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Aerobic Training Effect on Blood S-Klotho Levels in Coronary Artery Disease Patients

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

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

[2]*Department of Life Sciences, Wingate College, Wingate, Israel*

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

[*]**Corresponding author:** Moran Saghiv, Associate Professor, Department of Exercise Physiology, Harold C. Miller Center, Room 105A, University of Mary, 7500 University Drive, Bismarck, ND 58504, USA, Tel: 702-908-2390, 701-355-8103; Fax: 701-255-7687; E-mail: mssaghiv@umary.edu

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Abstract

Rationale: Aerobic exercise and Klotho gene expression reduce the risk of cardiovascular events in patients with prior coronary artery disease (CAD) thus, aerobic exercise may create a decreased risk of mortality.

Objective: The purpose of the present study was to compare the association between s-klotho serum levels and IGF-1 levels in 3 groups: 60 untrained coronary artery disease patients (CAD) age 52.6 ± 2 years, 60 active participants with CAD in supervised aerobic programs for at least 12 months (4-5 times•wk^{-1}) age 53.0 ± 2 years, and 40 untrained healthy males, age 53.6 ± 1.5 years to assess association of aerobic training and s-klotho activity.

Methods and results: 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. Significant ($p>0.05$) differences were noted between the aerobically trained CAD patients and both untrained groups: CAD patients and healthy subjects with regard to s-Klotho (491 ± 66, 386 ± 70 and 418 ± 81 pg•mL^{-1} respectively), IGF-1 (82 ± 12, 106 ± 21 and 98 ± 14 nmol•L^{-1} respectively) and maximal oxygen uptake (42.1 ± 4.5, 31.9 ± 3.9 and 35.8 ± 2.9 mL•kg^{-1}•min^{-1} respectively).

Conclusions: S-Klotho and aerobic exercise training are factors that may promote upgrading capacities of the CAD patients. Inflection of Klotho expression through aerobic training represents a relationship that may contribute to the explanation of the effects of aerobic activity on CAD patients. In addition, findings suggest that atenolol treatment does not influence s-Klotho and IGF-1 levels in CAD patients treated with atenolol.

Keywords: IGF-1; Aerobic exercise; Untrained aged adults; Klotho expression; Cardiovascular disease

Introduction

Aerobic exercise and Klotho gene expression could reduce the risk of cardiovascular events in patients with prior coronary artery disease (CAD), thus aerobic exercise may decrease the risk of mortality [1,2]. Aerobic exercise can decrease the incidence and severity of cardiac events during exercise among an unselected group of patients with stable CAD [3,4]. In addition, exercise training improves endothelium-dependent vasodilatation both in epicardial coronary vessels and in resistance vessels in patients with CAD [5].

Patients with significant CAD present lower soluble concentrations of α-Klotho (s-Klotho), as well as reduced levels of Klotho gene expression in the vascular wall. This protein is related to the attenuation of vascular calcification as well as prevention of cardiac hypertrophy. Reduced serum s-Klotho concentrations and decreased vascular Klotho gene expression were associated with the presence as well as the severity of CAD independently of other established cardiovascular risk factors [2]. S-Klotho, is a pleiotropic protein related to longevity, which acts as a co-receptor of the fibroblast growth factor 23, has been proposed as a key regulator of the development of cardiovascular disease. In the few published clinical studies, 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 [6]. Therefore, the purpose of the present study was to assess the effect of chronic aerobic exercise training on s-Klotho serum levels and IGF-1 levels in CAD patients following long lasting aerobic exercise training.

Methods

Subjects

60 untrained sedentary CAD patients (52.6 ± 2 years old), that were not active participants in supervised aerobic programs, sixty (53.0 ± 2 years old) active participants with CAD in supervised aerobic programs (60-75% work capacity) for at least 12 months (4-5 times•wk^{-1}) and forty untrained healthy males 53.6 ± 1.5 years old were recruited to the study. The untrained CAD patients and the healthy untrained subjects served as a control groups to the trained CAD patients.

