Research Article

Are S-Klotho’s Maximal concentrations dependent on Exercise Intensity and Time in young adult males?

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Abstract

The purpose of the present study was to define the period of time in which aerobic training does not increase further serum S-Klotho levels in untrained young adult males, and to examine the relation between plasma S-Klotho concentration and maximal oxygen uptake (VO2max).

Methods: Sixty (60) untrained subjects (27.05±1.1 years) were divided into 2 groups, both exercised six months 4×wk-1 for the duration of 45 min×session. One group (LTI) exercised below the anaerobic threshold at 40-50% of VO2max while the second group (HTI) worked above the anaerobic threshold at 65-70% of VO2max. Testing sessions were performed at 0, 2, 4, and 6 months. Blood samples were drawn after overnight fasting. S-Klotho was analyzed using an ELISA kit.

Results: Following 2 and 4 months, significant (p≤0.05) increases were noted in the HTI group, at the fourth testing session, S-Klotho leveled off. In the LTI group, S-Klotho remained almost unchanged. Findings of the present study, support emerging evidence suggesting that a relation between plasma S-Klotho concentration and VO2max exists.

Conclusions: Data suggest that increases in S-Klotho is tidally associated with VO2max levels. In addition, the S-Klotho increase levels-off following 4 months of aerobic training. Exercising below the anaerobic threshold does not increase VO2max and thus, does not increase S-Klotho.

Introduction

The α-Klotho protein circulates in the blood as Soluble-Klotho (s-Klotho), which in humans, decreases in the serum with aging [1]. S-Klotho in humans is a pleiotropic protein with a considerable influence on longevity [2]. Exercise is a very active approach averting leading reasons of disease and as relates to aging. Exercise and Klotho gene expression reduce the risk of cardiovascular events in patients with prior coronary artery disease (CAD), thus, aerobic exercise may decrease the risk of mortality [3,4]. However, an association between low levels of s-Klotho and the occurrence and severity of cardiovascular disease have been reported, as well as a reduction of cardiovascular risk when levels were high [5]. Untrained, physically inactive individuals present low blood s-Klotho levels [6]. Recent studies [6,7] suggested that aerobic exercise training, is associated with increase blood s-Klotho and maximal oxygen uptake (VO2) values. Therefore, the purpose of the present study was to assess the influence of time and intensity (below or above the anaerobic threshold) on s-Klotho serum levels in untrained individuals.

Methods

Subjects

Sixty (60) young (27.05±1.1 years), healthy, untrained, and male subjects, were
divided randomly into two even groups. All subjects were evaluated as of “poor” fitness according to aerobic capacity and age.

Pre-testing procedures

Approval for the study was achieved in accordance with the Helsinki declaration, approved by the Clinical Science Center Committee on Human Subjects. A written informed consent was obtained from each subject, a health history questionnaire was filled, and risk stratification followed.

Subjects judged free of coronary artery disease by clinical history, and the absence of major risk factors were included in the study.

Procedures

Subjects were seated for five minutes before having their resting values obtained, then proceeded to undergo a maximal exercise stress test. Height, weight, heart rate, blood pressure, and s-Klotho values were obtained at rest. Subjects with no counter indications for exercise due to the stress test continued as subjects in the study. None of the subjects had exercise counter indications.

Both groups exercised six months, 4×wk⁻¹ for the duration of 45 min×session⁻¹. The first group exercised below the anaerobic threshold (Low Training Intensity; LTI) at 40-50% VO₂max, while the second group (High training Intensity; HTI) worked above the anaerobic threshold at 65-70% of VO₂max. Testing sessions were performed at 0, 2, 4 and 6 months.

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 [8].

Following warm-up, subjects underwent a graded maximal treadmill test utilizing the standard Bruce Protocol [9]. 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 [10]. VO₂ 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 ethylenediamine-tetraacetate 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 for each visit.

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 [11-13]. Measurements were conducted...
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according to the manufacturer instructions. The intra- and inter-assay coefficients of variation ranged from 2.7 to 9.8%.

**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 for s-Klotho 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. Mean descriptive data for the two experimental groups are presented in tables 1,2. Figure 1 discloses that s-Klotho in the HTI group, increased significantly (p≤0.05) from baseline to 2 months, and from 2 months to 4 months, yet insignificantly increased thereafter. The LTI group as a control did not significantly differ in their values from pretesting values to the end of the study; namely the 6th month. Figure 2, reveals that VO₂ in the HTI group, increased significantly (p≤0.05) and gradually following the 2nd and 4th training months without significant differences from the 4th month to the 6th. The LTI group as a control did not significantly (p>0.05) differ in their VO₂ values from pretesting to the end of the study; namely the 6th month.

