Hypertension

REVIEW

Role of Klotho in the Development of Essential Hypertension

Mehmet Kanbay, Atalay Demiray, Baris Afsar, Adrian Covic, Laura Tapoi, Carina Ureche, Alberto Ortiz

ABSTRACT: Klotho has antiaging properties, and serum levels decrease with physiological aging and aging-related diseases, such as hypertension, cardiovascular, and chronic kidney disease. Klotho deficiency in mice results in accelerated aging and cardiovascular injury, whereas Klotho supplementation slows down the progression of aging-related diseases. The pleiotropic functions of Klotho include, but are not limited to, inhibition of insulin/IGF-1 (insulin-like growth factor 1) and WNT (wingless-related integration site) signaling pathways, suppression of oxidative stress and aldosterone secretion, regulation of calcium-phosphate homeostasis, and modulation of autophagy with inhibition of apoptosis, fibrosis, and cell senescence. Accumulating evidence shows an interconnection between Klotho deficiency and hypertension, and Klotho gene polymorphisms are associated with hypertension in humans. In this review, we critically review the current understanding of the role of Klotho in the development of essential hypertension and the most important underlying pathways involved, such as the FGF23 (fibroblast growth factor 23)/Klotho axis, aldosterone, Wnt5a/RhoA, and SIRT1 (Sirtuin1). Based on this critical review, we suggest avenues for further research.

Key Words: albuminuria ■ aldosterone ■ inflammation ■ kidney diseases ■ serum

Hypertension is a prevalent disease that determines a crucial public health burden at a global level. It is estimated that 29% of the world adult population (1.56 billion) will have this condition by 2025.[1,2] Hypertension significantly contributes to the development of cardiovascular and kidney disease and represents a major modifiable risk factor for morbi-mortality due to ischemic heart disease and stroke. These were the leading causes of death globally in 2010 and are projected to remain so in 2030.[3] While secondary hypertension has a specific underlying cause, primary (essential) hypertension, which constitutes almost 95% of adult hypertensive cases, has a multifactorial origin stemming from a complex interplay between susceptibility genes and environmental factors. Despite extensive research for decades, the mechanisms underlying the susceptibility and the progression of essential hypertension are not fully understood. The understanding of the pathogenesis of essential hypertension has evolved from a hemodynamic phenomenon related to defective kidney sodium excretion to a complex syndrome including genetic, metabolic, and immune system abnormalities that include abnormal fat tissue distribution, over-activation of the sympathetic nervous system and endothelial dysfunction.[4–6] The huge financial and physical burden of essential hypertension on the health care system justifies the continued focus on finding new management and treatment strategies.

Klotho protein was named after the Greek goddess Clotho, who was thought to spin the thread of life and control the destiny of humans.[7] It was first described in 1997, when Kuro-o et al[8] reported that an insertional mutation of a transgene in mice led to an extreme premature aging phenotype by disrupting the *Kl* gene encoding Klotho. Decreased *Kl* gene expression results in accelerated aging and shortened life span, whereas *Kl* overexpression slows down the progression of aging-related diseases and prolongs life span in mice.[9] The discovery of Klotho was part of a broader approach which was meant to investigate the mechanisms of human aging, to slow down or even stop the development of age-related diseases.[8] Its strong aging-suppressing properties triggered a great interest and extensive research about its

Correspondence to: Mehmet Kanbay, Division of Nephrology, Department of Medicine, Koc University School of Medicine, 34010, Istanbul, Turkey. Email mkanbay@ku.edu.tr

For Sources of Funding and Disclosures, see page 748.

Hypertension is available at www.ahajournals.org/journal/hyp

Nonstandard Abbreviations and Acronyms

AMPK-α	AMP-activated protein kinase α
AT1R	angiotensin II type-1 receptor
CCR2	CC chemokine receptor 2
CKD	chronic kidney disease
eNOS	endothelial NO synthase
ERK	extracellular signal-regulated kinase
FGF23	fibroblast growth factor 23
GFR	glomerular filtration rate
HFTC3	hyperphosphatemic familial tumoral calcinosis type 3
HIF	hypoxia-inducible factor
IGF-1	insulin-like growth factor 1
KLPH	Klotho/lactase-phlorizin hydrolase-related protein
LDL	low-density lipoprotein
MCP	monocyte chemotactic protein
MMP	matrix metalloproteinase
mTOR	mammalian target of rapamycin
NO	nitric oxide
PPAR-γ	peroxisome proliferator-activated receptor-γ
ROS	reactive oxygen species
RUNX2	runt-related transcription factor 2
SGK1	serum/glucocorticoid-regulated kinase 1
SIRT1	Sirtuin-1
SNPs	single-nucleotide polymorphisms
SOD2	superoxide dismutase 2
TGF	transforming growth factor
VSMCs	vascular smooth muscle cells
WNK4	with-no lysine kinase 4
WNT	wingless-related integration site

functions has been made. It has been noted that serum Klotho levels decrease in humans both with aging,[10] as well as with several comorbidities such as cardiovascular diseases (including hypertension), chronic kidney disease (CKD), cancer, or Alzheimer disease.[11] In addition, various physiological and pathological conditions modulate Klotho expression. For instance, FGF23 (fibroblast growth factor 23) or estrogen deficiency upregulates Klotho expression, whereas inflammation, oxidative stress, angiotensin II, aldosterone, and albuminuria suppress Klotho expression.[12,13]

The role of Klotho in the pathophysiology of secondary hypertension was also investigated. In a study that included patients with renovascular hypertension (N=12), essential hypertension (N=12), and healthy volunteers (N=12), Klotho levels were decreased in the first 2 categories but were normal in the nonhypertensive group, even after adjustment for glomerular filtration rate (GFR). Lower Klotho levels in both renovascular and essential hypertension, together with the direct correlation of Klotho with GFR levels, points toward an important renal component in essential hypertension. This could suggest that Klotho could serve as an useful biomarker for early detection of subclinical kidney injury in hypertension.[14] Several studies have proven that Klotho levels are also correlated with the risk of preeclampsia, the most common hypertensive disease in pregnancy. Additionally, they suggested that both placental and serum levels of Klotho could represent a potential biomarker for predicting the risk of developing preeclampsia.[15,16]

However, Klotho deficiency could hide behind an essential hypertension and partially explain the high prevalence of this disease. Understanding the nature of the causal relationship between Klotho deficiency and hypertension could provide insights into the pathogenesis of essential hypertension as well as new horizons in its management and treatment.

THE STRUCTURE AND FUNCTIONS OF KLOTHO

Detailed information about Klotho's structure and functions has been extensively discussed elsewhere.[10,11,17,18] Thus, we will only reiterate the main features that support the relationship between Klotho and hypertension.

The human Klotho gene, *KL*, encodes α-Klotho and belongs to a superfamily that also comprises *KLB* encoding β-Klotho and lactase-like protein (*LCTL*) encoding KLPH (Klotho/lactase-phlorizin hydrolase-related protein; KLrP or γ-Klotho).[10] In the present article, unless otherwise specified, the term Klotho is used to refer to α-Klotho.

Three forms of Klotho have been detected in humans and mice: the full-length transmembrane, the secreted, and the shed form.[10] The full-length transmembrane Klotho is composed of an extracellular domain consisting of 2 internal repeats (KL1 and KL2), a single-pass membrane domain and an intracellular domain. The main activity and functions of Klotho depend on the KL1 and KL2 extracellular domains[10,17] (Figure 1A). Secreted Klotho is the major product of the *KL* gene in humans and its main source is the kidney.[18,19]

Since the different forms of Klotho have highly similar sequences, currently available antibodies and commercial tests may recognize different forms of shed and secreted Klotho.[20,21] This contributes to the ambiguity in the literature with only a few articles assessing different Klotho molecules. We prefer using the terms circulating or soluble Klotho to refer to both secreted and all shed forms of Klotho. Future studies are warranted to elucidate whether physiological functions of different circulating Klotho proteins (secreted or shed) differ and how their concentrations change under different pathophysiological conditions.

