

Research Article

## Effects of Anaerobic Exercise Training on S-Klotho in Young and Elderly

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### Abstract

The purpose of the present study was to compare the association between s-klotho serum levels and IGF-1 levels in trained anaerobic sprinters and aerobically trained young adults.

### Methods

Thirty healthy sportsmen were recruited as sprinters and endurance athletes: aerobically well trained young adults and well trained anaerobic sprinters (24.7±1.0, 24.2±1.0 years respectively), underwent maximal oxygen uptake test. Blood samples were drawn from a forearm vein after overnight fasting, s-Klotho levels in the serum were analyzed using an  $\alpha$ -klotho Enzyme Linked Immunosorbent Assay ELISA kit, while, IGF-1 was measured by a chemiluminescent immunometric method.

**Results:** 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<sup>-1</sup> respectively), IGF-1 (70.2±10.9 and 94±21.5 nmol•L<sup>-1</sup> respectively) and maximal oxygen uptake (60.3±2.7 and 55.1±2.7 mL•kg<sup>-1</sup>•min<sup>-1</sup> respectively).

### Conclusions

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.

**Key words:** Lactic acid; IGF-1; aerobic training; untrained young adults;  $\alpha$ -Klotho

## Introduction

Anaerobic bouts can be limited by lactic acid levels in the blood and active muscles. It is characterized by exposing the subjects to a very high degree of sudden strenuous all-out exercise. Thus, increasing insulin-like growth factor-1 (IGF-1) levels in the blood [1], primarily due to a substantial major increase in plasma catecholamine concentrations [2]. When reactive oxygen species production overwhelms the protection and repair mechanisms, the net effect is oxidative stress and oxidative damage of DNA, membrane lipids, and proteins [3]. In mice, the anaerobic exercise bouts decreased the growth rate. Inhibition of weight gain may result from exercise intensity and duration or frequency [4]. Indirect data have supported the concept that IGF-1 may be atherogenic because it can induce vascular smooth muscle cell proliferation in vitro [5]. On the other hand, soluble-Klotho (s-Klotho) is a powerful protein that has been linked to the prevention of muscle atrophy, osteopenia, and cardiovascular disease. s-Klotho is a transmembrane protein which can be cleaved, shed and act as a circulating hormone [6]. The secreted s-Klotho protein can regulate multiple growth factor signaling pathways, including insulin/IGF-1 [7]. S-Klotho-deficient mice show a shortened life span and multiple disorders resembling human aging [6], while, over expression of s-klotho increases lifespan [8]. Similar anti-aging effects have also been ascribed to aerobic exercise and physical activity [9]. While an association between aerobic exercise and s-Klotho expression has been previously suggested from longitudinal cohort studies [10], a direct relationship between circulating s-Klotho and anaerobic exercise training has not been investigated. Therefore, the purpose of the present study was to assess whether the association between s-klotho serum levels and IGF-1 levels is different in long lasting aerobic exercise training from well-trained young adult sprinters.

## Methods

**Subjects:** Thirty healthy young sportsmen volunteered for this study. They consisted of two evenly divided groups of 15 well trained sprinters at the national level, age  $24.2 \pm 1.0$  years with maximal oxygen uptake ( $VO_{2max}$ ) of  $55.4 \pm 2.7$   $mL \cdot kg^{-1} \cdot min^{-1}$ , and 15 aerobically well trained elite runners aged  $24.7 \pm 1.0$  years with maximal oxygen uptake ( $VO_{2max}$ ) of  $60.3 \pm 2.6$   $mL \cdot kg^{-1} \cdot min^{-1}$ . All subjects were asked to run on a treadmill in an effort corresponding to 75% of their  $VO_{2max}$  for 60 min. All subjects were judged free from coronary artery disease by the clinical history, absence of major risk factors and by a normal exercise stress test up to  $VO_{2max}$ . A written informed consent was obtained from each subject, both, for taking of blood samples and for their medical records. The research was done in accordance with the Helsinki declaration, approved by the Clinical Science Center Committee on Human Subjects.

Adipose fat assessment included measurement of total body

weight ( $\pm 0.05$  kg), skin fold thicknesses at 8 sites ( $\pm 1$  mm) using the Lange Caliper (chest, axilla, triceps, subscapula, abdomen, suprailium, front thigh and circumferences at the shoulder). Anthropometric procedures followed the recommendations of Behnke and Wilmore [11].

