JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Physiology

Research Article

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

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, Exercise Physiology Department, Harold C. Miller Center, Room 105A, University of Mary, 7500 University Drive, Bismarck, ND 58504, USA, Tel: 702-908-2390; Tel: 701-355-8103; Fax: 701-255-7687;*

Email: mssaghiv@umary.edu

Received: 04-22-2015

Accepted: 05-28-2015

Published: 06-18-2015

Abstract

The purpose of the present study was to revise the association between s-klotho serum levels and IGF-1 levels in trained young adults and elderly.

Methods: Two hundred healthy subjects were divided evenly into 4 groups: untrained young adults, untrained elderly, aerobically well trained young adults and aerobically well trained elderly (24.5±1.0, 23.9±1.0**,** 58.6±1.1 and 58.1±1.1 years respectively)**,** 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 α-klotho Enzyme Linked Immunosorbent Assay ELISA kit, while, IGF-1 was measured by a chemiluminescent immunometric method.

Results: Significant ($p>0.005$) differences between the trained young adults and trained elderly and untrained young adults and untrained elderly in s-Klotho (682±106.0, 571±92.0, 435 ±89.0 and 321 ±96.2 $pg \bullet mL^{-1}$ respectively) and IGF-1 (62.6±17.4, 74.6±16.7, 82.6±23.2 and 97.8±29.2 $nmol \bullet L^{-1}$ respectively). In addition, significant ($p>0.005$) differences were noted between untrained young adults and untrained elderly for s-Klotho and Total IGF1.

Conclusions: S-Klotho is associated with younger age and aerobic exercise training, thus, probably related as well to other factors that promote health and postpone senescence . Being an aerobic athlete, especially at an elite level, seems to be associated with decreased risk for major chronic diseases. Inflection of s-Klotho expression through skeletal muscle contraction represents an interesting relationship that may help to explain the anti-aging effects of aerobic activity. Findings of the present study, support emerging evidence suggesting that such a relationship exists.

Keywords: Aging; IGF-1; Untrained Elderly; Untrained Young Adults; Well Trained Elderly; Long Lasting Training; Klotho Expression

Introduction

Age causes structural and functional changes in skeletal, cardiac and oxygen delivery ability in humans. No matter what genes one has inherited, the body is continuously undergoing complex biochemical reactions [1]. Some of these reactions cause damage and, ultimately, aging in the body. However, genetic component of longevity and aging helps a person to live longer. Because of its complexity, the aging process takes us into the area of integrative biology [2].

There are many proves that aging may be linked with a gene located on chromosome 13: Klotho gene facilitating functions as an aging-suppressor gene that extends life span. Soluble-Klotho (s-Klotho) is a powerful longevity protein that has been linked to the prevention of sarcopenia, muscle atrophy, osteopenia, and cardiovascular disease. s-Klotho is a transmembrane protein which can be cleaved, shed and act as a circulating hormone [3]. The secreted s-Klotho protein can regulate multiple growth factor signaling pathways, including insulin/IGF-1 [4]. S-Klotho-deficient mice show a shortened life span and multiple disorders resembling human aging [3], while, overexpression of s-klotho increases lifespan [5].

Similar anti-aging effects have also been ascribed to exercise and physical activity [6]. A study using a combination of transcriptomic and Genomic data identified a comprehensive map of the transcriptomic features important for aerobic exercise training–induced improvements in maximal oxygen consumption, but no genetic variants derived from candidate transcripts were associated with trainability [7]. While an association between muscle function and s-Klotho expression has been previously suggested from longitudinal cohort studies, a direct relationship between circulating s-Klotho and aerobic exercise training has not been investigated [8]. Therefore, the purpose of the present study was to revise the association between s-klotho serum levels and IGF-1 levels in long lasting aerobic exercise trained subjects, well trained young adults and elderly.