REVIEW

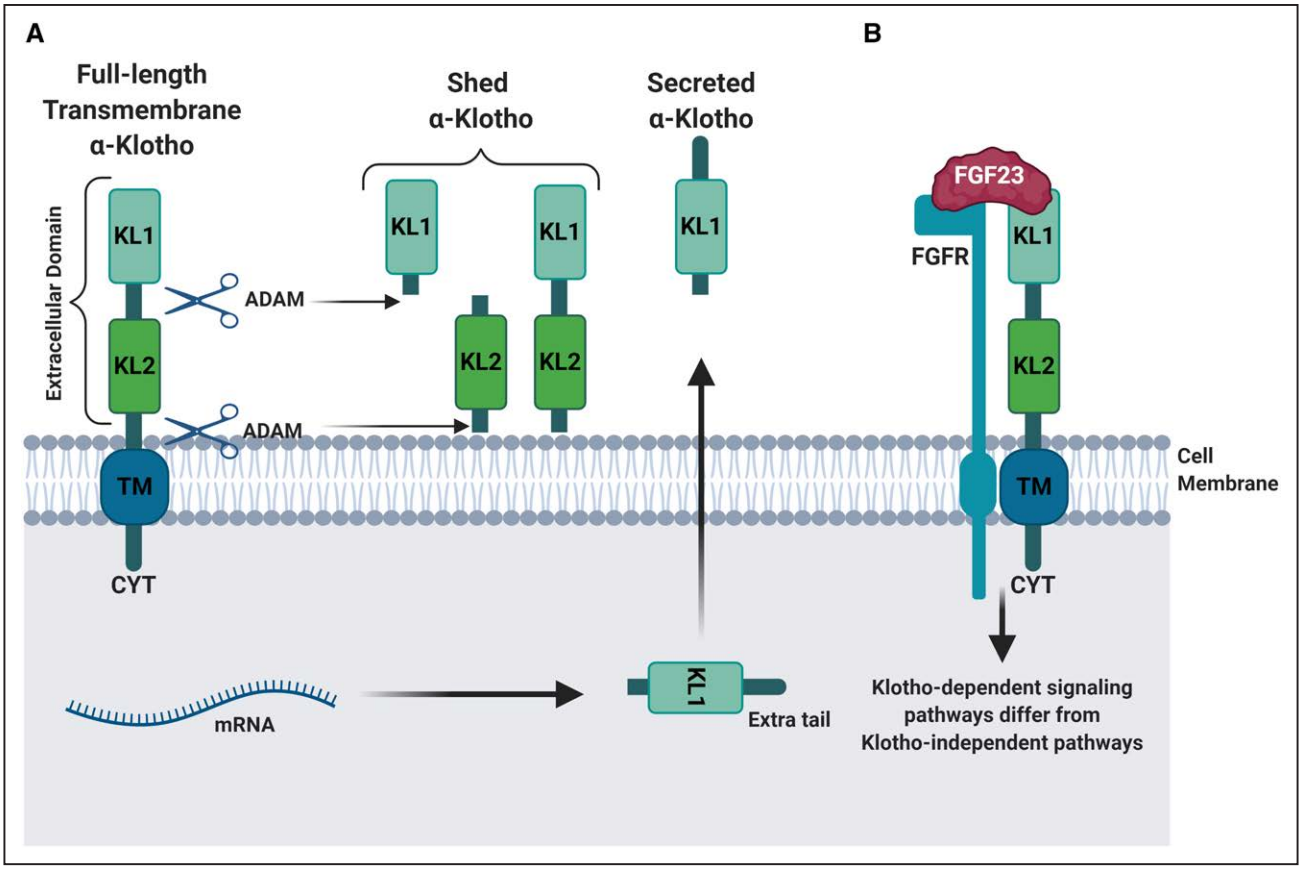

Figure 1. Schematic representation of full-length transmembrane and circulating (shed and secreted) forms of α-Klotho. **A**, The full-length transmembrane α-Klotho consists of 3 domains: cytoplasmic (CYT), transmembrane (TM), and extracellular. The extracellular domain has 2 internal repeats, KL1 and KL2. The extracellular domain of α-Klotho is cleaved by a disintegrin and metalloproteinase (ADAM) 10 and 17 from 2 different points to release 3 types of shed α-Klotho. Alternative mRNA splicing leads to the formation of secreted α-Klotho. **B**, Full-length transmembrane α-Klotho or soluble KL1-KL2 (130 kDa) Klotho form a complex with FGFR (fibroblast growth factor receptor) to create a high-affinity binding site for an FGF (fibroblast growth factor)23.

Full-length transmembrane Klotho and soluble 130 kDa Klotho function as obligatory coreceptors for FGF23.[22] Klotho increases the affinity of FGFRs selectively to FGF23 at target organs, by forming a complex with FGFR1c, FGFR3c, and FGFR4. Thus, Klotho converts canonical FGFRs into a specific receptor for FGF23[17] (Figure 1B). Through this interaction, Klotho is involved in the metabolism of phosphate, calcium, and vitamin D. The key mineral metabolism effect is the promotion of a negative phosphate balance through actions on kidney proximal tubular cells.[17] In Klotho-deficient mice, FGF23 resistance promotes the development of ectopic calcifications of blood vessels and soft tissues as a result of high serum levels of calcitriol which increase the absorption of dietary Ca^{2+} and P_i from the gut and impair urine excretion of phosphate.[23] Murine studies identified impaired urine phosphate excretion as a key driver of accelerated aging in Klotho-deficient mice since this was improved by proximal tubule deficiency in the main phosphate transporter NaPi2a and worsened by excess dietary phosphate.[24] Unfortunately, blood pressure was not assessed.

Similar physical and biochemical phenotypes are observed in Klotho-deficient mice and FGF23-knockout mice suggesting the function of FGF23 heavily depends on Klotho. Indeed, FGF23 was devoid of any impact on phosphate homeostasis in the absence of Klotho.[25] Klotho is also highly expressed in the parathyroid glands, another FGF23 target, leading to the suppression of parathyroid hormone secretion.[26] Klotho and FGF23 have additional actions on several organ systems by modulating inflammation and insulin resistance, iron metabolism, and erythropoiesis.[27]

Klotho likely has FGF23-independent actions since circulating forms of Klotho without FGFRs do not have a high affinity for FGF23.[17] However, no binding site or receptor for circulating Klotho has been identified. Circulating or urinary Klotho could regulate calcium-phosphate metabolism independently of FGF23 by modifying glycans via its β-glucuronidase activity. This modification possibly leads to the internalization of phosphate transporters from the apical membrane, which results in decreased transporter activity and tubular phosphate reabsorption.[17,28,29] Future studies

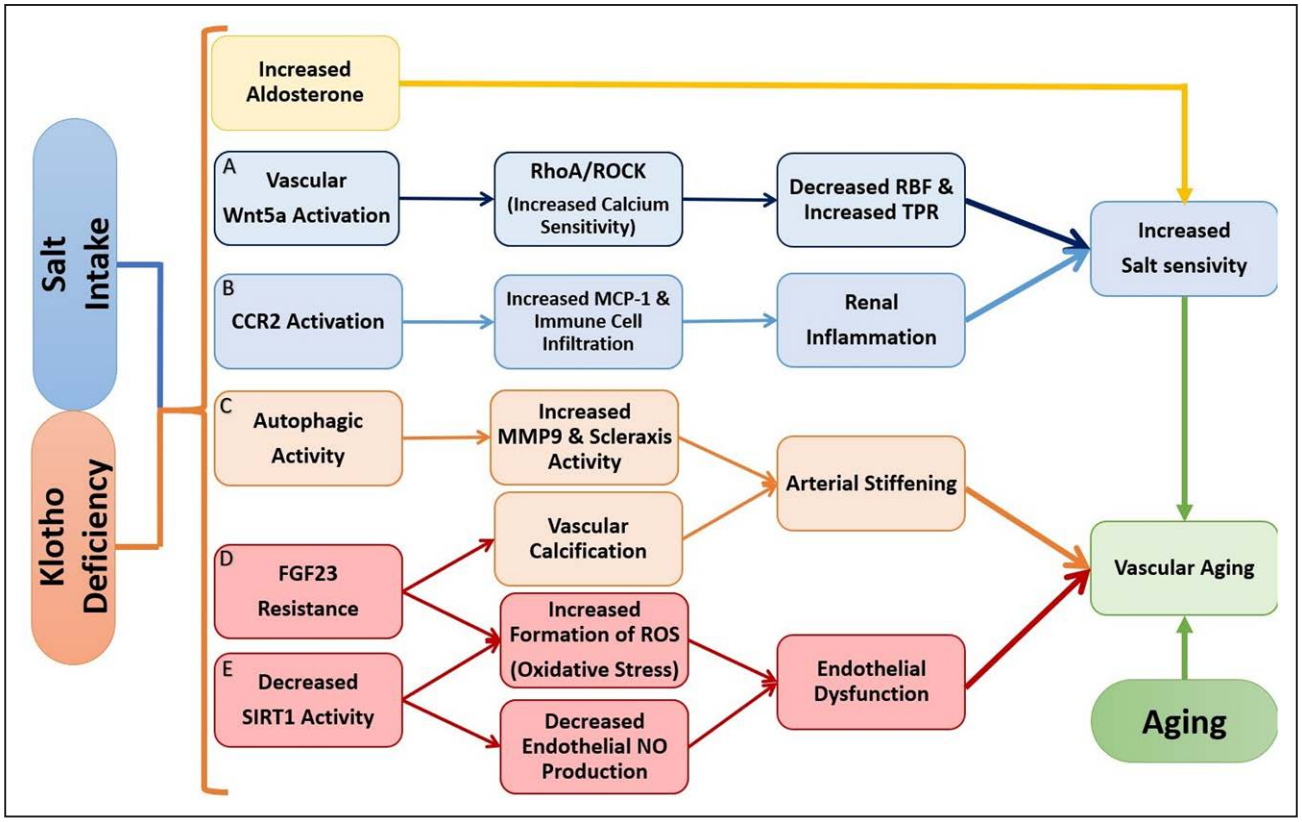

Figure 2. Mechanisms by which Klotho deficiency is involved in the development of hypertension.
Klotho deficiency is association with the following molecular consequences that overall promote arterial stiffening, vascular aging, and endothelial dysfunction which cumulatively contribute to the development of hypertension: (**A**) Activation of vascular ROCK (Rho kinase) via the noncanonical Wnt pathway and (**B**) activation of the CCR2 (CC chemokine receptor 2)-mediated inflammatory processes contribute to salt-sensitive hypertension. **C**, The upregulation of autophagy increases MMP (matrix metalloproteinase)-9 and scleraxis activities contributes to arterial stiffening and hypertension. **D**, Vascular calcification and increased oxidative stress due to FGF23 (fibroblast growth factor 23) resistance. **E**, Downregulation of SIRT1 (Sirtuin1) activity decreases endothelial nitric oxide (NO) production and increases prooxidative, proinflammatory, and proapoptotic activities. FGFR indicates fibroblast growth factor receptor; MCP-1, monocyte chemotactic protein-1; NO, nitric oxide; RBF, renal blood flow; ROS, reactive oxygen species; and TPR, total peripheral resistance.

should further characterize the mechanism of action of circulating Klotho.

