

## Effect of Chester-step Aerobic Exercise on the Levels of Oxidized Low-Density Lipoprotein and Adiponectin in Adult Subjects in Nnewi Metropolis.

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### ABSTRACT

**Background:** Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide with sedentary lifestyle, characterized by consistently low levels of physical activity, now recognized as a leading contributor to cardiovascular diseases. **Objective:** This was a longitudinal prospective study aimed at assessing the serum levels of adiponectin and oxidized low-density lipoprotein (Ox-LDL) in subjects undergoing chester-step aerobic exercise in Nnewi metropolis, Anambra State. **Materials and methods:** The aerobic exercise involved a standard chester step test bench protocol which was repeated for at least 3 times a week for a six-week period. A total of 30 subjects (18-50years) were recruited for this study. 5 ml of blood samples were collected before and after the stipulated period of aerobic exercise. Levels of adiponectin and Ox-LDL were determined using the Enzyme Linked Immunosorbent Assay (ELISA) technique. **Result:** The pre-exercise BMI mean value ( $24.07 \pm 3.64$ ) was not significantly different from the post-exercise BMI ( $24.02 \pm 3.94$ ) ( $p > 0.05$ ). There was also non-significant difference in the mean serum levels of adiponectin and Ox-LDL ( $p > 0.05$ ) in pre- and post-chester-step aerobic exercise subjects. A non-significant positive correlation between adiponectin ( $r = 0.324$ ,  $p = 0.081$ ) and Ox-LDL ( $r = 0.146$ ,  $P = 0.442$ ) with age. There was also a non-significant positive correlation between adiponectin and Ox-LDL with body mass index ( $r = 0.122$ ,  $p = 0.521$  and  $r = 0.197$ ,  $p = 0.29$ ) respectively. **Conclusion:** The non-significantly decreased mean levels of adiponectin and oxidized low density lipoprotein after chester-step aerobic exercise against pre-exercise values, suggest that chester-step aerobic exercise did not significantly alter cellular levels of adiponectin and Ox-LDL. However, the post-exercise decrease in mean levels of adiponectin and Ox-LDL against pre-exercise values (albeit non-significant), demonstrates that a more rigorous longitudinal aerobic exercise could yield a better outcome.

**Keywords:** cardiovascular diseases, adiponectin, oxidized low density lipoprotein, chester-step aerobic exercise,

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## INTRODUCTION

Aerobic exercise refers to the use of oxygen in muscles' energy-generating process. "Aerobic" means "relating to, involving, or requiring free oxygen"(1) and refers to the use of oxygen to adequately meet energy demands during exercise via aerobic metabolism (2). Aerobic exercise includes any type of exercise, typically those performed at moderate levels of intensity for extended periods of time that maintains an increased heart rate. In such exercise, oxygen is used to "burn" fats and glucose in order to produce adenosine triphosphate (ATP), the basic energy carrier for all cells. Aerobic exercise such as walking not only improves fitness but also improves overall quality of life and decreases all-cause mortality (2,3). Generally, light-to-moderate intensity activities that are sufficiently supported by aerobic metabolism can be performed for extended periods of time (4). Among the recognized health benefits of doing regular aerobic exercise are; strengthening the muscles involved in respiration, to facilitate the flow of air in and out of the lungs, enlarging the heart muscle, to improve its pumping efficiency and reduce the resting heart rate, known as aerobic conditioning, improving circulation efficiency and reducing blood pressure, increasing the total number of red blood cells in the body, facilitating transport of oxygen, reducing the risk for diabetes (One meta-analysis has shown, from multiple conducted studies, that aerobic exercise does help lower HbA<sub>1C</sub> levels for type 2 diabetics) (3) and reducing the risk of death due to cardiovascular problems (5).