Following warm-up, subjects underwent a graded maximal oxygen uptake ( $VO_{2max}$ ) treadmill test utilizing the standard Bruce protocol [12]. Maximal tests were terminated by the following criteria: a) leveling off or no further increase in  $VO_2$  with increasing work rate, according to the guidelines of the American College of Sports Medicine [13]. Oxygen uptake was determined breath by breath utilizing the Medical Graphics (St. Paul, MN) metabolic cart. The metabolic cart was calibrated before each test with known primary standard quality gases. Heart rate and electrocardiogram were monitored continuously, using a Burdick Eclipse 400 3-channel, 12-lead ECG recorder system, and oscilloscope. Five-second recordings were obtained at rest and at peak exercise. Blood pressure was taken using a standard sphygmomanometer cuff and mercury manometer mounted at eye level, at rest and at peak exercise.

**Blood sampling and procedures:** Peripheral venous blood samples (2.5 mL) were collected by sterile antecubital venipuncture techniques into ethylenediaminetetraacetate containing tubes. Time of day for blood sampling was in the morning and was kept consistent to control for problems associated with diurnal variation. Blood collection was obtained from each subject once.

**Analysis:** Blood samples were drawn from a forearm vein after overnight fasting, centrifuged for 15 minutes at 2700 rpm, separated and frozen at  $-70^\circ C$  until use. S-Klotho levels in the serum were analyzed using an  $\alpha$ -klotho Enzyme Linked Immunosorbent Assay ELISA kit (Immuno-Biological Laboratories Co, Japan). The kit has been validated and widely used for the measurement of klotho levels [14-16]. Measurements were conducted according to the manufacturer instructions. The intra- and interassay coefficients of variation ranged from 2.7 to 9.8%. IGF-1 was measured by a chemiluminescent immunometric method (Immulite 2000, Siemens Medical Solutions Diagnostics (Los Angeles, CA, USA). The analytical sensitivity of the assays was 2.6 nmol/L and the inter-assay coefficients of variation ranged from 3.7 to 8.1%. IGF-1 levels were transformed to natural logarithm ( $\ln$ ) in order to approximate normal distribution, and standard deviation scores (IGF-1-SDS) for each subject were calculated as explained elsewhere [17].

**Statistical methods:** Data are reported as mean  $\pm$  SD values. Sprinters and aerobically trained athletes were compared on physiological responses at rest, during maximal exercise, mean s-Klotho levels and mean IGF-1 levels, by means of two samples t-tests (SPSS version 22.0). The level of significance was set at  $\alpha < 0.05$ .

**Results**

All subjects completed the exercise challenge without difficulties or abnormal symptoms. Subjects' descriptive statistics are presented in Table 1. Table 2 summarizes physiological variables at rest and at maximal effort. It revealed no significant ( $p > 0.05$ ) differences between the groups at rest while at maximal exercise a significant ( $F_{(1,28 \text{ d.f.})} = 27.8, p > 0.05$ ) difference in  $VO_2\text{max}$  was found between the aerobic groups and anaerobic sprinters ( $60.3 \pm 2.7$  and  $55.1 \pm 2.7 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  respectively). Figure 1 reveals significant ( $p > 0.05$ ) differences between the aerobic group and the anaerobic sprinters in both s-Klotho ( $F_{(1,28 \text{ d.f.})} = 259.5, p < 0.05$ ) and IGF1 ( $F_{(1,28 \text{ d.f.})} = 14.6, p < 0.05$ ) with lower mean levels of s-Klotho and higher mean levels of IGF1 in the trained anaerobically athletes than in the aerobically trained.

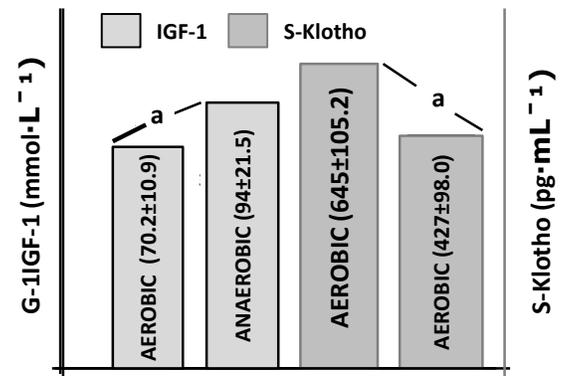
**Table 1.** Subjects' physical characteristics (mean  $\pm$  S.D).

Variables	Anaerobic trained	Aerobic trained
N of subjects	15	15
Age (years)	24.2 $\pm$ 1.0	24.7 $\pm$ 1.0
Weight (kg)	71.3 $\pm$ 1.7	70.5 $\pm$ 1.8
Height (cm)	180.4 $\pm$ 2.0	180.0 $\pm$ 2.1
Fat (%)	9.9 $\pm$ 1.6	10.4 $\pm$ 1.7

**Table 2.** Physiological responses at rest and maximal exercise in both groups (mean  $\pm$  S.D). a = significant ( $p < 0.05$ ) between anaerobic subjects and aerobic subjects.

