Research Article

Cardiology & Vascular Research

# The Impact of 12 Weeks Exercise Training on Circulating Soluble-Klotho and Pro - BNP in Coronary Artery Disease Patients

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## ABSTRACT

***Background:*** *Klotho protein is a membrane-based circulating protein that regulates cell metabolism, as well as the lifespan modulating activity of Fibroblast Growth Factors (FGFs). Higher plasma circulating Klotho levels reduce cardiovascular risk, suggesting Klotho has a protective role in cardiovascular diseases. Brain natriuretic peptide is a prognostic marker in coronary artery disease (CAD) patients, and in particular in CAD patients with heart failure. Aerobic exercise reduces the risk of cardiovascular events and mortality in patients with proven coronary artery disease (CAD), thus, S-Klotho serum levels were assessed in order to find out whether exercise can modulate its activity. Purpose: to assess the impact of 12 weeks exercise training program on S-Klotho and pro- BNP levels in coronary artery disease patients, and to assess possible correlation between S-Klotho and pro-BNP II.*

***Methods:*** *S-Klotho and pro-BNP serum were assessed in 2 groups: gr. A = 41 coronary artery disease patients (CAD), age 59.6 years ± 2.2 sd, all with recent (< 45 days) aorto coronary by- pass surgery (CABG) years, myocardial infarction (MI), or percutaneous intervention (PCI) who were recruited to a 12 weeks supervised aerobic exercise program (45 min/4-5 sessions/week), and gr. B, a control group consisting of 17 CAD patients, age 61 years ± 2.4sd who continued their usual treatment and lifestyle with no active exercise intervention. Assessment was done twice, prior to exercise program and at the end of 12 weeks intervention. 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 (IBL, Immuno-Biological Laboratories Co., Japan) and the pro- BNP was measured as well by an immunoassay method using the Cobas e - 411 analyzer, Roche Diagnostics, Mannheim, Germany.*

***Results:*** *No significant (p=0.27) difference was found at baseline for S-Klotho levels between the two groups, 770.49pg/ml ± 202.20 sd and 727.54pg/ml ± 207.83 sd respectively, while a significant difference was found following exercise intervention, 863.39 pg/ml ± 213.66 sd in gr. A compared to 677.71pg/ml ± 167.46 sd in gr. B, p <0.01. S-Klotho and BNP showed an inverse correlation at baseline in group A, r = -0.803, P<0.01 and in group B, r = - 0.850, p <0.01, with similar values post 12 weeks, r = -0.829 & -0.834 respectively.*

***Conclusions:*** *Aerobic exercise may modulate S-Klotho activity, thus conferring a possible mechanism for the enhanced survival of coronary artery patients participating in an exercise based cardiac rehabilitation program.*

## Keywords

Exercise, Coronary Artery, Cardiovascular disease, Rehabilitation program.

## Introduction

In 1997 the gene that seems to control aging was discovered by Kuro-o et al. in 1997 [1] and was named it Klotho. The name

derives from the Greek goddess of life who is involved in vascular disease. Klotho is one of the Greek Moirai, the goddesses of fate who controlled the ultimate destiny of man, the death. Klotho spins the thread of life, Lakhesis measured the thread of life, and Atropos cut the thread of life. Kuro-o in his pioneer work identified Klotho in a mutant mouse strain that could not express Klotho gene, developed multiple disorders resembling human aging, and had a shortened life span [2]. The aging phenotypes included atherosclerosis, endothelial dysfunction, sarcopenia, skin atrophy, and impaired cognition. Overexpression of Klotho resulted in transgenic mice resulted in a significantly longer life span [3]. In an atherosclerotic mouse model, the in vivo gene delivery of Klotho protected against endothelial dysfunction [4]. Vascular klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23 [5].

Arking [6], found association between a functional variant of the Klotho gene and high density lipoprotein cholesterol, blood pressure, stroke, and longevity. Similarly, in community dwelling adults, higher plasma Klotho concentrations were independently associated with a lower likelihood of having cardiovascular disease [7]. Reduced Klotho was found to be associated with presence and severity of coronary artery disease [8], Moe S, in her editorial in Circulation [9] suggested that Klotho might even be considered the master regulator of cardiovascular disease. The reduction of circulating levels of Klotho is not only associated with the presence and severity of CAD, it is also an independent marker of some forms of vascular dysfunction such as arterial stiffness and endothelial dysfunction. It is related as well to the attenuation of vascular calcification as well as prevention of cardiac hypertrophy [10-12]. Exercise and exercise training has been related with increased Klotho serum levels in several works [13-15], thus conferring potentially beneficial cardiovascular effects. Brain natriuretic peptide (BNP) as well as Pro- BNP has been shown to be reduced following exercise training in CAD patients, with heart failure in particular [16-18].

