Mitigations in Lipid Profile Levels with Duration-Dependent Exercise Intensities in Overweight and Obese Females

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ABSTRACT

Although known for its role in clogging arteries and pathogenesis of heart diseases and stroke, cholesterol (and similar lipids) is also useful in the bio-synthesis of sex and adrenal hormones. Studies have shown three forms of cholesterol-carrying proteins in the blood: high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL). With LDL and VLDL being the "bad cholesterol", this study investigated the effect(s) of a 10-week

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interval exercise training and a 10-week continuous exercise training on total body Cholesterol, HDL, LDL, VLDL and Triglycerides (TAGs) in obese and overweight females. To approach this, one hundred and eleven (111) overweight and obese female subjects (aged between 18-65 years) were ethically recruited and grouped into three of thirty-seven (37) subjects each; (CETG = Continuous Exercise Training Group, IETC = Interval Exercise Training Group, and CG = Control group). While CG had no form of exercise training, IETC received alternate intensity training in ratio 1:3 as 30 seconds of high-intensity exercise with an alternate 90 seconds of low-intensity exercise. Subjects in CETG received similar treatments as IETC but without the low-intensity phase. In both cases, exercise intensity was 65% of heart rate maximum for 20 minutes at the first 2 weeks and was subsequently increased by 5% intensity and 5 minutes duration every 2 weeks till the 10th week with proper profiling and record of serum lipid levels (cholesterol, LDL, VLDL, HDL and TAGs) in each case. Analysis of the differences in mean (ANOVA) for obtained records showed a statistically significant decrease in cholesterol, LDL, VLDL and TAGs after 10 weeks of high-intensity continuous exercise training and a statistically significant increase in HDL after 10 weeks of high-intensity interval exercise training. Thus, lipid profile levels were positively affected by continuous mode of aerobic exercise. Similar but advanced studies with dietetic implications are recommended for further explanation of the reasons for the observed differences.

1. INTRODUCTION

Though several health benefits, including increased energy levels per day, decreased blood pressure and cholesterol levels, strengthened bones, improved posture, and a lower level of stress have been linked with continuous aerobic exercise, Proper nutrition is also Jermaine to physical fitness [1-4]. This is because energy expenditure in exercise is dependent on nutrition. If the diet is inadequate, fitness level will drop. Overweight, underweight, and weak individuals will have below average fitness levels [2,3].

Studies have shown that both continuous endurance exercise and high-intensity interval exercise can increase fat oxidation post-training, but it is unclear which training protocol elicits the greater adaptation in overweight and obese females [5]. Thus, application of energy balance principles toward prevention of overweight and obesity is more subtle, and the results are less evident and less reinforcing than those for treatment of obesity [6,7]. Overweight or obese individuals have been reported to run a greater risk of developing diabetes mellitus, hypertension, coronary heart disease, stroke, arthritis, and some forms of cancer. This is because, weight build up causes deposition of arteriosclerotic plaques in blood vessels, initiating the pathogenesis of cardiovascular ailments [8,9].

Over time, a close relationship exists among levels of blood cholesterol in the body, those of other fats or lipids, and the development of atherosclerosis. In this disorder, plaques containing cholesterol are deposited on the walls of arteries, particularly arteries of small and medium sizes, reducing their inside diameter and the flow of blood [10]. Clotting of blood, such as may occur in the coronary arteries to cause a heart attack, is most likely to develop at places where arterial walls are roughened by such plaques [10]. Although many foods, particularly dairy products and meat fat, contain cholesterol, the body also synthesizes this sterol in the liver from cholesterol-free substances. Nevertheless, the investigation indicates that a cholesterol-rich diet causes abnormally high levels of cholesterol and the related fats and lipids in the blood. Evidence strongly indicates that people with such high levels are more likely to develop atherosclerosis and heart attacks than those with lower levels [11-13]. Scientists have identified three forms of cholesterol-carrying proteins in the blood: high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL). LDL and VLDL appear to promote atherosclerosis, and they are often referred to as "bad cholesterol." By contrast, HDL appears to retard atherosclerosis, earning it the nickname of the "good cholesterol."[13]

In a systematic review of the prevalence of overweight and obesity of adult Nigerians, Chukwuonye et al. (as cited by Mbada, 2009) reported prevalence between 8.1 and 22.2% [8]. According to the authors, the possible predisposing factors of overweight and obesity include female sex, high socioeconomic class, sedentary lifestyle, age above 40

years, and a high energy diet [13,14]. In another study, Oyeyemi et al. also showed that environmental factors play a great role in promoting overweight and obesity [10]. In view of this, this study investigated the possible physiological effects of a 10-week interval training programme and a corresponding 10-week continuous training programme on lipid profile levels. Specifically, study;

    i. Examined the effect(s) exercise on cholesterol level
    ii. Ascertained the changes in lipoprotein (HDL, LDL, VLDL) levels with mild to moderate exercise administration
    iii. Determined the impact(s) of exercise on triglyceride (TAG) levels.

