

Effect of Aerobic Exercise Training on Circulating s-Klotho in a Heart Transplant Recipient; A Case Report

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ABSTRACT

Reduced circulating s-Klotho has been seen in heart failure patients, However, its values are increased following aerobic exercise training. The purpose of the case study was to examine whether high intensity aerobic exercise training increase serum s-Klotho levels in untrained heart transplant recipient, and to examine the relation between plasma s-Klotho concentration and peak oxygen uptake (VO_{2peak}). Methods: Untrained heart recipient 25 years old trained for $60 \text{ min}^{-1} \times 4 \text{ times} \times \text{wk}^{-1}$ for 12 wks^{-1} at 85% of VO_{2peak} . Testing sessions were performed at 0, and 12 weeks. Blood samples were analyzed for s-Klotho by an ELISA kit. Results: Subject increased its VO_{2peak} from 25.1 to 31.2 $\text{mL} \times \text{kg}^{-1} \times \text{min}^{-1}$, s-Klotho increased from 349 to 467 $\text{pg} \times \text{mL}^{-1}$. Conclusions: This case report indicated that aerobic exercise was tolerated without any difficulties, along with significantly improve in s-Klotho and peak oxygen uptake values. In addition, it is apparent that training at 85% work capacity in young heart transplant recipients following heart failure benefits to reestablish functional of central oxygen delivery variables mainly; cardiac output. Thus, it may have a better prognosis for the entire heart transplant recipient's population.

Keywords: heart failure, ELISA, maximal oxygen uptake, work intolerance, cardiac output.

INTRODUCTION

Following heart transplantation, an increase occurrence of late difficulties is noted, along with a slow decline in physical work capacity due to the previous terminal syndrome of end-stage heart failure period (1). In the early post-transplant period, prior studies reported that cardiac transplant recipients have low exercise work capacity due to muscle dystrophy and work intolerance. Following myocardial transplantation, functional capacity is reduced by 30% of age-predicted normal values. (2,3), while, VO_{2peak} values are 50% -70%. VO_{2peak} level is generally associated with a poorer prognosis (1). These decrements are due to reduced heart rate and stroke volume and thus cardiac output, along with reduction in; arterial vasodilation, reduced muscle mass and strength (sarcopenia) therefore, declined oxidative capacity (4). Low circulating levels of soluble Klotho (s-Klotho), are related to increased risk of age-associated diseases, however, it increases following aerobic exercise in young and older normal individuals and in coronary artery disease patients (5,6). Long lasting aerobic exercise training is an effective tool to increase work capacity in heart transplant recipients, and may inverse at least in part pathophysiological responses (7). However, information is limited on the influence of aerobic exercise training on circulating s-Klotho in cardiac transplant recipients, who undergo aerobic exercise rehabilitation. Therefore, the present case study was designed to assess the effect of 12 weeks high intensity aerobic exercise training on circulating s-Klotho and VO_{2peak} in cardiac transplant recipient.

METHODS

Patient Characteristics

One patient age 25 years old, height 184 cm and weighing 75 kg, volunteered for this study, he was approximately 1year post-cardiac transplantation, due to ischemic dilated cardiomyopathy. Patient was treated with a calcineurin inhibitor and an antiproliferative agent, without treatment for hypertension. Patient had a very mild reduced LVEF (45%) without the presence of, left ventricular hypertrophy, and 1.4 plasma creatinine $mg \times dL^{-1}$. A written consent form, approved by the Clinical Science Center Committee on human subjects was obtained from the subject, after being fully informed of the details and possible discomforts associated with the experimental protocol.

Measurements and Treatment

Following warm up, subject underwent a graded maximal treadmill exercise test. Maximal test was terminated according to the guidelines of the American College of Sports Medicine (8), namely: a) leveling off or no further increase in VO_2 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 Patient underwent two maximal modified Balke graded exercise treadmill test at baseline and after 12 weeks up to peak oxygen uptake and cardiopulmonary responses. Time of day for the two tests was kept consistent to control for problems associated with diurnal variations. Following 5 minutes of warmup, treadmill initial testing speed was kept constant at $5.5 km \times h^{-1}$ while grad was increased by 3% each 2 min. VO_{2max} 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. A 12-lead electrocardiogram and heart rate were continuously monitored at rest, during exercise and recovery using a Burdick Eclipse 400 3-channel, 12-lead ECG recorder system, and oscilloscope. Five-second recordings were obtained at rest and at

VO₂peak obtained during the last minute of the test period. Heart rates were recorded with a 5 sec. interval throughout testing.

Patient completed a 12-week supervised swimming training period 60 min⁻¹×4 times×wk⁻¹ above at training heart rate of 145-150 beats×min⁻¹ which corresponded to 80% of VO₂max.

Lactate Measurement

A 25 µl fingertip blood sample was taken at rest and at the end of the 2nd minute post any sprint for the determination of lactate response to strenuous exercise. The sample was immediately transferred to a micro-tube containing 100 µl of 7% perchloric acid. The tubes were centrifuged after standing at least for an hour. Twenty- microliter aliquots of the supernatant were subsequently used for lactic acid analysis on the Analox LM3 analyzer (Analox Instruments, England; Reagent Kit # GMRD-071).

Blood Sampling and Procedures

Peripheral venous blood samples (2.5 mL) were collected by sterile antecubital venipuncture techniques into ethylenediaminetetraacetate 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 Immunosorbent Assay ELISA kit (Immuno-Biological Laboratories Co, Japan). The kit has been validated and widely used for the measurement of klotho levels (9-12). Measurements were conducted according to the manufacturer instructions. The intra- and intraassay coefficients of variation ranged from 2.7 to 9.8%.

