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The klotho gene and soluble klotho in health and disease: From 1997-2018; A review

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Introduction

The topic of aging and anti-aging has interested humanity from the start of time [1]. In Greek myth (According to Hesiod's Theogony), the goddess Clotho spins the thread of life; the goddess Lakhesis determines the length of the thread; and the goddess Atropos cuts the thread [2]. It is obvious that ageing is a multifactorial process that influences many aspects of our existence as humans and is influenced by many factors [3]. Such factors include biological, environmental, psychological, lifestyle, and medical. Logically, assuming that a person's lifespan is limited and that the age in which a person dies is the result of a balance between factors that prolong life and those that shorten it, then two major and powerful conclusions can be drawn. The first is that we do not know what "could have been" a person's longest lifespan, rather than what was actually their lifespan. The seconds is that while a greater lifespan is the balance of two types of factors (simplified), it is enough to practice decreasing negative factors (ageing accelerators) or increasing positive factors (ageing decelerators) to prolong life. It is of course obvious that maximizing both should result in the greatest life span [4]. Furthermore, the mutual effects of aging in the development of disease, and disease on the acceleration of the ageing process have been established [5]. It is for all these reasons that the Klotho gene and its protein, S-Klotho, interest the scientific community. While plenty of data exist regarding Klotho and S-Klotho in general, relatively few data exist regarding the mutual effects of exercise on S-Klotho and S-Klotho on exercisability. The Klotho gene was discovered in rats in the year 1997 by Kuro-o et al. [6]. Klotho has been identified a bit more than two decades ago as an ageing influencer (for better or for worse) [6,7].

The Klotho protein has two forms: One as a membrane protein, and the other in secreted form [8]. The researchers reported the gene had several ageing suppressing properties. The researchers indicated that the gene encodes for a membrane protein with similarities in its sequence to those of enzymes from the beta-glucosidase family [6]. The protein was suggested as part of a signaling pathway that is influential in age-related

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diseases' morbidity and the regulation of ageing *in vivo* [6]. Kuro-o et al. further indicated a defect in the gene may leads to shorter lifespan, infertility, Arteriosclerosis, skin atrophy, Osteoporosis, Emphysema, and a syndrome resembling that of human ageing [6].

Over expression of the Klotho gene has been associated with positive effects on health and longevity, while under expression with disease and a shorter lifespan in both mice and humans [4,9].

In mice, life was prolonged by 19-31% simply due to the over expression of the Klotho gene [10]. According to the PRB data sheet for the world's population in 2015 [11], men's average lifespan was 69.02±8.4 years, women's average lifespan was 73.82±9.31 years, and the average for both sexes together was 71.34±8.8 years. The average shortest lifespan for men was 43 years, 46 for women, and 44 for both sexes. The greatest average lifespan was 84 years for men, 89 years for women, and 87 for both sexes. If achieving the same increase in lifespan in humans as was achieved in mice becomes a reality, adding 19-31% to lifespan will result in an average lifespan of 100-110 years for men and 106-116 years for women. One may assume that these lifespans will be in relatively good health under the assumption that health care improves and so does our understanding of how to age better and healthier. It is also very important to remember that such an increase in lifespan is based on merely the over expression of the Klotho gene alone.

From a genetic standpoint, gene expression is affected by genetic and epigenetic factors. Simplified, genetic factors pertain to processes at the DNA level, whereas epigenetics do not [12]. Exercise in health and disease, creates potent combinations of influences that lead to adaptation and possibly to improvements of function and health [13]. The purpose of this review is to lay the foundations for the understanding of the Klotho gene, Soluble Klotho protein, and their interactions with exercise in health and disease.

Current published literature regarding the Klotho gene and Soluble Klotho (together and apart) include an estimated 3,599 resources of which 1,680 via NIH funded grants (data is accurate for the 15th of March 2018).

Reviewing all publications is not realistic, thus, this article has selected publications the author found important to indicate. In order to gradually increase the readers knowledge pertaining to the Klotho gene and protein, citations are hence forth cited according to their order of publication, in an attempt to convey the advancement of knowledge according to time. Thus, the purpose of this review is to educate the reader in regards to Klotho's role in ageing and anti-ageing, major findings in animal models (mice and rats) and in humans, as a basis to understanding the influence of exercise modalities on Klotho expression.

The klotho gene/protein in mice and rats

Shiraki-Iida et al. reported an advancement to the originally published article [6] by indicating the entire organization of the KL gene in mice [14]. In their report, the researchers noted the KL gene being of 50 kilo-bases and five exons [14]. Furthermore, they noted that a TATA-box is lacking in the promotor region yet includes "four potential binding sites for SP1". SP1 is a transcription factor influencing multiple processes such as cell differentiation, cell growth, apoptosis, immune responses, response to DNA damage, and chromatin remodeling. Post-translational modifications such as phosphorylation, acetylation, glycosylation, and proteolytic processing significantly affect the activity of this protein, which can be an activator or a repressor [15]. Shiraki-Iida et al. additionally reported that via alternative transcriptional termination "two kl gene transcripts encoding membrane or secreted protein are generated" [14]. Ohyama Y. et al. further indicated the suppression properties of the KL gene and its similarity of sequence with the beta-glucosidases of bacteria and plants [16]. Furthermore, they noted the protein to be of 1,014 amino acids with a 94% homologousto mouse Klotho protein and 85% homologous to human Klotho proteins. Substantial Klotho cDNA expression was detected in the Kidneys while low levels of expression were also detected in the brain, lungs, intestine, and ovaries [16]. A substantial decrease on Klotho expressions was contributed to the influence of Lipopolysaccharide (LPS) in vivo, interpreted as an effect of inflammation [16].

Shiraki-Iida T, et al. concluded that in rats, the traits (phenotypes) due to pathological abnormalities improved completely or partially as a result of the local expression of the klotho gene [17].

Aizawa H, et al. identified the down-regulation of kidney Klotho protein in rats under stress. They further indicated the possible role of Klotho as a protecting agent against endothelial dysfunction [18]. Since then, several studies have established the relationship between Klotho expression, S-Klotho copy number, and disease. Nagai R. et al. established a link between endothelial dysfunction and down regulation of the gene, as well as a decreased amount of protein copies in various animal models. These models included rats with vascular and metabolic diseases [19]. Saito et al. further strengthened the understanding of the role of S-Klotho in endothelial dysfunction and disease. In this study the authors claimed that "adenovirus-mediated klotho gene delivery can [1] ameliorate vascular endothelial dysfunction [2], increase nitric oxide production, [3] reduce elevated blood pressure, and [4] prevent medial hypertrophy and perivascular fibrosis" [20].

