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Original Article

Immune system response to isometric handgrip exercise and effects of duration and intensity of the exercise protocol on selected immune system parameters in prehypertensives

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Abstract: Background: Recent studies have shown that physical exercise significantly modulates immunocyte dynamics and possibly plays a significant role on immune function. This study examined the responses of some selected immune system parameters to isometric handgrip exercise and identified possible effects of intensity and duration of the exercise protocols. Methods: One hundred and ninety-two (N=192) sedentary pre-hypertensive subjects, aged between 30-50 years were recruited into the study. They were randomly distributed into three groups of 64 subjects each. A detailed explanation and a demonstration of the exercise protocol were given to the subjects and they were asked to report at the Exercise Physiology unit of the Physiotherapy department, Federal Medical Centre, Asaba, Delta State at 4.00 pm daily for the exercise practice. The training session for each day took place between the hours of 4.00 pm and 8.00 pm daily (FMC/ASB/A81.VOL.XII/101). The subjects performed a 24 consecutive day's isometric handgrip exercise at 30% Maximum Voluntary Contraction (MVC). At the end of the 24 days, group one (GP1) discontinued with the exercise protocol, while group two (GP2) and group three (GP3) continued with the exercise protocol for another 24 consecutive days nevertheless GP3 performed at an increased intensity of 50% MVC. The clinical trial was registered with Nigeria Clinical Trial Registry, Federal Ministry of Health, Abuja Nigeriawith Trial No: 1216582 (https://www.nctr.nhrec.net/viewTrials.php?TID=1216582). Results: At the end of the study, the result shows thatthe number of CD4 cells and CD4/CD8 ratio significantly ($P<0.05$) increased while the CD8 cell decreased in GP2 and GP3. It was further shown that increase in duration produced a more significant change compared to an increase in intensity of the isometric effort. Conclusion: The study established that isometric handgrip exercise alters the circulating levels of the immune system parameters which could have positive beneficial effects on the prehypertensive individuals as the number of CD4 cells and CD4/CD8 ratio increased especially when practiced over a longer duration.

Keywords: Handgrip exercise, immune system response, exercise protocol prehypertensives, maximum voluntary contraction (MVC)

Introduction

Over the years, several studies have demonstrated that physical exercise induces considerable physiological alterations in the immune system indices, thereby interfering with the physical state of health [1-4]. According to Rodrigo *et al*., physical exercise acutely and chronically affects many components of the immune system [5]. Exercise stress model has been proven in previous research to be applied experimentally towards achieving a positive health benefit. Epidemiological evidence suggests a link between the intensity of the exer-

cise and the occurrence of infections and diseases [6]. Physical exercise of moderate intensity has equally been shown to stimulate parameters related to cellular immunity, thereby decreasing the risk of infection, while high-intensity exercise promotes the risk of infectious diseases by decreasing these same parameters [5]. A previous study demonstrated that physical exercise is of utmost benefits on the physiological and immunological functions of HIV infected persons [7]. Veljkovic *et al*. stated that physical exercise acts as an immune stimulant by creating a type of natural vaccine depending on duration and intensity of such exercise protocol [8].

The American College of Sports Medicine recommends aerobic exercise 3-5 times per week for 30-60 minutes per session, at a moderate intensity, that maintains the heart rate between 65-85% of the maximum heart rate with emphasis on dynamic exercise with less attention paid to isometric effort. Despite all of the known benefits of exercise, there are also well-documented associations between acute episodes of exertion and sudden cardiac death and these deaths commonly occur in an unexpected fashion among those who appear quite healthy [11]. One promising regimen, currently being explored, is isometric hand grip exercise training. This type of study will help to provide evidence for time-efficient and cost-effective therapy [13]. Moreover, handgrip exercise is easy to perform and may be accomplished in various locations such as hospitals, schools, in transit and in the home, enabling increased adherence to treatment, in contrast with other forms of exercises. Thus, the need for this study is to assess the responses of some selected immune system parameters to isometric handgrip exercise protocols based on intensity and duration.