In the untrained CAD group, 36 patients had single-vessel disease and 24 had double-vessel disease, and inferior wall motion abnormalities were noted in three patients and posterior wall motion

abnormalities were also identified in three patients. The exercising group consisted of, 21 patients who had a single-vessel disease and 39 patients who had double-vessel disease. Inferior wall motion abnormalities were noted in three patients, posterior wall motion abnormalities in three patients and anterior wall motion abnormalities in three patients. All patients were treated solely with atenolol β-adrenergic blocking agent: the control group consisted of 36 patients who were prescribed with 25 $mg \bullet d^{-1}$ and 24 patients prescribed with 50 $mg \bullet d^{-1}$. In the exercise group, 27 patients were prescribed with 25 $mg \bullet d^{-1}$ and 33 patients were prescribed with 50 $mg \bullet d^{-1}$. Study exclusion criteria included hemodynamically significant mitral regurgitation, significant arrhythmias (rapid atrial fibrillation, frequent, multifocal ventricular premature beats, and documented ventricular tachycardia within the last 3 months) not well controlled arterial systemic hypertension, non-controlled congestive heart failure, and or known autoimmune diseases. In addition, Patients with heart transplantation and heart failure were excluded from the study. A written consent form was obtained from each subject, approved by the Clinical Science Center Committee on Human Subjects.

Procedures

Adipose fat assessment included measurement of total body weight (± 0.05 kg), skin fold thicknesses at 8 sites (chest, axilla, triceps, subscapula, abdomen, suprailium, quadriceps and circumferences at the shoulder) ± 1 mm using the Lange Caliper. Measurement protocol and body fat estimation were in accordance with the recommendations of Behnke and Wilmore [7].

Following warm-up, subjects underwent a symptom limited graded maximal oxygen uptake (VO_{2max}) on a treadmill utilizing the standard Bruce protocol [8]. 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 [9]. 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

Peripheral venous blood samples (2.5 mL) were collected before the stress test was performed, by sterile antecubital venipuncture techniques into ethylenediam-inotetraacetate containing tubes. Time of day for blood sampling was in the morning and was kept consistent to control for 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. S-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 [10-12]. 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 [13].

Statistical methods

Data are reported as mean ± SD values. The three samples were compared by means of a one way ANOVA for independent samples and *post hoc* multiple comparisons analysis was applied using the Tukey 2 procedure. The level of significance was set at $\alpha<0.05$.

Results

All subjects completed the symptom limited graded maximal oxygen uptake test without difficulty or abnormal response. Subjects' mean descriptive data are presented in Table 1. Significant ($p<0.05$) lower values in VO_{2max} were noted for both untrained groups; healthy subjects and CAD patients, compared to the trained CAD patients. Fat values were significantly ($p<0.005$) higher in the untrained groups; both healthy subjects and untrained CAD patients compared to the trained CAD patients. Significant differences ($p<0.05$) between the trained CAD patients and both untrained groups; healthy subjects and CAD patients, were found in s-Klotho and IGF1. Figure 1 defines the relationship between s-Klotho and fitness level in all three groups. Figure 2 describes the association between VO_{2max} and IGF-1 for the three groups.

Variable	Trained CAD	Untrained CAD	Healthy untrained
N of subjects	60	60	40
Age (years)	53.0 ± 2.0	52.6 ± 2.0	53.6 ± 1.5
Weight (kg)	74.2 ± 2.1	73.4 ± 2.5	72.3 ± 3.0
Height (cm)	177.4 ± 3.1	178 ± 3.1	176.8 ± 2.7
Fat (%)	16.3 ± 3.8[a]	23.1 ± 3.7	22.6 ± 4.4
VO_{2max} $mL \bullet kg^{-1} \bullet min^{-1}$	42.1 ± 4.5[a]	31.9 ± 3.9	35.8 ± 2.9

s-Klotho (pg•mL^{-1})	491 ± 66[a]	385 ± 70	418 ± 81
IGF-1 (nmol•L)	82 ± 12[a]	106 ± 21	98 ± 14
a= A significant ($p<0.05$) differences from untrained subjects			

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

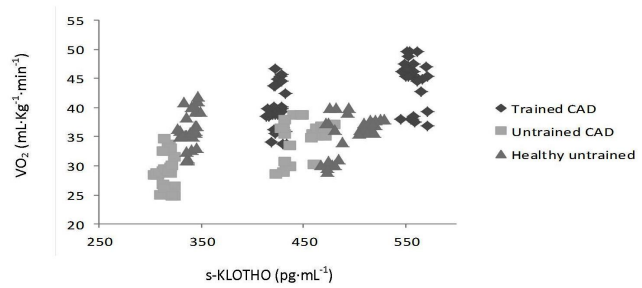

Figure 1: s-KLOTHO levels according to VO_2 in trained CAD subjects, untrained CAD subjects, and untrained healthy subjects.