Nabeshima Y. indicated that the creation of "Klotho mice" of "unique short lifespan" allowed for the association of Klotho expression mainly in tissue that are tightly linked to Calcium homeostasis [21]. Furthermore, the researchers reported that the most abnormal phenotypes are presented while Calcium metabolism is affected and/or impaired [21]. The researcher wrote "Klotho plays a critical role for the regulation of calcium and phosphorus homeostasis by negatively regulating the synthesis of active Vitamin D. The deficiency of the klotho gene results in degradation of cells by the activation of calcium-dependent proteolysis in kidney, lung and heart" [21]. An interesting finding was that the downregulation of Klotho expression in old mice occurred parallel to the activation of calcium-dependent proteolysis [21]. Mitani H, et al. noticed S-Klotho's ability to negate renal damage caused by Angiotensin II-induced renal damage. This study shade light on the relationship between S-Klotho and Angiotensin II [22]. The researcher wrote "This angiotensin II-induced renal klotho down regulation was an angiotensin type 1 receptor-dependent but pressor-independent event" [22]. Ishizaka N, et al. reported the regulatory properties of Angiotensin II on S-Klotho. The researchers indicated that S-Klotho influences the amelioration of tubulointerstitial damage induced by this octapeptitde, creatinine clearance, and the decrease in urinary protein excretion [23].

Pletcher and Stumpf, noted that mutations in the Klotho gene lead to premature aging in mice [24]. Manya H, et al. noted that increased activation of Calpain due a decrease in Klotho

protein results in the degradation of cytoskeletal elements such as alpha (II)-spectrin [25]. This in turn results in renal abnormalities. Kamemori M, et al. suggested Klotho has a key mediatory role in auditory function [26]. The researcher noted that "No obvious morphological abnormalities were detected in Klotho mice, although no expression of Klotho protein was detected, and there was an apparent hearing disorder. Taken together, these findings suggest that by contributing to the maintenance ion homeostasis in the endolymph, Klotho protein serves as a key mediator of auditory function" [27]. Saito Y, et al. suggested S-Klotho has "therapeutic potential" while treating atherosclerotic disease [28].

Imura A, et al. concluded that "The secreted as well as the membrane-bound Klotho proteins were suggested to form oligomerized complex. These results delineate post-translation processing of Klotho and possible regulatory mechanisms for secretion of Klotho *in vivo*" [29]. The suggestion of a regulatory role of S-Klotho would soon to be proven in many aspects of function and ageing.

Following the work of Imura and colleagues in 2004, the regulatory role of S-Klotho was investigated by other researchers. Kurosu H, et al. indicated the similarity of symptoms and acceleration of the ageing process between S-klotho and factor-23 (FGF23)-deficient mice [30]. The role of factor-23 in inherited disorders involving disturbances in phosphate homeostasis was first published in the year 2000 [31]. Hayashi Y, et al. established the cytosolic neutral beta-glycosylceramidase characteristics of S-Klotho in the brains of zebrafish, mice, rats, and humans [32]. The researchers further indicated the possible role of the protein in a non-lysosomal catabolic pathway of C6-NBD-glucosylceramide (GlcCer) [32].

In 2007, Kurosu H, et al. established the regulatory function of beta S-Klotho on the activity of both FGF19 and FGF21 [33]. The researchers wrote "The Fibroblast Growth Factor (FGF) 19 subfamily of ligands, FGF19, FGF21, and FGF23, function as hormones that regulate bile acid, fatty acid, glucose, and phosphate metabolism in target organs through activating FGF Receptors (FGFR1-4)" [33]. While at this point in time the activation of FGF 21 and FGF 23 by beta S-Klotho were established, the current study established the activation of FGF 19 by beta S-Klotho as well [33].

S-Klotho was further indicated as an influencer and regulator of white matter in monkeys as part of the ageing process [34]. Duce, et al. concluded that "the overall changes described in this study could provide an explanation for aging changes in white matter that might be initiated or enhanced by an altered expression of life span associated genes such as Klotho" [34]. The same researchers also indicated the role of S-Klotho in Insulin resistance, cell apoptosis, and battling oxidative stress [34].

Klotho was investigated as an influencer of blood pressure in cases of Hypertension (HTN) [35].Wang Y, and Sun Z. set out to prove that S-Klotho delays the onset of spontaneous hypertension in rats. The researcher suggested a possible therapeutic role for S-Klotho in the treatment of hypertension [35]. A first indication of the relationship between S-Klotho and TNF-α, was made by Thurston RD, et al. The researchers indicated in their study the interaction of S-Klotho with immune factors such as TNF-α, T-cell function, and impaired Calcium and inorganic Phosphate [36].

King GD. et al. identified a threshold of Klotho activation, whereas the activation of Klotho is dependent upon at least 30% expression of Luciferase [37]. Wang Y, et al. indicated the relationship of Klotho with Nox2 and ROSs in Rat Aorta Smooth Muscle (RASM) cells. In their study the researchers stated that "Klotho not only downregulated Nox2 protein expression and intracellular superoxide production but also attenuated AngII-induced superoxide production, oxidative damage, and apoptosis. The klotho-induced suppression of Nox2 protein expression may be mediated by the cAMP-PKA pathway" [38]. Hu MC, et al. reported the regulatory relationship between klotho and Erythropoietin. They concluded that "in the kidney, EpoR and its activity are downstream effectors of Klotho enabling it to function as a cytoprotective protein against oxidative injury". In their study EpoR was used as the abbreviation for the Erythropoietin receptor [39]. The role of rat secreted Klotho in Growth Hormone (GH) regulation was suggested by Shahmoon S, et al. The researchers concluded that "data indicate for the first time that klotho is a positive regulator of GH secretion and suggest the IGF-I and bFGF pathways as potential mediators of this effect" [40].

The protective role of alpha-Klotho in Pulmonary epithelia oxidative damage was indicated by Ravikumar P, et al. The researchers found it important to indicate the following: "We conclude that circulating α-Klotho protects the lung against oxidative damage and apoptosis partly via increasing endogenous antioxidative capacity in pulmonary epithelia. Cytoprotection by α-Klotho may play an important role in degenerative diseases of the lung" [41]. Deng M, et al. recently suggested a therapeutic role Klotho could have in the battle against Diabetes. The researchers wrote that "results indicated that klotho gene delivery ameliorated renal hypertrophy and fibrosis in diabetic rats, possibly by suppressing the ROCK signaling pathway. This may offer a novel approach for the long-term control and renoprotection of diabetes" [42].

The effect of Klotho upregulation on vascular calcification in rats was observed in 2015 by Zhao Y, et al. The researcher suggested a "vital association" between mTOR signaling and Klotho expression [43]. Additionally, they concluded that "Klotho has a critical role in mediating the observed decrease in calcification by rapamycin in vitro and *in vivo*" [43].

The effects of Klotho on atherosclerosis, high blood pressure, and metabolic syndrome, all of which are affected by Triglyceride levels in plasma, were investigated by Kamari Y, et al. The researchers mainly concluded as to the KL1 internal repeat of the extracellular domain of Klotho. The researchers wrote "In fructose-fed Sprague-Dawley rats, klotho treatment did not lower blood pressure or plasma triglyceride levels. Although KL1 treatment did not lower blood pressure or plasma insulin levels, it significantly reduced the elevation of total plasma triglyceride levels (from 2.3-fold to 1.6-fold, $p<0.05$) due to lower triglyceride-rich VLDL levels. Klotho did not show any beneficial effects on atherosclerosis and components of the metabolic syndrome and was associated with increased plasma cholesterol levels. On the other hand, treatment with KL1 may lower plasma triglyceride levels independent of insulin. Additional studies are required in order to decipher the complex role of klotho and its active domains in the regulation of plasma lipid levels" [44].