Methods

Study setting

This study was conducted at Delta State University, Abraka and Federal Medical Centre Asaba. The experimental bench work and exercise intervention was conducted in the Exercise Physiology unit of the Department of Physiotherapy, Federal Medical Centre, Asaba. The subjects were recruited from the consultant outpatient clinic of Federal Medical Centre, Asaba. Federal Medical Centre, Asaba is a Federal tertiary health establishment situated in the headquarters of Delta State. It occupies a strategic position as it receives referrals from all parts of the states and outside the states with an average of 1,500 patients daily.

Materials used

The key instruments used in this study included the following: dynamometer, mercury sphygmomanometer, automated sphygmomanometer, stethoscope, calendar, stop clock, weighing scale, stadiometer, and patients' case note and ballot box.

Study population

The population of this study was drawn from the prehypertensive patients that visited Federal Medical Centre, Asaba. Prehypertension was defined according to JNC 7 criteria as having a blood pressure of 120-139 mmHg and/or 80-89 mmHg of the systolic and diastolic blood pressure respectively in persons who were not on treatment for hypertension. It is a symptom of intensified risk for emerging hypertension. Persons in such classification consequently require preventive health associated behavioral or therapeutic lifestyle changes [14]. One hundred and ninety two prehypertensives [n=192, males =105 and females =87, age, 39.04±6.4 years; body mass index, 25.45±2.72 kg/m^2], were recruited into the study. All the subjects were diagnosed and referred by the physician with a blood pressure level classified as prehypertension were chosen for the study.

Experimental protocol

All procedure for this study was made to observe and conforms to the highest standard of practice as stipulated by various committees and regulatory bodies. The subjects were properly briefed and written informed consent was obtained. A screening session was conducted to assess the baseline parameters and blood pressure of the subjects and blood samples were collected for the analysis of some parameters of the immune system [CD4, CD8 and CD4/CD8 ratio]. The sample population was randomly selected into any of the three groups. The subjects were asked to pick from a ballot box, concealed papers marked G1, G2 or G3. A detailed procedure of the exercise was then

given to the subjects before commencement of the exercise training. A detailed medical examination including a 12 lead electrocardiogram was done on the participants by the consultant Physician to rule out contraindications to physical exercise. Medical records of the participants were reviewed from the case notes and assessment of the subjects. The succeeding designated health parameters were thereafter, measured to obtain the baseline values of the relevant parameters under study.

Exercise practice procedure

The subjects were made to observe a minimum of 15 minutes seated rest following their arrival at the clinic; subsequently their cardiovascular parameters were assessed. The subjects were then asked to squeeze the dynamometer with their dominant hand twice, for a maximum of 2 seconds with a one minute rest in between, so as to determine their individual maximum voluntary contraction [MVC] per session and the mean readings computed. Subjects were subsequently instructed to squeeze and sustain the dynamometer for 2 minutes at 30% MVC. The participants assumed an upright sitting position throughout the exercise training with upper limbs supported on a table. The exercise protocol was repeated twice with a five minutes rest in between for each day.At the end of the 24 days, the EG1 discontinued with the exercise protocol while the EG2 continued with the exercise protocol for the next 24 days and the EG3 continued the exercise protocol at a higher intensity of 50% MVC for the next 24 consecutive days.

Ethical approval

This study jointly received institutional ethical approval of Federal Medical Centre, Asaba, Delta State [FMC/ASB/A81.VOLXII/101] and Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State [REC/FBMS/DELSU/18/16/103] and conformed to the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Inclusion criteria

Subjects who are physically and clinically in good health, devoid of chronic diseases, hematological and clinical abnormalities were recruited. All the participants were untrained which was defined by a score of three or less using the Rapid Assessment of Physical Activity survey.

Exclusion criteria

Subjects were excluded from the study if their age is below 30 years and or above 50 years or have a queried health status with clinical evidence of chronic diseases, a blood pressure above or below the prehypertension level, and/or who declined to participate in the study. Subjects on medications were excluded from this study. Other exclusion criteria include; individuals suffering from debilitating arthritis, carpel tunnel syndrome, peripheral neuropathy, an aneurysm, or mitral valve complications.

