

Original Research Article**IMPACT OF CARDIO-EXERCISE ON ANTIBODY ISOTYPES OF
HEALTHY INDIVIDUALS IN EASTERN NIGERIA****ABSTRACT**

Background: Cardiovascular disease is a major public health problem & a leading cause of mortality in Nigeria, which has been largely attributed to the decline in physical exercise predisposing people to various forms of chronic ailments in general. The objective of this study was to determine the more preferred form of cardio-exercise and compare results of moderate and vigorous exercises on antibody-isotypes IgG & IgM before exercise, four weeks after exercise, eight weeks after exercise and twelve weeks after exercise. **Methods:** Serum concentration of antibody-isotypes IgG & IgM 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. **Results:** In the moderate exercise group, the mean serum Immunoglobulin M (IgM) was significantly increased ($P<0.05$) all through with exception of the 8 weeks result compared with the result 4 weeks after exercise while the mean serum Immunoglobulin G (IgG) was significantly increased ($P<0.05$) 12 weeks after exercise as compared with the result before exercise. In the vigorous exercise group, the mean serum IgM was significantly increased ($P<0.05$) all through while the mean serum IgG was significantly increased ($P<0.05$) 8 weeks & 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.

Conclusion: Physical exercise leads to transitory elevation in antibody-isotypes, though only after an extended period of physical exercise indicating that a moderate intensity cardio-exercise is preferred as it produces enhanced immune response and reduced risks of cardiovascular disease.

Keywords: Antibody, Exercise, Immune, Nigeria.

INTRODUCTION

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, prolonged sitting and long screen time, which have been restricted in ways that minimize human movement and muscular activities. This shift from a physically demanding life to reduced physical activities have exposed people to high risk of developing various health conditions such as obesity, hypertension, diabetes and cardiovascular diseases [1]. 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 [2]. Immunity is the defense mechanism or resistance of a host against a foreign body. It involves the recognition and disposal of such “foreign” or “non-self” material that wants to enter or that enters the body [3]. The immune system is a system of biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents. It protects the body from potentially harmful substances by recognizing and responding to antigens. The immune system includes certain types of white blood cells. It also includes chemicals and proteins in the blood, such as antibodies, complement proteins, and interferon [4]. Immunoglobulins are glycoprotein molecules that are produced by plasma cells in

response to an immunogen and which function as antibodies. An immunoglobulin (Ig), also known as an antibody (Ab), is a large Y-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. The antibody recognizes a unique part of the foreign target, called an antigen. There are five sub-classes of antibodies including immunoglobulin G (IgG) and immunoglobulin M (IgM). Immunoglobulin G (IgG) is the most abundant type of antibody, is found in all body fluids and protects against bacterial and viral infections while Immunoglobulin M (IgM), is found mainly in the blood and lymph fluid and it is the first to be made by the body to fight a new infection [5]. A research work on the effects of training time on serum immunoglobulin alterations in male athlete students, showed that there were no significant differences between the amounts of serum immunoglobulins of both groups in pre-test and post-test [6]. Another study on the influence of selected exercise on immunoglobulin showed that the levels of serum IgG and IgA reduced but not significantly during preparation and competition phases while serum IgM level increased significantly during the competition phase of exercise [7]. Also, a 2015 study on the effect of physical exercises on serum immunoglobulins G & M of healthy individuals, showed that a significant increase in immunoglobulin M (IgM) while immunoglobulin G (IgG) was not significantly increased four weeks after exercise [8]. The study was therefore carried out to determine and compare results of the effect of moderate and vigorous aerobic exercises on antibody-isotypes immunoglobulin G & immunoglobulin M before exercise, two weeks after exercise, eight weeks after exercise and twelve weeks after exercise and determine the preferred form of physical exercise in terms of duration, intensity and frequency most especially required to avoid sedentary lifestyle.

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/females), 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% [9]. The formula for sample size when population is more than 1000 is: $n = Z^2PQ/d^2$ [10].

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

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

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

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 immunoglobulin G (IgG) & immunoglobulin M (IgM) were analyzed by Enzyme Linked Immunosorbent Assay (ELISA) technique.