2. METHODOLOGY

2.1 Ethical Considerations

Ethical approval was obtained from the University of Nigeria Health and Research Ethics Committee. Participants' confidentiality was maintained by using code numbers instead of their names. Prior to commencement of the study, Subjects' informed consent was also obtained; following clear explanation of purpose, procedure, benefit, risks and alternatives for study as well as confidentiality and withdrawal information.

2.2 Study Design

The study adopted the pre-test/post-test control group experimental design with a 3x3x3 factorial matrix. Simple randomization method by "drawing from the hat" was used to randomize eligible participants into three groups that were tested prior to and after the intervention. These groups were:

   I. Continuous Exercise Training Group (CETG)
  II. Interval Exercise Training Group (IETG)
  III. The control group (CG)

2.3 Study Area

The study was conducted in two Gymnasia; Department of Physiotherapy, University of Nigeria Teaching Hospital, Ituku-Ozalla and the Gymnasium of the Department of Medical Rehabilitation, University of Nigeria, Enugu Campus.

2.4 Study Population

A population of overweight or obese females were targeted in the study. This populace comprised of a staff of the University of Nigeria Teaching Hospital and students and staff of the University of Nigeria Enugu campus. Adverts were placed for such volunteers with a detailed explanation of the goal of the study.

2.5 Sample and Sampling Technique

The simple random sampling technique was used to draw a total of 111 overweight and obese female volunteers who responded to the advertisement to participate in the study. To obtain a minimum sample size, the power analysis of 80% was used to obtain the difference in means of 0.35 (Based on the prevalence of overweight and obesity in Nigeria as reported by Chukwuonye et al., with a significance level of 0.05[8]. A sample size of 30 in each group was mathematically determined for each of the three groups (CETG, IETG and CG) that were studied. However, to allow for 15% dropout, the sample size was increased to 37 in each arm of the three groups, totalling 111 participants in the study.

2.6 Selection Criteria

Volunteers that met all criteria for selection were verbally informed of all procedures and, if willing to participate, read and signed a written consent form and a Physical Activity Readiness Questionnaire (PAR-Q). The PAR-Q was designed as a fitness appraisal form to determine participants' ability levels, safety and willingness to partake in physical activity prior to participation.

2.7 Inclusion Criteria

Participants were included based on the following eligibility criteria:

1. Ages between 18 and 65 years
2. A regular (28 – 35 days) menstrual cycle.
3. No pregnancy or lactation.
4. A Body Mass Index (BMI) > 25 kg.m2, defined as a BMI of 25 – 29.9 kg.m2 (overweight) and ≥30 kg.m2 (obese) [13].
5. Volunteers who must not have gained or lost more than 3 kg of weight within the previous three months

6. Volunteers who have not changed their diet dramatically with respect to caloric intake within the previous three months.
7. Volunteers that are sedentary and had not participated in a regular exercise program, defined as at least 2 hours of regular strenuous activity per week for the previous year, prior to testing.

2.8 Exclusion criteria

Volunteers who presented with the following conditions were excluded from the study:

1. High-level athletes or spinsters.
2. Subjects with any kind of orthopaedic disorder that limits the walking / running practice.
3. Individuals with Cardiovascular or cardiorespiratory disorders (like asthma).
4. Subjects with a too large variation of physical activity frequency.
5. Participants with acute illness or unstable chronic illness or recent injury.
6. Subjects on beta-blocking medications.

2.9 Procedure

First, the purpose of the study was explained to volunteer(s) who met the inclusion criteria, after which they signed to participate. Next, eligible volunteers were consecutively recruited but randomly allocated to one of the three groups for the study until they have all completed the 10-week exercise training. Next, participants attended an orientation session one week prior to the start of the exercise training program during which they visited the exercise laboratory twice in order to complete 2 pre-training procedures. For the initial visit, the investigator explained the exercise procedures, possible risks and benefits and also instructed the participants on how to properly perform all training exercises that are part of the programme. They were afterwards given the informed consent form and the willingness to participate questionnaire (PAR-Q) to fill. Selected health indicators (for this study) were then measured to obtain their pre-test or baseline values. They were measured again at the 5th week (mid training) and at the 10th week (post-training).