Yamamoto K, et al. suggested Klotho has a mediation role in the suppression of Calpain activation, resulting in neuro-protection [45]. In their main conclusion the researchers indicated that "the shed klotho fragments might contribute to the attenuation

of axonal injury-induced Calpain activation and oxidative stress, thereby protecting RGCs from post-traumatic neuronal degeneration"; whereas RGCs is the abbreviation of Retinal Ganglion Cell [45]. Additional neuroprotective characteristics of Klotho were associated with Ischemic Brain Injury. Zhou HJ, et al. suggested that the inhibition of RIG-I/NF-κB Signaling may result in neuro-protection of the brain [46]. The researchers concluded that "Klotho itself or enhancers of Klotho may compensate for its aging-related decline, thus providing a promising therapeutic approach for acute ischemic stroke during advanced age" [46].

Klotho gene/protein in healthy/diseased humans

The dynamic of utilizing animal models prior to application in humans is custom practice. The development of research pertaining to the Klotho gene and protein is no different. Matsumura Y, et al. reported the isolation of the human homologue for the KL gene [7]. The researchers indicated it has five exons and ranges over 50 kb on chromosome 13q12. Two transcripts were identified for membrane and secreted protein. Researchers noted that it is by means of RNA splicing that these options exist [7]. In addition, it was mentioned that the secreted form predominates that of the membrane form [7]. Nagai R, and Hoshino Y. reviewed the new knowledge (at the time of publishing their review) in regards to the genetics of cardiovascular disease [47]. In their article they wrote "We recently found a novel gene which seems to affect human aging phenotype and vascular endothelial function. It is important as a future study to clarify the regulatory mechanisms of the klotho gene in the cardiovascular system and the clinical significance of klotho gene polymorphisms" [47]. This study symbolizes the beginning of research in regards to Klotho and clinical conditions and populations. Saito Y, et al. reported regarding Klotho's influence of human vascular endothelial tissue, suggesting it has a protective role [48]. The researchers indicated the secreted form of Klotho is of cDNA, and the lack of knowledge as to its pathophysiological relevancy. In their conclusion the authors wrote "These results suggest that the Klotho protein protects the cardiovascular system through endothelium-derived NO production by humoral pathways" [48].

Kato Y, et al. detected Klotho within human kidneys. Utilizing the sandwich-type ELISA system, the existence of Klotho in the human kidney was established. Part of the purpose of the study was to establish the physiological roles of Klotho. A 130 kDa Klotho protein was identified in the kidney membrane of both mouse and human [49]. Takahashi Y, et al. suggested that the evolution of the human reproductive system came with the price of aging. The logical conclusion of which would be that Klotho may be a reminder and part of a mechanism of anti-aging from the area that precedes the development of the reproductive system [50].

Yahta K, et al. were able to isolate a novel human protein that they defined as "structurally related to klotho protein" [51]. Cytosolic beta-glucosidase-like protein-1 (cBGL1) and klotho were found to decrease and/or completely lose expression in malignant tumors [51]. Nabeshima Y. indicates the human homolog has 1,012 amino acid residues, with an 86% homology to that of the mouse protein [52]. In addition, the Klotho protein was characterized as a member of the beta-glucosidase family in humans as well, as it has been previously been indicated regarding mice and rats [52]. Klotho has been found to be severely reduced in people with Chronic Renal Failure (CRF) [53]. The researched reported that "The levels of kl mRNA expression were greatly reduced in all CRF kidneys. Moreover, the production of Klotho protein was also severely reduced in all CRF kidneys. These results suggest that the decrease in kl gene expression in CRF patients may underlie the deteriorating process of multiple complications in the CRF patients" [53]. Mizuno I, et al. suggested that Klotho has a role in adipose differentiation. Klotho gene expression in 3T3-L1 adipocytes was investigated using quantitative reverse transcription-polymerase chain reaction. The researchers observed a greater increase of the membrane form of Klotho during adipose differentiation, though the secreted form was expressed as well [54]. In a population-based association study, Arking DE, et al. applied two polymorphic microsatellite markers flanking the gene in order to determine whether variation in the human KLOTHO locus contributes to survival [55]. The researchers reported significant differences between newborn and elderly pertaining to selected marker allele frequencies [55]. In this study, the KL-VS allele was identified, including six sequence variants in complete linkage disequilibrium. Of the six, two presented a substitution in F352V and C370S. The researchers further reported that "In a transient transfection assay, secreted levels of klotho harboring V352 are reduced 6-fold, whereas extracellular levels of the S370 form are increased 2.9-fold" [55]. They concluded that "These results suggest that the KL-VS allele influences the trafficking and catalytic activity of klotho, and that variation in klotho function contributes to heterogeneity in the onset and severity of human age-related phenotypes" [55].

In 2002, Ogata N, et al. associated klotho gene polymorphism with lumbar spine bone density and spondylosis in women post menopause [56]. The researchers indicated that the association of osteopenia and subchondral sclerosis with Klotho in mice led them to investigate a possible similar association in humans with osteoporosis and spondylosis [56]. This study indicated Klotho as having a substantial role in the etiology of diseases. The researchers wrote "These findings indicate that the klotho gene may be a candidate for the genetic regulation of common age-related diseases like osteoporosis and spondylosis, and we provide the first evidence suggesting that this gene may be involved in the etiology of human diseases" [56].

Kawano K, et al. added to the existing knowledge regarding Klotho and bone tissue. Klotho polymorphism and bone density was compared in white vs Japanese. The researchers identified eleven polymorphisms; three of which were common to both populations [57]. The researchers indicated that a G-A substitution in the promotor region affected the protein-DNA interaction in kidney cells. The researcher thus concluded that the Klotho gene might be a factor influencing bone lose as part of the ageing of humans [57]. Yang J, et al. investigated the intracellular signal pathways of Klotho. The researchers reported that cAMP upregulation has a functional role in the signaling pathway of Klotho in endothelial cells [58]. In their conclusions the researchers concluded that their findings "suggest that mouse membrane-form Klotho protein acts as a humoral factor to increase ACE activity in HUVEC via a cAMP-PKA-dependent pathway"; whereas ACE stands for angiotensin I-converting enzyme, and HUVEC stands for human umbilical vein endothelial cells [58]. Arking DE, et al. associated the KL-VS allele with higher risk for the occurrence of early-onset (age <60 years) CAD [59]. The researcher additionally concluded that elevated blood pressure (hypertension; HTN; mmHg), smoking, and HDL-C concentrations increase the risk related to the KL-VS allele. In their conclusions, the researchers wrote that "results demonstrate that the KL-VS allele is an independent risk factor for occult CAD in two independent high-risk samples. Modifiable risk factors, in-

cluding hypertension, smoking status, and HDL-C level, appear to influence the risk imposed by this allele" [59].

