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Research Article

Comparison between aerobic and anaerobic training influence on s-klotho blood levels following 60 min aerobic bout

Moran Saghiv[1*], David Ben-Sira[2], Michael Sagiv[2]

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

[2]*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

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Abstract

The purpose of the present study was to revise and compare the association between s-Klotho serum and IGF-1 levels in elite anaerobic and aerobic trained young adult athletes, following 60 min of aerobic bout at 75% of their maximal work capacity.

Methods

Thirty elite athletes subjects were recruited: 15 elite anaerobically trained sprinters (24.4±1.0 years) and 15 elite matched group aerobically well trained (24.7±1.0 years). Subjects underwent maximal oxygen uptake test. Blood samples were drawn at rest and following 60 min of aerobic bout at 75% reserved heart rate, 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: At rest a significant ($p<0.01$) differences were noted between the aerobic trained and anaerobic trained athletes for s-Klotho (672±38 and 442±24 pg•mL^{-1} respectively) and IGF-1 (65±7 and 94±12 mmol•L^{-1} respectively). Following 60 min aerobic exercise, an interaction effect ($p<0.01$) was obtained for s-Klotho and IGF-1 between the groups: in the aerobic group both variables were changed significantly from base level while in the anaerobic athletes s-Klotho increased significantly ($p<0.05$) and IGF-1 was significantly ($p<0.05$) reduced (566±32 pg•mL^{-1} and 85±8 mmol•L^{-1} respectively).

Conclusions

S-Klotho and long lasting aerobic exercise training are factors that may promote upgrading capacities of the young adults. However, being a highly anaerobically active sprinter suggests that there is no association between anaerobic vigorous exercise training and decreased risk factors for major chronic diseases.

Key words: IGF-1; sprinters; anaerobic training; aerobic training

Introduction

Soluble-Klotho (s-Klotho) is a powerful longevity protein that has been linked to the prevention of muscle atrophy, osteopenia, and cardiovascular disease. S-Klotho is a transmembrane protein which can be cleaved, shed and act as a circulating hormone [1]. Strenuous all-out exercise training increase lactic acid levels in the blood and active muscles and increase insulin-like growth factor-1 (IGF-1) levels in the blood [2], primarily due to a substantial major increase in plasma catecholamine concentrations [3]. Indirect data have supported the concept that IGF-1 may be atherogenetic because it can induce vascular smooth muscle cell proliferation in vitro [4]. But, the secreted s-Klotho protein can regulate multiple growth factor signaling pathways, including insulin/IGF-1[5], while, overexpression of s-Klotho increases lifespan [6].

Avin et al. [7] investigated the relationship between circulating s-Klotho and an acute exercise bout. Their pilot clinical findings performed in young and aged individuals suggest that circulating s-Klotho levels are upregulated in response to a single aerobic exercise bout, but that the response may be dependent on fitness level. While an association between muscle function and s-Klotho expression has been previously suggested from longitudinal cohort studies, the effect of single aerobic exercise bout for 60 min on circulating s-Klotho in trained elite athletes has not been investigated previously [7]. Therefore, the purpose of the present study was to measure blood s-Klotho serum and IGF-1 levels in anaerobic and aerobic elite athletes, following single aerobic bout lasting 60 min at 75% of their maximal work capacity.

Methods

Subjects

Thirty healthy young males, volunteered for this study. They were recruited as elite athletes: 15 well anaerobic trained sprinters at the national level, age 24.4±1.0 years with VO2max of 56.2±2.5 $mL \bullet kg^{-1} \bullet min^{-1}$, and 15 well aerobically trained age 24.7±1.0 years with VO2max 63.3±2.2 $mL \bullet kg^{-1} \bullet min^{-1}$. 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.

Measurements and procedures

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 [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) respiratory exchange ratio > 1.1, and c) when the subject could not keep up with the load, according to the guidelines of the American College of Sports Medicine [10]. 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.

Following the VO2max test and 60 min of rest, subjects run on a treadmill for 60 min at a workload corresponding to their 75% reserved heart rate, calculated by the Karvonen equation [11], in which:

Target Heart Rate = [(max HR − resting HR) × %Intensity] + resting HR

Blood sampling and procedures

Peripheral venous blood samples (2.5 mL) were collected twice from each subject: at rest (rest) and immediately post aerobic exercise bout, 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.

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 (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 [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

All subjects completed the exercise challenge without difficulties or abnormal symptoms. Subjects' mean descriptive data are presented in Table 1.

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

Variables	Anaerobic trained	Aerobic trained
N of subjects	15	15
Age (years)	24.4±1.0	24.7±1.0
Weight (kg)	71.0±2.1	69.3±1.9
Height (cm)	179.8±2.0	180.2±2.1
Fat (%)	10.3±1.3	10.1±1.4
VO2max (mL•kg^{-1}•min^{-1})	56.2±2.5	63.3±2.2 a

a = A significant p<0.05 differences between groups

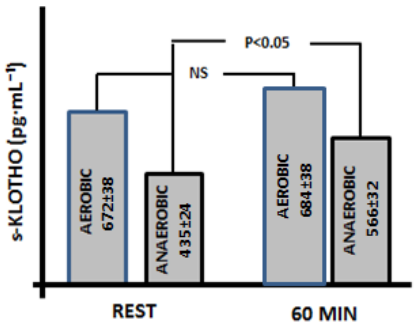
It exposes a significant (p<0.05) higher VO2max in the aerobic trained subjects compared to the anaerobically trained athletes. Table 2 summarizes physiological variables at rest and during exercise.

Table 2: physiological responses at rest and following 60 min aerobic bout in both groups (mean ± S.D.).