Body Composition Index: Percentage of body fat, Body water, Body muscle and Bone mass was estimated using the Omron Body Logic Bioelectrical Impedance Analyser (BIA; Omron Home Health Care, Bannockburn, IL) [15].

2.10 Exercise Testing

Subjects walked on a motorized treadmill (*YJ-8057D*) at 1.5 mph and 0% grade for 4 minutes to familiarize themselves with the exercise modality. Speed was increased to 2.0 and 3.0 mph at 0% grade during the next two 4-minute stages. A speed of 3.5 mph and 1% grade during the fourth stage. The treadmill gradient was increased by 1% and speed was increased by 0.5 mph every 4 minutes until 20 minutes is reached or volitional exhaustion is reported. All subjects were introduced to Borg's scale for a rating of perceived exertion. The 6 to 20 Borg scale of perceived exertion was used [16]. Subjects were asked to refrain from any other forms of structured exercise outside of the training program for the duration of the ten weeks. Participants were instructed to follow the Canadian Society for Exercise Physiology preliminary instructions (no eating, drinking caffeine, smoking, or drinking alcohol for 2, 2, 2, or 6hours, respectively, prior to training) [17]. Water may be taken as needed at any time. Subjects were dressed appropriately for the exercise, especially with regards to footwear. No unusual physical efforts were performed for at least 12 hours before exercise testing [16].

2.11 Testing Metabolic Data

Following an overnight fasting (8-12hours), about 5ml of venous blood was drawn from a prominent superficial vein (through a clean venipuncture) in the morning (7-9am) and placed in a tube containing EDTA, centrifuged and frozen. The tube was centrifuged at 250 revolutions per minute (rpm) for 15 minutes at 4°C and plasma samples were stored at -80°C until analysis was performed.

2.12 Blood Lipid Profile

Biochemical analyses for high and low-density lipoproteins (HDLC and LDLC), as well as cholesterol, were obtained by the precipitation method, while triglyceride (TAG) was measured by the enzymatic-calorimetric method.

High-Density Lipoprotein (HDLC): Dyslipidaemia was defined as HDLC <60 mg/dl.

Low-Density Lipoprotein (LDL-C): Dyslipidaemia was defined as LDL-C >100 mg/dl.

Triglycerides (TG): Dyslipidaemia was defined as triglycerides >150 mg/dl.

Total Cholesterol (TC): Hypercholesterolemia was defined as TCH >200 mg/dl. (McArdle, Katch, 2001).

2.13 Validity and Reliability of the Instruments

The psychometric properties of the instruments that were used in this study have been severally evaluated and are within acceptable limits.

2.14 Exercise Training Protocol

The protocol designed for this study is based on the recommendations that any activity performed for training should be assessed in terms of intensity, frequency, duration, mode, and progression [18]. They recommended that all healthy adults aged 18-65years need a moderate-intensity aerobic physical activity for a minimum of 30 minutes on five days each week or vigorous-intensity aerobic activity for a minimum of 20 minutes on three days each week or a combination of the two. This study involved a 10-week exercise training programme of three training sessions per week with at least 24-hour interval between training sessions on a standard treadmill.

The participants were randomly assigned into 2 training groups. The exercise intensity was between 65% to 85 % HR max. The exercise was individualized for each participant based on their training heart rate. The heart rate was monitored through heart rate monitor with a polar belt (sensor) attached to the chest of the clients and the monitor attached to the wrist. The heart rate of the individual was displayed on the monitor attached to the wrist. The perceived exertion was monitored through Borg scale of 20. Perceived exertion was at 12 to 14 for moderate intensity on the Borg 6 to 20 scale, and high intensity when the perceived exertion is 15 to 17[10]. Each session of the exercise started with 10 minutes warm-up which included pulse raiser exercises through the gradual movement of the lower limbs, joints flexibility and muscle stretch. A standard flexibility routine was included in the warm-up time to minimize the risk of injury or fatigue. A 10-minute cool down was allowed after each exercise training session.

