Klotho gene expression responses to long lasting aerobic training and aging

Moran Saghiv[1], Ehud Goldhammer[2], Michael Sagiv[3], David Ben-Sira[3]

[1]*Exercise Science Department, University of Mary, North Dakota, USA*

[2]*Heart Institute Bnai-Zion Haifa Medical Center, Technion, Israel*

[3]*Life Sciences Department, Wingate College, Wingate, Israel*

*Corresponding author: Dr. Moran S. Saghiv, Ph.D, Associate Professor, Exercise Physiology Department,Harold C. Miller Center, Room 105A, University of Mary, 7500 University Drive, Bismarck, ND 58504, USA. Tel: 702-908-2390 , Fax: 701-255-7687: Email: mssaghiv@umary.edu

Abstract

The purpose of the present study was to test the hypothesis that long lasting aerobic exercise training could prevent the age-associated reduction in s-klotho serum levels and the increase in IGF-1 levels.

Methods: Thirty healthy sportsmen were recruited: 15 young aerobically well trained elite athletes and 15 aerobically well trained master athletes (27.7±1.1 and 56.3±1.1 years respectively)**.** Blood samples were drawn from a forearm vein after overnight fasting, and s- Klotho serum levels were analyzed using an α-klotho Enzyme Linked Immunosorbent Assay ELISA kit, while, IGF-1 was measured by a chemiluminescent immunometric method. Following blood measurements, subjects underwent maximal oxygen uptake test.

Results: no significant differences were found between the young and masters subjects in s-Klotho (645.0 ±89.0 and 590.0 ±96.2 pg•mL^{-1} respectively) and IGF-1 (63.4±13.2 and 76.6±19.2 mmol•L^{-1} respectively).

Conclusions: The present study has shown that long lasting aerobic exercise training, is probably one of the antiaging factors that encounter the aging process by enhancement of high levels of s-Kotho and low levels of IGF-1 in the blood. The exact metabolic and physiological pathways involved in the activity of these aerobically well trained young and master sportsmen should be further studied and elucidated.

Keywords: IGF-1; s-Klotho; master athletes; elite athletes; ELISA

Introduction

The primary aging process which is genetically associated occurs independently of life style and the presence of disease [1].Therefore, successful aging is a function of both genetic and environmental factors [2]. Multiple age related structural and functional changes are involved in skeletal, cardiac and oxygen delivery ability during the human senescence. Aging results in a significant decline in aerobic capacity and in the expression of a gene located on chromosome 13: Klotho gene a suppressor of the aging phenomena, as well as the circulation of s-Klotho proteins [3].

The secreted s-Klotho protein can regulate multiple growth factor signaling pathways, including insulin and IGF-1[4]. S-Klotho-deficient mice show a shortened life span and multiple disorders resembling human aging [5], while, over expression of s-klotho increases lifespan [6].

Similar anti-aging effects have also been ascribed to aerobic

exercise training [7]. While an association between muscle function and s-Klotho expression has been previously suggested from longitudinal cohort studies [8], a direct relationship between circulating s-Klotho and aerobic exercise training has not been investigated [9], and since habitual exercise has antiaging effect the relationship between s-Klotho and long lasting aerobic exercise remains unclear. Therefore, the purpose of the present study was to test the hypothesis that long lasting aerobic exercise training could encounter the age-associated reduction in s-klotho serum levels and the increase in IGF-1 levels in young elite runners aerobically trained and aerobically master runners.

Methods

Subjects: Thirty healthy sportsmen all runners were recruited to participate in the present study: 15 young well trained aerobically elite athletes and 15 aerobically well trained master athletes (27.7±1.1 and 56.3±1.1 years respectively), with maximal oxygen uptake (VO2max) of 62.1±3.1 and 53.2±2.9 mL•kg^{-1}•min^{-1} respectively. All subjects were judged free from coronary artery disease by clinical history, absence of major risk factors and by a normal exercise stress test up to VO2max. A written informed consent was obtained from each subject, both, for taking 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 [± 0.05 kg], skin fold thicknesses at 8 sites (± 1 mm) using the Lange Caliper (chest, axilla, triceps, sub-scapula, abdomen, suprailium, front thigh and circumferences at the shoulder). Anthropometric procedures followed the recommendations of Behnke and Wilmore [10].