Data collection

Initial and final resting parameters: The initial resting parameters are the baseline parameters collected prior to the date of commencement of the intervention. The initial resting parameters were measured between 8.00-10.00 hours. The measurements were carried out after each subject had observed a 15 minutes seated rest on arrival. The final resting parameters were taken on the 49th day to the commencement of the exercise.

Biodata: This was collected via face-to-face interview of the participants. As the participants arrived at the clinic, they were made to rest in a chair for at least 10 minutes. The subjects then were formerly evaluated exclusively via a structured questionnaire.

Height: This was measured using Stadiometer [Ayron 226, USA]; which is a measuring scale for height calibrated in centimeters. The participants were instructed to stand barefooted on the platform of the height scale with knees flexed at 180° while the participants rested against the height scale with the back with the eyes looking forward. The measure of the height was taken as the space from the scale platform to the vertex of the head.

Weights: Measurement was done using Digital Weighing Scale [BEU-GS27-007, Beurer GmbH; Ulm, Germany]. Each participant was instructed to wear light clothing then stand barefooted, one foot on each side of the scale while standing straightforward on the weighing scale with the arms kept by the side.

Table 1. The descriptive demographic data of the exercise group participants

Parameters	N	Mean	Std. Deviation
Age	192	39.04	6.441
Height	192	1.7000	11299
Weight [kg]	192	73.3750	9.00975
BMI [kg/m^2]	192	25.4487	2.72359

Blood pressure measurement: The participants were screened to evaluate the blood pressure of prospective candidates. All blood pressure measurements were obtained for blood pressure measurement which required subjects to rest in a quiet environment for at least 10 minutes prior to the measurement. Subjects were made to rest in a sited position for at least 15 minutes, comfortably with the back supported, legs uncrossed.

Resting blood pressure and pulse rate measurements: These parameters were measured using an automated monitor [Dinamap Pro 300, GEMedical Systems, Berks, UK]. The Dinamap Pro 300 device was evaluated for accurateness and dependability of measurement by means of the mercury sphygmomanometer. The cuff was placed around the participant's left arm over the left brachial artery, about 1.5 cm directly above the antecubital fossa and leveled with the heart. Measuring instruction required that the subjects remained silent throughout the procedure.

Biochemical examination: Whole blood specimens were intravenously collected pre- and post-exercise. Blood sample was collection was done a day prior to the date of commencement of the isometric handgrip protocols at 8-10 hours and repeated again at 8-10 hours on the 49th day to the commencement of the exercise protocol. The blood specimen was collected from the antecubital vein by venipuncture after about 15 min of resting in a chair. The blood specimen was kept in Ethylenediamine tetraacetate [EDTA] vacutainer tubes. It was kept at room temperature while being moved to the heamatology unit of the laboratory for analysis. The blood specimen collected into the EDTA tube was assayed for CD4, CD8 and CD4/CD8 counts within six hours. Immunofluroscence analysis by flow cytometry was used for the lymphocytes measurements. Absolute CD4+, CD8+ and CD4+/CD8+ ratios were determined using the BD FACSCount™ flow cytometer [Becton Dickinson, San Jose, California, USA] and the BD FACSCount™ CD4 and CD8 reagents according to the manufacturer's instructions. Following procession of the samples on the machine, the outcomes were formerly documented in a worksheet.

Statistical analysis

The collected data were statistically analyzed using the descriptive and inferential statistics. The IBM SPSS 21.0 package program version 2010 was used in the analysis and the significance level has been determined as $P<0.05$. The descriptive statistics employed in this study were the mean and standard deviation. The inferential statistics used in the analysis of the data included a one tailed related t-test to determine the intra-groups differences in the initial and final measurements in the different groups. One way analysis of variance was further used to assess the variation between the three modes of the exercise protocols.

Results

The effects of isometric handgrip exercise training on the immune system parameters and potential impact of intensity and duration of the exercise protocols on selected immune system parameters were assessed. Three major immune systems parameters (CD4, CD8 and CD4/CD8 ratio) were selected based on their peculiar role/characteristics. Findings from the study are presented in tables and charts.

