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SERUM ADIPONECTIN CHANGES IN CORRELATION TO WEIGHT REDUCTION IN OBESE EGYPTIAN ADOLESCENTS

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ABSTRACT

Introduction: Obesity has become one of the most important public health concerns recently, posing significant long term health and economic risks.Aim of the study: To evaluate the effect of six month of balanced diet combined with supervised physical exercise on adiponectinin obese Egyptian adolescents.Methodology: An intervention study that included 60 obese adolescents, subdivided into two groups) A&B.(Both groups were collected from Nutritional clinic at the National Research Center were included in the study. GroupA: (30)were subjected to diet and exerciseprogram. GroupB: (30) were subjected to diet program only.The program was applied for 6 months. All adolescents were subjected to: hormonal assay(ELISA),Anthropometric assessment body composition by BIA analysis and lipid profile. Results:The results of the present study show that a six months weight reduction program including physical activity and diet restriction in obese adolescents showed a statistically significant elevation in the serum level of adiponectin in both groups A&B after the application of the program, where the mean adiponectin levels before the program were 6.7±5.6ug/ml &6.0±3.4ug/ml in groups A&B respectively. While the mean levels ofadiponectin in both groups A&B after the program were 37.4±22.3ug/ml& 37.7±19.4ug/ml respectively.In the same time there was no significant difference between the study groupsA& B.Statistically significant reduction in weight,BMI, WC, WHR for group A, their means before study were ± 81.7, ±33.2, ±98.2, ±1.5 respectively, and their means after the study were± 66.4, ±26.8, ±71.7, ±1.2 respectively. In group B the means before study were ±84.8, ±33.8, ±99.3, ±1.6 respectively and their means after the study were ±74.7, ±30.4, ±69.0, ±1.2 respectively.There was no statistical significant difference between both groups in weight reduction

however the reduction was more significant in group A. Regarding the body composition, results of our study showed reduction in Fat%, VF with slight reduction in FFM, no significant changes in MM In Group A their means before the study was ±41.0, ±14.1±50.7, ±47.4 respectively and their means after the study were ±33.8, ±9.5, ±34.7 ± 46.8, respectively.In group B their means before the study were ±42.4, ±13.9, ±51.3, ±48.4, respectively) and their means after the study were ±35.6, ±9.4, ±38.2, ±48.2 respectively.There was significant difference between both groupsA& B after the program with more reduction in fat% in group A. Regarding the lipid profile in our study there was statistically significant reduction in both groups in (TG, Cholesterol, LDL,). In Group A their means before the study were ±41.7, ±28.9, ±42.8 respectively and their means after the study were ±36.5, ±18.2, ±25.2 respectively, In Group B their means before the study were ±33.8, ±67.5,±75.6 respectively and their means after the study were ±29.5, ±29.1, ±27.4 respectively. No significant difference in both groups after the program. Whereas, the HDL levels showed no significant changes.Conclusion: After the intervention program there was a highly significant elevation in adiponectin hormone, significant reduction in BMI, FAT%, VF,TG and Cholesterol level.

KEYWORDS :*Egyptian adolescents-obesity- Adiponectin hormone-weight reduction program.*

INTRODUCTION :

Obesity in children and adolescents has become an increasing clinical and public health concern. Its prevalence has increased significantly worldwide with an alarming rise of its co-morbidities that elevate the cardiovascular risk of affected people (Windham et al., 2012) Obesity is a major risk factor for chronic diseases and plays a central role in "metabolic syndrome", which includes hyperinsulinism, hypertension, hyperlipidemia, diabetes mellitus, and an increased risk of atherosclerotic cardiovascular disease in adults,Obese children and adolescents may experience more mental health and psychological issues as depression and low self-esteem compared with non-obese children. Obese children are more likely of becoming obese adults in the future (Nebalet al., 2010). Obesity is defined as condition of excess body fat or adipose tissue that results when excess energy has accumulated, it occurs when energy intake chronically exceeds energy expenditure associated with a large number of debilitating and life threatening disorders .Obesity is diagnosed by body mass index equal to or greater than 95th percentile for age and sex in a given population where BMI is calculated by dividing the weight in kilograms by the height in meters squared (BMI = Weight (kg) / Height (m2) (Ursula et al., 2015). The prevalence of obesity has been increasing for several decades. It is now the most common nutritional disorder worldwide, and its medical, psychological, social and economic effects have major consequences for health (Jennifer et al., 2015)