We attest to withstanding the strict compliance with the ISHLT ethics statement.

RESULTS

Table 1 reveals that following 12 weeks of swimming training participant improved at rest, s-Klotho levels, minute ventilation, breathing frequency, heart rate, systolic and diastolic blood pressures and lactate. At peak exercise test, workload was increased notable, as reflected from the VO₂peak increase compared to pre-test. This was achieved with similar values of maximal heart rate, lower systolic and diastolic blood pressures, minute ventilation and higher lactate values.

Table 1. Cardiopulmonary exercise test results at rest and following 12 weeks of aerobic training.

Variables	Pre-rest	Post-rest	Pre-training	Post-training
VO ₂ (mL×kg ⁻¹ ×min ⁻¹)	5.2	5.1	26.1	42.2
s-Klotho pg×mL ⁻¹)	349	451	-----	-----
HR (beats×min ⁻¹)	86	78	172	176
SBP (mmHg)	132	124	238	202
DBP (mmHg)	89	82	95	83
VE (L×min ⁻¹)	27.3	24.5	147.1	166.4
F (breaths×min ⁻¹)	24	17	53	47
Lactate (mM×L ⁻¹)	2.5	1.7	11.1	12.2
Maximal workload	-----	-----	6 km×h ⁻¹ 5%	6 km×h ⁻¹ 16%

VO₂ = Oxygen uptake; HR = Heart rate; SBP = Systolic blood pressure, DBP = Diastolic blood pressure; VE = Minute ventilation; F = Breathing frequency.

DISCUSSION

This case study demonstrated that following 12 weeks of vigorous aerobic swimming training, circulating s-Klotho and VO₂peak levels increased in the young recipient similar to the values of untrained young healthy individuals (13). Secreted klotho regulates nitric oxide production in the endothelium. It has been suggested that Klotho affect nitric oxide which in turn benefits endothelial function. s-Klotho where it may function as a vasculo-protective hormone possibly by enhancing endothelial function (14,15), or direct inhibition of vascular calcification and atherosclerosis (16). Klotho appears to apply different functions in distinct cell types in a reliant or, none-reliant means.

It suggests that in healthy and cardiac patients, circulating s-Klotho levels were increased in response to long-lasting aerobic exercise training, and that the s-Klotho response depends on fitness level, since it increases in a similar pattern as VO₂max (17). Thus, long lasting aerobic training may be an appropriate model for mechanistically probing the role of aerobic exercise on s-Klotho expression in heart recipients (18).

The increase in s-Klotho in the heart recipient following swimming training, may be a response to oxidized free radical species that increase in muscle cells as a result of aerobic bout. Although the association between s-Klotho and aerobic exercise training is not clear, s-Klotho reduces programmed cell death (apoptosis) through the nitric oxide production and thus, suppress

oxidized free radical species (19). Previously, increase of circulating s-Klotho was parallel to increase in $VO_2\text{max}$ in response to an acute exercise in young and old mice (18).

Oxygen uptake during exercise depends on hemodynamics, peripheral vascular resistance, cardiac output (oxygen delivery), skeletal muscle (oxygen extraction) and matched ventilation-perfusion (20). Heart transplant recipients have a significant low and inadequate cardiac output due to the autonomic denervation that limits maximal chronotropic response, stroke volume and left ventricular contractility (21). Limited cardiac output response and pulmonary disorders, that are usual in patients with advanced heart failure, contributes to the lesser $VO_2\text{max}$, common seen in these patients (22,23). Indeed, evaluating findings detected on pre and postoperative assessment of heart failure patients, who underwent heart transplantation, suggested that following heart transplantation pulmonary complications are common (24), due to the inability of respiratory muscles, rather than by interstitial variations or diffusion irregularities (25). Thus, $VO_2\text{peak}$ was limited by delivering factors rather than peripheral aspects. This suggests that the physical inactivity during the terminal syndrome of end-stage heart failure period decrease the pulmonary capability. These changes that compromise the percentage of saturated hemoglobin in the arterial blood, could also contribute to the lower $VO_2\text{peak}$. Additional factor is the altered skeletal muscle histology due to the severe terminal syndrome of end-stage heart failure period characterized by a reduced muscle's oxygen extraction thus, oxidative capacity, and energy production are reduced (26). In the present the case study, achieved $VO_2\text{peak}$ was not limited by its muscular stamina.

Prolonged and high-intensity exercise may result in oxidative damage to macromolecules in both blood and skeletal muscle, however it increases circulating s-Klotho values for the oxidized free radical species production. On the contrary, low intensity of aerobic training does not produce high oxidized free radical species levels therefore, circulating s-Klotho levels does not increase (19,27).

Previously it has been suggested that aerobic training above the anaerobic threshold intensities increase $VO_2\text{max}$ (28). Aerobic exercise like running, cycling and swimming appear to benefit heart recipients and minimize the physiological alterations that occurred during the end-stage heart failure period and may contribute to improvements in health and well-being (29).

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, b. mechanisms causal the effects of aerobic exercise on Klotho was not investigated in this study and c. only one participant was trained.

CONCLUSIONS

This case report indicates that aerobic exercise was tolerated without any difficulties, along with significantly improve in s-Klotho and peak oxygen uptake values. In addition, it is apparent that training at 80% work capacity in young heart transplant recipients following heart failure benefits to reestablish functional of central oxygen delivery variables mainly; cardiac output. Thus, it may have a better prognosis for the entire heart transplant recipient's population. In addition to earlier studies, this case study may add that planned aerobic exercise training regime as a rehabilitation tool is harmless while leading to improved exercise tolerance.

CONFLICT OF INTEREST

The authors wish to acknowledge that no external funding was received for this study and that no conflict of interest of any sort exists.

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