The descriptive demographic data

Table 1 shows the descriptive demographic data of the group participants. Atotal of one hundred and ninety two (192) subjects with a mean age of 39.04±6.4 years, height 1.7±0.11 meters, weight 73.4±9.0 kg and body mass index of 25.4±2.7 kg/m^2 participated in the study.

Figure 1 is a pie chart showing the BMI distribution of the participants. The chart shows that 3.1%, 15.6%, 34.4% and 46.9% of the subjects were morbidly obese, obese, overweight and normal with their weight respectively. From this data it can be deduced that weight has an association with prehypertension though the focus of the study was not directed towards this

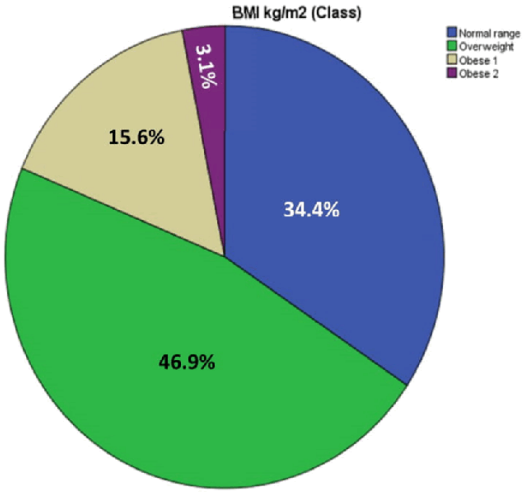

Figure 1. Classification based on body mass index [BMI classification].

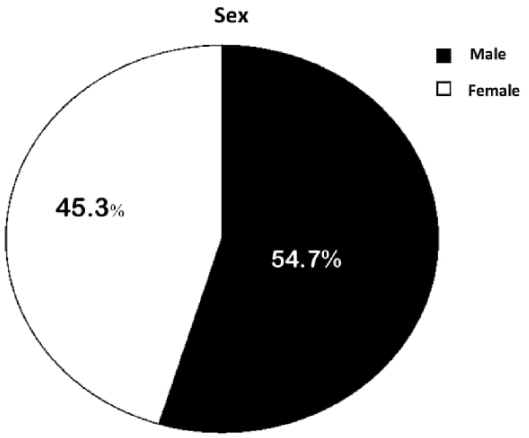

Figure 2. Classification of the participants based on sex.

goal. **Figure 2** is a pie chart showing the distribution of the participants based on their sex. A total of 54.7% were males while the rest 45.3% were females. Since the patients were randomly selected, it may be concluded that prehypertension may be more prevalent in males than in females though the focus of the study was not directed towards this goal.

Figure 3 is a bar chart showing the comparative Effect(s) of the various modes of exercise on CD4 counts. From the chart, the values of CD4 count slightly reduced at the end of 48 days in group I but increased maximally in group II while an increase was also noted in group III but in a lesser magnitude compared to

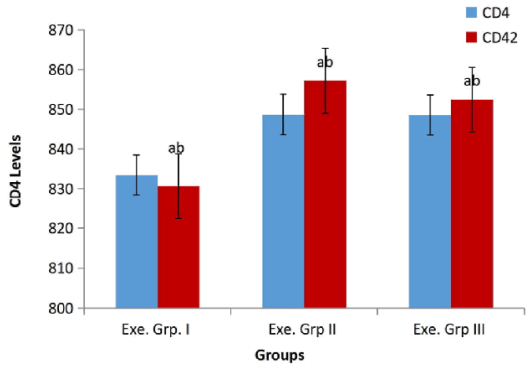

Figure 3. Responses of CD4 levels to the different mode of the exercise protocols. A higher significant increase (ab) in the level of CD4 was observed in group II in relation to group III shows that observing the exercise protocol at the same intensity for a longer duration produced a more significant increase in the levels of CD4 when compared to exercising at an increased intensity CD4. In group I, the chat shows that the exercise protocol could not produce a significant increase. It was possible that the duration was inadequate to affect an increase or that a reversal could have occurred following a cessation of the exercise protocol after 24 consecutive days.