Obesity in Egypt has risen markedly over the past 30 years. Statistics issued by the Egyptian Medical Association for the Study of Obesity in early 2010 estimate that 15 percent of Egyptian (school-age) children are obese, in comparison with its 1990 estimate of only six percent. Being overweight or obese is determined according to Body Mass Index with varying measurements for the different age groups and genders (WHO, 2010). The weight status can be quickly, simply, and inexpensively estimated using the Body Mass Index (BMI), as BMI correlates with the amount of body fat, as it is the preferred measurement for use among children and adolescents to determine their weight status. Child's weight status is determined based on an age- and sex-specific percentile for BMI rather than by the BMI categories used for adults. Classifications of overweight for children and adolescents are age and sex specific because children's body composition varies with age and gender, also the BMI growth charts varies according to ethnic differences so each country has to have its own charts (CDC, 2011).

Children and adolescents aged 2-18 years with a BMI percentile greater than the 5th but less than the 85th is considered normal weight for height; those in the 85th to 95th percentile are considered overweight; and those in greater than the 95th percentile are defined as obese (CDC, 2011).

Adipocyte differentiation (adipogenesis) plays an important role in obesity and energy homeostasis. It is well known that adipose tissue secretes multiple proteins that mediate various biological functions.These proteins are known as adipocytokine which include: tumor necrosis factoradiponectin,leptin, plasminogen activator inhibitor type-1(pal-1) and resistin (Xuet al., 2014).Several studies have demonstrated that there is a relation between plasma Adiponectin and plasma leptin concentrations with increase of visceral fat (Xuet al., 2014).There are several reports that argued about the effect of exercise and dietary-restriction on plasma level of Adiponectin hormone release (Thompson et al., 2014).Adolescents are tomorrow's adult population and their health and well-being are crucial(Katzmarzyket al., 2015).

During adolescence, the need for most nutrients including energy, protein, vitamins and minerals increases. As appetite is also likely to increase, it is important that food choices are made carefully. Many pediatricians view child and adolescent obesity with concern and feel that intervention is important.The US Preventive Services Task Force (USPSTF) identified a Comprehensive programs including: a dietary and physical activity component and using behavioral modification strategies. These programs significantly reduced BMI (-1.9 to -3.3 kg m-2) and improved metabolic outcomes in overweight and obese adolescents(Katzmarzyketal., 2015).Intervention with structured exercise and diet can play an important role in improving health for obese children and adolescents'.In addition to individual treatment modalities, slowing the national obesity trend will require social changes in the form of healthier policies, system reforms and environmental changes where children live learn and play. Health care clinicians and health care systems can be effective partners in formulating policy decisions and community design that nurture healthy children. An assessment of diet, physical activity and sedentary behaviors should be done annually; Clinicians may suggest that children get at least 60 minutes of moderate exercise daily counseling messages should be directed to all parents, regardless of the weight status of their child (Katzmarzyket al., 2015).

Aim of the study

To evaluate the effect of six month of balanced diet combined with supervised physical exercise on adiponectinhormone in obese Egyptian adolescents.

PATIENTS AND METHODS

An intervention study was carried on 60 adolescents aged from 12-14 were suffering from obesity based on their body mass index; which is greater than the 95th percentile for age and gender based on Egyptian Growth Charts were collected from Nutritional clinic at the National Research Center.Studied obese adolescents subdivided into two groups:Group A: 30 obese adolescents were subjected to exercise and nutrition program, Group B: 30 obese adolescents were subjected to diet program onlyboth groups (A&B). These obese adolescents were undergoing weight reduction program (diet &exercise) for 6 months. They were assessed before and after the intervention program for adiponectin hormonal assay, the body weight, body height (or stature), (BMI), waist and hip circumferences, waist/hip ratio, their body composition by BIA analysis and lipid profile.A written and verbal informed consent was taken from all children's parents before enrollment in the study and after full explanation of the objectives of the study and their role in it.