Methods

Subjects

Hundred healthy young males volunteered for this study. They were recruited as young active and young inactive subjects: 50 young men well trained aerobically active for at least 18 months (4-5 times•wk^{-1}) 23.9±1.0 years with maximal oxygen uptake (VO2max) of 57.1±3.1 mL•kg^{-1}•min^{-1} and 50 untrained young men 24.5±1.0 years and VO2max of 44.2±2.9 mL•kg^{-1}•min^{-1}. In addition 100 healthy elderly were also recruited as elder active and elder inactive subjects: 50 subjects were in good health, aerobically active for at least 18 months (4-5 times•wk^{-1}) age 58.1±1.1 years and VO2max of 48.2±3.2 mL•kg^{-1}•min^{-1} and 50 untrained controls 58.6±1.1 years and VO2max 36.7±2.9 mL•kg^{-1}•min^{-1}. 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 VO2max. 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 (± 0.05 kg), skin fold thicknesses at 8 sites (± 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 [9].

Following warm-up, subjects underwent a graded maximal treadmill test utilizing the standard Bruce protocol [10]. 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 [11]. 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 [12-14]. 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 (Im-

mulite 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 [15].

Statistical methods

Data are reported as mean ± SD values. Two ways ANOVA was performed for multiple comparisons, post hoc analysis was performed by using the Tukey 2 multiple comparison tests. The level of significance was set at alpha<0.05.

Results

Young and elderly subjects completed the exercise challenge without difficulties or abnormal symptoms. Subjects' mean descriptive data are presented in Table 1. Table 2 summarizes physiological variables at rest. It revealed significant (p>0.005) differences between the trained (young and elderly) and untrained (young and elderly) in heart rate, systolic and diastolic blood pressures. Differences (p>0.005) were seen between trained young adults and trained elderly for heart rate. In addition, significant (p>0.005) differences were noted between untrained young adults and untrained elderly in heart rate and systolic blood pressure. Table 3 report on the results achieved at maximal effort. It revealed significant (p>0.005) differences between the trained (young and elderly) and untrained (young and elderly) in VO2max, heart rate, systolic and diastolic blood pressures and lactic acid. Differences (p>0.005) were seen between trained young adults and trained elderly for VO2max, heart rate and lactic acid. In addition, significant (p>0.005) differences were noted between untrained young adults and untrained elderly in VO2max, heart rate, systolic and diastolic blood pressures and lactic acid.

Table 1. Subjects' physical characteristics (mean ± S.D).

Variables	Young		Elderly	
	untrained	trained	untrained	trained
N of subjects	50	50	50	50
Age (years)	23.9±1.0	24.5±1.0	58.6±1.1	58.1±1.1
Weight	75.6±3.1	70.9±2.4	72.3±3.0	78.7±4.1
Height (cm)	179.9±2.0	180.0±2.1	176.1±2.4	177.0±2.0
Fat (%)	15.1±3.1	9.4±0.7	22.6±4.1	12.7±2.5

Table 2. physiological responses at rest (mean ± S.D).

Variables	Young		Elderly	
	untrained	trained	untrained	trained
VO2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	3.3±0.3	3.2±0.3	3.3±0.3	3.5±0.3
Heart Rate ($beats \cdot min^{-1}$)	68.7±8.3	59±5.2 [a]	78.6±9.4 [b]	64.1±5.4 [a, b]
Systolic BP (mmHg)	117.2±7.9	108.1±9.5 [a]	124.0±7.0 [b]	114.0±9.1 [a]
Diastolic BP (mmHg)	80.9±4.0	72.1±2.1 [a]	85.1±3.7	76.0±2.4 [a]
Lactic acid ($mmol \cdot l^{-1}$)	1.3±0.3	1.2±0.2	1.4±0.3	1.3±0.2

a = A significant different from untrained (p>0.05).
b = A significant different from young adults (p>0.05).

Table 3. physiological responses at maximal effort (mean ± S.D).