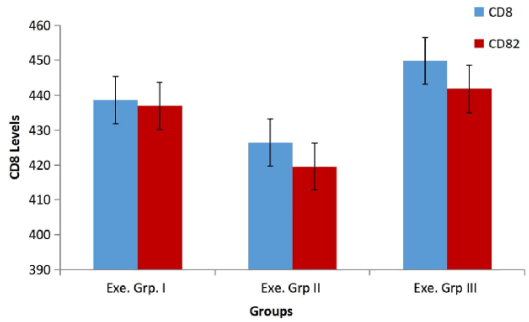

Figure 4. Responses of CD8 levels to the different mode of the exercise protocols. The chat shows reduction in the values of CD8 across the three groups. However, the reduction was more in group II and group III.

group II. **Figure 4** is a bar chart showing the comparative effect(s) of the various modes of exercise on CD8 counts. The chart shows that the values of CD8 count reduced at the end of 48 days across all the groups but with a less reduction noted in group I. **Figure 5** is a bar chart showing the comparative effect(s) of the various modes of exercise on CD4/CD8 counts. The chart shows that the values of CD4/CD8 count was reduced at the end of 48 days in group I but increased in both group II and group III.

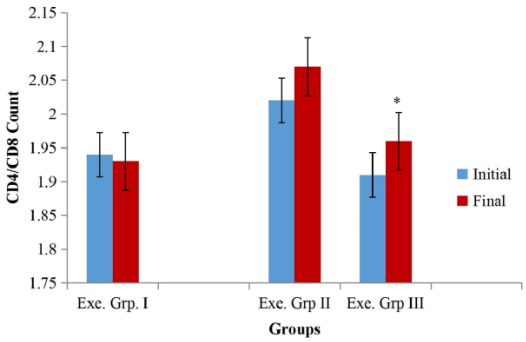

Figure 5. Comparative effect[s] of the exercise modes on CD4/CD8 ratios across groups. A significant increase (*) in the level of CD4/CD8 was observed in group II and group III which implies that observing the exercise protocol at an increased duration and intensity effect produced a more significant increase in the level of CD4/CD8 ratio. In group I, the chat shows that the exercise protocol could not produce any significant increase. It is possible that the duration was inadequate to affect an increase or that a reversal would have occurred following a cessation of the exercise protocol after 24 consecutive days.

Effects of cessation of the exercise protocol

Table 2 illustrates the pre-exercise and post-exercise mean values of the immune systems parameters of exercise group I. Results show a mean reduction though statistically insignificant of 2.81±8.90 cells/mm^3 (P>0.05), 1.68±3.34 cells/mm^3 (P>0.05) and 0.011±0.06 cells/mm^3 (P>0.05) in the CD4, CD8 and CD4/CD8 ratio respectively.

Effects of increase in duration of the exercise protocol

Table 3 shows the pre and post exercise mean values of the immune systems parameters of the exercise group II. The participants show a significant increase of 8.5±7.6 cells/mm^3 (P<0.05) and 0.053±0.03 cells/mm^3 (P<0.05) in the CD4 and CD4/CD8 ratio respectively and a significant reduction of 0.053±0.03 cells/mm^3 (P<0.05) in CD8 cells.

Effects of increase in intensity of the exercise protocol

Table 4 shows the pre and post exercise mean values of the immune systems parameters of the exercise group III. The participants had a mean increase of 3.87±16.94 cells/mm^3 and 0.049±0.037 cells/mm^3 in CD4 and CD4/CD8 ratio respectively and a reduction of 0.049±0.037 cells/mm^3 in the CD8. However, the increase noted in the values of CD4 was not significant (P>0.05), but the increase noted in CD4/CD8 ratios and the reduction noted in the values of CD8 was statistically significant (P<0.05).

Comparative effects of cessation and continuation of the exercise protocol

Table 5 shows the comparative effects of cessation (Exercise Group I), and continuation (Exercise Group II) of the exercise protocol at the end of 48 days on the immune system parameters. A mean value of 5.7±1.3 cells/mm^3, 5.25±1.37 cells/mm^3 and 0.042±0.03 cells/mm^3 was observed in favour of group II showing that continuing the exercise protocol produced a greater effect to the tune of the above values as compared to cessation of the exercise protocol at the end of 24 days. An independence t-Test on the observed mean difference shows no significant difference except with the CD8 (P>0.05).