Investigations

All obese adolescents in both groups (A&B) underwent physical examination including anthropometric measures .The body height was measured to the nearest 0.1 cm on a Holtain portable anthropometry, and the body weight was determined to the nearest 0.01kg on a Seca scale Balance with the subject wearing minimal clothing and no shoes. Body mass index (BMI) was calculated as body weight (in kilograms) divided by body height (in meters) squared. Waist circumference was measured at the level of the umbilicus with the subject standing and breathing normally, hip circumference at the level of the iliac crest, using non-stretchable plastic tape to the nearest 0.1 cm. The following indices were calculated:

- Body mass index (Kg/m^2).
- Waist/ Hip ratio (cm/cm).

Fat percentage: Visceral fat, Fat Free Mass, Muscle Mass was measured by a special body composition analyzer called (TANITA model BC-418 Segmental Body Composition Analyzer) (Competitive edge, 2009).

Measurement of serum Adiponectin level:(AviBionHuman Adiponectin (Acrp30) ELISA Kit).

Lipid Profile:

1.Total Cholesterol: measured using an enzymatic method with cholesterol esterase and cholesterol oxidase (Allainet al., 1974).

2.Triglycerides (TG): were measured using an enzymatic method with glycerol phosphate oxidase (Dryer, 1970)

3.High-density lipoprotein cholesterol(HDL-cholesterol):was measured after precipitation of apo-B containing lipoproteins with magnesium chloride/dextran reagent using the same enzymatic method (Finley, 1978)

4.Low-density lipoprotein cholesterol (LDL-cholesterol): was calculated in plasma samples using Friedwald formula: LDL-Cholesterol = total cholesterol – (HDL-Ch + triglyceride/ (Friedwald, 1972).

Intervention program (Diet and Physical activity):

A)Diet:

Before starting the program a dietary pattern was assessed to obtain information about the different items of foods and beverages consumed by every child. Repeated Twenty-four hour recall method, record food intake for three scattered days (3 recalls), includes one day as a holiday. Data was collected by qualified dietary staffs, by personal interview. During the 6-months weight reduction period, personalized diets was offered on the basis of the baseline basal metabolic rate (BMR) test and physical activity level for each adolescent (Lazzeret al., 2005). During weight-reduction period, adolescents had dietetic lessons (nutritional education program) including food pyramids, reading food labels, 5 - a day fruit and vegetables, the importance of eating breakfast and a balanced diet with regular physical exercise and the health hazards of soft drinks consumption and fast food eating behaviors (Aoronet al., 2005). A diet with moderate deficit balanced calories is a reduced-energy diet (-500 kcl/day) (less energy than required to maintain weight but not less than 1200 kcal / day) was established by the nutritional professional. it was selected from the usual 4 food groups in quantities thought to meet basic requirements of all macronutrients and micronutrients in a healthy proportion: (20-30% of total calories from fats,8-10% from saturated fats, up to 15% of total calories from

monounsaturated fatty acids, up to 10% from polyunsaturated fats< 300 mg/d cholesterol, approximately 15 -20 % of total calories from protein, 50-60 % from CHO, 20 - 30 gm/d fiber, no more than 1.5 gm/d Na , water not less than 2.5 liter /d , 1000 - 15000 mg/d calcium).

These diet supplies enough calories to enable the patients to proceed normally in their life & not feel hungry nor lacking energy. Also, it can be adherent to and safe in long and short time, the diet design was individualized, flexible allowing the patients to exchange items, so as not to be boring, and produce 1 lb or 0.5 kg loss per week (Lazzeret al., 2005). During weight-reduction period, adolescents had dietetic lessons (notional education program) including food pyramids, reading food labels, 5 - a day fruit and vegetables, the importance of eating breakfast, and a balanced diet with regular physical exercise and the health hazards of soft drinks consumption and fast food eating behaviors (Aaron et al., 2005).