Variables	AEROBIC	AEROBIC	ANAEROBIC	ANAEROBIC
	REST	EXERCISE	REST	EXERCISE
$VO_2$ ( $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ )	3.3 $\pm$ 0.3	60.3 $\pm$ 2.7	3.3 $\pm$ 0.3	55.1 $\pm$ 2.7a
Heart Rate ( $\text{beats} \cdot \text{min}^{-1}$ )	71.6 $\pm$ 8.4	199 $\pm$ 8.3	68.1 $\pm$ 9.3	198.4 $\pm$ 7.2
Systolic BP (mmHg)	109.2 $\pm$ 8.0	179.0 $\pm$ 7.1	107 $\pm$ 7.9	180.4 $\pm$ 9.5
Diastolic BP (mmHg)	73.2 $\pm$ 3.7	70.0 $\pm$ 2.7	72.1 $\pm$ 2.4	68.0 $\pm$ 2.1
Lactic acid ( $\text{mmol} \cdot \text{L}^{-1}$ )	1.4 $\pm$ 0.3	13.3 $\pm$ 1.1	1.3 $\pm$ 0.3	12.8 $\pm$ 1.2

**Figure 1.** s-Klotho and IGF-1 levels in the aerobic group and anaerobic group (mean $\pm$ SD). a = significant ( $p > 0.05$ ) differences between groups.



a = significant ( $p < 0.05$ ) between anaerobic subjects and aerobic subjects.

**Discussion**

In the current study, levels of s-Klotho in aerobic trained sportsmen were markedly higher compared to those measured in the anaerobic sprinters. Our findings on long lasting anaerobic exercise training suggest that circulating s-Klotho levels in sprinters are similar to those of sedentary young adults males [18], and that the response depends on aerobic fitness level [19], suggesting that this may be a poor model for mechanistically probing the role of aerobic training on s-Klotho expression. Sportsmen with aerobic capacity have longer life expectancies compared to inactive people [20], there are several studies proving the definitive role of life-long physical activity, which can be engaged at any age [21].

While in the well aerobic trained sportsmen s-Klotho levels were markedly elevated, IGF-1 levels were decreased. In the well anaerobically trained sprinters s-Klotho levels were as in sedentary young adults and IGF-1 levels were significantly higher compared to sedentary young adults [22]. IGF-1 is generally thought to be associated with positive attributes such as growth, health, youth and wellbeing, yet the bulk of the scientific evidence suggests that signaling through IGF-1 and insulin receptors is related to a shortened lifespan in adults [23].

The comparative analysis of biochemical indices measured showed that the long lasting anaerobic exercise training causes the significant increase in IGF-1 concentrations. Previous study indicated that increased circulating concentrations of IGF-1 are associated with increased risks for colorectal, prostate, and premenopausal breast cancers, and that increased concentrations of IGF binding protein 3 (IGFBP-3) are associated with increased risk of premenopausal breast cancer [24].

S-Klotho is a transmembrane protein which can act as a circulating hormone [25], it is a protein that has been reported to inhibit IGF-1 and insulin receptor, IGF-1R signaling by inhibiting tyrosine phosphorylation of both receptors and their downstream signaling proteins [26]. The reduced s-Klotho levels in the anaerobic sprinters may be associated in later life with increased mortality, increased rate of cardiovascular disease and disability in daily living activities [25-27].

## Conclusions

The differences in training intensity modes may confound the training mode differences in the response of Klotho to exercise. S-Klotho clearly counteracts the action of IGF-1. However, anaerobic exercise training is not a potent stimulus to increase plasma Klotho levels, thus, anaerobic exercise do not have positive relationship with s-Klotho to explain the support effects of anaerobic activity. However, being an aerobic athlete, especially at an elite level, seems to be associated with decreased risk factors for major chronic diseases.

