



SJMLS-3(2)-008

Effect of Cardiovascular Exercise on Cystatin C Level of Healthy Subjects in South Eastern NigeriaSaheed Opeyemi Usman*¹, Samuel Chukwuemeka Meludu², Ifeoma Joy Onuora¹, Chikaodili Nwando Obi-Ezeani¹, Chudi Emmanuel Dioka¹,*Department of Chemical Pathology, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi, Nigeria¹,
Department of Human Biochemistry, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi².**Author for Correspondence*: Senatorhopsy@yahoo.com/+234-803-467-6223***Abstract**

Sedentary lifestyle is an issue of great concern because of its deleterious health implications in developed and developing countries. It is associated with limited physical activity thus exposing people to high risk of developing various health conditions. The objective of this study was to determine and compare results of moderate and vigorous exercises on cystatin C before exercise, four weeks after exercise, eight weeks after exercise and twelve weeks after exercise. Serum concentration of cystatin C of both vigorous exercise group (30 male individuals who played football for 40 minutes daily for 3 days/week) and moderate exercise group (30 male individuals who engaged in mild jogging for 30 minutes daily for 5 days/week) were determined using Enzyme Linked Immunosorbent Assay (ELISA) technique. All data were expressed as Mean \pm Standard Deviation (SD) and analyzed with Analysis of Variance (ANOVA) while multiple comparisons were done using Post Hoc test. Pearson's correlation coefficient was used for correlational analysis. The mean age for subjects in moderate exercise group was 23.13 ± 2.11 years while mean age for subjects in vigorous exercise group was 22.63 ± 1.67 years ($p > 0.05$). In both moderate and vigorous exercise groups, the mean serum concentration of Cystatin C was significantly reduced ($p < 0.05$) 8 weeks and 12 weeks after exercise as compared with the result before exercise as well as 12 weeks after exercise as compared with the results 4 weeks after exercise. Cardiovascular exercise leads to reduced cystatin levels, indicating an enhanced glomerular filtration rate and reduced risks of cardiovascular disease thus contributing a beneficial effect of maintenance of sound health.

Keywords: Cystatin, Exercise, Cardiovascular, Nigeria.

Introduction

Physical inactivity and preventable deaths continue to be on the rise in Nigeria especially in the urban centers where it has become a growing problem. Physical inactivity, which contributes mainly to the high prevalence of most non-communicable diseases (NCDs) that predominate in adults alongside their high mortality, has been reported to be a major and leading risk factor for many chronic conditions and diseases (WHO, 2009). Physical exercise is any bodily activity that enhances or maintains physical fitness and overall health and wellness. It improves mental health, helps prevent depression and promote positive self-esteem (Kawano *et al.*, 2009). Cystatin C is a protein mainly used as a biomarker of kidney function. Recently, it has been studied for its role in predicting new-onset or deteriorating cardiovascular disease. Cystatin C is removed from the bloodstream by glomerular filtration in the kidneys. If kidney function and glomerular filtration rate decline, the blood levels of cystatin C rise, thus elevated levels of cystatin C indicate decreased GFR. Serum levels of cystatin C are a more precise test of kidney function than serum creatinine levels (Roos *et al.*, 2007). Cystatin C levels are less dependent on age, gender, race and muscle mass compared to creatinine. If kidney function and glomerular filtration rate decline, the blood levels of cystatin C rise (Stevens *et al.*, 2008).

In a 2008 study on the influence of physical activity on serum cystatin C, it was reported that there were no significant differences in the serum cystatin levels among the groups classified as sedentary, mild or moderate/intense physical activity (Baxmann *et al.*, 2008). A 2009 study in South-Western Nigeria on plasma biochemical changes during moderate and vigorous exercises reported that the level of Urea was significantly elevated while the levels of Sodium and

Potassium were significantly reduced in vigorous exercising subjects compared with the controls (Adedapo *et al.*, 2009). In the United States (US), a study on linking physical activity to lower serum cystatin C among US adults showed that higher physical activity is associated with lower serum cystatin C (Faramawi *et al.*, 2010). This study was therefore carried out to determine and compare results of the effect of moderate and vigorous cardiovascular exercises on cystatin C before exercise, two weeks after exercise, eight weeks after exercise and twelve weeks after exercise.