B) Physical activity:

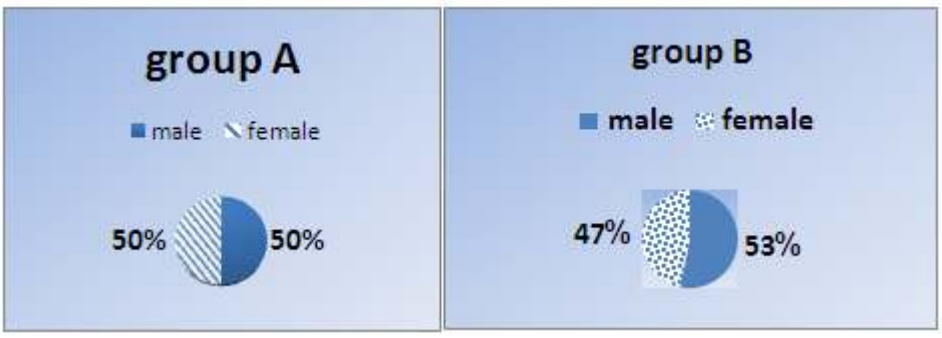
The exercise program was performed at the Faculty of Physical Education or Boys in Cairo under supervision of professional exercise trainer and medical supervision During the 6-month weight-reduction period, the adolescents was participated in an exercise-training program including: two 40 min endurance and strength training sessions (preceded and followed by 5–7 min stretching) per week under heart rate (HR) monitoring and medical supervision. Intensity of endurance exercises was set at an HR corresponding to 55–60% of the initial Oxygen maximum.Subjects had 2 h of physical education lessons (PEL) and 2 h of aerobic activities per week. The adolescents and their parents were also advised to practice leisure physical activities during the weekend and holidays(Lazzeret al., 2005).

Statistical Analysis:

Data analysis was done using SPSS version 16. Simple statistics and bivariate analysis were used. For comparing between two means, Student t-test of significance was done while one way analysis of variance was used to compare between more than two means. The Chi-square test of significance was used to compare frequency between two categorical variables. Correlation analysis using Pearson test was performed between different quantitative variables. In case of p value less than 0.05 the difference between two observations is considered statistically significant.Odds ratio was used to measure the magnitude of the risk factors related to obesity.

RESULTS

Sixty obese adolescents were enrolled in the study with BMI



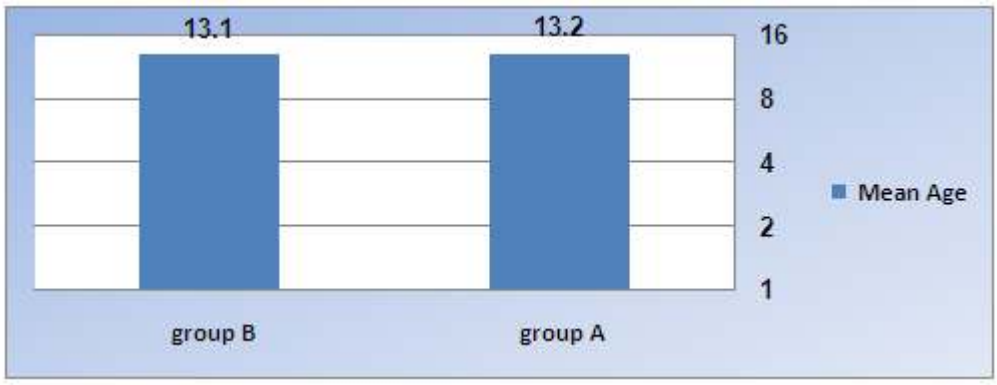


Fig. 1: Demographic characteristics(age&sex) of the studied groups(A&B).

Table 1:Comparison between groups of study as regardsAdiponectin hormone (µg/ml).

Time	Measure	Group A	Group B	ti	p
Before	Mean±SD	6.7±5.6	6.0±3.4	0.658	0.514
After	Mean±SD	37.4±22.3	37.7±19.4	0.061	0.952
Difference	Mean±SD	30.7±20.9	31.8±19.5	0.212	0.833
Before/After		tp= 8.026	tp= 8.903		
		P<0.001*	P<0.001*		

ti: Independent t-test, tp: Paired t-test, *Significant, Negative values indicate reduction.