VARIABLES	ANAEROBIC ATHLI		AEROBIC ATHLETES	
	Rest	60 min bout	Rest	60 min bout
Heart Rate (beats•min^{-1})	67.1±8.3	178±7.2	62.1±7.9	174±8.6
Systolic BP (mmHg)	120.0±8.2	174±9.9	110.9± 7.4	170±8.9
Diastolic BP (mmHg)	77.1±3.6	72.1±5.8	70.1± 5.9	69.4±6.7
Lactic acid (mmol•L^{-1})	1.2±0.3	8.2±1.0	1.3±0.3	7.6±0.8

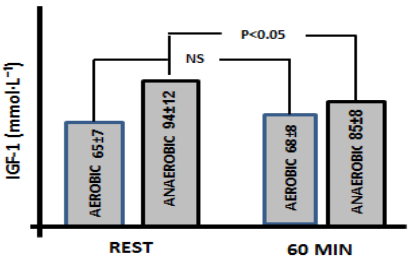
It revealed that there are no significant differences between the groups in all variables presented. Figure 1 discloses an interaction effect between rest - exercise conditions and training background of athletes in s-Klotho levels (F(1,28)=3860, p<0.01).

Figure 1: s-Klotho levels in aerobically trained and anaerobically sprinters (mean±SD). An interaction effect (p<0.05) was noted since anaerobically sprinters increased significantly (p<0.05) levels of s-Klotho from rest to exercise while in the aerobically trained subject it reminded unchanged.



At rest, anaerobic trained athletes demonstrated significantly lower mean values of s-Klotho than anaerobically elite trained athletes (435±24 and 672±38 pg•mL^{-1} respectively). In response to a 60 min of aerobic exercise bout the anaerobically trained mean values of s-Klotho levels were significantly increased compared with rest while in the aerobically trained athletes it has been slightly, though significantly, reduced (435±24 to 566±32 pg•mL^{-1} respectively). Figure 2 also discloses an interaction effect between rest - exercise conditions and training background of athletes in IGF-1 levels (F(1,28)=42.1, p<0.01).

Figure 2: IGF-1 levels in aerobically trained and anaerobically sprinters (mean±SD). An interaction effect (p<0.05) was noted since anaerobically sprinters decreased significantly (p<0.05) levels of IGF-1 from rest to exercise while in the aerobically trained subject it reminded unchanged.



At rest, anaerobic trained athletes demonstrated a significantly higher mean values of IGF-1 than aerobically elite trained athletes (94.0±11.5 and 65.0±7.1 mmol•L^{-1} respectively). Following 60 min of aerobic exercise the anaerobically trained mean values of IGF-1 levels were significantly reduced compared with rest while in the aerobically trained athletes it has been slightly, though significantly, increased (84.7±8.1 and 68.0±7.4 mmol•L^{-1} respectively).

Discussion

The relevant features of the present study are the positive relationship between plasma s-Klotho concentration and acute aerobic bout for 60 min in well trained elite anaerobically sprinters, while, IGF-1 levels were reduced. However, acute aerobic bout in long lasting well trained elite aerobic athletes induced a slightly increase in plasma s-Klotho concentration and a decrease in IGF-1 levels. Therefore, we propose that the increase in the plasmas s-Klotho concentration, after 60 min aerobic bout in the anaerobic trained athletes, might be responsible for the decreased IGF-1.

Secreted s-Klotho functions as a humoral factor is involved in suppression of oxidative stress [16], transforming growth factor (TGF)-β1 signaling [17] and insulin/IGF-I signaling [6]. Plasma s-Klotho concentrations decrease with advancing age in healthy humans [12, 18], this decrease in blood circulating s-Klotho may have important clinical significance in the pathophysiology of specific diseases. S-Klotho represses DAF-2 an insulin/IGF-like receptor under physiological conditions, thereby inducing increase the insulin pathway effector FOXO i.e. de-repression of DAF- 16 and subsequent overexpression of factors such as antioxidant enzymes that improve longevity and stress resistance [19]. S-Klotho increases resistance to oxidative stress by activating the FOXO fork head transcription factors that induce expression of manganese antioxidant enzyme superoxide dismutase [20].

Our findings at rest, on well trained aerobically elite athletes, suggest that circulating s-Klotho levels are soundly increased in response to long lasting aerobic exercise training. A similar increase of circulating s-Klotho at rest was not observed in response to a long lasting anaerobically training. Thus, following 60 min aerobic bout levels of s-Klotho and IGF-1 did not change in well trained aerobically elite athletes, due to the fact that the maximal effects of aerobic exercise were already exhausted. Aerobic bout lasting 60 min, in anaerobically sprinters, suggests that this may be a good model for systematically probing the role of physical activity on s-Klotho expression [7]. In addition from the literature this study may suggest that elite athletes with very high aerobic capacity may have longer life expectancies compared to the elite anaerobically sprinters [21]. Eleven case control studies on life expectancy in former athletes revealed consistently greater life expectancy in aerobic endurance athletes. It appears to benefit longer life expectancy than anaerobic exercise like power lifting [22]. 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 [23]. When aerobic elite athletes engaging in various sports are analyzed together, their mortality is lower than that of the general population. Thus, long-term aerobic exercise training is associated with increased survival rates of specific groups of athletes [24].

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 [25]. 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 [26]. The comparative analysis of biochemical indices measured at rest showed that following 60 min aerobic bout IGF-1 was decreased in the anaerobically athletes, suggesting that long lasting vigorous anaerobic training does not affect positively IGF-1 levels.

Conclusions

S-Klotho and long lasting aerobic exercise training are factors that may promote upgrading capacities of the aerobic trained young adults. 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. However, being a highly anaerobically active sprinter suggests that there is no association between anaerobic vigorous exercise training and decreased risk factors for major chronic diseases.

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