Although Klotho is mainly expressed in certain organs (the kidney's distal tubules, the choroid plexus of the brain, the parathyroid glands and is also detectable in the bone, placenta, prostate, and small intestine[12,18,22]), Klotho gene mutations cause dysfunctions in almost all organs and tissues. These pleiotropic actions with multisystemic effects of Klotho deficiency imply that circulating Klotho could act on several organ systems and may have direct actions on cells that do not express Klotho.

Besides the coreceptor function of full-length transmembrane or soluble 130 kDa Klotho to regulate ion metabolism, Klotho could also inhibit insulin/IGF-1, Wnt signaling pathways and suppress oxidative stress and aldosterone synthesis.[10,22] Klotho increases the expression of SOD (superoxide dismutase) by activating forkhead transcription factors, which are normally suppressed by insulin/IGF-1 signaling. Thus, Klotho inhibition of insulin/IGF-1 signaling increases resistance to oxidative stress.[30] Klotho's antioxidant properties potentially contribute to its antiaging effect. Additional beneficial actions of Klotho include promotion of nitric oxide (NO) production, inhibition of apoptosis, suppression of fibrogenesis, induction of autophagy, and preservation of stem cells.[17] These effects could contribute to the beneficial impact of Klotho on the progression of acute kidney injury to CKD, of cardiovascular diseases, and atherosclerosis.[30]

ASSOCIATION OF *KL* GENE POLYMORPHISMS WITH HYPERTENSION

Single-nucleotide polymorphisms (SNPs) in the *KL* gene were associated with increased risk of cardiovascular disease, stroke, and hypertension in different populations.[31–34] One well-studied *KL* SNP is the functional KL-VS variant which holds 2 amino acid changes (F352V; rs9536314 and C370S; rs9527025) that alter the metabolism and distribution of secreted Klotho.[35] KL-VS was an independent risk factor for early-onset coronary artery disease and was associated with traditional cardiovascular risk factors, including high systolic

blood pressure.[31] In this regard, the rs9536314 SNP was associated with salt-sensitive hypertension in treatment-naive hypertensive patients, and the G allele was associated to a less steep pressure-natriuresis curve. Thus, in carriers of the G allele, a higher blood pressure was needed to excrete the same quantity of salt as in salt-resistant subjects.[36]

The G-395A SNP (rs1207568) in the promoter region of the *KL* gene was associated with essential hypertension among 435 Chinese Han, especially in those aged over 60 years.[31] Specifically, the −395A allele was associated with a lower adjusted odds ratio of essential hypertension (0.593, *P*=0.024) and in vitro it showed a higher promoter activity than the −395G allele.[31] By contrast, in 251 healthy Korean women, the A allele was associated with higher mean systolic blood pressure than noncarriers, although both mean values were within the normotensive range.[32] No significant associations were found between the C1818T SNP (rs564481) in exon 4 of the *KL* gene with hypertension in 286 Iranians suspected of having coronary artery disease, although some statistically significant differences were found in certain subanalyses.[37] However, Japanese women over 60 years of age who were T carriers of C1818T had significantly higher systolic blood pressure in another study, and similar findings were observed in Korean women, although statistical significance was lost after adjusting for confounders.[32,38] These apparently contradictory results illustrate the need for large cohorts to confirm the impact of the genetic background on phenotypes.

In a 13-year-old patient, homozygous *KL* mutations caused HFTC3 (hyperphosphatemic familial tumoral calcinosis type 3), characterized by childhood osteopenia, calcifications of the dura and arteries, hyperphosphatemia, hypercalcemia and increased calcitriol, FGF23, and parathyroid hormone levels. At the age of the diagnosis, the patient was normotensive, but unfortunately, no later report on outcomes and evolution could be found.[39]

KLOTHO DOWNREGULATION: AN EARLY EVENT IN CKD PROGRESSION

CKD and hypertension have a bidirectional relationship. Hypertension is a well-established cause of kidney disease, although its impact as a cause of end-stage kidney disease is likely overestimated.[40] On the contrary, the contribution of CKD to essential hypertension is likely underestimated. In this regard, the concept that secondary hypertension (among others, CKD-related hypertension) represents around 5% of hypertensive individuals dates back to the 20th century, well before the CKD concept was developed. CKD encompasses either a decreased estimated GFR (below 60 mL/min per 1.73 m^2, ie, a milder loss of kidney function than that inherent to the serum creatinine values used for the diagnosis of chronic renal insufficiency in the 20th century) or evidence of kidney injury, such as pathological albuminuria or altered kidney imaging.[41] Thus, CKD can be diagnosed (and contribute to hypertension) in the presence of a perfectly normal GFR. Indeed, the concept of CKD playing a role in the development of what is now considered essential hypertension is supported by necropsy data from young Europeans killed in traffic accidents: individuals with essential hypertension had roughly half the total number of glomeruli, and glomeruli had evidence of compensatory hypertrophy when compared with normotensive individuals.[42] This compensatory hypertrophy was likely sufficient to maintain normal estimated GFR values. Since Klotho production is one of the first kidney functions to be lost, starting with CKD category G1 (ie, preserved estimated GFR),[43] acquired Klotho deficiency is well suited to be one of the drivers of hypertension in early CKD stages, in which hypertension may be considered essential. At this stage, Klotho downregulation is likely driven by pathological albuminuria and local inflammation, and not by loss of tubular cell mass.[13,44,45] In this regard, serum Klotho levels are decreased in long-term hypertension as well as in conditions associated with hypertension such as diabetes and CKD and other chronic conditions.[46–48]

THE ROLE OF KLOTHO DEFICIENCY IN THE DEVELOPMENT OF HYPERTENSION

As we already mentioned, essential hypertension is a worldwide prevalent disorder[49] and its prevalence increases with age. In addition, salt intake is a significant environmental modifiable risk factor for developing hypertension.[50] Factors contributing to the increase in blood pressure with aging include salt-induced increase in total peripheral resistance, impairment of kidney sodium excretion, vascular aging (eg, arterial stiffening), endothelial dysfunction, and increased aldosterone levels.[51–58] Serum Klotho levels are inversely associated with blood pressure salt sensitivity in patients with hypertension, and recent studies support an interplay between Klotho and hypertension in which Klotho deficiency facilitates the development of salt sensitivity, vascular aging, and endothelial dysfunction.[50,59]

Salt Sensitivity

In humans, after the age of 40, the salt sensitivity of blood pressure increases while circulating Klotho levels decrease.[60] This age-associated increased salt sensitivity is thought to result from a combination of sodium retention through impaired urinary sodium excretion and increased total peripheral resistance through vasoconstriction.

The increase in total peripheral vascular resistance through vasoconstriction involves both the G_q-G_{11} and G_{12}-G_{13}-LARG mediated signaling pathways. The G_q-G_{11} pathway is mainly responsible for the α1-adrenergic effects on vascular smooth muscle cells (VSMC) to maintain baseline blood pressure. Meanwhile, the G_{12}-G_{13}/RhoA pathway is responsible for the effects of angiotensin II, endothelin 1, and thromboxane A_2, which are closely related to salt-induced hypertension, without affecting baseline blood pressure regulation.[61] Angiotensin II–induced activation of the Wnt5a/RhoA pathway is suppressed by Klotho in human cultures of VSMCs.[62] In both Klotho-deficient aged mice and young *Kl*$^{+/-}$ mice, a high-salt diet magnified renal blood flow reduction and blood pressure elevation in response to the intra-arterial injection of angiotensin II and a thromboxane A_2 analog.[62] Klotho supplementation by gene overexpression reversed those responses in both groups of mice and prevented the increase in blood pressure, suggesting that in Klotho deficiency, an enhanced response to angiotensin II is associated with salt-induced hypertension. The Wnt inhibitor LGK974 and the Wnt5a antagonist box5 also reduced salt-induced hypertension in both groups of mice. Thus, Klotho deficiency caused aging-related salt-sensitive hypertension through activation of the noncanonical Wnt5a/RhoA pathway leading to decreased renal blood flow and increased total peripheral resistance via vasoconstriction.[62]

In *Kl*$^{+/-}$ mice, high salt intake increased the kidney expression of MCP-1 (monocyte chemotactic protein-1) and the infiltration by macrophages and T cells, as well as blood pressure. These responses were abolished by inhibiting the MCP-1 receptor CCR2 (CC chemokine receptor 2) with INCB3284. Thus, Klotho deficiency could contribute to the development of salt-sensitive hypertension also via the activation of CCR2-mediated inflammation in the kidney.[50]

Silencing of brain Klotho in rats via small interference hairpin RNA, increased endothelin production and sympathetic nervous activity, potentiating cold-induced blood pressure elevation.[63]

Vascular Aging

Vascular calcification and arterial stiffness are 2 interrelated vascular aging processes potentially modulated by Klotho availability.