2.15 Interval Training

A modified interval training protocol of Gibala (2009) was used for this study [15]. The interval training involved 30 seconds of high-intensity exercise followed by 90 seconds low-intensity exercise in the ratio 1: 3. In the first and second week, participants started with a constant walking speed of 3.3km/hr at 0.0% elevation. The exercise intensity was at 65% percentage of HR max for a period of 20 minutes for 2weeks. In the 3rd and 4th week the intensity of the exercise was increased to 70% of the HR max for the duration of 25 minutes while the low intensity was at 30% HR max resulting into 17 lapses. Subsequently, the exercise regimen was graduated every two weeks by 5% increase in the HR max for high intensity and also 5% increase for the low intensity. The duration of the exercise was increased by 5 minutes. The lapses of exercise also increased as the duration of the exercise increased.

2.16 Continuous Training

The intensity, duration and progression pattern of the exercise was similar to that of interval training except for the absence of low-intensity exercise phase. The exercise intensity was at 65 percentage of HR max for a period of 20 minutes. In the 3rd and 4th week the intensity of the exercise was increased to 70% of the HR max for the duration of 25 minutes. Subsequently, the exercise regimen was graduated every two weeks by 5% increase in the HR. The duration of the exercise was increased by 5 minutes.

2.17 Data Analyses

Data analyses were done using SPSS 21.0 version (*SPSS Inc.*, Chicago, IL). Data were analysed using both descriptive and inferential statistics. Values were expressed in Mean ± Standard Deviation, One Way Analysis of Variance (ANOVA) and Repeated Measures ANOVA were used to compare means. Turkey HSD Post Hoc analysis was used for the one way ANOVA while Bonferroni Post Hoc Test was used for the repeated measures ANOVA to ascertain the direction where ANOVA result was significant. The significance level was set at $P < 0.05$ for all statistical analyses.

3. RESULTS

One hundred and eleven subjects were recruited for this study out of which ninety-seven concluded the study. The results of the ninety-seven participants were analysed and presented as follows:

Table 1. Shows body composition indices of participants at baseline

| Variables	CETG n=32	IETG n=29	CG n=36	f-ratio	p-value
BF (%)	37.48±5.11	39.34±4.34	38.34±6.47	0.886	0.416
BW (%)	44.81±4.75	44.01±3.40	45.29±4.56	0.714	0.492
BMS (%)	31.28±1.62	30.44±2.11	31.07±5.57	0.420	0.658
BM (kg)	9.43±1.57	8.86±1.57	8.07±1.33	7.167	0.001*

Significant at p<0.05; BF = Body Fat; BW = Body water; BMS=Body Muscle; BM=Bone Mass. Results in CET, IET, and Control (C) groups are presented as Mean ± Standard Deviation

Table 2. Shows Lipid profile of participants at baseline

Variables	CETG n=32	IETG n=29	CG n=36	f-ratio	p-value
LDL	2.11±0.73	1.71±0.86	2.33±0.83	4.694	0.011*
HDL	1.55±0.40	1.31±0.44	1.37±0.44	2.703	0.072
VLDL	0.28±0.11	0.22±0.56	0.24±0.10	3.261	0.043*
TG	1.07±0.43	0.78±0.25	0.89±0.41	4.400	0.015*
TC	4.17±1.04	3.40±1.04	4.12±1.02	5.238	0.007*

