Nephro Urol Mon. 2016 January; 8(1): e30245. doi: 10.5812/numonthly.30245

Published online 2016 January 9. **Brief Report**

Serum Klotho Levels in Trained Athletes

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Received 2015 July 17; **Revised** 2015 August 15; **Accepted** 2015 November 10.

Abstract

Background: Klotho is an anti-aging protein that is predominantly secreted by the kidneys.
Objectives: The aim of the study was to measure and compare the circulating Klotho levels in the serum of trained athletes and in healthy, non-athlete controls.
Materials and Methods: Thirty trained football players were enrolled and their serum Klotho levels were measured the morning after their last evening exercise training.
Results: The plasma free Klotho concentration was significantly higher in the athlete group (3.375 ± 1.48 ng/mL) compared to the non-athletes (1.39 ± 0.43 ng/mL) ($P < 0.05$). Serum levels of cholesterol, triglycerides, calcium, and phosphorus were not significantly different between the two groups.
Conclusions: Regular aerobic exercise could increase plasma Klotho levels, and this could be an explanation for exercise-related anti-aging effects.

Keywords: Serum Klotho, Kidney, Aging, Exercise

1. Background

The Klotho gene and its related protein were identified as a putative aging factors in 1997, when the aging process was aggravated in a group of Klotho knockout mice (1). Klotho is expressed mainly in the kidneys, parathyroid glands, brain choroid plexus, and testes (2-4). Studies have confirmed Klotho expression in other tissues, including the aorta, colon, thyroid gland, and pancreas, but the kidney remains the strongest Klotho-producing organ (5).

There are two types of Klotho: circulating and membrane-bound. The latter functions as a co-receptor for fibroblast growth factor-23 (FGF23). The membrane-bound form, after losing its membrane domain, enters into the circulation as soluble Klotho (sKl), acting as a hormone with anti-aging and anti-oxidative stress properties; sKl can also be directly generated by alterative splicing of the Klotho transcript (2, 5). Klotho deficiency is an early biomarker for chronic kidney disease, and its upregulation could protect the kidney from fibrosis progression (6). The beneficial effect of physical activity in preventing premature mortality has been established by epidemiological studies showing that exercise may delay aging through various mechanisms. Exercise-induced Klotho upregulation could be one explanation. Klotho upregulates nitrous oxide (NO) production and inhibits angiotensin II-induced reactive oxygen species production within endothelial cells (7). In an epidemiological study, handgrip strength, an indicator of total body muscle strength, was correlated with plasma Klotho concentration (8).

2. Objectives

The purpose of this study was to determine whether plasma Klotho levels are influenced by aerobic exercise. For this purpose, plasma Klotho levels were measured in a group of trained athletes.

3. Materials and Methods

In this study, 30 healthy football players (males aged 18 – 22 years) participated. All participants were performing daily morning and evening exercise training. The controls were 28 healthy young males (age range 18 – 27 years). All subjects were nonsmokers and free of cardiovascular disease, as indicated by their medical history. None of the subjects took cardiovascular medications or

hormone replacement therapy, and they all maintained routine eating habits. In the experimental group, Klotho concentration was measured the morning after a session of afternoon training, with blood samples collected from the antecubital vein. All participants had abstained from caffeine and fasted for at least 8 h before sampling. The measurements were performed at constant room temperature (22°C). We did not measure the level of physical activity in the control group; they were healthy young males with normal daily physical activity, but none were trained athletes. Serum concentrations of cholesterol and triglycerides were also measured in both groups. Plasma Klotho concentrations were measured with the ELISA technique, using a soluble Klotho ELISA assay kit based on the manufacturer's instructions (Human Klotho ELISA Kit; Hangzhou Eastbiopharm Co., Ltd., Hangzhou, China).

4. Results

The demographic characteristics and measurements of the athlete group and the controls, respectively, were as follows: age, 18 – 22 versus 18 – 27 years; body mass index, 22.3 ± 1.4 versus 24.9 ± 1.3 kg/mg; total cholesterol, 5.3 ± 0.4 versus 5.7 ± 0.3 mmol/L; triglycerides, 1.5 ± 0.1 versus 1.7 ± 0.15 mmol/L; serum calcium, 9.8 ± 0.8 versus 9.7 ± 0.6; serum phosphorus, 4.4 ± 0.3 versus 4.93 ± 0.34; systolic blood pressure, 117 ± 5 versus 119 ± 6 mmHg; diastolic blood pressure, 70 ± 4 versus 71 ± 3 mmHg; and plasma Klotho, ng/mL 3.375 ± 1.48 ng/mL versus 1.39 ± 0.43 ng/mL ($P < 0.05$).

We found no significant differences between the groups for total cholesterol, triglycerides, and blood pressure. 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.