Arterial stiffness is an independent predictor of poor cardiovascular outcomes such as stroke, myocardial infarction, and hypertension, representing an aging-related process associated with the replacement of elastin fibers by stiffer collagen fibers.[54,64] Stiffness of large arteries such as the aorta preceded the development of hypertension in the Framingham study.[65]

Human aortic VSMCs and the medial layer of both kidney and epigastric arteries express Klotho. Klotho deficiency in human aortic-VSMC increases the sensitivity to vascular calcification.[66] Thus, vascular Klotho expression is crucial to prevent pathological vascular calcification. High levels of FGF23 have off-target actions by activating FGFR in the absence of Klotho. Indeed, high FGF23 levels are an independent risk factor for cardiovascular disease in patients with renal failure and are related to coronary artery disease, heart failure, and cardiovascular mortality even in patients with normal kidney function.[67,68] In Klotho-deficient mice, the administration of magnesium[69] or eicosapentaenoic acid[70] prevented vascular calcifications.

There are observational clinical links between Klotho expression and arterial stiffness as well as preclinical evidence of a cause-and-effect relationship. Serum Klotho levels were reduced by 45% in patients with arterial stiffness and hypertension[64] and low serum soluble Klotho was independently associated with arterial stiffness in patients with CKD.[71] In this regard, aortas of *Kl* haplodeficient (*Kl*$^{+/-}$) mice had increased levels of β-gal and p53 suggesting that Klotho deficiency promotes vascular aging[48] and arterial stiffness, a process regulated by increased autophagy and SIRT1 (Sirtuin-1) downregulation.[48,72]

Kl haplo deficiency may facilitate hypertension through induction of autophagic activity leading to arterial stiffening. Autophagy is significantly increased in the aortas of Klotho-deficient mice as evidenced by high light chain 3b-II expression and autophagy flux.[48] Autophagy is thought to contribute to arterial stiffening by increasing MMP (matrix metalloproteinase)-9 activity, the expression of TGF (transforming growth factor)-β1 and the transcription factors RUNX2 (runt-related transcription factor 2) and scleraxis. The latter is a promoter of collagen expression since suppression of autophagy with chloroquine abolished both these molecular changes and also Klotho deficiency-induced arterial stiffness and hypertension within 2 weeks in *Kl*$^{+/-}$ mice. This time-course suggests a very active ongoing process. However, chloroquine increases lysosomal pH, thus potentially interfering with other lysosomal functions. Studies in cultured murine VSMC confirmed a direct activity of soluble Klotho in preventing autophagy and identified beclin-1 upregulation as the trigger of autophagy when soluble Klotho was absent from the cell culture medium. Thus, Klotho deficiency could enhance autophagy in the vasculature, promoting arterial stiffening and hypertension through modulation of MMP-9 activity and scleraxis expression.[48]

SIRT1 is a nicotinamide adenine dinucleotide-dependent deacetylase with antiinflammatory, antioxidant, and antiapoptotic effects on the endothelium that prevents endothelial senescence and dysfunction.[72] SIRT1 overexpression suppresses angiotensin II–induced vascular remodeling and hypertension in mice.[73] However, SIRT1 expression and activity are reduced in the aorta of *Kl*$^{+/-}$ mice.[64] Moreover, pharmacological activation of SIRT1 by SRT1720 suppresses the elevation in blood pressure

within 3 days, decreases arterial collagen deposition and elastin degradation, and increases AMPK-α (AMP-activated protein kinase α) and eNOS (endothelial NO synthase) activity.[64] Thus, the downregulation of SIRT1 activity in endothelial and VSMC by Klotho deficiency could contribute to the development of arterial stiffness and hypertension. However, the rapid onset of the blood pressure-lowering effect of SRT1720 suggests a functional rather than structural impact on vascular wall composition as a key driver of the response.

Endothelial Dysfunction

Klotho antioxidant function may improve endothelial function. Human coronary artery endothelial cells express Klotho and FGFR1.[74] FGF23 activation of FGFR1/Klotho activates the Akt/eNOS signaling pathway in these cells, leading to the production of NO, a key vasodilator factor. FGF23 increases the expression of NADPH oxidase 2, which contributes to the generation of reactive oxygen species (ROS) and oxidative stress and of SOD2 and catalase, which contribute to ROS degradation. Klotho inhibition interferes with Akt/eNOS activation and NO synthesis, blocks SOD2 and catalase, while the activity of NADPH oxidase 2 remains unchanged suggesting that FGF23-induced ROS production is counterbalanced by increased ROS degradation in the presence of Klotho.[74] However, overproduction of ROS is not counterbalanced by ROS degradation in Klotho deficiency, and FGF23-induced NO synthesis by eNOS is blunted.[74] Thus, high FGF23 concentrations in the settings of Klotho deficiency induce oxidative stress and contribute to endothelial dysfunction. Another study confirmed that Klotho could strengthen antioxidant defenses through SOD and eNOS via PI3K/Akt/eNOS pathway activation as Klotho suppressed oxidized-LDL (low-density lipoprotein)-induced oxidative stress in human umbilical vein endothelial cells.[75]

All in all, the presence of Klotho in the vascular wall is an important element for maintaining their integrity by preventing pathological calcification, arterial stiffness and endothelial dysfunction, key processes for the development of hypertension.

Hyperaldosteronism

Aging is associated with increased aldosterone-producing cell clusters, autonomous aldosteronism, and decreased suppression of aldosterone by high salt intake as well as poor response to low dietary salt.[57] Hyperaldosteronism is closely linked to salt sensitivity[76] and there is evidence for a close link between Klotho and aldosterone regulation. On one hand, aldosterone significantly decreases Klotho transcription and protein expression in HEK293 cells.[77] On the other, Klotho deficiency leads to hyperaldosteronism.

Haplodeficient $Kl^{+/-}$ mice have increased plasma aldosterone by 16 weeks of age, which leads to spontaneous and persistent hypertension.[78] This is associated with increased adrenal expression of Cyp11b2 encoding aldosterone synthase, a direct effect of Klotho deficiency as judged by Klotho regulation of CYP11B2 expression in human adrenocortical cells through the coordinated modulation of the transcription factors steroidogenic factor-1, a negative regulator of CYP11B2, and ATF2, a positive regulator. The mineralocorticoid receptor antagonist eplerenone reverses Klotho deficiency-induced hypertension and attenuates kidney damage.[78] Furthermore, eplerenone abolishes Klotho deficiency-induced arterial stiffening in $Kl^{+/-}$ mice as well as vessel wall upregulation of MMP-9, TGF-β1, scleraxis, and evidence of autophagy and increases collagen-1 expression and decreased elastin levels, respectively.[79]

By contrast, Klotho-hypomorphic mice (kl/kl), the original model in which Klotho function was described and that is not completely Klotho deficient (it is not $Kl^{-/-}$)[8] also suffer from hyperaldosteronism, excessive vascular calcification, accelerated aging, and early death. Spironolactone decreases vascular and soft tissue calcification and increases the life span, without significant effects on 1,25(OH)2D3, FGF23, calcium, phosphate plasma concentrations, or blood pressure (which was low in this model).[80] In this model, decreased plasma volume, dependent on high calcitriol levels, appears to be a driver of hyperaldosteronism.[81]

Hyperaldosteronism was also noted in Six2-$Kl^{-/-}$ mice, which were deficient in kidney Klotho and, as consequence, had an ≈80% reduction in circulating Klotho.[82]