Significant at p<0.05; LDL=Low Density Lipoprotein; HDL=High Density Lipoprotein; VLDL=Very Low Density Lipoprotein; TG=Triglyceride; TC=Total Cholesterol. Results in CETG, IETG, and CG columns are presented as Mean ± Standard Deviation
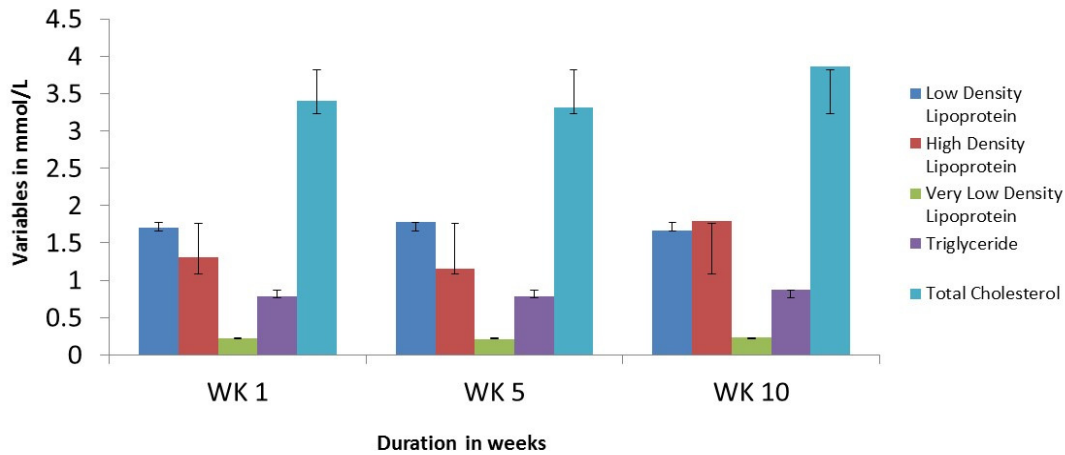
Lipid Profile (mmol/L)
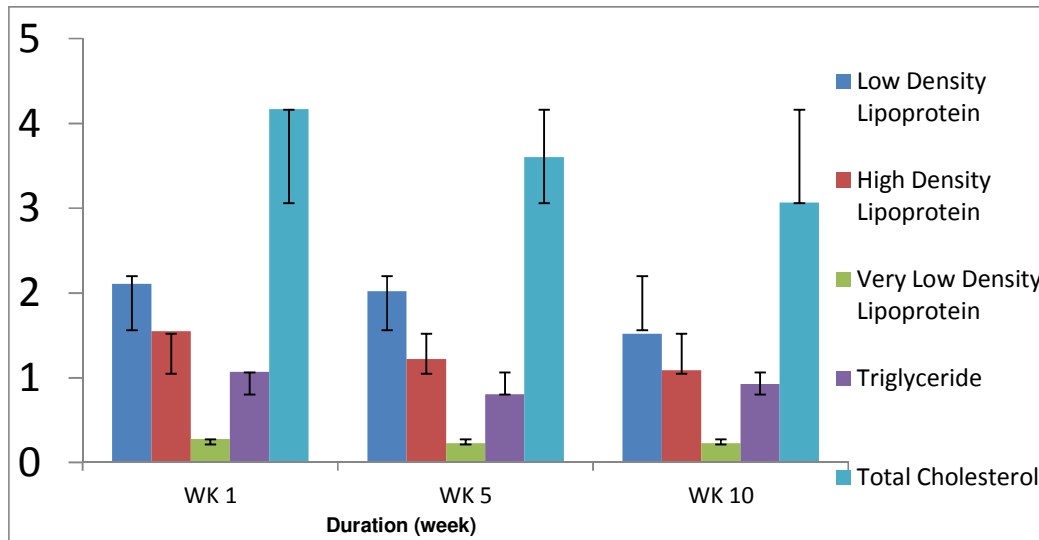
Chart 1. Shows effect of 10 weeks continuous training on LDL, HDL, VLDL, triglyceride and total cholesterol

Table 3. Comparisons of the effects of a ten-week continuous and interval training and a ten week of no exercise training on lipid profile

Variables (Mmol/L)	Continuous (CETG)			Interval (IETG)			Control		
	Pre	Post	MD	Pre	Post	MD	Pre	Post	MD
LDL	2.11	1.52	-0.59	1.7	1.67	-0.03	2.33	2.13	-0.2
HDL	1.55	1.09	-0.46	1.31	1.8	0.49	1.37	1.38	0.01
VLDL	0.28	0.23	-0.05	0.22	0.23	0.01	0.24	0.25	0.01
TG	1.07	0.93	-0.14	0.78	0.87	0.09	0.89	0.88	-0.01
TC	4.17	3.07	-1.1	3.4	3.86	0.46	4.12	3.93	-0.19

LDL=Low Density Lipoprotein; HDL=High Density Lipoprotein; VLDL=Very Low-Density Lipoprotein; TG=Triglyceride; TC=Total Cholesterol; MD=Mean Difference.