Yamada Y, et al. investigated the possible influences of genetic variants of the androgen receptor and klotho protein on bone density and the occurrence of Osteoporosis in Japanese women [60]. The researchers indicate in their study the difference in influence for the different genotypes pertaining to the KL gene. In their conclusions, the researchers indicated the results "suggest that AR is a susceptibility gene for reduced BMD in premenopausal Japanese women, and that KL is a susceptibility gene for reduced BMD in all women" [60].

An association of a SNP in Klotho with priapism in people with Sickle Cell Anaemia was investigated by Nolan VG, et al. The researchers indicated priapism as being a symptom in 30% of men with Sickle Cell Anaemia [61]. The authors indeed indicated an association between a certain SNP in the KL gene and priapism. The Authors furthermore concluded that "These findings may have broader implications in sickle cell disease, as KL encodes a membrane protein that regulates many vascular functions, including vascular endothelial growth factor expression and endothelial nitric oxide release" [61].

Arking DE, et al. established the association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity [61]. The researchers concluded that "ross-sectional and prospective studies confirm a genetic model in which the KL-VS allele confers a heterozygous advantage in conjunction with a marked homozygous disadvantage for HDL-C levels, SBP, stroke, and longevity" [62]. While comparing the role of Klotho in cognitive ability at age 11 years, 64 years, and 79 years, Deary IJ, et al. concluded that "Variation in the KLOTHO gene is a possible contributor to life-long reasoning differences in humans and/or to the ageing of non-verbal reasoning, especially in women" [63]. Low AF, et al. hypothesized that Klotho has a role in the occurrence of premature CAD. The KL-VS variant of the KLOTHO gene was typed using restriction digest of a PCR amplicon. The researchers concluded that "data do not support the hypothesis that premature CAD is associated with common variants in the progeroid syndrome genes LMNA and KLOTHO" [64].

Ikushima M, et al. investigated the influence of Klotho on apoptosis and cellular senescence in Human Umbilical Vascular Endothelial Cells (HUVEC). Researchers noted that indeed Klotho decreased apoptosis in several cell type [65]. Researchers concluded that "study suggests that Klotho acts as a humoral factor to reduce H(2)O(2)-induced apoptosis and cellular senescence in vascular cells" [65].

Unger HR. suggested an advantage to Klotho-mediated Insulin resistance. The author wrote "A reduction in insulin-stimulated intracellular glucose availability may prevent intracellular lipid overload and lipotoxicity, a proposed mechanism of hte life-shortening metabolic syndrome" [66]. Chang Q, et al. as well as Lewin E, and Olgaard K. suggested Klotho is an important factor in the activity of Calcium channels. Researchers indicated Klotho as the link between Calcium homeostasis, ageing and uraemia [67,68].

Nolan VG, et al. studies the involvement of the Klotho gene in the existence of Sickle cell leg ulcers in people undergoing haemolysis. The researchers indicated a higher likelihood of experiencing ulcers the more extensive the haemolysis is [69]. In addition, the researchers indicate that "215 SNPs in more than 100 candidate genes were studied. Associations were found with SNPs in Klotho, TEK and several genes in the TGF-beta/BMP signalling pathway by genotypic association analyses. KL directly or indirectly promotes endothelial Nitric Oxide (NO) production" [69].

The question of rather or not Klotho is associated with Type II Diabetes in humans was investigated by Freathy RM, et al. Since Klotho has been identified as having a role in Insulin resistance [70], the researchers hypothesized Klotho might have a role in Type II Diabetes as well. The researchers reported no association between the KL-VS variant and fasting insulin, glucose, triglycerides, HDL- or LDL-cholesterol [70]. In their conclusions the researchers wrote "We have found no evidence that the functional KL-VS variant is a risk factor for type 2 diabetes in a large UK Caucasian case-control and family-based study" [70].

Kim Y, et al. have identified Klotho as a risk factor for ischemic stroke and vascular dementia via cardio-embolism in Korean women. A non-significant association was found for the G-395A and C1818T polymorphisms with ischemic stroke and vascular dementia [71]. A significant association was established for the cardio-embolic subtype of ischemic stroke with the A allele of the G-395A polymorphism. The researchers concluded that "The sequence variant of G-395A in klotho might be a genetic risk factor for CE in females" whereas CE stands for Cardio-embolic subtype of ischemic stroke [71].

The relationship between Klotho and Bone Mineral Density (BMD) was investigated by Riancho JA, et al. These researchers compared men and women with and without Osteoporosis and the existence of certain Klotho alleles. In their conclusions, the researchers state that "he Klotho genotype was not associated to BMD in premenopausal women. In conclusion, the F352V Klotho polymorphism is associated with BMD in postmenopausal women, suggesting that Klotho gene variants influence skeletal aging" [72]. Zarrabeitia MT, et al. reported similar findings in men [73].

The down-regulation of Klotho at the mRNA, protein, and enzymatic levels as part of the mechanism inducing Rheumatoid Arthritis-related aging of human CD4+ lymphocytes was investigated by Witkowski JM, et al. The same down-regulation to at a decreased extent was reported for the elderly without Rheumatoid Arthritis [74]. The researchers suggest an anti-inflammatory role for Klotho in the young and healthy which is somehow deactivated in the elderly and in people with RA [74]. The researcher wrote "To support this hypothesis, we show here that the reduction of Klotho expression and activity in both elderly and patients' lymphocytes occurs in concert with the down-regulation of T cell costimulatory molecule CD28, the latter known to be dependent on increased levels of TNF-alpha. Thus, a common mechanism of KLOTHO down-regulation, but executed at various times in life, may underlie both physiological and disease-related T cell aging" [74]. The researchers' data may lead to Klotho being part of the treatment for people with RA.

Lu L, et al. studied the role of Klotho in women with ovarian cancer. The authors indicated the counteracting effect of Klotho on IGF-I, which in high concentrations may induce cancer. The researchers indicated that "High expression of secreted Klotho was associated with increased risk of disease progression and death" [75]. These associations were indicated as independent of the subjects' clinical and pathological traits. The authors concluded that "Klotho expression is associated with epithelial

ovarian cancer progression, and the protein may serve as an independent marker for ovarian cancer prognosis" [75].

Following the findings that Klotho has a role in Calcium and Phosphate hemostasis, Tsezou A, et al. investigated the influence of Klotho on the occurrence of Osteoarthritis (OA). Researchers reported a significant genotypic and allelic association for SNP C2998T and knee OAin both men and women [76]. The GA genotype for SNP G395A was significantly associated with knee OA in women only. This study implicated polymorphisms of Klotho in the pathogenesis of OA.

Lu P, et al. concluded that Klotho has an exclusive role in the activation of kidney Calcium channels TRPV5 and TRPV6. Both Klotho and beta-glucuronidase significantly increased the activity of TRPV5 and TRPV6 [77]. The researcher found no effect on TRPV4 and TRPM6. The researchers concluded that "results suggest a modulating effect for klotho primarily restricted to the epithelial Ca(2+) channels TRPV5 and TRPV6" [77].