Following warm-up, spoetsmen underwent a graded maximal treadmill test utilizing the standard Bruce Protocol [11]. Maximal tests were terminated by the following criteria: a) leveling off or no further increase in VO2 with increasing work rate, b) attainment of the age predicted maximum heart rate, c) respiratory exchange ratio > 1.1, and d) when the subject could not keep up with the load, according to the guidelines of the American College of Sports Medicine [12]. 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 ethylenediam-inotetraacetate containing tubes. Time of day for blood sampling 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°C until use. Klotho levels in the serum were analyzed using an α-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 [13-16]. 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 CV ranged from 3.7 to 8.1%. IGF-1 levels were transformed to natural logarithm [ln] in order to achieve normal distribution, and standard deviation scores (IGF-1-SDS) for each subject were calculated as explained elsewhere [16].

Statistical methods: Data are reported as mean ± SD values. Physiological responses at rest and maximal exercise between the two groups were statistically assessed by a one way ANOVA with repeated measure on the exercise-rest main effect. Post hoc analysis was performed by using the Tukey 2 multiple comparison tests. Comparisons between the groups in s-Klotho and IGF-1 levels were based on t-tests for unpaired samples. 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. Although s-Klotho was higher and IGF-1 lower in the young athletes however, it did not differ significantly from that of the masters. Table 2 summarizes physiological variables responses at rest and at maximal oxygen uptake. No significant differences were observed at rest in all variables. At maximal oxygen uptake a significant higher (p<0.05) values were noted for the young athletes compared to the masters with regard to VO2max and heart rate.

Table 1. Subjects' physical characteristics [mean ± S.D].

Variables	Young	Masters
N of subjects	15	15
Age [years]	27.7±1.1	56.3±1.1
Weight [kg]	71.3±1.7	70.5±1.8
Height [cm]	180.4±2.0	178.0±2.1
Fat [%]	9.9± 1.6	10.4± 1.7
S-Klotho [pg•mL^{-1}]	645.0 ±89.0	590.0 ±96.2
IGF-1 [mmol•L^{-1}]	63.4±13.2	76.6±19.2

Table 2. physiological responses at rest and maximal exercise in both groups [mean ± S.D].

Variables	Young	Young	Masters	Masters
	Rest	Exercise	Rest	Exercise
VO2[mL•kg^{-1}•min^{-1}]	3.3±0.3	62.1±3.1	3.3±0.3	53.2±2.9a
Heart Rate [beats•min^{-1}]	67.1±9.3	198±7.2	70.6±8.4	190.4±8.2a
Systolic BP [mmHg]	109.2±6.8	180.4±7.6	110±8.0	182±6.4
Diastolic BP [mmHg]	70.6±2.7	68.0±2.2	72.2±3.3	71.0±2.4
Lactic acid [mmol•L^{-1}]	1.3±0.3	12.8±1.2	1.4±0.3	12.1± 1.1

a = significant [a<0.05] between anaerobic subjects and aerobic subjects.

Discussion

This study demonstrated that circulating s-Klotho levels are similar for young healthy well trained elite runners and master athletes. It seems that the response depends on aerobic fitness level [17]. In addition, levels of s-Klotho were significantly higher in both groups if compared with untrained subjects reported earlier by Lee et al. [18], suggesting that long lasting aerobic training may be an appropriate model for mechanistically probing the role of physical activity on s-Klotho expression.

Since aging is an independent risk factor of the age related diseases and mortality, there are growing efforts in gerontology research to slow aging and extend healthy lifespan. The population aged from 0 to 91 years screened previously by ELISA revealed that the level of s-Klotho which is a serum factor related to human declines with aging. [19]. On the other hand, elderly 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 in at any age [21].

While in the well aerobic trained young runners and master athletes s-Klotho levels were markedly elevated, IGF-1 levels were decreased compared with sedentary young and old subjects [22]. IGF-1 is generally thought to be associated with positive attributes such as growth, health, youth and wellbeing [23], yet the bulk of the scientific evidence suggests that signaling through IGF-1 and insulin receptors is related to a shortened lifespan in adults [24]. A meta-analysis study indicated that increased circulating concentrations of IGF-1 are associated with increased risks for colorectal, prostate, and premenopausal breast cancers [25].

S-Klotho is a transmembrane protein which can act as a circulating hormone [26] 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 [27]. The mechanism by which secreted s- Klotho suppresses insulin/IGF-1 signaling remains to be determined. One suggested possibility is that s-Klotho protects against several pathogenic processes in a FGF23-independent manner. The higher s-Klotho levels in the young runners and masters may be associated in later life with reduce mortality, rate of cardiovascular disease and disability in daily living activities [26,28].

Conclusions

The present study have shown that long lasting aerobic exercise training, brings about similar s-Klotho and IGF-1 levels in young elite aerobically trained runners and aerobically trained master runners regardless of age. Aerobic training demonstrated its antiaging benefits, through its versatile metabolic and physiological pathways, by means of encounter acting the aging process in keeping high levels of s-Kotho and low levels of IGF-1. Accordingly, being a long lasting aerobically trained athlete, especially at an elite level, seems to be associated with decreased risk factors for major chronic diseases with aging.

