA methods	15 Controls	Serum Concentration	n.s.d. Between new cases and control (P = 0.859) Chronic group: Higher Klotho levels vs control (P = 0.037)
Aleagha., *et al.* in 2015 [153]	Case Control Level III-2	44	22 Patients with RRMS	Lumbar puncture to collect samples. ELISA to measure Klotho	22 Controls (other Neuro conditions)	CSF concentration of α-Klotho	Klotho Concentration in patients with RRMS was severely lower than controls (P < 0.0001). Ferric reducing anti-oxidant power (FRAP) - 19/22 measured. Result: 1.5-fold decline in MS patients vs control (P < 0.01) A significant negative correlation was observed between EDSS (disability) and Klotho Concentration. n.s.d between EDSS and FRAP levels. However, it was positive.

Ushigusia., *et al*. in 2016 [57]	Retrospective case control Level III-2	84	NPSLE - 34	Lumbar puncture to collect CSF. ELISA - in the CSF	SLE - 25 VM - 19 RRMS - 20 NMP - 20	CSF & Serum concentration	CSF α-Klotho in NPSLE patient group was sig. lower than SLE and VM group. Serum α-Klotho levels of NPSLE were sig. lower than VM, MS and NMO and similar to SLE. P < 0.05. Cut-off value was 230.2 pg/ml with 82.4% sensitivity and 94.0% specificity for NPSLE (p- = 0.0004)

Table 3: *Summary of included studies - Multiple Sclerosis SR.*

Summary of selected studies (See table 3)

Ellidag, Hamit Yasar., *et al*. in 2016 [151] recruited 32 patients who were admitted to hospital for relapsing-remitting MS (RM-MS). All patients were diagnosed by the standard McDonalds Criteria. While no or little significant difference was found between disease duration or disability scores (weak negative correlation), there was a significant difference seen for FGF-23, Klotho and Phosphate serum levels which were significantly higher in the RM-MS patient group compared with risk free healthy controls [151]. On the contrary Vitamin-D levels were significantly lower in the RM-MS group. A correlation was seen between Klotho and Vit-D as well as FGF-23 and Vit-D in the control group but not in the MS group.

Choroid Plexus and Klotho: Klotho may allow leukocytes through the Blood Brain Barrier (BBB) due to its inhibiting role of the Wnt signaling pathway implicated in tight junction regularity [151]. This is explained by the gene expression similarities between the Choroid Plexus and kidneys and the functions of the proteins expressed (i.e. ionic exchange).

Oligodendrocytes and Klotho: Nerve damage leads to OPCs proliferation and Klotho secretion as seen in MS [151]. Therefore, Klotho secretion could be a response to inflammation in the CNS and could change the specificity of the BBB [151].

FGF-23 and MS: Similarly, to Klotho, FGF-23 secretion from neurons or Choroid Plexus could also lead to the disruption of BBB integrity and later the phosphate metabolism in CSF.

Klt-FGF-23-Vitamin D Axis: The author concludes that the Klth-FGF-23-VitaminD axis is altered in MS patients. They believe that lower vitamin D levels are not the cause rather the result of elevated FGF-23, which in turn exacerbates inflammation and creates a vicious cycle [151]. The conflicting results of this study are attributed to possible underlying mechanism of MS or rather a response to the chronic inflammation in MS [151].

Likewise, another study by Ahmadi, Mona., *et al*. in 2016 reported a significant negative correlation with Klotho levels and the expanded disability status scale (EDSS) [8]. Again, it has been elucidated by independent authors that there is a significant difference in Klotho levels and MS pathophysiology. Furthermore, looking at Klotho levels in different stages of patients with MS, and so 15 new cases with no prior history of immunosuppression and or vitamin D treatment were recruited as well as 15 chronic cases and controls [8]. The whole protocol was presented in methods and statistical analysis was performed. No significant differences were observed between demographics of the participants in all groups, including the EDSS. There was a slight increased score in the new cases group (but not statistically different) [8].

The study presented a histogram indicating that serum Klotho concentration in new cases (585.56 pg/ml ± 153.99) was higher than that of the control group (556.81 pg/ml ± 120.36). This difference, however, was not statistically significant (P = 0.859). On the other hand, there was a significant increase in the concentration of serum Klotho of the chronic group (696.94 pg/ml ± 170.52) when compared to the control group (P = 0.037). No significant difference was observed between the new cases and the chronic group in this regard (P = 0.116) [151]. However, a change is observed, thus Klotho may show as a potential biomarker for MS patients in serum or CSF.