Jo SH, et al. associated the Klotho G395A gene polymorphism with coronary artery stenosis. In a study aimed at investigating the role or the lack of Klotho on coronary artery stenosis and calcification, the Authors wrote "Homozygotes or heterozygotes for G395A were significantly more common in the CAD patients than in the controls" and that "Using multivariate analysis, we identified the KLOTHO gene G395A mutant as an independent risk factor of CAD" [78]. last, the authors indicate the lack of association of the same Klotho polymorphism with coronary calcification in a Korean population [78].

The association of a number of Klotho variants with mortality in people undergoing hemodialysis was researched by Friedman DJ, et al. The authors indicate the highest mortality rate being in people diagnosed with End Stage Renal Disease (ESRD) during their first year of chronic hemodialysis [79]. The researchers believed that Klotho may have a role to play in the mortality of people with ESRD due to the previous findings whereas vitamin D and Fibroblast Growth Factor (FGF)-23 had such a role, and further findings of the relationship between Klotho, vitamin D, and (FGF)-23 [79]. 12 SNPs of the Klotho gene were tested for an association with mortality in the first year of hemodialysis. A significant association was found between the CC genotype of the rs577912 tag SNP, which was worsened by lack of activated vitamin D [79]. The researchers concluded that "data suggest that a specific Klotho variant (rs577912) is linked to survival in ESRD patients initiating chronic hemodialysis and that therapy with activated vitamin D may modify this risk" [79].

The existence of a specific "time window" for Klotho KL-SV to have an effect has been suggested by Invidia L, et al. In their study, the researchers based their hypothesis upon prior data published whereas Klotho expression and influences may differ at different ages, suggesting a "time window" for the KL-SV Klotho to have a greater effect [80]. Aged 19 to 109 years, the cohort included 1,089 men and women from northern and central Italy. Subjects were divided into three age groups (young, elderly, and extremely old/centenarians). Researcher wrote "We found a significant increase of the heterozygous Klotho genotype in the class of elderly people compared to young controls. On the contrary, no difference was present between centenarians and young controls" [80]. The researchers concluded that "such unusual age-related changes in the Klotho KL-VS genotype frequency is compatible with the hypothesis that alleles and genotypes involved in aging and longevity may exert their biological effect at specific time windows" [80].

Oguro R, et al. investigated the association of carotid atherosclerosis with genetic polymorphisms of the klotho gene in patients with hypertension. Previous studies suggested an association yet did not study it [81]. Researchers measured the common carotid artery Intima-Media Thickness (IMT) and studied its association with four Klotho haplotype block SNPs. The rs650439 SNP was found to be significantly associated with common carotid artery atherosclerosis in people with hypertension, and with the mean IMT values. No associations were found in the general population [81].

The question of Klotho being a predictor of metabolic syndrome was investigated by Majumdar V, and Christopher R. The study cross-sectional study aimed at evaluating the association of the Klotho genetic variants with metabolic syndrome and surrogates of insulin resistance in Asian Indians [82]. The KL-SV variant was significantly associated with the metabolic syndrome while the C1818T variant was not. The researchers concluded that "the genetic variants of Klotho might predict risk for metabolic syndrome and insulin resistance in Asian Indians. However, larger studies in other ethnic populations are warranted to determine the role of these gene variants in the etiology of metabolic syndrome" [82].

The role of prenatal Klotho was investigated by Ohata Y, et al. Researchers conducted a cross-sectional evaluation of healthy babies (at birth and/or at 4 d after birth), their mothers, and adult volunteers. Serum levels of soluble α-Klotho and fibroblast growth factor 23 (FGF23) were measured. Klotho concentrations in cord-blood in comparison to all other groups, and was negatively correlated with those of FGF23 [83]. While Ohata Y, et al. suggested alpha Klotho to be "a useful biomarker of for mineral metabolism in the fetus", the author of this review suggest the possibility of cord blood as a rich source of alpha Klotho.

Nzietchueng R, et al. studied the involvement of the KL-SV Klotho variant in the regulation of blood pressure, namely its decreasing as a prime aim, and secondary investigate its relationship with anti-hypertensive treatment, arterial stiffness and carotid artery parameters [84]. The researchers reported Klotho KL-VS to be significantly associated with lower systolic blood pressure and pulse-pressure compared to those of homozygous and heterozygous. An association between anti-hypertensive treatment and the KL-SV Klotho variant was reported as well. In their conclusions, the researchers wrote "Klotho KL-VS/KL-VS genotype may be associated with decreased cardio-vascular risk and may interact with antihypertensive treatment in order to reduce blood pressure. This finding could lead to identify subgroups of hypertensive adults who might benefit antihypertensive drug therapies" [84].

Paroni G, et al. suggested sex/gender influencing the association between Klotho locus, serum levels of hemoglobin, albumin, and High-Density Lipoprotein Cholesterol (HDL-C), fasting insulin, and fasting glucose. In both genders, independently, such an association was established [85]. Furthermore, the authors indicate that "The association of KL genotypes with creatinine levels was found only in females, while the association with Insulin-like Growth Factor-1 (IGF-1) and Lymphocytes Count (LC) was found only in males" [85].

Acromegaly is a disease characterized by high phosphate levels and reduced life expectancy, usually originating from a pituitary adenoma. Sze L, et al. examined the relationship between serum levels of Klotho in patients with acromegaly and excess

Growth Hormone (GH). Soluble Klotho levels were extremely elevated at baseline, and were correlated with GH as well as tumor size [86]. Soluble Klotho concentrations were associated with GH after correction for levels of creatinine and phosphate, age, and gender. Post-surgery, S-Klotho levels returned to normal. This study was first to show high concentrations of S-Klotho in an acquired clinical condition in humans, returning to normal post-operation [86].

Amitani M, et al. investigated the influence of obesity and Anorexia Nervosa on Klotho plasma levels. The Authors reported a much lower concentration of plasma Klotho in the obese subjects and those with Anorexia Nervosa compared to controls [87]. Weight loss and decreased BMI in subjects with Anorexia Nervosa were significantly associated with an increase of plasma Klotho levels [87]. No relationships were found between klotho and total adiponectin levels nor between klotho and respective adiponectin isoform levels. Researchers concluded that "klotho may reflect normal nutritional state, and that the decrease of klotho in r-AN and obesity may underlie the deteriorating processes of these disorders" [87].

Shibata K, et al. investigated the association between circulating fibroblast growth factor 23 and α-Klotho with left ventricular ejection fraction and mass in one hundred cardiology inpatients who were not undergoing chronic hemodialysis. The authors reported an association between fibroblast growth factor 23, left ventricular ejection fraction, and mass. No association was found between alpha Klotho, left ventricular ejection fraction, and mass [88].

Lim SC, et al. indicated that the blockage of losartan, an angiotensin II receptor blocker, reduces Albuminuria in type 2 diabetic patients and elevates circulating alpha-klotho [89]. An average increase of 23% was observed in circulating alpha-klotho with the use of losartan. Lim SC, et al. concluded that "The angiotensin receptor blocker losartan increases circulating α-klotho in T2DM with albuminuria. The clinical significance of this rise in α-klotho associated with losartan intervention deserves further investigation" [89].