References

1. Hawkins S, Wiswell R. Rate and mechanism of maximal oxygen consumption decline with aging: implications for exercise training. 2003, 33(12): 877-888.

2. Sebastiani P, Solovieff N, Puca A. Genetic Signatures of Exceptional Longevity in Humans. Science. 2010.

3. Matsubara T, Miyaki A, Akazawa N 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): H348-355.

4. Kuro-o M. Klotho as a regulator of oxidative stress and senescence. Biol Chem. 2008, 389(3): 233-241.

5. Kuro-o M, Matsumura Y, Aizawa H et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature. 1997, 390(6655): 45-51.

6. Kurosu H, Yamamoto M, Clark JD et al. Suppression of aging in mice by the hormone klotho. Science. 2005, 309(5742): 1829-1833.

7. Castillo-Garzon MJ. Anti-aging therapy through fitness enhancement Clin Interv Aging. 2006,1(3): 213-220.

8. Koltai E, Hart N, Taylor AW, Goto S, Ngo JK et al. Age-associated declines in mitochondrial biogenesis and protein quality control factors are minimized by exercise training. Am J Physiol Regul Integr Comp Physiol. 2012, 303(2): R127-134.

9. Avin KG, Coen PM, Huang W et al. Skeletal muscle as a

regulator of the longevity protein, Klotho Front. Physiol. 2014.

10. Behenke AR, Wilmore J. Evaluation and regulation of body build and composion.1974, Englewood Cliffs NJ Prentile Hall, inc.

11. Pollock ML, Bohannon RL, Cooper KH et al. A comparative analysis of four protocols for maximal treadmill stress testing. Am Heart J. 1976, 92(1): 39-46.

12. American College of Sports Medicine. ACSM's Guidelines for Exercise Testing and Prescription, 9th edition. 2014, 145-147 and 165-199.

13. Yamazaki Y, Imura A, Urakawa I 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.

14. Pedersen L, Pedersen SMl, Brasen CL, Rasmussen LM. Soluble serum Klotho levels in healthy subjects. Comparison of two different immunoassays. Clin Biochem. 2013, 46(12): 1079–1083.

15. Heijboer AC, Blankenstein MA, Hoenderop J, de Borst MH, Vervloet MG et al. NIGRAM consortium. Laboratory aspects of circulating alpha-Klotho. Nephrol Dial Transplan. 2013, 28(9): 2283-2287.

16. Ranke MB, Schweizer R, Elmlinger MW 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.

17. Reimers CD, Knapp G, Reimers AK. Does physical activity increase life expectancy? A review of the literature. J Aging Res. 2012.

18. Lee EY, Kim SS, Lee JS et al. Soluble α-Klotho as a Novel Biomarker in the Early Stage of Nephropathy in Patients with Type 2 Diabetes. Plos One. 2014, 1;9(8): e102984.

19. 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.

20. Paffenbarger RS Jr, Kampert JB, Lee IM. Physical activity and health of college men: longitudinal observations. Int J Sports Med. 1997, 18 Suppl: 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. Manetta J, Brun JF, Fedou C, Maïmoun L, Prefaut C et al. Serum levels of insulin-like growth factor-I [IGF-I], and IGF-binding proteins-1 and -3 in middle-aged and young athletes versus sedentary men: relationship with glucose disposal. Metabolism. 2003, 52(7): 821-826.

23. Bonnefoy M, Patricot MC, Lacour JR, Rahmani A, Berthouze S et al. [Relation between physical activity, muscle function and IGF-1, testosterone and DHEAS concentrations in the elderly]. [Article in French] Rev Med Interne. 2002, 23(10): 819-827.

24. Berryman DE, Christiansen JS, Johannsson G, Thorner MO, Kopchick JJ et al. 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.

25. Ahima RS, Prabakaran D, Mantzoros C et al. Role of leptin in the neuroendocrine response to fasting. Nature. 1996, 382(6588): 250-252.

26. Semba RD, Cappola AR, Sun K et al. Plasma klotho and cardiovascular disease in adults. J Am Geriatr Soc. 2011, 59(9): 1596-1601.

27. 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.

28. Neidert MC, Sze L, Zwimpfer C, Sarnthein J et al. Soluble alpha-Klotho: a novel serum biomarker for the activity of GH-producing pituitary adenomas. Eur J Endocrinol. 2013, 168(4): 575-583.