Methods

Study site/ subject selection/study design

The study was conducted at Okofia playing ground on the Nnamdi Azikiwe University, Okofia, Nnewi, Anambra State, Nigeria. Total study size of 60 subjects but 240 serum samples were used. They were divided into two groups: Group 1 (Vigorous Exercise) - This group consisted of 30 individuals who played football for 40 minutes per day (3 days/week). Group 2 (Moderate Exercise) -This group consisted of 30 individuals who engaged in mild jogging for 30 minutes daily (5 days/week). A baseline specimen was obtained from each subject before exercise. After four weeks, eight weeks and twelve weeks of respective training, fresh samples were collected from each subject.

Inclusion and exclusion criteria

Inclusion criteria for subjects were: physically healthy male individuals' ages 18-35 years, occasional or non-alcohol consumers, non-smokers, as well as, those not on drugs especially that will interfere with the parameter studied. Subjects physically unhealthy (males), outside the age range, regular alcohol consumers, smokers and those on drugs that will interfere with the parameter studied, were all excluded.

Sample size

Sample size calculation was done using 95% confidence interval, 0.05 precision and prevalence rate. There seem to be no data available as regards the proportion of Anambra State residents that participate in various forms of physical exercise, but, high physical activity levels assessed in Ibadan, Western Nigeria, reported 1.7% (Odunaiya *et al.*, 2010). The formula for sample size when population is more than 1000 is: $n = Z^2PQ/d^2$ (Alexander *et al.*, 2013).

$$n = Z^2PQ/d^2$$

Where: n = sample size, d = degree of precision (taken as 0.05), Z = standard normal deviation at 95%

confidence interval which is 1.96, P = proportion of the target population (estimated at 1.7% which is $1.7/100 = 0.017$), Q = alternate proportion (1-P) which is $1-0.017=0.983$

$$n = (1.96)^2 (0.017)(0.983) = 26 \\ (0.05)^2$$

Sample collection, storage and analysis

A 5ml fasting blood sample was aseptically collected into plain sample containers from each of the participating individuals by venipuncture on each of the three occasions sample was withdrawn between 7.30am and 10am. Blood samples were centrifuged at 4000 Revolution per Minute (RPM) for 10 minutes and the serum of each sample was extracted into fresh plain bottle for analysis. Serum samples were analyzed promptly after centrifugation while those not analyzed immediately analyzed were stored at - 20 degree Celsius until analysis few days later. Serum cystatin C was analyzed by Enzyme Linked Immunosorbent Assay (ELISA) technique.

Principle of enzyme linked Immunosorbent assay (ELISA)

The antigens or antibodies present in patient's sample were allowed to bind to a polyvinyl plate and then the microtiter plate was then washed to separate antigens or antibodies from remaining sample components. To this plate, a corresponding second antigen or antibody was added to get fixed to the already adhered first antigen in the plate. A tagged enzyme and a suitable substrate is added. The enzyme reacts with the substrate to produce a color. This color produced is measurable as a function of antigens or antibodies present in the given sample.

Ethical Consideration

Ethical approval was obtained from the Ethical Research Committee of the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria.

Statistical analysis

Data was statistically analyzed using Statistical Package for the Social Sciences (SPSS) for windows version 23.0 software. Data were expressed as Mean \pm Standard Deviation (SD). Statistical analysis of the data before exercise, four weeks after exercise, eight weeks after exercise and twelve weeks after exercise was performed by Analysis of Variance (ANOVA) while multiple comparisons were done using Post Hoc test. Significance was fixed at $p < 0.05$ and highly significant if $p < 0.01$. Pearson's correlation coefficient was used for correlational analysis of the test.