Principle of enzyme linked Immunosorbent assay (ELISA)

The antigens or antibodies present in patient's sample are allowed to stick to a polyvinyl plate and then plate is washed to separate antigens or antibodies from remaining sample components. To this plate, a corresponding second antigen or antibody is added to get fixed to the already adhered first antigen in the plate. A tagged enzyme is added, then, a suitable substrate is added, the enzyme reacts with the substrate to produce a colour. This colour 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.

In the moderate exercise group, the mean serum concentration of Immunoglobulin M (IgM) was significantly increased ($P < 0.05$) all through the study with exception of the 8 weeks result compared with the result 4 weeks after exercise while the mean serum concentration of Immunoglobulin G (IgG) was significantly increased ($P < 0.05$) 12 weeks after exercise as compared with the result before exercise (Table 1). In the vigorous exercise group, the mean serum concentration of Immunoglobulin M (IgM) was significantly increased ($P < 0.05$) all through the study while the mean serum concentration of Immunoglobulin G (IgG) was significantly increased ($P < 0.05$) 8 weeks & 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 2).

TABLE 1: COMPARISON OF SERUM IgM & IgG RESULTS FOR MODERATE EXERCISE GROUP BEFORE EXERCISE, 4-WEEKS AFTER EXERCISE, 8-WEEKS AFTER EXERCISE AND 12-WEEKS AFTER EXERCISE

Groups	Parameters	
	IgM (g/L)	IgG (g/L)
Before exercise	0.88 ± 0.15	10.22 ± 1.11
4 weeks after exercise	0.98 ± 0.14	10.36 ± 1.07
8 weeks after exercise	1.07 ± 0.14	10.89 ± 1.20
12 weeks after exercise	1.17 ± 0.09	10.99 ± 1.21
F-value	26.227	3.314
P-value	0.001*	0.023*
POST HOC		
a/b	0.023*	1.000
a/c	0.001*	0.152
a/d	0.001*	0.010*
b/c	0.074	0.469
b/d	0.001*	0.220
c/d	0.020*	1.000

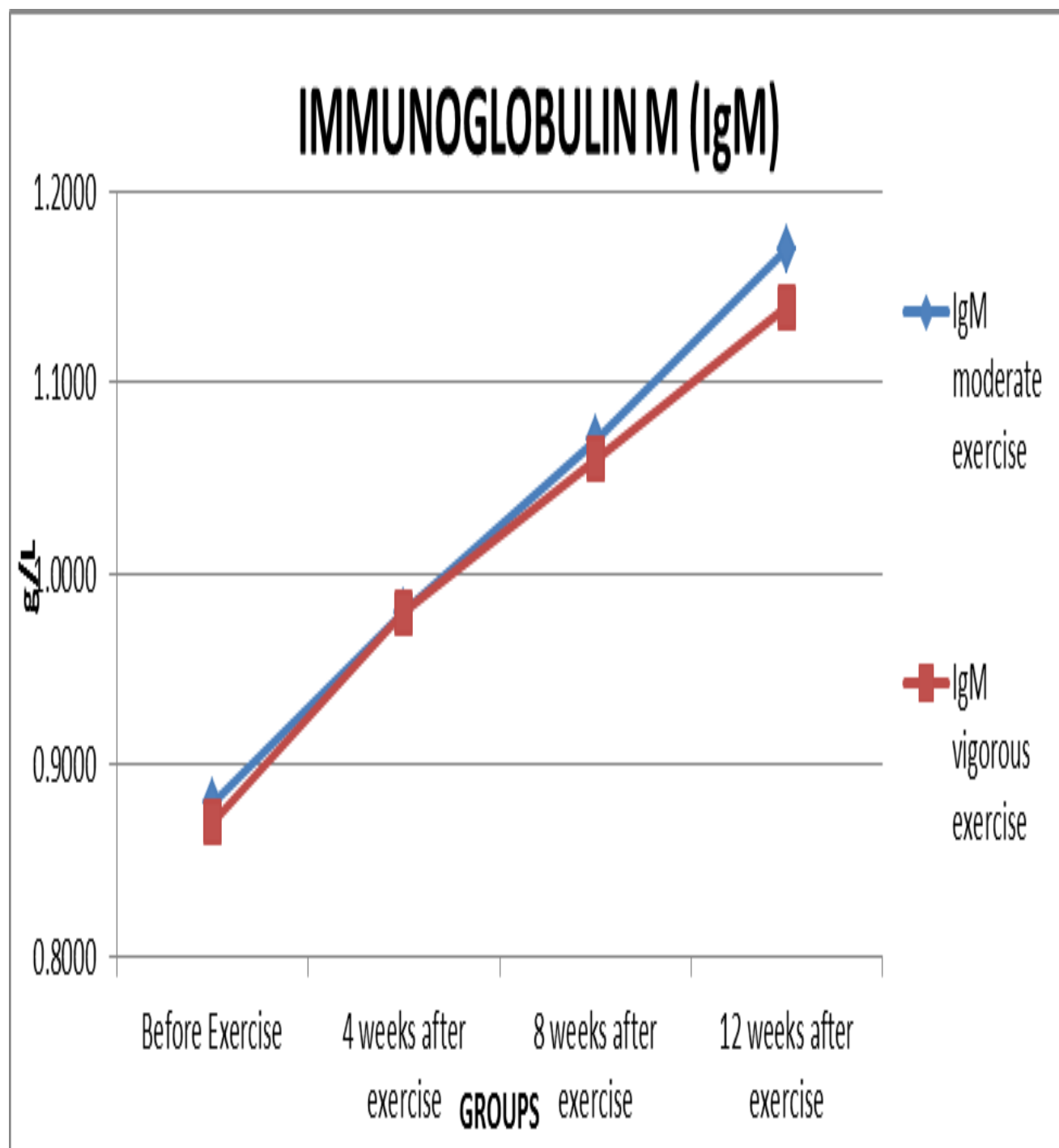
KEY:
a – before exercise b – 4 weeks after exercise c – 8 weeks after exercise d – 12 weeks after exercise
IgM – Immunoglobulin M IgG – Immunoglobulin G
* = Results compared are significantly different at P-value < 0.05 (P < 0.05).