Table 2: Comparison between both groups(A&B) of study as regards BMI for age percentile (cross tabulation meaning comparison the distribution of frequency between groups).

Time	Percentile	Group A	Group B	X2	P	Significant
Before	<90	0 (0.0%)	0 (0.0%)	*	1.000	
	≥90	30 (100.0%)	30 (100.0%)	0.000		
After	<90	30 (100.0%)	23 (76.7%)	7.925	0.005*	*
	≥90	0 (0.0%)	7 (23.3%)			
^PBefore/ After		1.000	1.000			

χ2: Chi*Significant *not applicable.

Table 3:Comparison between groups of study as regards Triglycerides (mg/dl).

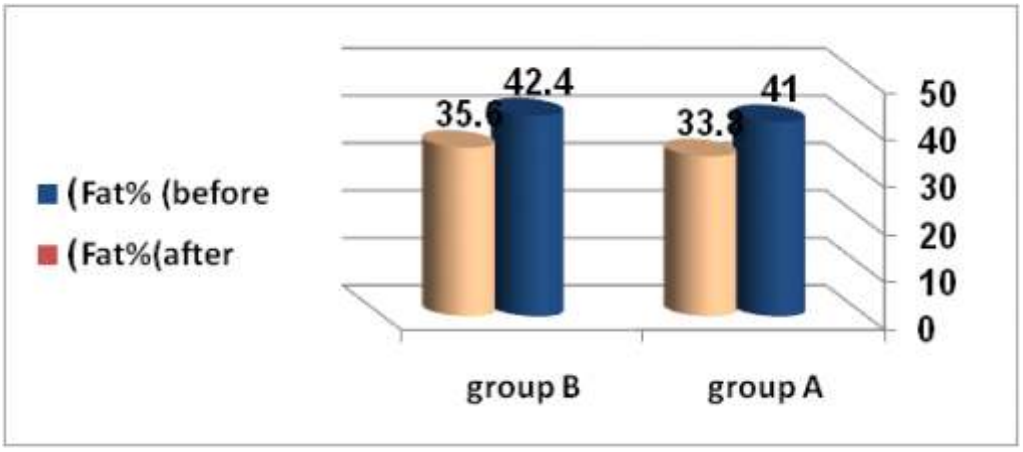
Time	Measure	Group A	Group B	ti	p
Before	Mean±SD	150.3±36.5	143.6±33.8	0.730	0.468
After	Mean±SD	74.8±41.7	86.8±29.5	1.283	0.205
Difference	Mean±SD	-75.5±49.3	-56.9±40.8	1.592	0.117
Before/ After		tp= 8.382	tp= 7.640		
		P<0.001*	P<0.001*		

ti: Independent t-test, tp: Paired t-test, *Significant, Negative values indicate reduction

Table 4:Comparison between groups of study as regards Cholesterol (mg/dl).

Time	Measure	Group A	Group B	ti	p
Before	Mean±SD	277.5±28.9	268.0±67.5	0.709	0.483
After	Mean±SD	148.1±18.2	148.6±29.1	0.080	0.937
Difference	Mean±SD	-129.4±39.1	-119.4±74.4	0.651	0.518
Before/ After		tp= 18.121	tp= 8.783		
		P<0.001*	P<0.001*		

ti: Independent t-test, tp: Paired t-test, *Significant, Negative values indicate reduction.



(P value <0.001*, *=significant)

Fig. 2: Comparison between groups of study as regards Fat percentage.

Table 5:Comparison between groups of study as regards Free Fat Mass (FFM).