Therefore, the purpose of the present study was to assess the effect of a 12 weeks exercise based cardiac rehabilitation programs in CAD patients with recent MI, PCI, and CABG on S-Klotho serum levels, and to assess possible association with BNP activity.

## Methods

Subjects - 41 CAD patients, age - 59.6 years ± 2.2 s.d., 30 males & 11 females who had a recent (<45 days) MI, PCI, or CABG were included in gr. A and underwent a supervised 12 weeks aerobic exercise based cardiac rehabilitation (45 min/4-5 sessions/week) and 17 age matched patients (age 61 years ± 2.4 s.d.12 males & 5 females) with proven CAD (recent or old MI, previous PCI or CABG) were included in the control group, gr. B. and required to continue their usual medical treatment and their usual lifestyle habits without a supervised exercise intervention. Gr. A patients were prescribed an individually tailored exercise program according to 75%-80% of their maximal heart rate achieved in a symptom limited exercise test performed at baseline, 24 hours prior to exercise program initiation. All patients, in both groups were in NYHA class I – II, mean left ventricular ejection fraction % (LVEF%) was 36.5 ± 4.1 in gr. A, and 35 ± 3.3 in gr. B (p=ns), risk factors prevalence was similar in both groups, with diabetes mellitus being more frequent in gr. A (11 patients, 26.8% vs 3 in gr. B, 17.6%), while smoking more in Gr. B (17.6% vs 14.6%).

Exclusion criteria included renal failure with creatinine levels ≥ 2.0 mg/dL, liver dysfunction, non-controlled hypertension, neurological or orthopedic diseases limiting patient ability to perform aerobic exercise.

Blood sampling - Peripheral venous blood samples (5 mL) were collected twice, at baseline, (in gr. A before the stress test was performed), and following 12 weeks, by an antecubital venipuncture technique into ethylenediam-inotetraacetate containing tubes. Time of day for blood sampling was in the morning and was kept consistent to avoid possible diurnal variation.

### Analysis

Blood samples were centrifuged for 15 minutes at 2700 rpm, separated and frozen at –70°C until use. S-Klotho levels in the serum were analyzed using a α-klotho enzyme linked Immunosorbent assay eliza kit (IBL, Immuno- Biological Laboratories Co, Japan). The kit has been validated and widely used for the measurement of klotho levels [10-12]. The intra- and interassay coefficients of variation ranged from 2.7 to 9.8%. Pro – BNP II was measured by an immunoassay method using the Cobas e - 411 analyzer, Roche Diagnostics, Mannheim, Germany.

All subjects (both groups) underwent a symptom limited sub maximal exercise test on a treadmill utilizing the standard Bruce protocol. Rest and peak exercise heart rate (rHR & pHR), rest and peak exercise systolic and diastolic blood pressure (rSBP, pSBP, rDBP, & pDBP), as well as the metabolic equivalents (METS) were determined. A written consent form was obtained from each subject, approved by the Clinical Science Center Committee on Human Subjects.

### Statistical methods

Continuous variables are expressed as the mean ± SD, and comparisons between groups were made using the Student's t-test for continuous variables. To achieve a normal distribution, the Klotho and Pro-BNP-II values were log transformed prior to the analysis. Single and multiple regression analysis were performed in order to find out possible relationship between S-Klotho as dependent variable and the various coronary risk factors, METS, HR, SBP, DBP, and LVEF% being independent variables. A p value of<0.05 was considered to be statistically significant, and all data were statistically analyzed using the Statistix software, version 10.

## Results

Both groups did not differ in baseline characteristics (Table 1) except for diabetes incidence that was higher in the exercise intervention group (p <0.05) and smoking status being more frequent in the control group (p<0.05).

| Variable | Gr. A | Gr. B |
|---|---|---|
| No. of subjects | 41 | 17 |
| Age (years) ± s.d. | 59.6 ± 2.2 | 61 ± 2.4 |
| Male/female ratio | 2.7 | 2.4 |
| LVEF% ± s.d. | 36.5 ± 4.1 | 35 ± 3.3 |
| Smoking % and no. | 14.6 (6) | 23.5 (4) ^^ |
| Diabetes % and no. | 26.8 (11) ± s.d. ^^ | 17.6 (3) |
| Hypertension % and no. | 36.6 (15) | 35.3 (6) |
| r-HR (bpm) ± s.d. | 71 ± 3.3 | 69 ± 2.6 |
| r – SBP ± s.d. | 119 ± 4.5 | 123 ± 3.9 |
| METS ± s.d. | 6.54 ± 1.36 | 6.91 ± 1.61 |

**Table 1:** Baseline characteristics.

^^ = p<0.05 (r-HR =rest heart rate, r-SBP = rest systolic blood pressure).