Miranda J, et al. investigated alpha Klotho levels in non-pregnant women, and women during a healthy vs unhealthy pregnancy. Authors reported levels to be higher during pregnancy compared to while non-pregnant, and higher in healthy pregnancies compared to unhealthy pregnancies [90].

Semba RD, et al. compared Cerebrospinal Fluid (CSF) Klotho levels in people with Alzheimer's disease with that of old and young people free of Alzheimer's disease. The comparison included both sexes. The study's design aimed at investigating circadian fluctuations in CSF Klotho levels [91]. No significant influence was reported for the influences of the circadian cycle on CSF Klotho levels, and that concentrations were higher in men compared to women, in people without Alzheimer's disease compared to with Alzheimer's disease, and lower in the elderly compared to the young [91].

Growth Hormone Deficiency (GHD) results in short stature. Klotho deficiency has been proven to induce lesser growth in mice and in humans [92]. Wolf I, et al. set out to investigate if Klotho deficiency influences children' and adolescents' stature with GHD. Furthermore, subjects with organic GHD were compared to those with idiopathic GHD and non-GHD subject [93]. Klotho levels were found to be lower in organic GHD subjects compared to all non-GHD subjects of any age. No significant differences were found between subjects with GHD. Klotho levels were not associated with gender, pubertal status, age or anthropometric measurements [93].

Okamoto Y, et al. investigated the association between alpha Klotho levels and left ventricular diastolic dysfunction among patients with preserved ejection fraction. A significant negative association between alpha Klotho concentrations and left ventricular diastolic dysfunction among patients with preserved ejection fraction was found [94]. The authors indicated that "Whether modulation of serum levels α-Klotho will ameliorate cardiac diastolic function among patients with this disorder awaits further investigation" [94].

Paula RS, et al. reported on their findings related to the possible association of serum Klotho levels with post-Myocardial Infarction (MI) status of older adults. Most risk factors for a vascular event were not associated with serum Klotho levels. An interesting association was found between serum Klotho levels and a prior history of at least one MI occurrence for both rs9536314 and rs9527025 genotypes [95]. The researchers concluded the following "results suggest that serum Klotho is higher in individuals with a clinical history of myocardial infarction but not with a history of coronary artery disease or stroke. None of the Klotho haplotypes were associated with the variables investigated herein" [95].

Talotta R, et al. study's aim was to "evaluate the role of klotho in the pathogenesis Of Systemic Sclerosis (SSc), through the measurement of its serum concentration in SSc patients compared to healthy controls, and to assess the association with cutaneous and visceral involvement" [96]. Researchers reported a lower median biomarker concentration in the clinical group in comparison to the control group (0.23ng/mL vs 0.60ng/mL; respectively; $p<0.001$) [96]. The researchers concluded that "data show a significant deficit of klotho in SSc patients although any significant association was detected between klotho serum concentration and the clinical, laboratory or instrumental features of the disease" [96].

Li P, et al. indicated the role of Klotho Beta (KLB) in the prevention of breast cancer. The researchers wrote "through an immunohistochemical analysis of invasive ductal carcinoma tissue arrays, low KLB expression was identified in invasive ductal carcinoma samples compared with paired adjacent non-tumorous breast tissues (82 cases)" [97]. The researchers offered to further examine Klotho beta as a therapeutic option for the prevention of breast cancer [97].

Liang H, et al. indicated that "miR-130a protects against lipopolysaccharide-induced glomerular cell injury by upregulating Klotho expression". In this study, HK-2 cells (human renal proximal tubule cells) were used for detecting miR-130a levels. Cells were divided into scramble, miR-130 mimic, siNC, si-miR-130a and si-Klotho groups. Researcher utilized apoptosis and CCK-8 assays in order to establish the proliferation and apoptosis rates [98].

Słomiński B, et al. investigated the relationship between the expression of the Klotho KL-VS variant and type 1 Diabetes. While the researchers did not find an association between the two, they indicated that "the incidence of KL-VS genotype is lower in a group with retinopathy in comparison to diabetic patients without this complication. Moreover, we established that KL-VS carriers had the lowest levels of inflammatory markers, pro-angiogenic factors and adhesion molecules. Simultane-

ously, the KL-VS carriers had increased serum levels of anti-inflammatory and anti-angiogenic cytokines than holders bearing wild type genotype" [99].

Cho NJ, et al. aimed there research at proving that urine and serum S-Klotho levels can act as a biomarker for the prediction of renal fibrosis and podocyte injury. Soluble serum/urinary klotho and urinary angiotensinogen were assessed by enzyme-linked immunosorbent assays, and tissue klotho expression was assessed by immune-histochemical staining [100]. In their conclusions the researchers wrote "Our data suggested that soluble serum and urinary klotho levels represent a potential biomarker to predict renal fibrosis and podocyte injury in humans" [100]. Skrzypkowska M, et al. suggested a possible mechanism explaining Klotho KL-VS polymorphism's influence on ageing and cardiovascular disease development. These researchers suggested that individuals possessing at least one KL-VS allele are characterized by greater number of CD34+ and CD34+VEGFR2+ and their various subpopulations (CD34+CD133+, CD34+c-Kit+, CD34+CXCR4+ and CD34+VEGFR2+c-Kit+) than wild-type" [101]. The researchers came to the conclusion that "One of the mechanisms that are responsible for previously described KL-VS heterozygote advantage may be connected with maintaining greater size of hematopoietic and endothelial progenitor cells population" [101]. Gazdhar A, et al. set out to investigate rather or not Klotho alpha Klotho has antioxidant protective properties in people with acute lung injury via Pluripotent Stem Cells (iPSCs). The researchers stated that "we hypothesized that cell-free Conditioned Media (CM) containing the secretome of iPSCs possess antioxidative constituents that can alleviate pulmonary oxidant stress damage" [102]. Thus, the researchers harvested the Continued Media (CM) of human dermal fibroblasts. The researchers indicated that "In both the *in vitro* and *in vivo* models, iPSC CM ameliorated oxidative damage to DNA, lipid, and protein, and activated the nuclear factor (erythroid 2)-related factor 2 (Nrf2) network of endogenous antioxidant proteins. Compared with control fibroblast-conditioned or cell-free media, iPSC CM is highly enriched with αKlotho at a concentration up to more than 10-fold of that in normal serum. αKlotho is an essential antioxidative cell maintenance and protective factor and an activator of the Nrf2 network. Immunodepletion of αKlotho reduced iPSC CM-mediated cytoprotection by ~50%. Thus, the abundant αKlotho content significantly contributes to iPSC-mediated antioxidation and cytoprotection" [102].

Klotho gene/protein and exercise in humans

This section of the review is dedicated to inform of published knowledge pertaining to the mutual effects of exercise modalities (in humans only) and Klotho (i.e. the effects aerobic and resistance training, physical activity etc. on Klotho levels and/or effects of Klotho on the ability to exercise, perform and compete). The section does not include studies that do not include exercise as an intervention in an experimental design. Thus, very few published work exists on the topic and the author wished to utilize this publication as a call for further research. Notice that contrary to previous sections of this review, the author chooses to go more in-depth regarding the design of the studies in this section as there are fewer to review and the author's relation to the field of exercise.