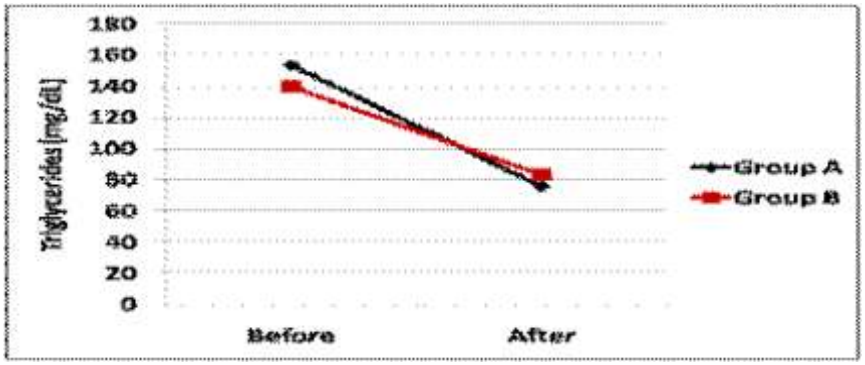
Time	Measure	Group A	Group B	ti	P	Significant
Before	Mean±SD	50.7±7.3	51.3±6.3	0.337	0.738	
After	Mean±SD	34.7±2.0	38.2±4.0	4.240	<0.001*	*
Difference	Mean±SD	-16.0±7.6	-13.1±6.3	1.598	0.115	
Before/After		tp=11.532	tp=11.518			
		P<0.001*	P<0.001*			

ti: Independent t-test, tp: Paired t-test, *Significant, Negative values indicate reduction

Table 6:Comparison between groups of study as regards Muscle Mass.

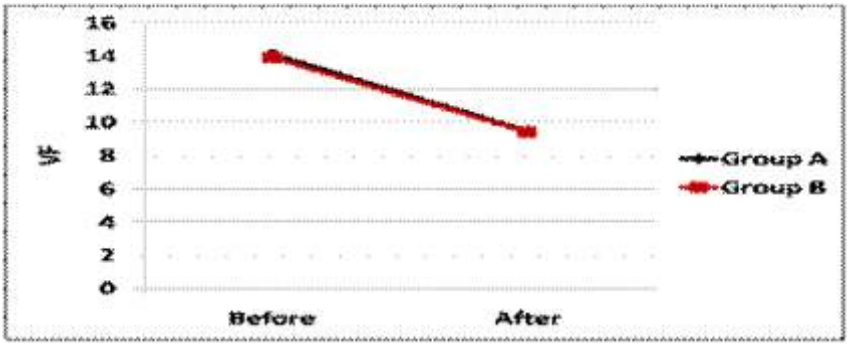
Time	Measure	Group A	Group B	ti	P	Significant
Before	Mean±SD	47.4±7.1	48.4±6.0	0.575	0.567	
After	Mean±SD	46.8±8.6	48.2±6.9	0.713	0.279	
Difference	Mean±SD	-0.6±2.9	-0.1±2.9	0.610	0.544	
Before/ After		tp=1.090	tp=0.232			
		p=0.285	p=0.818			

ti: Independent t-test, tp: Paired t-test, *Significant, Negative values indicate reduction



(P value <0.001*, *=significant)

Fig. 3: Comparison between groups of study as regards Triglycerides (mg/dl).



(P value <0.001*, *=significant)

Fig. 4: Comparison between groups of study as regards Visceral Fat (VF).

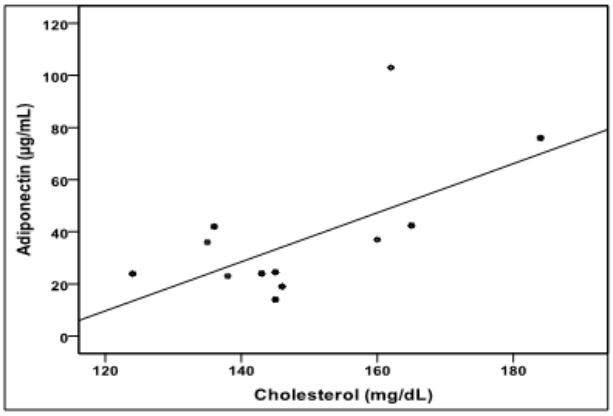


Fig. 5: Correlation between Adiponectin and cholesterol in group A.