TABLE 2: COMPARISON OF SERUM IgM & IgG RESULTS FOR VIGOROUS EXERCISE GROUP BEFORE EXERCISE, 4-WEEKS AFTER EXERCISE, 8-WEEKS AFTER EXERCISE AND 12-WEEKS AFTER EXERCISE

Parameters

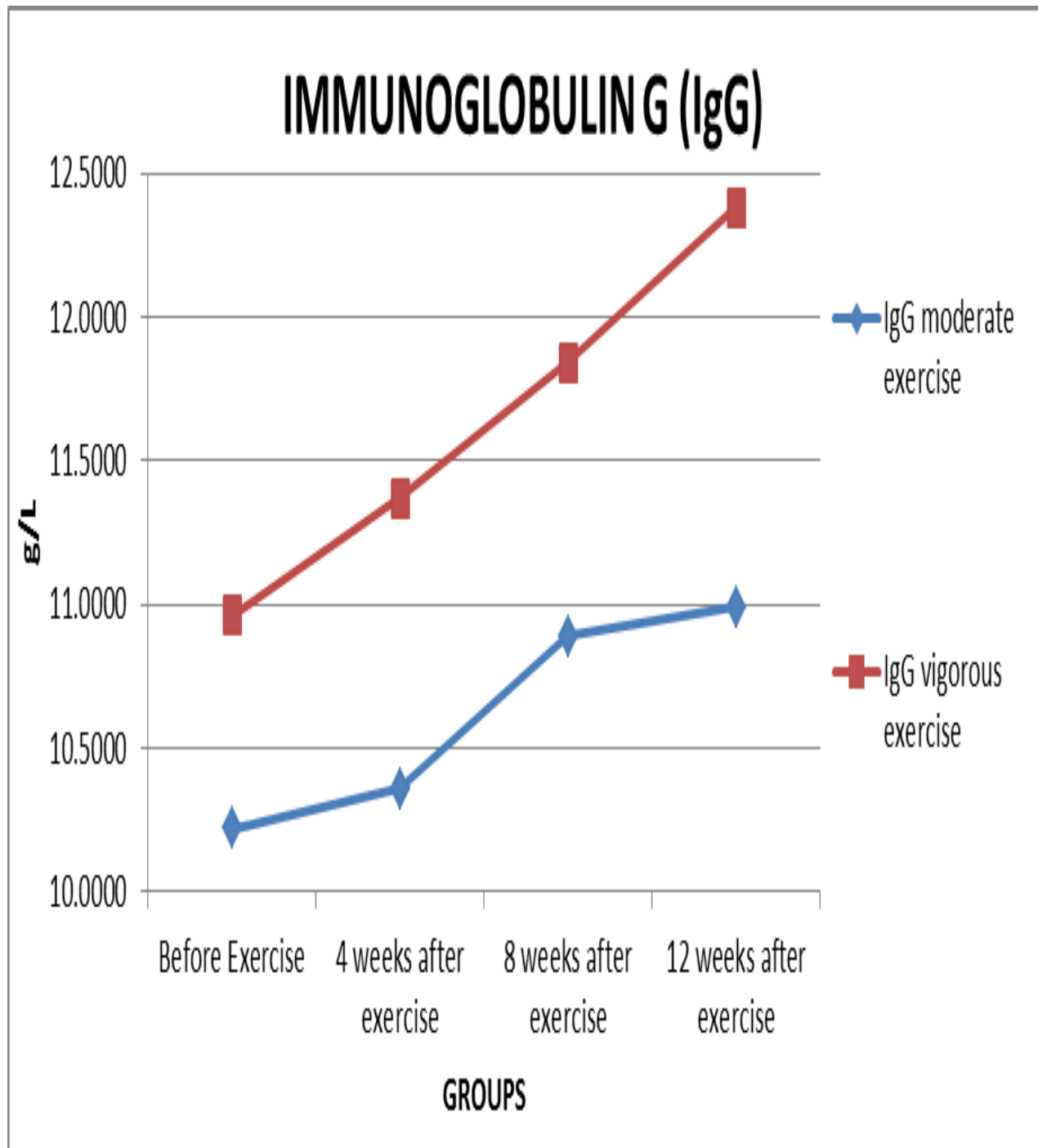
* = Results compared are significantly different at P-value < 0.05 (P < 0.05).