Le Gall JY, and Ardaillou R. state in their manuscript that Physical exercise and dietary measures are currently the only known ways of slowing the aging process" [103]. Though prior sources used in this review stated that there are possible treatments to consider when it pertains to Klotho, Le Gall JY, and Ardaillou R's statement remains greatly true [103].

Matsubara T, et al. investigated the influence of aerobic exercise training on plasma Klotho levels and arterial stiffness in postmenopausal women. Researchers first correlated Klotho levels with arterial stiffness (via carotid artery compliance and β-stiffness index), as well as with aerobic exercise capacity (via oxygen uptake at ventilatory threshold). Data collection was divided into two periods of time. The first cohort (experiment I) included sixty-nine (n=69) healthy, postmenopausal women ages 50-76 years, while the second cohort included nineteen (n=19) [104]. A cross-sectional design was utilized for the first cohort (without exercise intervention), while the design for the second cohort included an exercise intervention. The second cohort (experiment II) was divided into a control (n=8) and exercise group (n=11). Researchers indicated the exercise intervention included the following "Subjects in the exercise group underwent aerobic exercise training for >3 days/wk (two to three supervised sessions and additional home-based training) for 12 wk. Initially, the training included cycling and walking for 30 min/day at a relatively low intensity (60% of their individually determined maximal heart rate). As their exercise tolerance improved, the intensity and duration of the aerobic exercise were increased to 40–60 min/day at an intensity of 70–80% of the maximal heart rate. Subjects in the control group were instructed not to change their level of physical activity" [104].

In experiment I, Klotho plasma concentrations were 281 to 770 $pg \cdot mL^{-1}$. In experiment II, no significant differences were found between groups in regards to age, height, body mass, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, daily step counts, or physical activity level. While no significant differences were found between groups for Klotho levels at baseline, a significant difference was found post-exercise (414 ± 28 vs. 453 ± 36 $pg \cdot mL^{-1}$; $P < 0.05$).

A significant difference was observed in intercepts but not in slopes between plasma Klotho concentrations and beta-stiffness index after adjusting for age, BMI, and pulse pressure.

Changes in the plasma Klotho concentration were positively correlated with the changes in carotid arterial compliance after adjusting for age, BMI, and pulse pressure. Changes in plasma Klotho concentration were negatively correlated with the changes in beta-stiffness index after adjusting for age and BMI. No correlation was observed between the changes in the plasma Klotho concentration and those in cholesterol levels (total, HDL, and LDL) [104].

Saghiv M, et al. investigated the influences of aerobic and anaerobic training and a sixty-minute aerobic bout on blood s-Klotho levels [105]. Fifteen elite anaerobic cyclists were compared to fifteen elite aerobic athletes. Both groups underwent a maximal oxygen uptake test to establish VO_2max. Thereafter, s-Klotho samples were obtained at rest and after a sixty-minute aerobic bout at 75% of heart rate reserve; blood samples were analyzed via ELISA and IGF-1 was measured by a chemiluminescent immunometric method [105]. At rest a significant ($p<0.01$) difference was noted between the aerobically trained and anaerobically trained athletes for s-Klotho (672 ± 38 and 442 ± 24 $pg \cdot mL^{-1}$ respectively) and IGF-1 (65 ± 7 and 94 ± 12 $mmol \cdot L^{-1}$ respectively). Following sixty minutes of aerobic exercise, an interaction effect ($p<0.01$) was obtained for s-Klotho and IGF-1 between the groups: in the aerobic group both variables were changed significantly from base level while in the anaerobic ath-

letes s-Klotho increased significantly ($p<0.05$) and IGF-1 was significantly ($p<0.05$) reduced (566±32pg·mL^{-1} and 85±8mmol·L^{-1} respectively) [105]. Researchers wrote in their conclusions that "S-Klotho and long lasting aerobic exercise training are factors that may promote upgrading capacities of the young adults. However, being a highly anaerobically active sprinter suggests that there is no association between anaerobic vigorous exercise training and decreased risk factors for major chronic diseases" [105].

Saghiv, et al. additionally investigated the influences of aerobic training on s-Klotho while comparing young adults to the elderly [106]. Two hundred (n=200) healthy subjects were divided evenly into four groups: untrained young adults, untrained elderly, aerobically well-trained young adults, and aerobically well-trained elderly (24.5±1.0, 23.9±1.0, 58.6±1.1 and 58.1±1.1 years respectively). All subjects underwent a maximal oxygen uptake test (VO_{2max}). S-Klotho levels in the serum were analyzed using an alpha-klotho ELISA kit, while, IGF-1 was measured by a chemiluminescent immunometric method [106]. Significant ($p>0.005$) differences were found between the trained young adults and trained elderly as well as between untrained young adults and untrained elderly in s-Klotho (682±106.0, 571±92.0, 435±89.0 and 321±96.2 pg·mL^{-1} respectively) and IGF-1 (62.6±17.4, 74.6±16.7, 82.6±23.2 and 97.8±29.2 nmol·L^{-1} respectively). In addition, significant ($p>0.005$) differences were noted between untrained young adults and untrained elderly for s-Klotho and Total IGF-1 [106]. The researchers concluded that "S-Klotho is associated with younger age and aerobic exercise training, thus, probably related as well to other factors that promote health and postpone senescence. Being an aerobic athlete, especially at an elite level, seems to be associated with decreased risk for major chronic diseases. Inflection of s-Klotho expression through skeletal muscle contraction represents an interesting relationship that may help to explain the anti-aging effects of aerobic activity. Findings of the present study, support emerging evidence suggesting that such a relationship exists" [106].

Saghiv, et al. investigated the influence of anaerobic training on alpha-Klotho and IGF-1 levels in trained athletes. Fifteen well aerobically trained young adults and fifteen well anaerobically trained short distance sprinters were tested for maximal oxygen uptake (VO_{2max}). Following a warm-up, subjects underwent a maximal Bruce aerobic protocol, and had their blood drawn immediate-post [107]. Significant ($p>0.05$) differences were noted between the aerobic group and anaerobic sprinters with regard to s-Klotho (645±105.2 and 427±92.0 pg·mL^{-1} respectively), IGF-1 (70.2±10.9 and 94±21.5 nmol·L^{-1} respectively) and maximal oxygen uptake (60.3±2.7 and 55.1±2.7 mL·kg^{-1}·min^{-1} respectively). The researchers concluded that "Anaerobic exercise training is not a potent stimulus to increase plasma s-Klotho levels. Being an aerobic athlete, especially at an elite level, seems to be associated with decreased risk factors for major chronic diseases" [107].