DISCUSSION:

Obesity in children and adolescents is a global public health concern and is associated with a range of short- and long-term health complications. Although prevention of obesity is important, so too are effective treatments for those already affected. Lifestyle interventions, involving a combination of diet, exercise, and/or behavior modification, are an essential element of obesity management. Several systematic reviews of childhood obesity have been published and lifestyle interventions targeting treatment of child and adolescent obesity are reported as efficacious in weight loss in the short to mediumterm (Kelly & Kirshenbaum, 2011). Obesity is increasing rapidly all over the world not only in adults but also among children. The prevalence of obesity has reached alarming levels. Obesity is affecting virtually both developed and developing countries of all socioeconomic groups including all age groups thereby posing an alarming problem, described by the World Health Organization (WHO) as an "escalating global epidemic" (Yevgeniyet al., 2015).

Worldwide, over 22 million children under the age of 5 are severely overweight, as are 155 million children of school age. This implies that one in 10 children world- wide is overweight. The dramatic increase in the prevalence of obesity in the past few decades can only be due to significant changes in lifestyle influencing children and adults (Hassan et al.,2011).Adolescents who are overweight or obese are more likely to remain so in adulthood than pre-adolescents aged 10–14, unless the latter obtain treatment(Katzmarzyket al., 2015). Management of obesity has a multi-factorial approach, including dietary modification, exercise, psychotherapy and medication. There are several reports in about exercise programs for adolescents with obesity. However, the focus of most programs is on long-lasting endurance activities, which in our opinion are boring for the pediatric population. Furthermore, most programs are not easily reproducible due to lack of detail or requirement of special equipment.(Katzmarzyket al., 2015).Therefore, our aim was to encourage adolescents to do a moderate physical exercise activity which is accessible to everyone and motivates adolescents by walking with their colleagues.

The present research demonstrated a statistically significant elevation in the serum level of adiponectin in both groups A and B before and after the application of the program, where the mean adiponectin levels before the program were 6.7±5.6ug/ml &6.0±3.4ug/ml in groups A&B respectively. While the mean levels of adiponectin in both groups A&B after the program were 37.4v ±22.3ug/ml& 37.7±19.4ug/ml respectively.In the same time there was no significant difference between the study groups A and B. The obtained results were in line with Numao et al.(2011) which reported increase in the serum level of adiponectin after 12 weeks of exercise and diet intervention. Jae etal.(2013)reported that serum adiponectin level is increased after flexibility exercise and aerobic exercise intervention. However, the findings of Nicklas and Beavers(2010) research were different as they stated that the serum level of adiponectin wasn't affected by moderate exercise, this discrepancy can be attributed to the differences among the studied groups in terms of race, training period, intensity, duration and type of training.Also Hu Hung(2006) found that adiponectinlevel is negatively associated with fat cell size and he suggested that exercise training had no effect on adiponectin production.The Current study showed there was a positive correlation between serum adiponectin and cholesterol in group A after the intervention (p=<0.001;r=0.673). The same correlation between adiponectin and cholesterol level after 6 months weight loss program were found by Sigalet al.(2012)it is well established that losing weight, especially from abdominal fat stores, increases adiponectin concentration in blood and in turn improves all lipid parameters including cholesterol.The current study showed reduction in (weight,BMI,WC,WHR) for group A, their means before study were (± 81.7, ±33.2, ±98.2, ±1.5respectively), and their means after the study were± 66.4, ±26.8, ±71.7, ±1.2 respectively. In group

B the means before study were ±84.8, ±33.8, ±99.3, ±1.6 respectively, and their means after the study were ±74.7, ±30.4, ±69.0, ±1.2 respectively.There was no statistical significant difference between both groups in weight reduction however the reduction was more significant in group A.

Moreover like our resultsHassan et al.(2011) showed in their study on obese adolescents with 6 month weight reduction program that there was a reduction in weight, WC, and WHR. Wittmeieret al.(2008)also, reported that lower durations of both moderate physical activity (MPA) and vigorous physical activity (VPA) was associated with increased odds of overweight and adiposity. They concluded that forty-five minutes of MPA and fifteen minutes of VPA were associated with reduced body fat and BMI. A total of one hour per day of moderate-intensity activity, such as walking on most days of the week, is probably needed to maintain a healthy body weight (WHO, 2008).