Variable	Young		Elderly	
	untrained	trained	untrained	trained
VO2 ($mL \cdot kg^{-} \cdot min^{-1}$)	44.2±2.9	57.1±3.1 [a]	36.7±2.9 [b]	48.2±3.2 [a, b]
Heart Rate ($beats \cdot min^{-1}$)	212.2±7.9	198.1±8.6 [a]	189.0±6.9	177±9.9 [a, b]
Systolic BP (mmHg)	194.3±7.9	180.1±8.6 [a]	219±7.0 [b]	182±9.7 [a]
Diastolic BP (mmHg)	86.7±9.8	76.1±9.2.0 [a]	94.0±7.3 [b]	84.0±9.1 [a]
Lactic acid ($mmol \cdot l^{-1}$)	11.0±0.3	12.6±1.1 [a]	9.7±0.3 [b]	11.5±0.7 [a, b]

a = A significant different from untrained (p>0.05).
b = A significant different from young adults (p>0.05).

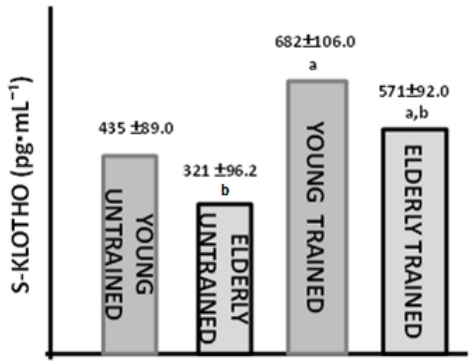

Figure 1. s-Klotho levels in trained and untrained young and elderly subjects (mean±SD). a = A significant difference from untrained (p>0.05). b = A significant difference from young adults (p>0.05).

Figures 1 and 2 reveals significant (p>0.005) differences between the trained (young and elderly) and untrained (young and elderly) in s-Klotho and IGF1. Differences (p>0.005) were seen between trained young adults and trained elderly for s-klotho and Total IGF1. In addition, significant (p>0.005) dif-

ferences were noted between untrained young adults and untrained elderly in s-Klotho and IGF1.

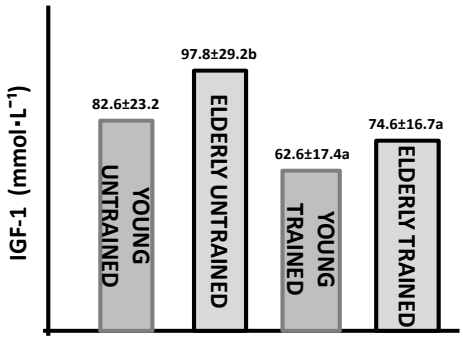

Figure 2. IGF-1 levels in trained and untrained young and elderly subjects (mean±SD). a = A significant difference from untrained ($p>0.05$). b = A significant difference from young adults ($p>0.05$).

Discussion

This study demonstrates that circulating s-Klotho levels are significantly higher in healthy well trained young and elderly subjects compared to the untrained counter partners with significantly higher values in the well trained young adults. Our cross sectional study findings in young and aged individuals suggest that circulating s-Klotho levels are increased in response to long lasting aerobic exercise training, and that the response depends on fitness level. A similar increase of circulating s-Klotho is also observed in response to an acute exercise in young and old mice, suggesting that this may be a good model for mechanistically probing the role of physical activity on Klotho expression [8].

Subjects engaged in higher aerobic capacity have longer life expectancies compared to inactive people [16]. There are several studies proving the definitive role of life-long physical activity, which can be engaged in at any age [17], even those in their 80s or 90s [18, 19]. Accordingly, regular physical activity or exercise participation promotes older adult health and disease prevention [20]. Eleven case control studies on life expectancy in former athletes revealed consistently greater life expectancy in aerobic endurance athletes. Endurance exercise like running, appear to benefit longer life expectancy than anaerobic exercise like power lifting [21]. Regular participation in physical activity and/or exercise training programs can minimize the physiological alterations that occur during aging and may contribute to improvements in health and well-being [20]. When elite athletes engaging in various sports are analyzed together, their mortality is lower than that of the general population. Thus, long-term vigorous exercise training is associated with increased survival rates of specific groups of athletes [22].