Further insights into the relationship between Klotho and hyperaldosteronism are derived from the transgenic α-Klotho longevity mouse model.[22] In this model, both membrane Klotho and soluble 130 kDa Klotho are increased. Membrane Klotho (but not soluble Klotho) increases FGF23 levels, favoring hypertension development despite low aldosterone levels, which explained why the mice were protected from left ventricular hypertrophy. Mechanistically, membrane Klotho and soluble Klotho 130 kDa are essential cofactors for FGF23-mediated ERK (extracellular signal-regulated kinase) activation but inhibit FGF23 stimulation of PLC-γ and PI3K/AKT signaling. Thus, even when the simultaneous increase in membrane and soluble Klotho resulted in increased FGF23 and hypertension, mice were protected from target organ damage and lifespan increased.[22] Additionally, we have to keep in mind that any therapeutic intervention that increases Klotho levels uses genetic means to increase soluble 130 kDa Klotho or actually provide recombinant soluble 130 kDa Klotho, which is devoid of the effect to increase FGF23 levels. In this regard, FGF23 directly upregulates the membrane abundance of the Na(+):Cl(−) cotransporter NCC in distal renal tubules by a signaling mechanism involving the FGFR/α-Klotho

complex, ERK1/2, SGK1 (serum/glucocorticoid-regulated kinase 1), and WNK4 (with-no lysine kinase 4). Thus, FGF23 increases distal tubular Na(+) reabsorption leading to volume expansion, hypertension, and heart hypertrophy in presence of high dietary salt.[83]

Klotho Deficiency and Metabolic Disorders in Hypertension

Data about the impact of Klotho deficiency on the development of hypertension in the setting of a metabolic disorder points towards 2 other metabolic pathways, the 5′ AMPK and the PPAR-γ (peroxisome proliferator-activated receptor-γ) pathways.[84] In animal studies, Klotho deficiency promotes arterial stiffening and hypertension in mice fed a high-fat diet by inhibiting the AMPK and without affecting the metabolic parameters.[85] Additionally, Klotho promotes the expression of adipogenic factors, adipocyte maturation and it may have a role in intracellular lipid accumulation.[86,87] This hypothesis is further supported by the fact that in leptin-deficient and obese mice, Klotho inhibition reduces both the body weight and the visceral fat.[87] A recent study on 2238 subjects with CKD showed that serum levels of Klotho are inversely associated with the presence of metabolic syndrome.[88] Thus, Klotho deficiency may represent a cornerstone imbalance in the pathophysiology of metabolic syndrome and hypertension.

In conclusion, there is convincing data suggesting that low levels of Klotho predispose to the development of hypertension by influencing several pathological pathways, as mentioned above. Still, a cause-and-effect relationship between Klotho and hypertension needs further validation in large clinical studies.

THE BENEFICIAL EFFECTS OF KLOTHO SUPPLEMENTATION

Overexpression of the *Kl* gene is associated with a longer lifespan and slows down the progression of aging-related conditions in mice. Exogenous Klotho supplementation to stroke-prone spontaneously hypertensive rats reduced both blood pressure and angiotensin II levels in plasma and kidney. Klotho suppressed the activity of the renin-angiotensin system by inhibiting the Wnt signaling pathway and increasing AT1R (angiotensin II type-1 receptor) internalization.[46] Klotho supplementation also enhanced pressure natriuresis and reduced blood pressure by inhibiting HIF (hypoxia-inducible factor)-1α activation and limiting medullary fibrosis. Furthermore, it restored the autoregulation of GFR and decreased kidney hypertrophy by inhibiting Akt-mTOR (mammalian target of rapamycin) signaling.[46] Klotho supplementation also reduced blood pressure and albuminuria in *db/db* diabetic mice and DBA/2-pcy polycystic kidney disease mice.[89,90]

Adenovirus-mediated Klotho gene delivery to Otsuka Long-Evans Tokushima Fatty rats, an experimental model for atherosclerotic disease, promoted NO production and endothelial-dependent aortic dilation, improving endothelial dysfunction and decreasing blood pressure.[91] An adeno-associated virus carrying mouse Klotho full-length cDNA increased Klotho expression in spontaneous hypertensive rats, which display a spontaneous suppression of Klotho expression. Additionally, a single dose of adeno-associated virus carrying mouse Klotho full-length cDNA prevented the development of spontaneous hypertension and renal damage in spontaneously hypertensive rats for 12 weeks (length of study).[92] Soluble Klotho also reduced serum phosphate levels and reduced aortic calcification in murine models.[93] To our knowledge, there are no studies that analyze the impact of Klotho administration on different age groups.

CONCLUSIONS

Recent studies have linked Klotho to essential hypertension. As the mechanisms of essential hypertension have not been fully clarified, current treatment focuses on lowering blood pressure rather than targeting the cause. Preclinical studies on diverse animal models of hypertension support a complex interaction between Klotho and blood pressure, in which Klotho deficiency may have a possible causal relationship with essential hypertension, and Klotho supplementation may be therapeutic.

Table. Current Research Needs Regarding the Relationship of Klotho to Hypertension

Assess the relationship between Klotho deficiency in early human CKD or in patients with eGFR below 90 m/min per 1.73 m² or reduced nephron number (eg, low birth weight or born prematurely) and hypertension
Assess the relationship between Klotho deficiency in early human CKD or in patients with eGFR below 90 m/min per 1.73 m² or reduced nephron number (eg, low birth weight or born prematurely) and Klotho-related pathways that regulate blood pressure and have been characterized in preclinical animal models
Assess the impact of recently characterized nephroprotective drugs that also lower blood pressure on circulating, urinary or kidney tissue Klotho. Examples include SGLT2 inhibitors and novel mineralocorticoid receptor antagonists
Develop therapeutic modalities to increase Klotho levels in humans, either through increased endogenous production or by means of gene therapy or genetic/epigenetic modulation or administration of recombinant human Klotho proteins
Assess both the acute and chronic impact of such therapies on healthy volunteers in phase 1 clinical trials
Incorporate blood pressure as a secondary outcome measure in any phase 2 or 3 clinical trial of therapeutic approaches to increase Klotho availability
Characterize molecular mechanisms by which increased Klotho availability modulates blood pressure in such clinical studies and compare with preclinical evidence on the topic

CKD indicates chronic kidney disease; and eGFR, estimated glomerular filtration rate.

These observations bring to the forefront of hypertension research once more the potential causative role of subclinical kidney dysfunction, evidenced by Klotho deficiency even before estimated GFR decreases. Future research should unravel the role of Klotho deficiency as observed in early CKD in human hypertension and its impact on sympathetic nervous system activation, the renin-angiotensin-aldosterone system, salt sensitivity, and circadian blood pressure control. Recombinant Klotho proteins for human use or further modalities of therapy that increase Klotho expression should be developed to test their impact on human hypertension. The possibilities range from genetic engineering[94] to drug repurposing.[95] A key research need arising from the successful use of Sodium-glucose Cotransporter-2 inhibitors to improve kidney and cardiovascular outcomes[96] is to unravel the interaction between Sodium-glucose Cotransporter-2 inhibitors, their cardio- and renal-protective effects, their blood pressure-lowering effect and Klotho regulation. The Table lists current research needs regarding the relationship of Klotho to hypertension.

ARTICLE INFORMATION

Affiliations

From the Division of Nephrology, Department of Medicine (M.K.) and Department of Medicine (A.D.), Koc University School of Medicine, Istanbul, Turkey; Division of Nephrology, Department of Internal Medicine, Suleyman Demirel University School of Medicine, Isparta Turkey (B.A.); Department of Nephrology, Grigore T. Popa University of Medicine, Iasi, Romania (A.C., L.T., C.U.); Cardiovascular Diseases Institute, Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania (A.O.); and IIS-Fundacion Jimenez Diaz, Department of Medicine, School of Medicine, Universidad Autonoma de Madrid, Spain (A.O.).

Acknowledgments

M. Kanbay gratefully acknowledges the use of the services and facilities of the Koc University Research Center for Translational Medicine (KUTTAM), funded by the Presidency of Turkey, Presidency of Strategy and Budget. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Presidency of Strategy and Budget. Contributed substantially to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work: A. Demiray, B. Afsar, and M. Kanbay. Drafted the work or revised it critically for important intellectual content: A. Demiray, B. Afsar, L. Tapoi, C. Ureche, A. Ortiz, A. Covic, and M. Kanbay. Approved the final version to be published: M. Kanbay, A. Ortiz, and A. Covic.

Sources of Funding

None.

Disclosures

None.