Chart 2. Shows Effect of 10 weeks Interval exercise training on LDL, HDL VLDL, triglyceride and total cholesterol
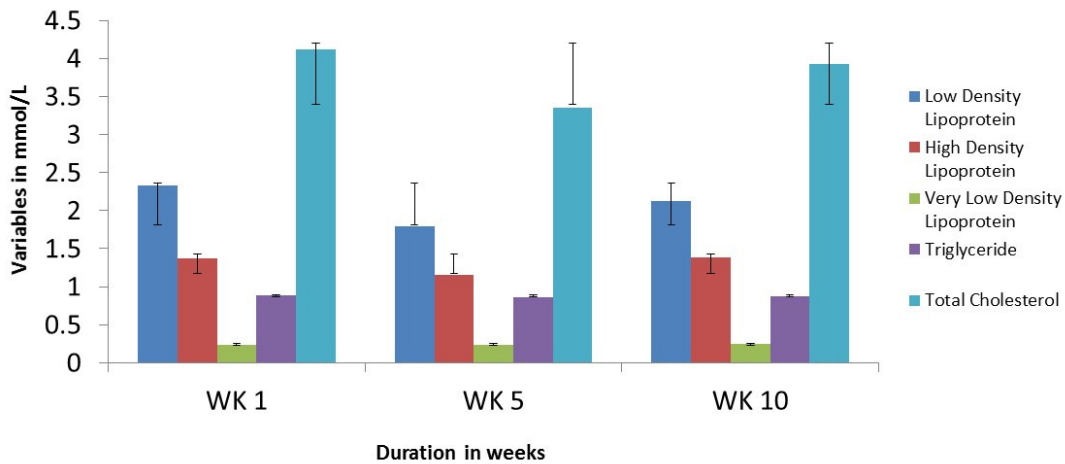
Chart 3. Shows effect of 10 weeks of no exercise training on LDL, HDL, VLDL, triglyceride and total cholesterol of control group

4. DISCUSSION

This study aimed at investigating the possible physiological effects of a 10-week Continuous exercise training programme and a corresponding 10-week Interval exercise training programme on metabolic health indicators (Lipid Profile) in overweight and obese untrained females.

From the study, Cholesterol profile levels of participants (in the 3 groups) met the desirable lipid levels of individuals at high risk of CVD in accordance with the recent European guidelines stated by Chapman et al. (2011). The guideline stated the following as the desirable lipid levels:

LDL-C, 2.5 mmol/L (100 mg/dL) in high risk; and 2.0 mmol/L (80 mg/dL) in very high risk. Triglycerides, 1.7 mmol/L (150 mg/dL); HDL-C .1.0 mmol/L (40 mg/dL) in men; 1.2 mmol/L (45 mg/dL) in women and Non-HDL-C, 2.5 mmol/L (100 mg/dL).

Large observational studies have shown that elevated levels of Total Cholesterol (TC), triglyceride (TG) (fasting or non-fasting) and low-density lipoprotein (LDL) are associated with high risk of cardiovascular disease while increased plasma levels of high-density lipoprotein (HDL) is regarded as cardio protective [19]. Several Studies have reported that Regular physical activity improves the blood lipid profile.

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Endurance exercise training has been shown to bring about favourable modifications to the blood lipid profile of sedentary adults. The result of this present study shows a statistically significant reduction in the LDL, very LDL, TG and TC of the CETG. However, HDL in this group was also seen to decrease following 10 weeks of continuous exercise training. The IETG in this study showed a reduction in the LDL and TG which was not statistically significant, following 10 weeks of interval training. However, there was a progressive statistically significant increase in the HDL. The variation in the response of the interventions to the lipid profile has been postulated by Fletcher et al., to result partly from the heterogeneity of study methods and exercise intervention [16]. The study of Tran and Weltman (1985) as reported by Fletcher et al. concluded that exercise leads to a reduction in total cholesterol, LDL cholesterol, and total/HDL cholesterol ratio and increase in HDL [16]. This is in line with the result of this present study.

Philips (2009) in his study titled 'Effects of Exercise Training Modalities on Fat Oxidation in Overweight and Obese Women ' reported that past studies have suggested lack of positive change in lipid profiles following exercise training in individuals with 'normal' baseline lipid profiles. Consequently, positive changes in the lipid profile are more likely to occur when individuals display an unfavourable lipid profile at the baseline level. This observation is in contrast to the result of this present study which reported the Cholesterol profile levels of the participants in the 3 groups of study to have met the desirable lipid levels of individuals, according to recent European guidelines stated by Chapman et al. (2011). This study thus suggests an improvement in lipid profile with exercise training, despite the level being within the normal range at baseline.