FIGURE 1: GRAPH SHOWING MEAN SERUM CONCENTRATION OF IMMUNOGLOBULIN M (IgM) FOR BOTH MODERATE & VIGOROUS EXERCISE GROUPS



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FIGURE 2: GRAPH SHOWING MEAN SERUM CONCENTRATION OF IMMUNOGLOBULIN G (IgG) FOR BOTH MODERATE & VIGOROUS EXERCISE GROUPS



221 **DISCUSSION**

222 In the moderate exercise group, the mean serum concentration of Immunoglobulin M
223 (IgM) was significantly increased ($P<0.05$) all through the study with exception of the 8 weeks
224 result compared with the result 4 weeks after exercise while the mean serum concentration of
225 Immunoglobulin G (IgG) was significantly increased ($P<0.05$) 12 weeks after exercise as
226 compared with the result before exercise. In the vigorous exercise group, the mean serum
227 concentration of Immunoglobulin M (IgM) was significantly increased ($P<0.05$) all through the
228 study while the mean serum concentration of Immunoglobulin G (IgG) was significantly
229 increased ($P<0.05$) 8 weeks & 12 weeks after exercise as compared with the result before
230 exercise as well as 12 weeks after exercise as compared with the results 4 weeks after exercise.
231 This research outcome is not in agreement with previous studies that showed there were no
232 significant differences between the amounts of serum immunoglobulins pre-and post-exercise
233 [6], another study that revealed that serum IgG level reduced but not significantly during
234 preparation and competition phases while serum IgM level increased significantly during the
235 competition phase of exercise [7] and even our previous research that showed that a significant
236 increase in immunoglobulin M (IgM) while immunoglobulin G (IgG) was not significantly
237 increased four weeks after exercise [8]. The increases in the immunoglobulin levels might be
238 attributed to the fact that generally in exercise immunology, an increase in immunoglobulin
239 concentration has usually been interpreted to represent enhanced immunity, and a decrease is
240 usually interpreted as immunosuppression, with the immunoglobulin alterations likely to be a
241 result of the antigen stimulation through airborne microbes gaining access through increased
242 lungs ventilation rates or breakdown of natural mucosal immunity via drying of airway
243 secretions, during the exercise, thus implying that, physical exercise strengthens the immune

system by protecting against foreign antigens in the event of enhanced immunoglobulin levels [5], [11].

CONCLUSION

Physical exercise leads to transitory elevation in antibody-isotypes, though only after an extended period of physical exercise as evidenced mainly in the vigorous exercise group indicating that a moderate intensity cardio-exercise is preferred as it produces enhanced immune response and reduced risks of cardiovascular disease, with exercise training having significant effect on antibody isotypes in general.

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