Saghiv, et al. investigated the influences of aerobic training on s-Klotho and IGF-1 levels in people with CAD. Biomarker levels were compared in sixty untrained people with CAD, sixty age and sex matching trained people with CAD, and forty healthy untrained sex and age matching subjects [108]. All subjects were 50-60 years old. Training program for the second group included twelve weeks of aerobic training at 60-75% of their work capacity; 4-5 times per week. Following warm-up, subjects underwent a symptom limited graded maximal oxygen uptake (VO_{2max}) on a treadmill utilizing the standard Bruce protocol. Significant differences ($p<0.05$) between the trained CAD patients and both untrained groups; healthy subjects and CAD patients, were found in s-Klotho and IGF-1 [108]. Biomarker results for the trained unhealthy, untrained unhealthy, and healthy were 491±66, 385±70, and 418±81 for s-Klotho (pg·mL^{-1}) and 82±12, 106±21, 98±14 for IGF-1 (nmol·L), respectively. Researchers indicated the following as their conclusions "S-Klotho with regard to life expectancy, encounters the IGF-1 action. Klotho and aerobic exercise training are factors that may promote upgrading capacities of the CAD patients. Being aerobically active seems to be associated with a decreased risk for cardiovascular diseases. Findings of the present study, endorse emerging evidence suggesting that such a relationship exists. In addition, the similar values of s-Klotho and IGF-1 in the untrained CAD patients and the healthy untrained, suggests that atenolol does not influence s-Klotho and IGF-1 levels in CAD patients treated with this drug" [108].

Mostafidi, et al. researched the influence of exercise on Klotho levels in soccer (football) players compared to healthy non-athletes. Klotho levels of thirty soccer players, ages 18-22 years, were obtained the morning after their last evening practice session [109]. The control group included twenty-eight healthy young males, ages 18–27 years. Plasma Klotho concentrations were measured with the ELISA technique, using a soluble Klotho ELISA assay kit based on the manufacturer's instructions [109]. Results reported showed plasma Klotho levels for the athletes compared to non-athletes were 3.375±1.48 vs 1.39±0.43ng·mL^{-1}, respectively, $P<0.05$. Researchers indicate that "The control subjects were within close range of the previously proposed Klotho concentrations for normal individuals, while the athlete group had significantly higher plasma Klotho concentrations" [109]. Researchers concluded that "Further studies are needed in order to clarify the dynamics of Klotho production and secretion, and to understand the mechanisms of exercise-induced Klotho secretion or shedding" [109].

Boeselt T, et al. investigated the effects of high intensity exercise training on several serum biomarkers (including alpha Klotho) in people with COPD [110]. Forty-nine subjects in total (n=49) were divided into a control group (n = 18) and a training group (n = 31). Exercised included two sessions a week of ninety minutes in duration with consecutively increasing loads. A Six Minute Walk Test (6MWT) was performed upon enrollment, three months, and six months thereafter. Data on health-related quality of life, femoral muscle thickness, and various serum markers were obtained.

In their results, researchers indicated that "Serum levels of myostatin, irisin, resistin, and α-Klotho did not change significantly within the training period. Of note, the exercise group showed an inverse relationship between serum levels of resistin and those of α-Klotho after 6 months" [110].

Saghiv, et al. studies the influences of long lasting chronic resistive training S-Klotho and IGF-1. Fifty national level powerlifters and fifty age matched untrained young adults (27.1±1.0 and 26.5±1.0 years respectively). Following overnight fasting forearm vein blood samples were taken, circulating s- Klotho were examined by means of a-klotho Enzyme Linked Immunosorbent Assay ELISA kit. A chemiluminescent immunometric method was applied in order to define serum IGF-1 levels [111]. Results showed no significant differences were seen between the weightlifters and untrained young adults for s-Klotho (421.0±76.0 and 435.2±89.0 pg·mL^{-1} respectively). However,

IGF-1 levels were significantly ($p<0.05$) higher in the weight lifters compared to the untrained subjects (110.6±16.4 and 77.6±23.2mmol·L^{-1} respectively). The researchers concluded that "long lasting resistive training did not influence circulating levels of s-Klotho, while the increased circulating IGF-I may, at least in part, mediate increases in strength, power and muscle hypertrophy" [111].

Saghiv, et al. investigated the influences of twelve weeks of exercise training on circulating soluble-Klotho and Pro - BNP in coronary artery disease patients. S-Klotho and pro-BNP serum were assessed in two groups. Group A included coronary artery disease patients (n=41), age 59.6±2.2 years, all with recent (<45 days) Aorto-Coronary Bypass surgery (CABG), Myocardial Infarction (MI), or Percutaneous Intervention (PCI) who were recruited to a twelve weeks supervised aerobic exercise program (45 min/4-5 sessions/week). Group B included seventeen CAD patients, age 61±2.4 years, who continued their usual treatment and lifestyle with no active exercise intervention. Group B acted as the control group [112].

Assessment was done twice, prior to exercise program and at the end of twelve weeks intervention. Blood samples were drawn from a forearm vein after overnight fasting, s-Klotho levels in the serum were analyzed using an α-klotho enzyme linked immunosorbent assay Elisa kit and the pro- BNP was measured as well by an immunoassay method [112]. Results showed that no significant ($p= 0.27$) difference was found at baseline for S-Klotho levels between group A and group B (770.49±202.20 vs 727.54±207.83pg·ml^{-1} respectively), while a significant difference was found between group A and group B following exercise intervention (863.39±213.66 vs 677.71±167.46pg·ml^{-1}, respectively, $p <0.01$). S-Klotho and BNP showed an inverse correlation at baseline in group A ($r = -0.803$, $P<0.01$) and in group B ($r = - 0.850$, $p <0.01$), with similar values post twelve weeks ($r = -0.829$ and 0.834 respectively) [112]. Researchers concluded that "Aerobic exercise may modulate S-Klotho activity, thus conferring a possible mechanism for the enhanced survival of coronary artery patients participating in an exercise based cardiac rehabilitation program" [112].

Pako, et al. investigated the influence of three weeks of respiratory rehabilitation on klotho levels in people with COPD. Respiratory function, 6-Minute Walking Distance (6MWD), impact of disease (CAT), dyspnea, grip strength, chest expansion and breath holding time, smoking history, and Body Mass Index (BMI) were also evaluated [113]. Blood samples for the analysis of Klotho levels were obtain at day 1, 3 and last day of the rehabilitation program. Researchers reported that Klotho levels showed no correlation with clinical parameters (FEV1%, 6MWD, grip strength, CAT, smoking history, $p > 0.05$)". Overall, klotho levels did not change significantly (510.1±149.9 vs. 504.2±139.8 pg·ml^{-1}, $p > 0.05$) [113]. The authors concluded that "Plasma klotho concentration can be reliably measured in stable COPD; however, its levels are not correlated with clinical parameters of patients. Despite functional improvement, klotho level remains unchanged during the rehabilitation program" [114].

Summary

Klotho is a relatively new biomarker, indicated as influential in the aging process, in diseases of different nature, in exercise, and in health.

Multiple studies have shown a trend whereas klotho levels are decreased during and due to disease, while elevated in the healthy and trained.

Furthermore, it has been shown the over-expression of Klotho is associated with a longer life expectancy compared to a shorter life expectancy while under-expressed.

Aerobic modalities of exercise have been proven to increase Klotho concentrations, while anaerobic modalities have little to no significant effect.

In light the body of knowledge and data that exists thus far, Klotho is and should be regarded as a possible component of future therapeutic processes.

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