Table 1 summarises the body composition indices of participants at baseline. The baseline comparison of the Body composition parameters showed there were no significant differences (p>0.05) in the percentage body fat (BF), percentage body water (BW) and percentage body muscle (BMS). The results revealed a significant difference (p<0.05) in the bone mass (BM) of participants across the groups. Again, Table 2 Summarizes the Metabolic parameters (lipid profile) of participants at baseline. Here, baseline comparison of cholesterol profile revealed significant differences (p < 0.05) in the Low-Density Lipoprotein (LDL), Very Low-

Density Lipoprotein VLDL), Triglyceride (TG) and Total Cholesterol (TC) of participants across the groups. The results also revealed significant differences (p < 0.05) in the High-Density Lipoproteins (HDL) of the participants across the groups.

Chart 1 shows the results of repeated measures (ANOVA and Bonferoni Post Hoc comparison) on the lipid profile of participants after 5 and 10 weeks of continuous exercise training. The repeated measures (ANOVA) revealed a significant difference in the mean LDL [F(2, 62) = 9.696; p < 0.001], HDL [F(1.648, 51.082) = 7.457; p = 0.003] ,VLDL[F(2, 62) = 4.769; p = 0.012] ,TG[F(2, 62) = 6.173; p = 0.004] and TC [F(1.618, 50.162) = 18.562; p < 0.001] of participants after 5 weeks and 10 weeks of continuous exercise training. Post hoc tests using the Bonferroni correction shows the pattern of the significant differences in metabolic variables of participants within the weeks. It showed a significant decrease in LDL of participants from week1 to week 10; a significant decrease in HDL is between week 1 and week 10; a significant decrease in VLDL and TG between week 1 and week 5.However, there was a steady significant decrease in TC of participants in the CETG from pre-training through mid-training to post-training (P < 0.001).

Chart 2 shows the results of repeated measures (ANOVA and Bonferroni Post Hoc comparison) on the lipid profile of participants after 5 and 10 weeks of Interval exercise training. The chart showed there was no significant difference in the LDL, VLDL and TG of participants in the IETG after 10 weeks of interval training. However, there is a significant difference in the mean HDL [F (1.427, 39.963) = 14.472; p = 0.001] and TC [F (1.227, 35.361) = 6.187; p < 0.013] of participants after 10 weeks of Interval exercise training. The Post hoc tests showed a significant increase (P < 0.05) in the HDL of participants between week1 and week 10; and also a significant increase in TC (P < 0.05) between week 5 and week 10.

Chart 3 shows the results of repeated measures ANOVA and Bonferoni Post Hoc comparison of the metabolic parameters of participants from week 1 to week 10 of no exercise training (CG). The table showed no significant difference in the VLDL and TG. However, the table showed a significant difference in the mean values of LDL [F (2, 70) = 4.463; p = 0.015]; HDL [F (2, 70) =

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3.512; p = 0.035] and TC [F (2, 70) = 6.289; p = 0.003] of CG participants. A Post hoc test to probe the direction of the significance revealed a significant decrease (p< 0.05) in the mean values of LDL, HDL and TC of participants between week 1 and week 5. It also revealed a significant increase in the mean values of LDL, HDL and TC from week 5 to week 10.

5. RELEVANCE OF STUDY

The study will be of great relevance and guide to clinicians and physical therapists (physiotherapists) through improvements of skills for further exploration of the effective mechanism of exercise training in weight management, enabling in extrapolation into their subjects' risk of possible cardio-vascular disease. Result from this study will also contribute to the already existing Protocols and guidelines for exercise prescription in weight management which had been reported by Pallock et al. (1995), Tjonna et al. (2008) and Manning [5] to lack precision in the aspect of training intensity, frequency, duration, mode and progression. The study will also encourage health workers to integrate obesity management using structured exercise into their intervention programmes. It can also serve as an academic guide for Scholars focusing on physical activities and cardio-vascular risk prevention.

6. CONCLUSION

Continuous and Interval exercise training programs have shown from this study to beneficially alter metabolic (lipid profile) parameters in untrained overweight and obese females. Hitherto, continuous and interval aerobic training programs may be incorporated as an effective adjunct, non-pharmacological and non-dietary management of overweight and obesity, and abnormal lipid profile levels.

7. RECOMMENDATIONS

We strongly recommend continuous and interval exercise training programs as alternating management plans for overweight and obese individuals. Similar but advanced studies with dietetic implications for LDL, HDL, VLDL, Cholesterol and TAG build-ups are recommended for further explanation of the reasons for and relevance of the observed differences seen in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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