This raises the question whether a study could be conducted to determine if people with a known high risk of developing MS (e.g. relatives) have different levels of Klotho to a low risk population. A longitudinal study could be conducted to determine if Klotho levels change and whether this corresponds to pathophysiological states. In general, to detect changes in Klotho pre-clinical phase and development of clinical symptoms. This could be beneficial for MS patients to detect the onset of symptoms with aim to delay or prevent illness.

Ahmadi, Mona., *et al*. in 2016 demonstrated that the secreted form of Klotho in CSF of patients with RM-MS was reduced in comparison to controls [8]. This finding could be attributed to the treatment with immunomodulatory drugs or a compensatory response to enhance CNS regeneration and/or vitamin D biosynthesis [8]. Oxidative stress and inflammatory conditions which are able to decrease Klotho expression have been shown as contributing factors to the MS pathogenesis [8]. Another study cited within this one was reported to have found that vitamin D supplementation decreased the serum Klotho concentration in hemodialysis patients. Ahmadi, Mona., *et al*. supports the hypothesis that serum Klotho levels might increase in MS patients due to Vitamin D deficiency. Connecting the fact that Klotho helps oligodendrocytes maturate for the purposes of remyelination this may explain the prolonged (chronic) course of MS and therefore the increased serum Klotho levels [8].

Another study by Berridge Michael J., in 2015 focused on vitamin D deficiency and its relation to MS [134]. Vitamin D deficiency is a major public health problem and has been linked with many human diseases such as Alzheimers Disease, Parkinson 's disease, cardio vascular disease, rickets and MS and many others. In general, the active form of Vitamin D is a hormone that regulates many different cellular processes, e.g. calcium homeostasis, antioxidant defences, inhibiting inflammation and cell proliferation [134]. It is formed by a series of reactions taking place in a number of different tissues [134]. Klotho is now known to inhibit the formation of Vitamin-D active form (by inhibiting the converting enzyme 1a-hydroxylase). Vitamin D also stimulates Klotho expression to regulate homeostasis [134].

Linking vitamin D and MS

Autoimmunity occurs when Th-1 cells attack self-cells. Vitamin D suppresses the proliferation of these T Cells and also reduces IF-gamma and IL-2 [134]. The active demyelination and neuro-degeneration process in MS is exacerbated by oxidative stresses resulting from Nitrogen Oxides (NOX) activation generating Reactive Oxygen Species (ROS), and via a pathway excitotoxic damage and eventual apoptosis from too much Ca^{2+} increase. Sunlight exposure and subsequent Vitamin D production have been linked with decreased incidence of MS due to Vitamin D being toxic to the ROS pathway [134]. However, in a Sardinian population it has been shown that there is a high level of MS, but there is also a high level of vitamin D [134]. However, this is due to a lack in a gene (Ifng) in Sardinian people encoding Interferon-gamma that is required for Vitamin D action [134]. Hence the true nature of Vitamin D and MS remains somewhat elusive and not yet completely clear.

A study conducted by Emami Aleagha, Mohammad Sajad., *et al*. in 2015 regarding Klotho and MS aimed at investigating the status of CNS-derived secretory form of Klotho in the CSF of patients with RR-MS [153]. The Sub-Aim was to elucidate the correlation between total anti-oxidant capacity and the concentration of secreted Klotho of which it claims to be the only paper to assess this in MS. Emami Aleagha, Mohammad Sajad., *et al*. results show potential for the use of Klotho in the CSF as a biomarker for severity and diagnosis. The results showed that the secretory form of Klotho was significantly lower in CSF of MS patients when compared to control [153]. Moreover, a negative correlation was observed between Klotho and EDSS, thus a predictor of severity.

A recent review by Berridge, Michael J., in 2015 on the role of Klotho with Vitamin D describes how there is a growing body of evidence that Vitamin D deficiency is a risk factor for neurodegenerative disease with particular emphasis on Multiple Sclerosis [135]. Although there is scepticism about the true link between Vitamin D and MS as challenged by the fact that a known high MS risk Sardinian population have shown to have normal Vitamin D levels. However, the population was also shown to have a decreased expression of Nrf2 gene required for the full function of vitamin D [135].