## References

- Vale RG, de Oliveira RD, Pernambuco CS, de Meneses YP, Novaes Jda S, et al. Effects of muscle strength and aerobic training on basal serum levels of IGF-1 and cortisol in elderly women. *Arch Gerontol Geriatr.* 2009, 49(3): 343-347.
- Crostill D L, Barnett A, Sharp R. Leg muscle pH following sprint running. *Med Sci Sports Exerc* 1983, 15(4): 325-329.
- Beckman K B, Ames B N. The free radical theory of aging matures. *Physiol Rev.* 1998, 78(2): 547-581.
- D Qiao, L Hou, X Liu. Influence of intermittent anaerobic exercise on mouse physical endurance and antioxidant components. *Br J Sports Med.* 2006, 40(3): 214-218.
- Pfeifle B, Hamann H, Fussganger R. Insulin as a growth regulator of arterial smooth muscle cells: effect of insulin and IGF-1. *Diabetes Metab.* 1987,13(3.2): 326-330.
1. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 1997, 390(6655): 45-51.
- Kuro-o M. Klotho as a regulator of oxidative stress and senescence. *Biol Chem.* 2008, 389(3): 233-241.
- Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, et al. Suppression of aging in mice by the hormone klotho. *Science* 2005,309(5742): 1829-1833.
- Castillo-Garzon MJ. Anti-aging therapy through fitness enhancement. *Clin Interv Aging.* 2006, 1(3):213-220.
- Crasto CL, Semba RD, Sun K, Cappola AR, Bandinelli S, et al. Relationship of low-circulating “anti-aging” klotho hormone with disability in activities of daily living among older community-dwelling adults. *Rejuvenation Res.* 2012, 15(3): 295-301.
- Behenke AR, Wilmore Evaluation and Regulation of Body Build and Composition. *Human Biology.* 1976, 48(2): 405-409.
- Pollock ML, Bohannon RL, Cooper KH, Ayres JJ, Ward A, et al. A comparative analysis of four protocols for maximal treadmill stress testing. *Am Heart J.* 1976, 92(1): 39-46.
- American College of Sports Medicine. ACSM’s Guidelines for Exercise Testing and Prescription, 9th edition, Philadelphia, PA: Lippincott Williams & Wilkins; 2014; pp. 145-147 and 165-199.
- Yamazaki Y, Imura A, Urakawa I, Shimada T, Murakami J, 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(3): 513-518.
- Pedersen L, Pedersen SMI, Brasen CL, Rasmussen LM. Soluble serum Klotho levels in healthy subjects. Comparison of two different immunoassays. *Clin Biochem.* 2013, 46(12):1079-1083.
- Heijboer AC, Blankenstein MA, Hoenderop J, de Borst MH, Vervloet MG. NIGRAM consortium. Laboratory aspects of circulating alpha-Klotho. *Nephrol Dial Transplan.* 2013, 28(9): 2283-2287.
- Ranke MB, Schweizer R, Elmlinger MW, Weber K, Binder G, et al. Significance of basal IGF-I, IGFBP-3 and IGFBP-2 measurements in the diagnostics of short stature in children. *Horm Res.* 2000, 54(2): 60-68.
- Devaraj S, Syed B, Chien A, Jialal I. Validation of an immunoassay for soluble Klotho protein: decreased levels in diabetes and increased levels in chronic kidney disease. *Am J Clin Pathol.* 2012, 137(3): 479-485.
- Reimers CD, Knapp G, Reimers AK. Does physical activity increase life expectancy? A review of the literature. *J Aging Res.* 2012.
- Paffenbarger RS Jr, Kampert JB, Lee IM. Physical activity and health of college men: longitudinal observations. *Int J*

- Sports Med. 1997, 18 Suppl 3: S200-203.
21. Apor P, Radi A. Master sportsmen. Health status and life expectancies of physically active elderly. *Orv Hetil Article in Hungarian*. 2010, 151(3):110-13.
  22. Eliakim A, Nemet D, Most G, Rakover N, Pantanowitz M, et al. Effect of gender on the GH-IGF-I response to anaerobic exercise in young adults. *J Strength Cond Res*. 2014, 28(12): 3411-3415.
  23. Berryman DE, Christiansen JS, Johannsson G, Thorner MO, Kopchick JJ. Role of the GH/ IGF-1 axis in lifespan and health span: lessons from animal models. *Growth Horm IGF Res*. 2008, 18(6): 455-471.
  24. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, et al. Role of leptin in the neuroendocrine response to fasting. *Nature*. 1996, 382(6588): 250-252.
  25. Semba RD, Cappola AR, Sun K, Bandinelli S, Dalal M, et al. Plasma klotho and cardiovascular disease in adults. *J Am Geriatr Soc*. 2011, 59(9):1596-1601.
  26. Chateau MT, Araiz C, Descamps S, Galas S. Klotho interferes with a novel FGF-signalling pathway and insulin/Igf-like signalling to improve longevity and stress resistance in *Caenorhabditis elegans*. *Aging*. 2010, 2(9): 567-581.
  27. Neidert MC, Sze L, Zwimpfer C, Sarnthein J, Seifert B, et al. Soluble alpha-Klotho: a novel serum biomarker for the activity of GH-producing pituitary adenomas. *European Journal of Endocrinology*. 2013, 168(4): 575-583.