While in the well trained subjects s-Klotho levels were elevated, IGF-1 levels were decreased. 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 aerobic exercise training causes the significant decrease in IGF-1 concentrations, while no differences were noted in both untrained groups. The finding on the reduced IGF-1 in the present study clarifies previously reported study [24] in which it was not possible to determine if exercise affects IGF levels. It seems that important methodological differences among studies, as well as concerns about study quality, limit the ability to draw firm conclusions in that mentioned study. A meta-analysis 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 [25].

S-Klotho is a transmembrane protein which can act as a circulating hormone [26]. 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 [27]. Disruption of the insulin and IGF-1 signaling attenuates aging-like phenotypes in s-klotho-deficient mice [5], suggesting that klotho suppresses aging probably *via* inhibition of insulin and IGF-1 signaling. Therefore, klotho functions possible as an anti-aging gene in mammals and as a potential tumor suppressor and as an activator of the FGF pathway in humans. Among elderly subjects, reduced klotho levels may be associated with increased mortality, increased rate of cardiovascular disease and disability in daily living activities [28-30].

Conclusions

S-Klotho with regard to life expectancy, encounters the IGF-1 action. Klotho and aerobic exercise training are factors that may promote upgrading capacities of the elderly. In addition to such improving factors, aerobic exercise training has long been acknowledged for its anti-aging effects and of the indisputable impact of muscle contraction on longevity. Being a highly aerobically active, seems to be associated with decreased risk factors for major chronic diseases. Inflection of Klotho expression through skeletal muscle contraction represents an interesting relationship that may contribute to the explanation of the anti-aging effects of aerobic activity. Findings of the present study, demonstrated emerging evidence to suggest that such a relationship exists.

References

1. Masoro E J. Bziochemical and molecular mechanisms of aging: from model systems to human longevity. Preface. Biochim

Biophys Acta. 2009, 1790(10): 949-950.

2. Jazwinski SM. Longevity, genes, and aging. Science. 1996, 273(5271): 54-39.

3. 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.

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

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

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

7. Roth SM, Rankinen T, Hagberg JM, Loos RJF, Russe LPE, et al. Advances in Exercise, Fitness, and Performance Genomics. Med Sci Sports Exerc. 2012, 44(5): 809-817.

8. Avin KG, Coen PM, Huang W, Stolz DB, Sowa GA, et al. Skeletal muscle as a regulator of the longevity protein, Klotho Front. Physiol, 2014, 5: 189.

9. Behenke AR, Wilmore J. Evaluation and regulation of body build and composion. Englewood Cliffs, N.J Prentile Hall, inc.

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

11. American College of Sports Medicine. ACSM's Guidelines for Exercise Testing and Prescription, 9th edition, Philadelphia, PA: Lippincott Williams & Wilkins. 2014, 58(3): 328.

12. 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.

13. 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.

14. 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.

15. 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.

16. 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.

17. 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.

18. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med. 1994, 330(25): 1769-1775.

19. Binder EF, Schechtman KB, Ehsani AA, Steger-May K, Brown M, et al Effects of exercise training on frailty in community-dwelling elderly adults: Results of a randomized, controlled trial. J Am Geriatr Soc. 2002, 50(12): 1921-1928.

20. American College of Sports Medicine, Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, et al. American College of Sports Medicine position stand. Exercise and physical activity for older adults. Med Sci Sports Exerc. 2009, 41(7): 1510-1530.

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

22. Teramoto M, Bungum TJ. Mortality and longevity of elite athletes. J Sci Med Sport. 2010, 13(4): 410-416.

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. Orenstein MR, Friedenreich CM. Review of Physical Activity and the IGF Family. J Physic Act Health 2004, 1(4): 291-320.

25. 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.

26. 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(66550): 45-51.

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. 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.

29. 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.

30. 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.