On establishing a comparison between male and female result of our study showed that females in group A revealed statistically significant decreased in (Weight,BMI,WC,WHR)., (P = >0.001,>0.001,>0.004,>0.001 respectively), Where females in group B revealed statistically significant decreased in Weight,BMI,WC,HC at P =>0.001,>0.005,>0.032,>0.014 respectively. Malgorzataet al.(2011)in their study after 1-year weight reduction program (diet &exercise),they found that females reduced their BMI%, waist circumference, triglycerides, triglycerides/HDL and increased HDL, while males reduced total cholesterol. It had been demonstrated that there was statistically significant decrease in systolic and diastolic blood pressure in both groups after the intervention program. GroupA:the means of SBP were 118.7 ±10.0, 109.2 ± 7.7 before and after the program respectively, and the means of DBP were83.7 ±6.3, 75.2 ± 4.4 before and after the program respectively.Group B: the means of SBP were 117.3 ±5.6, 110.8 ± 5.0 before and after the program respectively, and the means of DBP were 83.2 ±6.1, 73.7 ± 4.1 before and after the program respectively with no significant difference between both groups A and B.

Regarding body composition , The results of the present study show that a 6 months weight reduction program including physical activity and diet restriction in obese adolescents showed reduction in Fat%, VF, FFM, MM at Group A their mean before the study were ±41.0, ±14.1±50.7, ±47.4 respectively and their mean after the study were ±33.8 , ±9.5, ±34.7 ± 46.8, respectively.In group B their mean before the study were ±42.4, ±13.9, ±51.3, ±48.4, respectively and their mean after the study were ±35.6, ±9.4, ±38.2,± 48.2 respectively.There was significant difference between both groups A& B after the more reduction in fat% in group A. In accordance, Hassanet al.(2011) showed in their study on obese adolescents with 6 month weight reduction program that there was a decrease in the fat mass percent with preserved muscle mass. Moreover, it is not only the total amount of fat that is important, but also the distribution of fat in the body, with central fatness being most related to health risks. Kuanget al. (2010) examined the effect of 12 week exercise program on Chinese adolescents he found significant reduction in fat%. Sameresult was found by Jae et al.(2013).However some studies reported no changes in body composition after completion of an exercise program whereas others observed exercise induced fat mass reduction and preserved fat free mass(Lang etal.,2008).Comparing fat% between males and females in our study, itwas found that females in group B revealed statistically significant decrease in fat% than males some results were found by Malgorzataet al.(2011).

Regarding the lipid profile in our study there was statistically significant reduction in both groups in (TG, Cholesterol, LDL). In Group A their mean before the study were±41.7, ±28.9, ±42.8, respectively and their mean after the study were ±36.5, ±18.2, ±25.2 respectively, In Group B their mean before the study were±33.8, ±67.5, ±75.6 respectively and their mean after the study were±29.5, ±29.1, ±27.4 respectively. No significant difference in both groups after the program. Whereas, the HDL levelsshowed no significant changes.In agreement with our study,Robert (2010) stated that Because

dietary approaches tend to lower total cholesterol, LDL cholesterol, and triglyceride levels, and exercise tends to raise HDL and lower cholesterol levels and triglyceride levels, it seems logical to combine these approaches., combining diet and exercise interventions seems additive, or at least synergistic.However, Mandy et al.(2013) stated that diet-only intervention caused greater reductions in levels of triglycerides (at the end of active intervention) and low-density lipoprotein cholesterol.Susana et al.(2014)showed that measures of central adiposity such as WC and WHR significantly correlated with serum lipid levels in obese children and adolescents but not in leaner individuals.

CONCLUSION

In conclusion the body of research reviewed suggests that lifestyle interventions incorporating a dietary component along with an exercise are effective in treating childhood obesity and improving their anthropometric measurements,adiponectin level, body composition, and lipid profile

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