Klotho gene expression responses to long lasting aerobic training and aging

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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 encourage the aging process by enhancement of high levels of s-Klotho 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, supraillium, front thigh and circumferences at the shoulder). Anthropometric procedures followed the recommendations of Behnke and Wilmore [10].

Following warm-up, sportsmen 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 ethylenediaminetetraacetic acid 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 Immunosorbsent 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 [Immulette 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 α<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>
<thead>
<tr>
<th>Table 1. Subjects’ physical characteristics [mean ± S.D].</th>
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<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>N of subjects</td>
</tr>
<tr>
<td>Age [years]</td>
</tr>
<tr>
<td>Weight [kg]</td>
</tr>
<tr>
<td>Height [cm]</td>
</tr>
<tr>
<td>Fat [%]</td>
</tr>
<tr>
<td>S-Klotho [pg•mL⁻¹]</td>
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<td>IGF-1 [mmol•L⁻¹]</td>
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S-Klotho is a transmembrane protein which can act as a prostate, and premenopausal breast cancers [25]. of IGF-1 are associated with increased risks for colorectal, a meta-analysis indicated that increased circulating concentrations related to a shortened lifespan in adults [24]. A meta-analyzes that signaling through IGF-1 and insulin receptors is wellbeing [23], yet the bulk of the scientific evidence sug- gests that positive attributes such as growth, health, youth and old subjects [22]. IGF-1 is generally thought to be associated with decreased risk factors for major chronic diseases and mortality, there are growing efforts in geron- tology research to slow aging and extend healthy lifespan. 

Since aging is an independent risk factor of the age related diseases 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 lifelong physical activity in s-Klotho expression.

While in the well aerobic trained young runners and mas- ter 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 sug- gests that signaling through IGF-1 and insulin receptors is related to a shortened lifespan in adults [24]. A meta-analy- 
sis 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 down- stream signaling proteins [27]. The mechanism by which se- creted 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 encour- aging the acting process in keeping high levels of s-Klotho 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.

Discussion

This study demonstrated that circulating s-Klotho levels are similar for young healthy well trained elite runners and mas- ter athletes. It seems that the response depends on aerobic fitness level [17]. In addition, levels of s-Klotho were signifi- cantly 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 geron- tology 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 se- rum 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].

<table>
<thead>
<tr>
<th>Variables</th>
<th>Young Rest</th>
<th>Young Exercise</th>
<th>Masters Rest</th>
<th>Masters Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2[mL•kg⁻¹•min⁻¹]</td>
<td>3.3±0.3</td>
<td>62±1.3</td>
<td>7.3±0.3</td>
<td>53±2.9a</td>
</tr>
<tr>
<td>Heart Rate [beats•min⁻¹]</td>
<td>67.1±9.3</td>
<td>198±7.2</td>
<td>70.6±8.4</td>
<td>190.4±8.2a</td>
</tr>
<tr>
<td>Systolic BP [mmHg]</td>
<td>109.2±6.8</td>
<td>180.4±7.6</td>
<td>110±8.0</td>
<td>182±6.4</td>
</tr>
<tr>
<td>Diastolic BP [mmHg]</td>
<td>70.6±2.7</td>
<td>68.0±2.2</td>
<td>72.2±3.3</td>
<td>71.8±2.4</td>
</tr>
<tr>
<td>Lactic acid [mmol•L⁻¹]</td>
<td>1.3±0.3</td>
<td>12.8±1.2</td>
<td>1.4±0.3</td>
<td>12.1±1.1</td>
</tr>
</tbody>
</table>

a = significant [a<0.05] between anaerobic subjects and aero- 

bic subjects.