5. Discussion

The results of this study showed that aerobic exercise training induces an increase in plasma Klotho levels. Plasma Klotho levels were only measured one time, the day after exercise in the athlete group; therefore, it is not known whether this elevation continues over time. Our study population and the controls were healthy young adult males, and their serum calcium and phosphate levels were within the normal range. In the study group, we collected the blood samples the morning after the last evening exercise, so we cannot rule out the acute effect of exercise on plasma Klotho levels. It has been shown that aerobic exercise training induces increased plasma Klotho concentrations and decreased arterial stiffness in postmenopausal women (9). Exercise training might increase circulating Klotho due to increases in peroxisome proliferator-activated receptors (PPAR) and decreases in angiotensin II type I receptor (AT1R) signaling (10). Aerobic exercise-induced increases in plasma Klotho concentrations could be responsible for exercise-induced decreases in arterial stiffness (11), enhancing vascular protection and ameliorating endothelin-induced arterial stiffness. Secreted Klotho protects endothelial cells and smooth muscle cells through NO production (12) and suppression of oxidative stress (13-15). Klotho-induced endothelial NO production regulates endothelial cell calcium influx (9). Transforming growth factor beta-1 (TGF-β1) and endothelin-1 (ET-1) receptor activation negatively affect arterial stiffness, and their levels are decreased by exercise training (16). Interestingly, in a cross-sectional study, low plasma Klotho concentrations were independently associated with disability among the elderly (17). Exercise-induced increment of serum Klotho could be due to increased Klotho secretion or increased splicing of membrane-bound Klotho (9).

The kidney is the major source of sKl production (18), and membrane-bound Klotho is also a co-activator of FGF23, which is prominently expressed in distal convoluted tubule (DCT) and proximal convoluted tubule (PCT) cells; these locations are essential for its function as a phosphaturic substance (11). Klotho deficiency is an early biomarker for chronic kidney disease (CKD), and a progressive decline in urine Klotho occurs with CKD progression (6, 11, 19). Endogenous Klotho may influence the processes of inflammation, oxidative stress, and vascular calcification and remodeling (20). Secreted Klotho directly blocks phosphate-induced dedifferentiation of vascular smooth muscle cells into osteoblast-like cells. Secreted Klotho also prevents the transformation of endothelial cells to osteoblast-like cells (21, 22).

Klotho production is affected by many physiological and non-physiological conditions. Angiotensin II downregulates renal Klotho protein expression (23), and AT1R blockade increases circulating Klotho. Conversely, oxidative stress downregulates Klotho production (23).

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.

Acknowledgments

There are no direct acknowledgments to any specific person, other than recognition and favorable notice to all participants.

Footnotes

Authors' Contribution: Mohammadreza Ardalan, study design and conduc; Elmira Mostafidi, helping in study design and laboratory measurments; Amir Ghorbani Hagjo, helping in laboratory measurments; Akbar Moeen, sample collections and data gathering; Hamid Nasri, helping in study design.

Funding/Support: This study was conducted as a research project of the kidney research center of Tabriz University of Medical Sciences in Tabriz, Iran.