The Chester step test (CST) is one of many tests designed to provide a safe and practical means of assessing aerobic fitness under submaximal conditions and was originally developed by Kevin Sykes at University College Chester to assess aerobic fitness by predicting maximal aerobic power (VO<sub>2</sub>max) in fire brigades in Britain, Europe, USA, and Asia, and

more recently for work with airport firefighters, the ambulance service, health authorities, and corporate institutions (6). Examples of other similar tests include the Astrand-Ryhming nomogram cycle ergometer protocol (7). To date, the validity of the CST has only been assessed in terms of its ability to predict VO<sub>2</sub>max compared with an actual VO<sub>2</sub>max, with the error in this ranging from 5% to 15% (8,9). Body mass index (BMI) is a value derived from the mass (weight) and height of a person. The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m<sup>2</sup>, resulting from mass in kilograms and height in meters. The body mass index (BMI) is the metric currently in use for defining anthropometric height/weight characteristics in adults and for categorizing them into groups (10). Overweight and obesity are clearly associated with alterations in the lipid profile and raise in the markers of systemic inflammation, including C-reactive protein (CRP) and pro-inflammatory cytokines, such as interleukin- (IL-) 1 beta (IL-1 $\beta$ ), IL-6, and tumor necrosis factor alpha (TNF- $\alpha$ ) (11,12).

Adiponectin (also referred to as GBP-28) is a protein hormone produced by adipocytes that regulates metabolic processes and improves insulin sensitivity (13). It is a hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation (14). It is the most abundantly secreted product of adipocytes and is strongly correlated with cardio-metabolic risk (14). The mechanisms whereby adiponectin exerts its favorable cardio-metabolic effects are not fully understood, but could involve improvement in insulin action, decreased inflammatory tone, interaction with fibroblast growth factor (FGF)-21 and amelioration of lipotoxicity, among others (15). In rodent models, over expression of adiponectin is associated with several beneficial effects, including improved metabolic flexibility, decreased

inflammatory markers, decreased steatosis, improved insulin sensitivity, decreased apoptosis and preservation of  $\beta$ -cell function(15,16).

Oxidized low-density lipoprotein (Ox-LDL) is a critical factor in the initiation and progression of atherosclerosis and contributes to endothelial dysfunction and plaque destabilization through multiple mechanisms (17). Ruptured plaques are rich in lipids, which usually occupy more than 40% of plaque volume (18). Human studies have confirmed that Ox-LDL and oxidized lipid byproducts are present within atherosclerotic plaques (19). Lipoprotein oxidation is known to enhance atherogenesis by several different mechanisms (20,21). During oxidation, a variety of highly reactive breakdown products, such as malondialdehyde (MDA), (22) are generated, which additionally modify closely associated lipids and proteins into immunogenic epitopes. Atherogenesis starts when native LDL (nLDL) accumulates in the vessel wall at sites of injury and is oxidized by products from macrophages, smooth muscle cells, and endothelial cells (22). Oxidized LDL (Ox-LDL) accumulates in monocytes that have infiltrated the sub-endothelium and differentiated into macrophages. The resulting foam cells are characteristic for the early atherosclerotic lesion (20). Ox-LDL further contributes to atherosclerosis because it contains lysophosphatidic acid (LPA), which starts platelet shape change and aggregation (20). Cardiovascular disease (CVD) are diseases that affect the heart or blood vessels with a prevalence of about 18.2 million adults age 20 and older (about 6.7%) (23) and these include, coronary artery diseases (CAD) such as angina and myocardial infarction, heart attack (24). There are lots of risk factors that predisposes CVDs most commonly is sedentary lifestyle, characterized by consistently low levels of physical activity (2), as a result, cardiovascular disease has become a leading cause of morbidity and

mortality worldwide. Regular exercise and physical activity are associated with remarkable widespread of health benefits and a significantly lower CVD risk. It has been reported that physical inactivity may be a predictor of not only cardiovascular but also total mortality in middle-aged men and women (2). Aerobic exercise involves light-to-moderate intensity activities performed for extended periods of time with resultant aerobic metabolism (23). Oxidative modification of low-density lipoprotein (LDL) is a key step in the pathogenesis of atherosclerosis (24). Since, adiponectin possesses insulin-sensitizing and anti-atherosclerotic properties and Oxidized low-density lipoprotein (Ox-LDL) being implicated in the initiation and progression of atherosclerosis, contributing to endothelial dysfunction and plaque destabilization through multiple mechanisms, it will be pertinent to ascertain the impact of aerobic exercise on the levels of adiponectin and oxidized low density lipoprotein. Hence, this study was designed to evaluate the levels of adiponectin and oxidized low density lipoprotein in subjects undergoing aerobic exercise in Nnewi Metropolis

## Materials and methods

### Study design

This was a longitudinal prospective study designed to evaluate the serum levels of adiponectin and oxidized low density lipoprotein in subjects undergoing aerobic exercise in Nnewi metropolis. The aerobic exercise involved a standard chester step test bench protocol carried out for a period of six weeks. 5 ml of blood samples were collected before and after exercise and a total of 30 male subjects were recruited for the study. The baseline samples served as controls while the post exercise specimen were used as test samples.