Comparative effects of constant and increase in intensity of the exercise protocol

Table 6 shows the effects of intensity of the exercise protocol at the end of 48 days on the immune system parameters. A mean difference of 4.6±3.58 cells/mm^3 and 0.04±0.01 cells/mm^3 was observed in the values of CD4 and CD4/CD8 ratio in favour of group II and 1.1±1.70 cells/mm^3 in the values of CD8 in favour of group III showing that continuing the exercise protocol on the same intensity produced a greater effect in the values of the CD4 and CD4/CD8 ratio to the tune of the above values as compared to increasing the intensity after 24 days but reverse is the case with regard to the levels of CD8. The values however did not show any statistical significance (P>0.05). **Table 7** is a one-way analysis of variance for the three exercise groups. The results show that a significant difference exists only in CD8 (P<0.05) but insignificant in the values of CD4 and CD4/CD8 ratio (P>0.05).

Discussion

In this study, an attempt was made to elucidate the effects of isometric exercise on some selected parameters of the immune system

Table 2. Pre and post exercise mean values of the parameters of the immune system of the exercise group I

Parameters	Pre-Exercise	Post-Exercise	Diff.	Df	*P*-value [2-tailed]	Remarks
CD4 [cells/mm^3]	833.43±74.23	830.62±77.12	-2.81±8.90	63	0.226	Insignificant
CD8 [cells/mm^3]	438.62±50.60	436.93±51.74	-1.68±3.34	63	0.062	Insignificant
CD4/CD8 [cells/mm^3]	1.94±0.31	1.93±0.32	-0.011±0.06	63	0.426	Insignificant

Values are expressed as mean ± Standard Deviation [S.D], n=64. *P<0.05.

Table 3. Pre and post exercise mean values of the parameters of the immune system of the exercise group II

Parameters	Pre-Exercise	Post-Exercise	Diff.	Df	*P*-value [2-tailed]	Remarks
CD4 [cells/mm^3]	848.68±61.64	857.18±58.63	8.5±7.6	63	<0.001*	Significant
CD8 [cells/mm^3]	426.5±45.98	419.56±445.68	-6.93±4.71	63	<0.001*	Significant
CD4/CD8 [cells/mm^3]	2.01±0.30	2.07±0.31	0.053±0.03	63	<0.001*	Significant

Values are expressed as Mean ± Standard Deviation [S.D], n=64. *P<0.05.

Table 4. Pre and post exercise mean values of the parameters of the immune system parameters of the exercise group III

Parameters	Pre-Exercise	Post-Exercise	Diff.	Df	*P*-value [2-tailed]	Remarks
CD4 [cells/mm^3]	848.56±41.99	852.43±40.35	3.87±16.94	63	0.375	Insignificant
CD8 [cells/mm^3]	449.81±45.49	441.81±45.02	-8.0±4.95	63	<0.001*	Significant
CD4/CD8 [cells/mm^3]	1.91±0.26	1.95±0.26	0.049±0.037	63	<0.001*	Significant

Values are expressed as Mean ± Standard Deviation [S.D], n=64. *P<0.05.

Table 5. The Comparative effects of cessation and continuation of the exercise protocol at the end of 48 days on the immune system parameters

Parameters	Exercise Group I	Exercise Group II	Diff.	Df	*P*-value [2-tailed]	Remarks
CD4 [cells/mm^3]	-2.8±8.90	8.5±7.6	5.7±1.30	127	0.287	Insignificant
CD8 [cells/mm^3]	-1.68±3.34	-6.93±4.71	5.25±1.37	127	0.007*	Significant
CD4/CD8 [cells/mm^3]	-0.01±0.06	0.05±0.03	0.042±0.03	127	0.111	Insignificant

Values are expressed as Mean ± Standard Deviation [S.D], n=128. *P<0.05.