REFERENCES

1. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet.* 2005;365:217–223. doi: 10.1016/S0140-6736(05)17741-1
2. Rossignol P, Massy ZA, Azizi M, Bakris G, Ritz E, Covic A, Goldsmith D, Heine GH, Jager KJ, Kanbay M, et al; ERA-EDTA EURECA-m working group; Red de Investigación Renal (REDINREN) network; Cardiovascular and Renal Clinical Trialists (F-CRIN INI-CRCT) network. The double challenge of resistant hypertension and chronic kidney disease. *Lancet.* 2015;386:1588–1598. doi: 10.1016/S0140-6736(15)00418-3
3. Shahaj O, Denneny D, Schwappach A, Pearce G, Epiphaniou E, Parke HL, Taylor SJC, Pinnock H. Supporting self-management for people with hypertension: a meta-review of quantitative and qualitative systematic reviews. *J Hypertens.* 2019;37:264–279. doi: 10.1097/HJH.0000000000001867
4. Litwin M, Feber J, Niemirska A, Michałkiewicz J. Primary hypertension is a disease of premature vascular aging associated with neuro-immuno-metabolic abnormalities. *Pediatr Nephrol.* 2016;31:185–194. doi: 10.1007/s00467-015-3065-y
5. Johnson RJ, Lanaspa MA, Gabriela Sánchez-Lozada L, Rodriguez-Iturbe B. The discovery of hypertension: evolving views on the role of the kidneys, and current hot topics. *Am J Physiol Renal Physiol.* 2015;308:F167–F178. doi: 10.1152/ajprenal.00503.2014
6. Solak Y, Afsar B, Vaziri ND, Aslan G, Yalcin CE, Covic A, Kanbay M. Hypertension as an autoimmune and inflammatory disease. *Hypertens Res.* 2016;39:567–573. doi: 10.1038/hr.2016.35
7. Lewin E, Olgaard K. The vascular secret of Klotho. *Kidney Int.* 2015;87:1089–1091. doi: 10.1038/ki.2015.80
8. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature.* 1997;390:45–51. doi: 10.1038/36285
9. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, et al. Suppression of aging in mice by the hormone Klotho. *Science.* 2005;309:1829–1833. doi: 10.1126/science.1112766
10. Xu Y, Sun Z. Molecular basis of Klotho: from gene to function in aging. *Endocr Rev.* 2015;36:174–193. doi: 10.1210/er.2013-1079
11. Neyra JA, Hu MC, Moe OW. Klotho in clinical nephrology. *Clin J Am Soc Nephrol.* 2020.
12. Wang Y, Sun Z. Current understanding of klotho. *Ageing Res Rev.* 2009;8:43–51. doi: 10.1016/j.arr.2008.10.002
13. Fernández-Fernández B, Valiño-Rivas L, Sánchez-Niño MD, Ortiz A. Albuminuria downregulation of the anti-aging factor klotho: the missing link potentially explaining the association of pathological albuminuria with premature death. *Adv Ther.* 2020;37(suppl 2):62–72. doi: 10.1007/s12325-019-01180-5
14. Park MY, Herrmann SM, Saad A, Eirin A, Tang H, Lerman A, Textor SC, Lerman LO. Biomarkers of kidney injury and klotho in patients with atherosclerotic renovascular disease. *Clin J Am Soc Nephrol.* 2015;10:443–451. doi: 10.2215/CJN.07290714
15. Uzun Cilingir I, Varol F, Gurkan H, Sutcu H, Atli E, Eker D, Inan C, Erzincan S, Sayin C. Placental and serum levels of human Klotho in severe preeclampsia: a potential sensitive biomarker. *Placenta.* 2019;85:49–55. doi: 10.1016/j.placenta.2019.08.084
16. Loichinger MH, Towner D, Thompson KS, Ahn HJ, Bryant-Greenwood GD. Systemic and placental α-klotho: Effects of preeclampsia in the last trimester of gestation. *Placenta.* 2016;41:53–61. doi: 10.1016/j.placenta.2016.03.004
17. Kim JH, Hwang KH, Park KS, Kong ID, Cha SK. Biological role of anti-aging protein klotho. *J Lifestyle Med.* 2015;5:1–6. doi: 10.15280/jlm.2015.5.1.1
18. Olauson H, Mencke R, Hillebrands JL, Larsson TE. Tissue expression and source of circulating αKlotho. *Bone.* 2017;100:19–35. doi: 10.1016/j.bone.2017.03.043
19. Li SA, Watanabe M, Yamada H, Nagai A, Kinuta M, Takei K. Immunohistochemical localization of Klotho protein in brain, kidney, and reproductive organs of mice. *Cell Struct Funct.* 2004;29:91–99. doi: 10.1247/csf.29.91
20. Sanchez-Niño MD, Fernandez-Fernandez B, Ortiz A. Klotho, the elusive kidney-derived anti-ageing factor. *Clin Kidney J.* 2020;13:125–127. doi: 10.1093/ckj/sfz125
21. Neyra JA, Moe OW, Pastor J, Gianella F, Sidhu SS, Sarnak MJ, Ix JH, Drew DA. Performance of soluble Klotho assays in clinical samples of kidney disease. *Clin Kidney J.* 2020;13:235–244. doi: 10.1093/ckj/sfz085
22. Xiao Z, King G, Mancarella S, Munkhsaikhan U, Cao L, Cai C, Quarles LD. FGF23 expression is stimulated in transgenic alpha-Klotho longevity mouse model. *JCI Insight.* 2019;4:e132820.
23. Tsujikawa H, Kurotaki Y, Fujimori T, Fukuda K, Nabeshima Y. Klotho, a gene related to a syndrome resembling human premature aging, functions in a negative regulatory circuit of vitamin D endocrine system. *Mol Endocrinol.* 2003;17:2393–2403. doi: 10.1210/me.2003-0048
24. Ohnishi M, Razzaque MS. Dietary and genetic evidence for phosphate toxicity accelerating mammalian aging. *FASEB J.* 2010;24:3562–3571. doi: 10.1096/fj.09-152488
25. Nakatani T, Sarraj B, Ohnishi M, Densmore MJ, Taguchi T, Goetz R, Mohammadi M, Lanske B, Razzaque MS. *In vivo* genetic evidence for klotho-dependent, fibroblast growth factor 23 (Fgf23) -mediated regulation of