Results

Physical and biochemical parameters

The mean age for subjects in moderate exercise group was 23.13 ± 2.11 years while mean age for subjects in vigorous exercise group was 22.63 ± 1.67 years, with the mean age of both groups not significantly different ($p < 0.05$).

In both moderate and vigorous exercise groups, the mean serum concentration of Cystatin C was significantly reduced ($p < 0.05$) 8 weeks and 12 weeks after exercise as compared with the result before exercise as well as 12 weeks after exercise as compared with the results 4 weeks after exercise.

Table 1: Comparison of Serum Cyst C Levels for Moderate Exercise Group Before and after 4, 8 and 12-Weeks Exercise

Groups	Parameters
	CYST C (mg/L)
Before exercise	0.71 ± 0.09
4 weeks after exercise	0.68 ± 0.07
8 weeks after exercise	0.65 ± 0.07
12 weeks after exercise	0.61 ± 0.04
F-value	10.495 2.182
p-value	0.001*
Post Hoc	
a/b	0.645
a/c	0.012*
a/d	0.001*
b/c	0.744
b/d	0.002*
c/d	0.176

Key:

a – before exercise

b – 4 weeks after exercise

c – 8 weeks after exercise

d– 12 weeks after exercise

Cyst C – Cystatin C

** = Results compared are significantly different at P-value < 0.05 (p < 0.05).*

Table 2: Comparison of Serum Cyst C Result for Vigorous Exercise Group at baseline and after 4, 8 and 12-Weeks of Exercise

Groups	Parameters
CYST C (mg/L)	
Before exercise	0.67 ± 0.08
4 weeks after exercise	0.64 ± 0.07
8 weeks after exercise	0.60 ± 0.06
12 weeks after exercise	0.56 ± 0.05
F-value	17.122 2.182
P-value	0.001*
Post Hoc	
a/b	0.376
a/c	0.001*
a/d	0.001*
b/c	0.118
b/d	0.001*
c/d	0.082

Key:

a – before exercise

b – 4 weeks after exercise

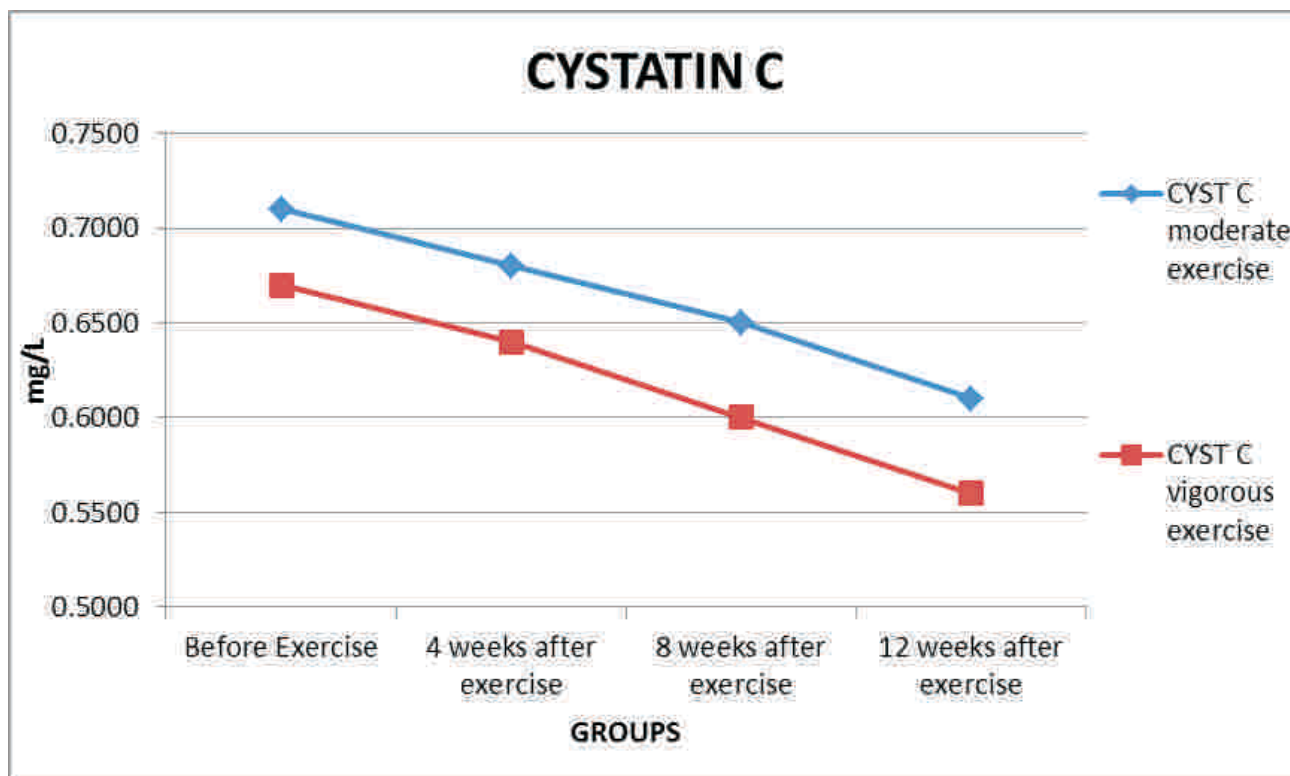
c – 8 weeks after exercise

d – 12 weeks after exercise

Cyst C – Cystatin C

** = Results compared are significantly different at P-value < 0.05 (p < 0.05).*

Figure 1: Graph Showing Mean Serum Concentration of Cystatin C (CYST C) for Both Moderate and Vigorous Exercise Groups



Discussion

In both moderate and vigorous exercise groups, the mean serum concentration of Cystatin C was significantly reduced ($p < 0.05$) 8 weeks and 12 weeks after exercise as compared with the result before exercise as well as 12 weeks after exercise as compared with the results 4 weeks after exercise. This research outcome is very similar to that carried out in the United States (US) where higher physical activity was reported to be associated with lower serum cystatin C (Faramawi *et al.*, 2010). Our finding is however at variance with some other studies that showed that serum urea was significantly elevated following vigorous exercise compared with controls (Adedapo *et al.*, 2009). Our finding is also at variance with another study which indicated that there were no significant differences in the serum cystatin levels among the groups classified as sedentary, mild or moderate/intense physical activity (Baxmann *et al.*, 2008). The significant reduction in cystatin C observed in both exercise groups mostly 8 weeks and 12 weeks after exercise might be attributed to the fact that cystatin C, a low molecular weight protein, is removed from the bloodstream by glomerular filtration and reabsorbed and metabolized at tubular level in the kidneys, as cystatin C is removed from the bloodstream by glomerular filtration in the kidneys.

Perhaps, this infers that whenever kidney function and glomerular filtration rate decline, the blood levels of cystatin C rise, thus elevated levels of cystatin C indicate decreased GFR and vice-versa. Thus, the implication of a reduced serum cystatin C concentration in this study is an indication for an enhanced glomerular filtration rate (GFR) (Roos *et al.*, 2007).

Conclusion

Cardiovascular exercise leads to reduced cystatin levels, indicating an enhanced glomerular filtration rate and reduced risks of cardiovascular disease, contributing a beneficial effect of maintenance of sound health.

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Citation: Saheed Opeyemi Usman, Samuel Chukwuemeka Meludu, Ifeoma Joy Onuora, Chikaodili Nwando Obi-Ezeani, Chudi Emmanuel Dioka.. *Effect of Cardiovascular Exercise on Cystatin C Level of Healthy Subjects in South Eastern Nigeria.* *Sokoto Journal of Medical Laboratory Science*;3(2):65- 70.

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