Table 6. The comparative effects of continuation and increase in intensity of the exercise protocol at the end of 48 days on the immune system parameters

Parameters	Exercise Group I	Exercise Group III	Diff.	Df	*P*-value [2-tailed]	Remarks
CD4 [cells/mm^3]	8.5±7.6	3.9±16.9	-4.6±3.58	127	0.418	Insignificant
CD8 [cells/mm^3]	-6.93±4.71	-8.0±4.95	1.1±1.70	127	0.611	Insignificant
CD4/CD8 [cells/mm^3]	0.053±0.03	0.049±0.04	-0.04±0.01	127	0.313	Insignificant

Values are expressed as Mean ± Standard Deviation [S.D], n=128.

and to assess the effects of variation of the duration and intensity of the isometric exercise protocol.

The effects of isometric handgrip exercise on the parameters of the immune system have not been extensively studied. However, related Previous studies support findings of this study. In a comprehensive review by Adrian *et al.*, [15] the relationship between physical exercise and immune system function was examined in Cancer Survivors. It was found that of the six empirical studies published between 1994 and 2000, four out of the six studies reported sta-

Table 7. Analyses of variance for the three exercise groups Effects on the immune systems parameters

		Sum of Squares	Df	Mean Square	F	*P*-value
CD4 [cells/mm^3]	Between Groups	307.125	2	153.563	1.791	0.178
	Within Groups	3858.188	189	85.738		
	Total	4165.313	191			
CD8 [cells/mm^3]	Between Groups	193.292	2	96.646	5.890	0.005*
	Within Groups	738.375	189	16.408		
	Total	931.667	191			
CD4/CD8 [cells/mm^3]	Between Groups	.005	2	.002	1.606	0.212
	Within Groups	.069	189	.002		
	Total	.074	191			

Values are expressed as Mean ± Standard Deviation [S.D], n=192. *P<0.05.

tistically significant improvements in a number of immune system components as a result of exercise. El-Kader and Al-Shreef, reported that the mean values of CD3+, CD4+ and CD8+ T cells count were significantly increased, whereas the mean values of CD4/CD8 ratio was significantly decreased [16].

Sixty sedentary subjects with age ranged from 61-66 years, participated in their study. All Subjects were randomly assigned to supervised aerobic exercise intervention group or resistance exercise group. It was further reported that a significant difference exists between mean levels of the investigated parameters in both groups after treatment. Ezema *et al.,* [17] reported significant increase of CD4 cell counts in contrast with the control group following physical exercise of moderate intensity by jogging in a treadmill three times per week for eight weeks. Maduagwu *et al.* also reported similar findings following moderate intensity treadmill aerobic exercise for 12 weeks [7].

The effect of aerobic training on the immune system of aging men was investigated and it was found that the number of CD4 and CD8 cells in the training group had increased significantly as compared to the control group while CD4/CD8 ratio in training group was significantly lower than the control group [18]. However, these studies utilized a non-specific exercise protocol with several flawed designs such as standardization and objective quantification of the exercise protocols; this has resulted in difficulties with drawing conclusions from the studies. This study utilized a simple isometric exercise protocol which can be replicated in any location thereby enhancing compliance and enabling conclusions to be drawn. MacArthur *et al.* linked lower CD4 cell counts to noncompliance with prescribed exercise [19]. Data involving a quantifiable isometric exercise and immune system parameters is not available to this researcher.

Free radicals production has been proposed as a mechanism of action of physical exercise on immune system improvement. Oxygen demand and consumption increases maximally during physical exercise and this in turn leads to increase in production and circulation of free radicals [20]. This process brings about the adjustment of antioxidant enzymes performances and cell-mediated immune response. Thus, the immune system acquires more capacity to combat harmful free radicals circulating in blood and subsequent increase in the production of antioxidant enzymes and the numbers of CD4 and CD8 cells [21-23].

Conclusion

This study results showed that isometric hand grip exercise significantly improved immune systems parameters following a 48 consecutive days practice at 30% MVC. It further revealed that exercising at a constant intensity over a longer duration produces a greater impact compared to alteration in the intensity.

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Disclosure of conflict of interest

None.

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