systemic phosphate homeostasis. *FASEB J.* 2009;23:433–441. doi: 10.1096/fj.08-114397
26. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, Goetz R, Kuro-o M, Mohammadi M, Sirkis R, Naveh-Many T, Silver J. The parathyroid is a target organ for FGF23 in rats. *J Clin Invest.* 2007;117:4003–4008. doi: 10.1172/JCI32409
27. Kanbay M, Vervloet M, Cozzolino M, Siriopol D, Covic A, Goldsmith D, Solak Y. Novel Faces of Fibroblast Growth Factor 23 (FGF23): iron deficiency, inflammation, insulin resistance, left ventricular hypertrophy, proteinuria and acute kidney injury. *Calcif Tissue Int.* 2017;100:217–228. doi: 10.1007/s00223-016-0206-7
28. Hu MC, Shi M, Zhang J, Pastor J, Nakatani T, Lanske B, Razzaque MS, Rosenblatt KP, Baum MG, Kuro-o M, et al. Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J.* 2010;24:3438–3450. doi: 10.1096/fj.10-154765
29. Cha SK, Ortega B, Kurosu H, Rosenblatt KP, Kuro-O M, Huang CL. Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1. *Proc Natl Acad Sci USA.* 2008;105:9805–9810. doi: 10.1073/pnas.0803223105
30. Olejnik A, Franczak A, Krzywonos-Zawadzka A, Kałuźna-Oleksy M, Bil-Lula I. The biological role of klotho protein in the development of cardiovascular diseases. *Biomed Res Int.* 2018;2018:5171945. doi: 10.1155/2018/5171945
31. Wang HL, Xu Q, Wang Z, Zhang YH, Si LY, Li XJ, Yang QH, Xiao H. A potential regulatory single nucleotide polymorphism in the promoter of the Klotho gene may be associated with essential hypertension in the Chinese Han population. *Clin Chim Acta.* 2010;411:386–390. doi: 10.1016/j.cca.2009.12.004
32. Rhee EJ, Oh KW, Yun EJ, Jung CH, Lee WY, Kim SW, Baek KH, Kang MI, Park SW. Relationship between polymorphisms G395A in promoter and C1818T in exon 4 of the KLOTHO gene with glucose metabolism and cardiovascular risk factors in Korean women. *J Endocrinol Invest.* 2006;29:613–618. doi: 10.1007/BF03344160
33. Nzietchueng R, El Shamieh S, Benachour H, Labat C, Herbeth B, Ndiaye NC, Masson C, Visvikis-Siest S, Benetos A. Klotho KL-VS genotype is involved in blood pressure regulation. *Clin Chim Acta.* 2011;412:1773–1777. doi: 10.1016/j.cca.2011.05.032
34. Gao LL, Ding X, Xie DM, Yang M, Dong BR. G-395A polymorphism in the promoter region of the KLOTHO gene and hypertension among elderly (90 years and older) Chinese individuals. *Genet Mol Res.* 2015;14:15444–15452. doi: 10.4238/2015.November.30.22
35. Wu PH, Westerberg PA, Kindmark A, Tivesten Å, Karlsson MK, Mellström D, Ohlsson C, Fellström B, Linde T, Ljunggren Ö. The association between single nucleotide polymorphisms of klotho gene and mortality in elderly men: The MrOS Sweden Study. *Sci Rep.* 2020;10:10243. doi: 10.1038/s41598-020-66517-5
36. Citterio L, Delli Carpini S, Lupoli S, Brioni E, Simonini M, Fontana S, Zagato L, Messaggio E, Barlassina C, Cusi D, et al. Klotho gene in human salt-sensitive hypertension. *Clin J Am Soc Nephrol.* 2020;15:375–383. doi: 10.2215/CJN.08620719
37. Akbari H, Asadikaram G, Aria H, Fooladi S, Vakili S, Masoumi M. Association of Klotho gene polymorphism with hypertension and coronary artery disease in an Iranian population. *BMC Cardiovasc Disord.* 2018;18:237. doi: 10.1186/s12872-018-0971-5
38. Shimoyama Y, Nishio K, Hamajima N, Niwa T. KLOTHO gene polymorphisms G-395A and C1818T are associated with lipid and glucose metabolism, bone mineral density and systolic blood pressure in Japanese healthy subjects. *Clin Chim Acta.* 2009;406:134–138. doi: 10.1016/j.cca.2009.06.011
39. Ichikawa S, Imel EA, Kreiter ML, Yu X, Mackenzie DS, Sorenson AH, Goetz R, Mohammadi M, White KE, Econs MJ. A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. *J Clin Invest.* 2007;117:2684–2691. doi: 10.1172/JCI31330
40. Carriazo S, Vanessa Perez-Gomez M, Ortiz A. Hypertensive nephropathy: a major roadblock hindering the advance of precision nephrology. *Clin Kidney J.* 2020;13:504–509. doi: 10.1093/ckj/sfaa162
41. Perez-Gomez MV, Bartsch LA, Castillo-Rodriguez E, Fernandez-Prado R, Fernandez-Fernandez B, Martin-Cleary C, Gracia-Iguacel C, Ortiz A. Clarifying the concept of chronic kidney disease for non-nephrologists. *Clin Kidney J.* 2019;12:258–261. doi: 10.1093/ckj/sfz007
42. Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron number in patients with primary hypertension. *N Engl J Med.* 2003;348:101–108. doi: 10.1056/NEJMoa020549
43. Hu MC, Shi M, Zhang J, Quiñones H, Griffith C, Kuro-o M, Moe OW. Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol.* 2011;22:124–136. doi: 10.1681/ASN.2009121311
44. Moreno JA, Izquierdo MC, Sanchez-Niño MD, Suárez-Alvarez B, Lopez-Larrea C, Jakubowski A, Blanco J, Ramirez R, Selgas R, Ruiz-Ortega M, et al. The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFκB. *J Am Soc Nephrol.* 2011;22:1315–1325. doi: 10.1681/ASN.2010101073
45. Fernandez-Fernandez B, Izquierdo MC, Valiño-Rivas L, Nastou D, Sanz AB, Ortiz A, Sanchez-Niño MD. Albumin downregulates Klotho in tubular cells. *Nephrol Dial Transplant.* 2018;33:1712–1722. doi: 10.1093/ndt/gfx376
46. Takenaka T, Inoue T, Miyazaki T, Kobori H, Nishiyama A, Ishii N, Hayashi M, Suzuki H. Klotho ameliorates medullary fibrosis and pressure natriuresis in hypertensive rat kidneys. *Hypertension.* 2018;72:1151–1159. doi: 10.1161/HYPERTENSIONAHA.118.11176
47. Zhou X, Wang X. Klotho: a novel biomarker for cancer. *J Cancer Res Clin Oncol.* 2015;141:961–969. doi: 10.1007/s00432-014-1788-y
48. Chen K, Sun Z. Autophagy plays a critical role in Klotho gene deficiency-induced arterial stiffening and hypertension. *J Mol Med (Berl).* 2019;97:1615–1625. doi: 10.1007/s00109-019-01841-6
49. Kanbay M, Girerd N, Machu JL, Bozec E, Duarte K, Boivin JM, Wagner S, Ferreira JP, Zannad F, Rossignol P. Impact of uric acid on hypertension occurrence and target organ damage: insights from the stanislas cohort with a 20-year follow-up. *Am J Hypertens.* 2020;33:869–878. doi: 10.1093/ajh/hpaa030
50. Zhou X, Chen K, Lei H, Sun Z. Klotho gene deficiency causes salt-sensitive hypertension via monocyte chemotactic protein-1/CC chemokine receptor 2-mediated inflammation. *J Am Soc Nephrol.* 2015;26:121–132. doi: 10.1681/ASN.2013101033
51. Meneton P, Jeunemaitre X, de Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. *Physiol Rev.* 2005;85:679–715. doi: 10.1152/physrev.00056.2003
52. Dumor K, Shoemaker-Moyle M, Nistala R, Whaley-Connell A. Arterial stiffness in hypertension: an update. *Curr Hypertens Rep.* 2018;20:72. doi: 10.1007/s11906-018-0867-x
53. Grillo A, Salvi L, Coruzzi P, Salvi P, Parati G. Sodium intake and hypertension. *Nutrients.* 2019;11:1970.
54. Sun Z. Aging, arterial stiffness, and hypertension. *Hypertension.* 2015;65:252–256. doi: 10.1161/HYPERTENSIONAHA.114.03617
55. Siriopol D, Covic A, Iliescu R, Kanbay M, Tautu O, Radulescu L, Mitu O, Salaru D, Dorobantu M. Arterial stiffness mediates the effect of salt intake on systolic blood pressure. *J Clin Hypertens (Greenwich).* 2018;20:1587–1594. doi: 10.1111/jch.13399
56. Kanbay M, Afsar B, Gusbeth-Tatomir P, Covic A. Arterial stiffness in dialysis patients: where are we now? *Int Urol Nephrol.* 2010;42:741–752. doi: 10.1007/s11255-009-9675-1
57. Nanba K, Vaidya A, Williams GH, Zheng I, Else T, Rainey WE. Age-related autonomous aldosteronism. *Circulation.* 2017;136:347–355. doi: 10.1161/CIRCULATIONAHA.117.028201
58. Kanbay M, Chen Y, Solak Y, Sanders PW. Mechanisms and consequences of salt sensitivity and dietary salt intake. *Curr Opin Nephrol Hypertens.* 2011;20:37–43. doi: 10.1097/MNH.0b013e32834122f1
59. Chen K, Sun Z. Activation of DNA demethylases attenuates aging-associated arterial stiffening and hypertension. *Aging Cell.* 2018;17:e12762. doi: 10.1111/acel.12762
60. Yamazaki Y, Imura A, Urakawa I, Shimada T, Murakami J, Aono Y, Hasegawa H, Yamashita T, Nakatani K, Saito Y, et al. Establishment of sandwich ELISA for soluble alpha-Klotho measurement: age-dependent change of soluble alpha-Klotho levels in healthy subjects. *Biochem Biophys Res Commun.* 2010;398:513–518. doi: 10.1016/j.bbrc.2010.06.110
61. Wirth A, Benyó Z, Lukasova M, Leutgeb B, Wettschureck N, Gorbey S, Orsy P, Horváth B, Maser-Gluth C, Greiner E, et al. G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension. *Nat Med.* 2008;14:64–68. doi: 10.1038/nm1666
62. Kawarazaki W, Mizuno R, Nishimoto M, Ayuzawa N, Hirohama D, Ueda K, Kawakami-Mori F, Oba S, Marumo T, Fujita T. Salt causes aging-associated hypertension via vascular Wnt5a under Klotho deficiency. *J Clin Invest.* 2020;130:4152–4166. doi: 10.1172/JCI134431
63. Wang X, Sun Z. RNAi silencing of brain klotho potentiates cold-induced elevation of blood pressure via the endothelin pathway. *Physiol Genomics.* 2010;41:120–126. doi: 10.1152/physiolgenomics.00192.2009
64. Gao D, Zuo Z, Tian J, Ali Q, Lin Y, Lei H, Sun Z. Activation of SIRT1 attenuates klotho deficiency-induced arterial stiffness and hypertension by enhancing AMP-activated protein kinase activity. *Hypertension.* 2016;68:1191–1199. doi: 10.1161/HYPERTENSIONAHA.116.07709