Subjects undergoing chester-step aerobic exercise between the ages of 18-50 years of age were included. While subjects outside the age range of 18-50 years, smokers, subjects with known cardiac

diseases, other related ailments such as diabetes and alcoholics were excluded from the study.

#### **Ethical approval**

The ethical approval for this research was obtained from the Ethics committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State of Nigeria, with the reference number: **NAUTH/CS/66/VOL.12/086/2019/046.C** onsent of the subjects was sought and obtained prior to study. 5ml of venous blood was aseptically collected from each participant before and after aerobic exercise by venipuncture; dispensed into a plain container and allowed to clot. Serum obtained after centrifugation at 3000rpm for 5minutes was refrigerated until analyzed.

#### **Determination of Oxidized Low Density Lipoprotein (Ox-LDL).**

The levels of oxidized low density lipoprotein were evaluated by sandwich enzyme linked immunoassay (ELISA) technique using anti-oxidized phosphatidycholine monoclonal antibody and antihuman apolipoprotein-B antibody as described by Itabe and Ueda (25).

Serial dilution of the standard was made on 10 standard wells on the micro-titre plate by pipetting 100ul of standard each into wells 1 and 2 and diluting with 50ul of standard dilution to wells 1 and 2. The same trend was continued up to wells 9 and 10 from where 50ul each were pipetted out and discarded leaving about 50ul of working solution in each well (density-180ug/L, 120ug/L, 60ug/L, 30ug/L, 15ug/L). Blank and sample wells were set with the blank well containing chromogen A and B and stock solutions only whereas 45ul of sample dilution was added to sample well followed by the addition of 10ul of specimen. 50ul of HRP conjugate reagent was further added to each well except blank well, followed by 30 minutes incubation at 37°C. After incubation, the micro-titre wells were washed with wash solution for 3 times

consecutively. 50ul of chromogen A and B was added to each well, covered and incubated for 10 minutes at 37°C. The reaction was stopped by the addition of 50ul of stop solution into each well. The reaction complex formed (yellow colour) was measured photometrically at 450nm wavelength.

#### **Determination of adiponectin**

The levels of adiponectin were evaluated using the sandwich enzyme linked immune sorbent assay (ELISA) method as described by Nishimura and Sawai, (26). Serial dilution of the standard was made on 10 standard wells on the micro-titre plate by pipetting 100ul of standard each into wells 1 and 2 and diluting with 50ul of standard dilution to wells 1 and 2. The same trend was continued up to wells 9 and 10 from where 50ul each were pipetted out and discarded leaving about 50ul of working solution in each well (density: 900ug/L, 600ug/L, 300ug/L, 150ug/L, 75ug/L). Blank and sample wells were set with the blank well containing chromogen A and B and stock solutions only whereas 40ul of sample dilution was added to sample well followed by the addition of 10ul of specimen. 50ul of HRP conjugate reagent was further added to each well except blank well, followed by 30 minutes incubation at 37°C. After incubation, the micro-titre wells were washed with wash solution for 3 times consecutively. 50ul of chromogen A and B was added to each well, covered and incubated for 10 minutes at 37°C. The reaction was stopped by the addition of 50ul of stop solution into each well. The reaction complex formed (yellow colour) was measured photometrically at 450nm wavelength.

#### **Determination of body mass index (BMI)**

BMI was evaluated by anthropometric method as described by Janssen *et al.*, (27).

Height (m) was measured using a stadiometer. Whole body weight (kg) was

taken using a body weight weighing scale with the subject wearing light clothing and without shoes. Body mass index (BMI) was calculated as the ratio of weight (kg) to the square of height (m<sup>2</sup>)

**Chester step test bench exercise protocol**

To begin, blood sample was firstly collected before exercise as baseline (control) from each subject. A heart rate monitor (Omron blood pressure monitor) was used to check resting blood pressure (mmHg) and the pulse rate according to the manufacturer’s instructions. BMI (height and weight) and age of subjects were taken and recorded. Subjects were then given instructions by the test administrator (researcher) on how to carry out the test, including a brief demonstration. Using a standard metronome counter software which was set at 94bpm (94 steps), subjects then commenced the exercise which was done till visible exhaustion. Once subject reached exhaustion, blood pressure and

pulse rate wasand then checked. Exercise protocol was repeated for at least 3 times a week for 5 weeks period with blood pressure and pulse rate checked after every routine exercise, after which BMI data was then recorded.