In respect to remyelination a novel study by Zeldich Ella., *et al*. 2015 [154] looking at the effects of enhanced Klotho expression on demyelination showed that mice that were induced with Cuprizone, a known demyelinating toxin were able to recover compared to the non-over expressing wild type. The mice were overexpressing the transmembrane form. The number of myelinated axons in Klotho overexpression mice per unit length of the Corpus Callosum (white matter) was 1.88 times higher ($p = 0.019$) than in the wild type mice [154], but did not show remyelination in grey matter, specifically the hippocampus. Zeldich, Ella., *et al*. shows promise to the future for the transmembrane type Klotho and its effects on demyelination in the CNS, in particular for demyelinating diseases such as MS.

Ushigusa, Takeshi., *et al*. in 2016 demonstrated α-Klotho to be a biomarker for the diagnosis of Neuropsychiatric Systemic Lupus Erythmatosus (NPSLE) [57]. In this study, it was additionally recognised Klotho as to be down regulated in CSF and up regulated in blood serum in a group of 20 people with MS. The patients with MS had serum values from 500 - 900 pg/ml. However, P values compared to a healthy population were not provided, as the studies primary aim was comparing NPSLE with diseased states. Serum α-Klotho levels of NPSLE were significantly lower than Viral Meningitis(VM), MS and Neuralmyelitis Optica (NMO) and similar to Systemic Lupus Erythematosus (SLE) $P < 0.05$ [57].

A decrease of α-Klotho level is associated with neuronal damage [57]. Ushigusa, Takeshi., *et al*. in 2016, investigated whether α-Klotho in CSF could be a candidate marker for the diagnosis of neuropsychiatric systemic lupus erythematosus (NPSLE) [57]. The multivariable analyses revealed that higher serum C3 (mg/dL), lower serum anti-Smith antibodies (U/mL) and lower CSF α-Klotho level were significant factors for predicting NPSLE [57]. The NPSLE patients CSF α-Klotho levels were inversely related with the level of granulocyte/macrophage-colony stimulating factor [57]. Ushigusa, Takeshi., *et al*. concluded that the determination of CSF α-Klotho levels will aid to the diagnosis of NPSLE and assist reveal the mechanisms of disease [57].

In summarising the studies [57,135,153,154] related to MS and Klotho (as seen in the table 3) it seems there is a relationship between Klotho in the Serum and CSF. α-Klotho levels appear to be elevated in the Serum during acute and chronic MS while decreased in the CSF. However, Ahmadi., *et al*. in 2016 showed that Klotho serum levels were significantly higher in the chronic state vs the Acute [8]. Whereas, when looking at MS compared to other miscellaneous neurological conditions including: Neuropsychiatric Systemic Lupus Erythmatosus (NPSLE); Systemic Lupus Erythematosus (SLE); Viral Meningitis(VM); Neuralmyelitis Optica(NMO); RR-MS showed less specificity for higher levels of α-Klotho in the serum. However, concurrent with other studies α-Klotho is higher in the serum in patients with MS, thus further showing the potential for a biomarker in MS. Furthermore, NPSLE was shown to be a better candidate for α-Klotho Biomarker in regard to α-Klotho in CSF.

Based on these studies alone, further research should be conducted in order to better understand the mechanism of why Klotho is upregulated in various neurological and demyelinating diseases such as MS and AD. Answering the question of which comes first, Klotho upregulation or demyelination associated with MS? Additionally, further research could be conducted to better understand the relation of psychiatric conditions with Klotho.

Concluding Remarks

This literature review has synthesized much of the current evidence in regard to Klotho and various pathologies. Klotho has been shown in various studies as mentioned to have wide effects and be implicated in many pathologies. However, Klotho does not yet appear to be specific to any one condition but indicates that changes in the levels of Klotho is associated with a number of pathological disorders.

Thus, this warrants further investigation into its use as a potential biomarker. Also, as a possible future treatment Klotho has shown promising results in mouse models such as changing the levels of Klotho to improve diseased states.

Overall this review brings conflicting evidence regarding Klotho and the body's utilization of this protein. Much evidence suggests Klotho is protective. However, as indicated above for the association of MS and Klotho, there is an over expression of Klotho in the serum, in particular in the chronic state of MS, whereas there is a decrease in the CSF. This may indicate that in MS the body over produces Klotho as a result of the demyelination in order to exert its effects on OPCs. However, the Klotho produced outside of the CNS is unable to enter the BBB and due to the inability to remyelinate the CNS, the damaged myelin further increases Klotho to remain high, which would explain why in the chronic state there is a constant upregulation. Why Klotho levels are decreased in the CSF still needs to be investigated.

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