65. Kaess BM, Rong J, Larson MG, Hamburg NM, Vita JA, Levy D, Benjamin EJ, Vasan RS, Mitchell GF. Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA.* 2012;308:875–881. doi: 10.1001/2012.jama.10503
66. Lim K, Lu TS, Molostvov G, Lee C, Lam FT, Zehnder D, Hsiao LL. Vascular Klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation.* 2012;125:2243–2255. doi: 10.1161/CIRCULATIONAHA.111.053405
67. Lutsey PL, Alonso A, Selvin E, Pankow JS, Michos ED, Agarwal SK, Loehr LR, Eckfeldt JH, Coresh J. Fibroblast growth factor-23 and incident coronary heart disease, heart failure, and cardiovascular mortality: the Atherosclerosis Risk in Communities study. *J Am Heart Assoc.* 2014;3:e000936. doi: 10.1161/JAHA.114.000936
68. Kanbay M, Nicoleta M, Selcoki Y, Ikizek M, Aydin M, Eryonucu B, Duranay M, Akcay A, Armutcu F, Covic A. Fibroblast growth factor 23 and fetuin A are independent predictors for the coronary artery disease extent in mild chronic kidney disease. *Clin J Am Soc Nephrol.* 2010;5:1780–1786. doi: 10.2215/CJN.02560310
69. Ter Braake AD, Smit AE, Bos C, van Herwaarden AE, Alkema W, van Essen HW, Bravenboer N, Vervloet MG, Hoenderop JGJ, de Baaij JHF. Magnesium prevents vascular calcification in Klotho deficiency. *Kidney Int.* 2020;97:487–501. doi: 10.1016/j.kint.2019.09.034
70. Nakamura K, Miura D, Saito Y, Yunoki K, Koyama Y, Satoh M, Kondo M, Osawa K, Hatipoglu OF, Miyoshi T, et al. Eicosapentaenoic acid prevents arterial calcification in klotho mutant mice. *PLoS One.* 2017;12:e0181009. doi: 10.1371/journal.pone.0181009
71. Kitagawa M, Sugiyama H, Morinaga H, Inoue T, Takiue K, Ogawa A, Yamanari T, Kikumoto Y, Uchida HA, Kitamura S, et al. A decreased level of serum soluble Klotho is an independent biomarker associated with arterial stiffness in patients with chronic kidney disease. *PLoS One.* 2013;8:e56695. doi: 10.1371/journal.pone.0056695
72. Zu Y, Liu L, Lee MY, Xu C, Liang Y, Man RY, Vanhoutte PM, Wang Y. SIRT1 promotes proliferation and prevents senescence through targeting LKB1 in primary porcine aortic endothelial cells. *Circ Res.* 2010;106:1384–1393. doi: 10.1161/CIRCRESAHA.109.215483
73. Gao P, Xu TT, Lu J, Li L, Xu J, Hao DL, Chen HZ, Liu DP. Overexpression of SIRT1 in vascular smooth muscle cells attenuates angiotensin II-induced vascular remodeling and hypertension in mice. *J Mol Med (Berl).* 2014;92:347–357. doi: 10.1007/s00109-013-1111-4
74. Richter B, Haller J, Haffner D, Leifheit-Nestler M. Klotho modulates FGF23-mediated NO synthesis and oxidative stress in human coronary artery endothelial cells. *Pflugers Arch.* 2016;468:1621–1635. doi: 10.1007/s00424-016-1858-x
75. Yao Y, Wang Y, Zhang Y, Liu C. Klotho ameliorates oxidized low density lipoprotein (ox-LDL)-induced oxidative stress via regulating LOX-1 and PI3K/Akt/eNOS pathways. *Lipids Health Dis.* 2017;16:77. doi: 10.1186/s12944-017-0447-0
76. Pedrinelli R, Bruschi G, Graziadei L, Taddei S, Panarace G, Orlandini G, Natali A, Borghetti A, Salvetti A. Dietary sodium change in primary aldosteronism. Atrial natriuretic factor, hormonal, and vascular responses. *Hypertension.* 1988;12:192–198. doi: 10.1161/01.hyp.12.2.192
77. Tang C, Pathare G, Michael D, Fajol A, Eichenmüller M, Lang F. Downregulation of Klotho expression by dehydration. *Am J Physiol Renal Physiol.* 2011;301:F745–F750. doi: 10.1152/ajprenal.00037.2011
78. Zhou X, Chen K, Wang Y, Schuman M, Lei H, Sun Z. Antiaging gene klotho regulates adrenal CYP11B2 expression and aldosterone synthesis. *J Am Soc Nephrol.* 2016;27:1765–1776. doi: 10.1681/ASN.2015010093
79. Chen K, Zhou X, Sun Z. Haplodeficiency of Klotho gene causes arterial stiffening via upregulation of scleraxis expression and induction of autophagy. *Hypertension.* 2015;66:1006–1013. doi: 10.1161/HYPERTENSIONAHA.115.06033
80. Voelkl J, Alesutan I, Leibrock CB, Quintanilla-Martinez L, Kuhn V, Feger M, Mia S, Ahmed MS, Rosenblatt KP, Kuro-O M, et al. Spironolactone ameliorates PIT1-dependent vascular osteoinduction in klotho-hypomorphic mice. *J Clin Invest.* 2013;123:812–822. doi: 10.1172/JCI64093
81. Fischer SS, Kempe DS, Leibrock CB, Rexhepaj R, Siraskar B, Boini KM, Ackermann TF, Föller M, Hocher B, Rosenblatt KP, et al. Hyperaldosteronism in Klotho-deficient mice. *Am J Physiol Renal Physiol.* 2010;299:F1171–F1177. doi: 10.1152/ajprenal.00233.2010
82. Lindberg K, Amin R, Moe OW, Hu MC, Erben RG, Östman Wernerson A, Lanske B, Olauson H, Larsson TE. The kidney is the principal organ mediating klotho effects. *J Am Soc Nephrol.* 2014;25:2169–2175. doi: 10.1681/ASN.2013111209
83. Andrukhova O, Slavic S, Smorodchenko A, Zeitz U, Shalhoub V, Lanske B, Pohl EE, Erben RG. FGF23 regulates renal sodium handling and blood pressure. *EMBO Mol Med.* 2014;6:744–759. doi: 10.1002/emmm.201303716
84. Zhang H, Li Y, Fan Y, Wu J, Zhao B, Guan Y, Chien S, Wang N. Klotho is a target gene of PPAR-gamma. *Kidney Int.* 2008;74:732–739. doi: 10.1038/ki.2008.244
85. Lin Y, Chen J, Sun Z. Antiaging gene klotho deficiency promoted high-fat diet–induced arterial stiffening via inactivation of AMP-activated protein kinase. *Hypertension.* 2016;67:564–73.
86. Chihara Y, Rakugi H, Ishikawa K, Ikushima M, Maekawa Y, Ohta J, Kida I, Ogihara T. Klotho protein promotes adipocyte differentiation. *Endocrinology.* 2006;147:3835–3842. doi: 10.1210/en.2005-1529
87. Ohnishi M, Kato S, Akiyoshi J, Atfi A, Razzaque MS. Dietary and genetic evidence for enhancing glucose metabolism and reducing obesity by inhibiting klotho functions. *FASEB J.* 2011;25:2031–2039. doi: 10.1096/fj.10-167056
88. Kim HJ, Lee J, Chae DW, Lee KB, Sung SA, Yoo TH, Han SH, Ahn C, Oh KH. Serum klotho is inversely associated with metabolic syndrome in chronic kidney disease: results from the KNOW-CKD study. *BMC Nephrol.* 2019;20:119. doi: 10.1186/s12882-019-1297-y
89. Takenaka T, Kobori H, Miyazaki T, Suzuki H, Nishiyama A, Ishii N, Yamashita M, Hayashi M. Klotho protein supplementation reduces blood pressure and renal hypertrophy in db/db mice, a model of type 2 diabetes. *Acta Physiol (Oxf).* 2019;225:e13190. doi: 10.1111/apha.13190
90. Takenaka T, Kobori H, Inoue T, Miyazaki T, Suzuki H, Nishiyama A, Ishii N, Hayashi M. Klotho supplementation ameliorates blood pressure and renal function in DBA/2-pcy mice, a model of polycystic kidney disease. *Am J Physiol Renal Physiol.* 2020;318:F557–F564. doi: 10.1152/ajprenal.00299.2019
91. Saito Y, Nakamura T, Ohyama Y, Suzuki T, Iida A, Shiraki-Iida T, Kuro-o M, Nabeshima Y, Kurabayashi M, Nagai R. *In vivo* klotho gene delivery protects against endothelial dysfunction in multiple risk factor syndrome. *Biochem Biophys Res Commun.* 2000;276:767–772. doi: 10.1006/bbrc.2000.3470
92. Wang Y, Sun Z. Klotho gene delivery prevents the progression of spontaneous hypertension and renal damage. *Hypertension.* 2009;54:810–817. doi: 10.1161/HYPERTENSIONAHA.109.134320
93. Hum JM, O'Bryan LM, Tatiparthi AK, Cass TA, Clinkenbeard EL, Cramer MS, Bhaskaran M, Johnson RL, Wilson JM, Smith RC, et al. Chronic hyperphosphatemia and vascular calcification are reduced by stable delivery of soluble klotho. *J Am Soc Nephrol.* 2017;28:1162–1174. doi: 10.1681/ASN.2015111266
94. Liao HK, Hatanaka F, Araoka T, Reddy P, Wu MZ, Sui Y, Yamauchi T, Sakurai M, O'Keefe DD, Núñez-Delicado E, et al. *In Vivo* target gene activation via CRISPR/Cas9-mediated trans-epigenetic modulation. *Cell.* 2017;171:1495–1507.e15. doi: 10.1016/j.cell.2017.10.025
95. Navarro-González JF, Sánchez-Niño MD, Donate-Correa J, Martín-Núñez E, Ferri C, Pérez-Delgado N, Górriz JL, Martínez-Castelao A, Ortiz A, Mora-Fernández C. Effects of pentoxifylline on soluble klotho concentrations and renal tubular cell expression in diabetic kidney disease. *Diabetes Care.* 2018;41:1817–1820. doi: 10.2337/dc18-0078
96. Fernandez-Fernandez B, Sarafidis P, Kanbay M, Navarro-González JF, Soler MJ, Górriz JL, Ortiz A. SGLT2 inhibitors for non-diabetic kidney disease: drugs to treat CKD that also improve glycaemia. *Clin Kidney J.* 2020;13:728–733. doi: 10.1093/ckj/sfaa198