**Statistical analysis**

The statistical analysis was performed using the paired student’s t-test. Values were deemed significant at p<0.05. Correlation studies were performed using the Pearson’s correlation coefficient. Statistical package for social sciences (SPSS) 21.0 was used for the statistical analysis.

**Results**

Table 1 shows the Demographic and anthropometric characteristics in pre and post aerobic exercise subjects.

There was no significant difference in the mean body mass index (p=0.194) and age (p=0.286) in pre and post aerobic exercise subjects.

**Table 1 Demographic and anthropometric characteristics in pre and post aerobic exercise of subjects (Mean±SD.)**

| Parameters              | Pre-Chester step exercise | Post-Chester step exercise | Wilcoxon sign Rank | P-value |
|-------------------------|---------------------------|----------------------------|--------------------|---------|
| BMI(kg/m <sup>2</sup> ) | 24.07±3.64                | 24.02± <b>3.94</b>         | -1.299             | 0.194   |
| AGE(years)              | 24.07±2.98                | —                          | <b>-1.067</b>      | 0.286   |

\*Statistically significant at p<0.05

**Table 2 Levels of adiponectin and oxidized LDL in pre and post aerobic exercise subjects. (Mean±SD)**

| Parameters   | Pre-Chester step exercise | Post-Chester step exercise | Wilcoxon sign rank | P-Value |
|--------------|---------------------------|----------------------------|--------------------|---------|
| Adiponectin  | 139.14±228.64             | 134.08± <b>311.43</b>      | <b>-1.173</b>      | 0.241   |
| Oxidized LDL | 22.82±38.27               | 22.09±56.86                | -1.491             | 0.136   |

Table 2 shows the mean levels of adiponectin and oxidized LDL in pre and post aerobic exercise subjects.

There was no significant difference in the mean serum levels of adiponectin (p>0.05) in pre and post aerobic exercise subjects.

No significant difference was also observed in the mean serum levels of oxidized LDL in pre and post aerobic exercise subjects (p>0.05).

Table 3 shows the correlation of the mean levels of adiponectin and oxidized low density lipoprotein with age in post exercise subjects.

A non-significant positive correlation between the mean levels of adiponectin with age ( $r=0.324$ ,  $p=0.081$ ) and mean levels of oxidized LDL with age ( $R=0.146$ ,  $P=0.442$ ) in post aerobic exercise subjects.

Table 4 shows the association between the mean levels of adiponectin and oxidized low density lipoprotein, with BMI in post aerobic exercise subjects.

A non-significant positive correlation existed between the mean serum levels of oxidized low density lipoprotein with BMI ( $r=0.197$ ,  $p=0.297$ ) and the mean levels of adiponectin with BMI ( $r=0.122$ ,  $p=0.521$ ) in post aerobic exercise subjects.

**Table 3 Correlation of the mean levels of adiponectin and oxidized low density lipoprotein with age in post exercise subjects.**

| Parameters          | N  | R     | P-value |
|---------------------|----|-------|---------|
| Adiponectin vs Age  | 30 | 0.324 | 0.081   |
| Oxidized LDL vs Age | 30 | 0.146 | 0.442   |

**Table 4 Correlation of the levels of adiponectin and oxidized low density lipoprotein, with BMI in post aerobic exercise samples.**

| Parameters          | n  | R     | P-value |
|---------------------|----|-------|---------|
| Adiponectin vs BMI  | 30 | 0.122 | 0.521   |
| Oxidized LDL vs BMI | 30 | 0.197 | 0.297   |

## DISCUSSION

Cardiovascular disease is among the leading cause of death globally (24) with increasing incidence in the developing countries. Aerobic exercise involving aerobic oxidation of low-density lipoprotein, is considered a key factor in the pathogenesis of atherosclerosis and adiponectin suggested to possessing insulin-sensitizing and anti-atherosclerotic properties (28). In this study, the levels of adiponectin and oxidized low density lipoprotein were evaluated in subjects undergoing aerobic exercise using chester-step aerobic exercise.