References

1. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature.* 1997;**390**(6655):45–51. doi: 10.1038/36285. [PubMed: 9363890]
2. Imura A, Iwano A, Tohyama O, Tsuji Y, Nozaki K, Hashimoto N, et al. Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. *FEBS Lett.* 2004;**565**(1-3):143–7. doi: 10.1016/j.febslet.2004.03.090. [PubMed: 15135068]
3. John GB, Cheng CY, Kuro-o M. Role of Klotho in aging, phosphate metabolism, and CKD. *Am J Kidney Dis.* 2011;**58**(1):127–34. doi: 10.1053/j.ajkd.2010.12.027. [PubMed: 21496980]
4. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, et al. Suppression of aging in mice by the hormone Klotho. *Science.* 2005;**309**(5742):1829–33. doi: 10.1126/science.1112766. [PubMed: 16123266]
5. Takeshita K, Fujimori T, Kurotaki Y, Honjo H, Tsujikawa H, Yasui K, et al. Sinoatrial node dysfunction and early unexpected death of mice with a defect of klotho gene expression. *Circulation.* 2004;**109**(14):1776–82. doi: 10.1161/01.CIR.0000124224.48962.32. [PubMed: 15037532]
6. Hu MC, Kuro-o M, Moe OW. Secreted klotho and chronic kidney disease. *Adv Exp Med Biol.* 2012;**728**:126–57. doi: 10.1007/978-1-4614-0887-1_9. [PubMed: 22396167]
7. Rakugi H, Matsukawa N, Ishikawa K, Yang J, Imai M, Ikushima M, et al. Anti-oxidative effect of Klotho on endothelial cells through cAMP activation. *Endocrine.* 2007;**31**(1):82–7. [PubMed: 17709902]
8. Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, Crasto C, et al. Relationship of low plasma klotho with poor grip strength in older community-dwelling adults: the InCHIANTI study. *Eur J Appl Physiol.* 2012;**112**(4):1215–20. doi: 10.1007/s00421-011-2072-3. [PubMed: 21769735]
9. Matsubara T, Miyaki A, Akazawa N, Choi Y, Ra SG, Tanahashi K, et al. Aerobic exercise training increases plasma Klotho levels and reduces arterial stiffness in postmenopausal women. *Am J Physiol Heart Circ Physiol.* 2014;**306**(3):H348–55. doi: 10.1152/ajpheart.00429.2013. [PubMed: 24322608]
10. Ciampone S, Borges R, de Lima IP, Mesquita FF, Cambiucci EC, Gontijo JA. Long-term exercise attenuates blood pressure responsiveness and modulates kidney angiotensin II signalling and urinary sodium excretion in SHR. *J Renin Angiotensin Aldosterone Syst.* 2011;**12**(4):394–403. doi: 10.1177/1470320311408750. [PubMed: 21628355]
11. Hu MC, Shi M, Zhang J, Quinones H, Griffith C, Kuro-o M, et al. Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol.* 2011;**22**(1):124–36. doi: 10.1681/ASN.2009121311. [PubMed: 21115613]
12. Saito Y, Yamagishi T, Nakamura T, Ohyama Y, Aizawa H, Suga T, et al. Klotho protein protects against endothelial dysfunction. *Biochem Biophys Res Commun.* 1998;**248**(2):324–9. [PubMed: 9675134]
13. Lesniewski LA, Durrant JR, Connell ML, Henson GD, Black AD, Donato AJ, et al. Aerobic exercise reverses arterial inflammation with aging in mice. *Am J Physiol Heart Circ Physiol.* 2011;**301**(3):H1025–32. doi: 10.1152/ajpheart.01276.2010. [PubMed: 21622824]
14. Tanabe T, Maeda S, Miyauchi T, Iemitsu M, Takanashi M, Irukayama-Tomobe Y, et al. Exercise training improves ageing-induced decrease in eNOS expression of the aorta. *Acta Physiol Scand.* 2003;**178**(1):3–10. doi: 10.1046/j.1365-201X.2003.01100.x. [PubMed: 12713509]
15. Maeda S, Sugawara J, Yoshizawa M, Otsuki T, Shimojo N, Jesmin S, et al. Involvement of endothelin-1 in habitual exercise-induced increase in arterial compliance. *Acta Physiol (Oxf).* 2009;**196**(2):223–9. doi: 10.1111/j.1748-1716.2008.01909.x. [PubMed: 18945274]
16. Doi S, Zou Y, Togao O, Pastor JV, John GB, Wang L, et al. Klotho inhibits transforming growth factor-beta1 (TGF-beta1) signaling and suppresses renal fibrosis and cancer metastasis in mice. *J Biol Chem.* 2011;**286**(10):8655–65. doi: 10.1074/jbc.M110.174037. [PubMed: 21209102]
17. Otsuki T, Maeda S, Iemitsu M, Saito Y, Tanimura Y, Ajisaka R, et al. Vascular endothelium-derived factors and arterial stiffness in strength- and endurance-trained men. *Am J Physiol Heart Circ Physiol.* 2007;**292**(2):H786–91. doi: 10.1152/ajpheart.00678.2006. [PubMed: 16997889]
18. Razzaque MS. The role of Klotho in energy metabolism. *Nat Rev Endocrinol.* 2012;**8**(10):579–87. doi: 10.1038/nrendo.2012.75. [PubMed: 22641000]
19. Zununi Vahed S, Nikasa P, Ardalan M. Klotho and renal fibrosis. *Nephrourol Mon.* 2013;**5**(5):946–8. doi: 10.5812/numonthly.16179. [PubMed: 24693499]
20. Lim K, Lu TS, Molostvov G, Lee C, Lam FT, Zehnder D, et al. Vascular Klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation.* 2012;**125**(18):2243–55. doi: 10.1161/CIRCULATIONAHA.111.053405. [PubMed: 22492635]
21. Nakano-Kurimoto R, Ikeda K, Uraoka M, Nakagawa Y, Yutaka K, Koide M, et al. Replicative senescence of vascular smooth muscle cells enhances the calcification through initiating the osteoblastic transition. *Am J Physiol Heart Circ Physiol.* 2009;**297**(5):H1673–84. doi: 10.1152/ajpheart.00455.2009. [PubMed: 19749165]
22. Fleenor BS, Marshall KD, Durrant JR, Lesniewski LA, Seals DR. Arterial stiffening with ageing is associated with transforming growth factor-beta1-related changes in adventitial collagen: reversal by aerobic exercise. *J Physiol.* 2010;**588**(Pt 20):3971–82. doi: 10.1113/jphysiol.2010.194753. [PubMed: 20807791]
23. Mitani H, Ishizaka N, Aizawa T, Ohno M, Usui S, Suzuki T, et al. In vivo klotho gene transfer ameliorates angiotensin II-induced renal damage. *Hypertension.* 2002;**39**(4):838–43. [PubMed: 11967236]