**Table 1: Subjects’ Physical Characteristics at Rest. (mean ± S.D).**

<table>
<thead>
<tr>
<th>Variables</th>
<th>LTI</th>
<th>HTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>N of subjects</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.9±1.1</td>
<td>27.2±1.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.3±1.7</td>
<td>70.1±1.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.4±2.0</td>
<td>179.8±2.1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>12.1±1.6</td>
<td>12.4±1.7</td>
</tr>
<tr>
<td>VO₂ (mL·kg⁻¹·min⁻¹)</td>
<td>3.4±0.3</td>
<td>3.4±0.3</td>
</tr>
</tbody>
</table>

**Table 2: Physiological Responses at Rest and Pre-Post Training at Maximal Exercise in Both Groups (mean ± S.D).**

<table>
<thead>
<tr>
<th>Variables</th>
<th>HTI Rest</th>
<th>HTI Exercise</th>
<th>LTI Rest</th>
<th>LTI Exercise</th>
</tr>
</thead>
<tbody>
<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>
<tr>
<td>Heart Rate (beats·min⁻¹)</td>
<td>67.1±9.3</td>
<td>198±7.2</td>
<td>79.6±8.4</td>
<td>194.4±8.2</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>71.0±2.4</td>
</tr>
</tbody>
</table>

HTI = Exercising group; LTI = Inactive group; a = significant (p>0.05) differences between both groups.

**Figure 1:** S-Klotho levels in HTI and LTI (mean±SD); a. = Significant differences between HTI and LTI (p>0.05); b = Significant difference between months of training.
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Discussion

This study demonstrated that aerobic exercise training induced a significant increase in plasma s-Klotho concentration following six months of aerobic training. Additionally, it revealed that following four months of aerobic training S-Klotho values leveled off. In the LTI group no significant changes in s-Klotho and VO$_{2\text{max}}$ were found. In the HTI group, s-Klotho was increased in response to long-lasting aerobic exercise training, and that the response depends on the subjects’ fitness level, since s-Klotho increased in a similar pattern as VO$_{2\text{max}}$. Maximal oxygen uptake (VO$_{2\text{max}}$) is largely used as the best single physiological variable to evaluate the cardiopulmonary function and is powerful tool for investigational and clinical evaluations. However, the mechanism by which VO$_2$ level influence blood s-Klotho values is yet unclear.

Previously it has been reported that oxidizing free radical species (ORS) are generated during aerobic bouts [13]. Skeletal muscles generate Super Oxide and Nitric Oxide which is increased by muscle contraction activity [14]. ROS is essential for skeletal muscle force generation, however, ROS in high values may reduce muscle contraction properties and thus, result in early exhaustion [15,16]. The increase in s-Klotho following aerobic exercise training may be a response to ROS that increase in muscle cells as a result of aerobic training.

Although the association between s-Klotho and aerobic exercise training is not clear, s-Klotho reduces programed cell death (apoptosis) through Nitric Oxide production and thus, suppresses ROS [17]. Previously, a parallel increase of circulating s-Klotho was 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 aerobic exercise on Klotho expression [18]. Endurance exercise such as running, cycling and swimming appear to benefit and minimize the physiological alterations that occur during aging and may contribute to improvements in health and well-being [19]. Previously it has been suggested that aerobic training above the anaerobic threshold intensities increase VO$_{2\text{max}}$ [20]. The unchanged VO$_{2\text{max}}$ in the LTI group and s-Klotho, is probably due to the low training intensity; namely below the anaerobic threshold and below of heart rate reserve [21]. Prolonged or high-intensity exercise result in oxidative damage to macromolecules in both blood and skeletal muscles. However, low intensity aerobic training does not produce ROS thus, S-Klotho levels did not increase in the LTI group, since s-Klotho protein suppresses oxidative stress [17,22].

Study’s limitations

There are limitations in the present study that should be well-thought-out when...
understanding the results. a) Only s-Klotho in the plasma was measure and, b) mechanisms causial of the effects of aerobic exercise on Klotho were not investigated in this study.

In conclusion, data suggest that increases in s-Klotho is close associated with VO\textsubscript{2}\text{max} levels. In addition, the s-Klotho increase levels-off following 4 months of aerobic training. Exercising below the anaerobic threshold does not increase VO\textsubscript{2}\text{max} and thus, does not increase s-Klotho.

References


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