The mean value of BMI before and after aerobic exercise was non-significantly related with the mean value of age. This implies that the test subjects were uniformly distributed between the groups and was in accordance with the findings of Wonisch *et al.* (29), which states that an unbalanced lipid profile is associated with increase in BMI and age. Thus, the BMI values found in the test group and also the

non-significant positive correlation found in our study between Ox-LDL and Adiponectin both with BMI and Age validates the Wonisch *et al.* findings amongst aerobic exercise individuals (29).

The mean serum level of adiponectin was non-significantly lower in post-chester step exercise when compared with the pre-chester step exercise mean value. The same trend was also observed in the mean serum levels of oxidized low density lipoprotein in post exercise subjects compared with pre exercise subjects. Adiponectin is exclusively secreted by adipocytes and circulates in the blood at the concentration of 5-to-30  $\mu\text{g/ml}$  as 3 oligomeric forms: trimer, hexamer, and high molecular weight multimer. Therefore, the non-significant difference observed in this study suggests that chester-step aerobic exercise do not significantly alter adiponectin blood levels. The non-significant decrease recorded after chester step aerobic exercise however demonstrates that aerobic exercise has a

cellular reduction potentials to adiponectin levels. This is in accordance to the findings of Huiet *al.* and Kimet *al.* which stated that adiponectin levels are affected by *ADIPOQ* polymorphisms and lifestyle risk factors (e.g. diet, exercise, and adiposity) (30,31). And that, adiponectin levels influenced by acquired lifestyle risk factors might be more important than genetically determined levels. Furthermore, Ox-LDL was not significantly decreased in the post-chester exercise group than in the pre-chester exercise subjects, which is in accordance with studies showing that several modalities of exercise do not significantly alter Ox-LDL cellular levels (32,33). However, regardless of the lack of effect on Ox-LDL levels, exercise training can improve the LDL metabolism by increasing the LDL removal from the plasma and the lipoprotein turnover, as shown in the study by Vinagre *et al.* (34).

A non-significant positive correlation existed between the mean levels of adiponectin with age. The same was also observed when the mean level of oxidized low density lipoprotein was correlated with age in post aerobic exercise subjects. The effects of aerobic exercise training on the lipid profile, oxidative stress, and inflammatory markers of atherosclerosis have been scarcely explored in aged subjects. However, it is widely accepted that the regular practice of exercise training, including aerobic exercise, is one of the most effective non-pharmacological interventions that can partially reverse the effects of vascular dysfunction, thereby decreasing the risk of death and consequently increase longevity (11–13). Furthermore, a non-significant positive correlation between the levels of oxidized low density lipoprotein with BMI and a non-significant positive correlation between the mean levels of adiponectin with BMI were recorded in post aerobic exercise subjects. Overweight and obesity are clearly associated with alterations in the lipid profile and raise in the markers of

systemic inflammation, including C-reactive protein (CRP) and pro-inflammatory cytokines, such as adiponectin, interleukin- (IL-) 1 beta (IL-1 $\beta$ ), IL-6, and tumor necrosis factor alpha (TNF- $\alpha$ ) (11,12). Thus, the non-significant difference recorded could due to the relative normal body mass index of the study participants used in this present study. According to Chodzko-Zajko *et al.* and Mann *et al.* aerobic exercise preferentially improves cardiovascular fitness, resistance training increases the muscle mass and both training modalities promote loss of body fat mass (9, 10).

### Conclusion

The non-significantly decreased mean levels of adiponectin and oxidized low density lipoprotein after chester-step aerobic exercise against pre-exercise values, suggest that chester-step aerobic exercise did not significantly alter cellular levels of adiponectin and Ox-LDL. However, the post-exercise decrease in mean levels of adiponectin and Ox-LDL against pre-exercise values (albeit non-significant), demonstrates that a more rigorous longitudinal aerobic exercise could yield a better outcome. Hence, as a limitation of this study, it should be mentioned that a longitudinal interventional study would be rigorously more appropriate to establish the bio-humoral changes elicited by chester-step aerobic exercise.

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