Baseline Klotho levels did not differ, 770.49 pg/ml ± 202.20sd in Gr. A compared to 727.54pg/ml ± 207.83sd in Gr. B (p=ns), while baseline BNP was higher in the control group, Gr. B compared to Gr. A, 992.49 g/ml ± 503.82sd vs 823.20pg/ml ± 499.67sd and 823.20pg/ml ± 499.67sd respectively, p<0.05.

Following 12 weeks of exercise training Klotho levels increased significantly in Gr. A, to 863.39pg/ml ± 213.66sd, p<0.05, while no change was noticed in the control group (Table 2). BNP levels were significantly reduced following exercise training program, from 823.20pg.ml ± 499.67sd to 684.90pg/ml ± 349.26sd, p<0.05, while no such change was noticed in the control group (Table 2). A single regression and multiple regression analysis showed that the Klotho levels were not correlated with LVEF%, smoking status, diabetes, and hypertension (p = ns), respectively, whereas there was an inverse correlation between the Klotho levels with the Pro-BNP (P<0.01). Another single regression analysis showed that the Klotho levels continued improving in proportion to increasing METS values, which is a sensitive survival predictor in CAD. A multiple regression analysis (Table 3) reaffirmed these results.

| variable | Baseline | Post exercise |
|---|---|---|
| **Klotho – Gr. A pg/ml** | 770.49 ± 202.20 | 863.39 ± 213.66 ^^ |
| **Pro – BNP Gr. A pg/ml** | 823.20 ± 499.67 | 684.90 ± 349.26 ^^ |
| **Klotho – Gr. B pg/ml** | 727.54 ± 207.83 | 677.71 ± 167.46 |
| **Pro – BNP Gr. B pg/ml** | 992.49 ± 503.82 ^^ | 1025.5 ± 506.47 |

^^ = p<0.05

| Explanatory variable | Regression coefficient | Standard regression coefficient | P value | 95% CI | VIF |
|---|---|---|---|---|---|
| **Pro-BNP** | 0.040 | 0.183 | < 0.01 | 0.024–0.057 | 1.286 |
| **LVEF%** | 0.012 | 0.009 | NS (0.09) | 0.007–0.018 | 1.257 |
| **Dyslipidemia** | 0.201 | 0.004 | NS | 0.070–0.332 | 1.085 |
| **Smoking** | 0.180 | 0.009 | NS (p=0.1) | 0.051–0.309 | 1.201 |
| **Hypertension** | 0.014 | 0.007 | NS (p = 0.84) | − 0.121 to 0.149 | 1.111 |

**Table 3:** Results of the multiple regression analysis of Klotho levels in all patients (=58).

## Discussion

Exercise capacity is known to be an important prognostic factor in patients with cardiovascular disease, and considered even to be a more powerful predictor of mortality among men than other established risk factors for cardiovascular disease [19-21]. Exercise training has been found in this study to have a possible modulatory effect on S-Klotho serum activity in CAD patients with a recent myocardial infarction or recent coronary artery perfusion procedure. This potentially beneficial effect has not proven in the control group that did not undergo an exercise training program. Regression analysis showed that the Klotho levels continued improving in the exercise intervention group in proportion to increasing METS values, thus, in accordance with the fact that exercise capacity is a powerful predictor of mortality in CAD patients. Furthermore, Klotho levels had a significant negative correlation with Pro-BNP II serum levels in both groups at baseline and following 12 weeks exercise program in Gr. A patients, thus, conferring an additional plausible explanation to the mechanisms aerobic exercise training improve survival and reduces cardiovascular morbidity in these patients. The study has two drawbacks, one, its small sample size, thus, not powered enough statistically, limiting conclusions, the second, derives from the information included in the informed consent. Patients enrolled to study were fully aware of the triad, S-Klotho, exercise, and longevity, thus, all were hyper motivated. In fact most of them reported additional exercise sessions at home.

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