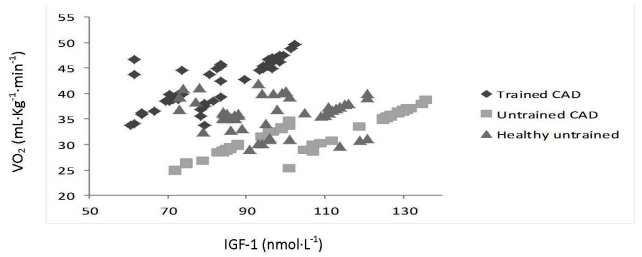

Figure 2: IGF-1 levels according to VO_2 in trained CAD subjects, untrained CAD subjects, and untrained healthy subjects.

Discussion

This study demonstrated that circulating s-Klotho levels are significantly higher while IGF-1 is significantly lower in CAD aerobically trained patients compared to untrained CAD patients and healthy counter partners. Our findings in trained CAD patients suggest that circulating s-Klotho levels are augmented in response to long lasting aerobic exercise training of at least 12 months. A similar increase of circulating s-Klotho was also observed in response to acute aerobic exercise in young and old mice, suggesting that experimentation with mice may be a good model for elucidation the role of physical activity on Klotho gene expression [14]. In addition, no significant differences between the untrained CAD patients and the healthy untrained subjects in s-Klotho and IGF-1 levels were found.

Subjects who demonstrated higher levels of aerobic capacity may have longer life expectancies compared to inactive people [15]. Regular participation in aerobic exercise training programs can minimize the physiological alterations that occur during aging and may contribute to improvements in health and well-being [16]. While the trained CAD patients'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 animals [17].

A reduction in s-Klotho levels was observed in CAD patients, similar to other premature vascular aging diseases, such as hypertension or diabetes mellitus. Even normal aging is associated with a reduction in serum concentration of s-Klotho [18]. CAD is a highly prevalent disease in the general adult population and is among a major cause of death. Several clinical studies have suggested that Klotho gene exerts strong cardioprotective effects. For instance, Klotho gene has been shown to protect against vascular calcifications in rodent models of CAD while in humans without CAD, higher s-Klotho levels have been related to a lower incidence of mortality and CAD [2,18]. Patients with significant CAD present lower soluble concentrations of Klotho, as well as reduced levels of Klotho gene expression in the vascular wall. Reduced serum Klotho concentrations and decreased vascular Klotho gene expression were associated with the presence and severity of CAD independently of established cardiovascular risk factors [2]. Moreover, low s-Klotho levels have been associated with increased arterial stiffness in chronic kidney disease patients [19]. Further support for a direct role of Klotho gene in vascular homeostasis comes from *in vitro* studies showing endogenous expression of Klotho gene in human vascular smooth muscle cells [20]. Additionally, several experimental studies indicate that s-Klotho facilitates the maintenance of vascular homeostasis. S-Klotho improves endothelial dysfunction through promotion of nitric oxide production and mediates anti-inflammatory and anti-aging effects such as suppression of adhesion molecules expression, attenuation of nuclear factor-kappa B or inhibition of Wnt signaling, thus, limits Wnt-mediated cellular senescence [21,22].

Beta-blockers are medications prescribed to treat CAD patients and patients with high blood pressure. Beta-blockers suppress the effects of the sympathetic nervous system on the heart [23], thus reduces the work of the heart so that it requires less blood and oxygen. As a result, the workload of the heart is reduced, which in turn lowers oxygen demand and blood pressure [24]. Beta-blockers help control heart rate and are also used in the treatment of abnormal heart rhythms that are too fast or irregular. However, differences of about 8% were noted between the untrained CAD patients and the healthy untrained subjects in IGF-1 levels. These results did not have a significant difference suggesting that atenolol treatment does not significantly influence IGF-1 and s-Klotho blood levels in untrained CAD patients.

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 CAD patients. Being aerobically active seems to be associated with a decreased risk for cardiovascular diseases. Findings of the present study, endorse emerging evidence

suggesting that such a relationship exists. In addition, the similar values of s-Klotho and IGF-1 in the untrained CAD patients and the healthy untrained, suggests that atenolol does not influence s-Klotho and IGF-1 levels in CAD patients treated with this drug.

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