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Impact Of Treadmill Training on Immunoglobulin G And C- Reactive Protein in Obese Adolescent Girls: Randomized Controlled Clinical Trial

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ABSTRACT
Background: Obesity is linked to metabolic disorders that result in tissue damage and malfunction. In addition to impairing immunity, obesity is associated with low-grade chronic inflammation. **Objective:** The aim of the current study is to assess the effect of treadmill training on immunoglobulin G and C-reactive protein (CRP), body mass index (BMI) and waist circumference (WC) in obese adolescents' girls. **Patients and methods:** A total of 60 obese adolescent girls were selected from National Nutrition Institute aged from 12 to 18 years old. All girls were randomly assigned into two groups. Control group included 30 girls who received diet control therapy only. Study group included 30 girls who received treadmill gait training (3 sessions/per week) in addition to diet control therapy. All girls in both groups were assessed before and after three successive months as Immunoglobulin G and CRP were measured using blood analysis, BMI calculated by weight in kilograms (Kg) divided by the girls' height in meter squared (m^2) while waist circumference by Graduated measuring tape. **Results:** There was a statistically significant increase in IgG level, and a statistically significant decrease in CRP, BMI and WC in both groups; these changes were more in the study than the control, but there was no statistically significant difference in IgG, CRP, BMI and WC when comparing post-treatment results of both groups. **Conclusion:** Treadmill gait training is an effective modality that can be used in the rehabilitation program in obese adolescents for improving their immune functions and reducing inflammatory reactions.
Keywords: Treadmill training, Obese adolescent girls, Immunoglobin G, C-reactive protein.

INTRODUCTION
Obesity and related metabolic disorders have become a major global health concern. It is a state defined by a pathological increase in adipose tissue that can lead to a variety of metabolic disorders, including type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease, and some cancers [1].

Since the 1980s, the prevalence of obesity has more than doubled worldwide, and in 2014, 13% of adults were classified as obese and over 39% as overweight. 1.9 billion adults are predicted to be overweight, with more than 600 million of them being obese. Globally, childhood obesity is on the rise. Overweight/obesity affects 200 million school-age children, of which 40–50 million are obese. Seventy percent of obese adolescents will go on to become obese adults [2].

There is evidence that childhood obesity can persist into adulthood. Obesity in adults is associated with a significantly increased risk of morbidity, such as type 2 diabetes, cardiovascular disease (CVD), and cancer [3].

Adolescent obesity has psychosocial consequences such as body dissatisfaction and lower educational attainment, as well as medical complications such as cardiovascular disease, colon cancer, and diabetes mellitus [4]. Obese people have a poorer immune response to the hepatitis B vaccine than people of normal weight and compared to children of normal weight, the anti-tetanus IgG antibodies in the overweight children were considerably lower [5].

Regular physical activity has been shown to improve physiological, psychological, and immune functions. Exercise-induced changes in immunological responses have been linked to increased levels of cortisol, catecholamine, and neuropeptides. Also, exercises have been associated with changing in consistency of serum immunoglobulin IgG, IgA and IgM, levels [6].

The concentrations of adrenaline and cortisol rise during exercise when the VO2max is greater than 60% [7]. Exercise has anti-inflammatory effects, therefore moderate amount of exercise improves immune function and reduce resting C-reactive protein (CRP) levels [8]. Therefore, this study aimed to determine the effect of treadmill gait training program on immunoglobulin G and C-reactive protein, body mass index and waist circumference in obese adolescent girls.

PATIENTS AND METHODS
Study design and sample: A total of 60 obese adolescent girls participated in this randomized controlled clinical trial.

Inclusion Criteria:
1. Age ranged from 12 to 18 years.
2. Body mass index (BMI) equal or greater than 95th percentile according to CDC growth curves [9].
3. All girls were clinically and medically stable.

Exclusion Criteria
1. Autoimmune diseases.
2. Metabolic disorders.
3. Neurological or genetic disorders (e.g. Down syndrome, hypothyroidism, endocrinal cause of obesity).
4. Cardiac disease, liver or kidney problems.
5. Severe visual or hearing problems.
6. Respiratory disorders.

All girls were randomly assigned into 2 groups using sealed envelopes. Control group included 30 girls who received diet control therapy only. Study group included 30 girls who received treadmill gait tanning in addition to diet control therapy.

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Figure 1 showed flow chart of recruitment and study design.

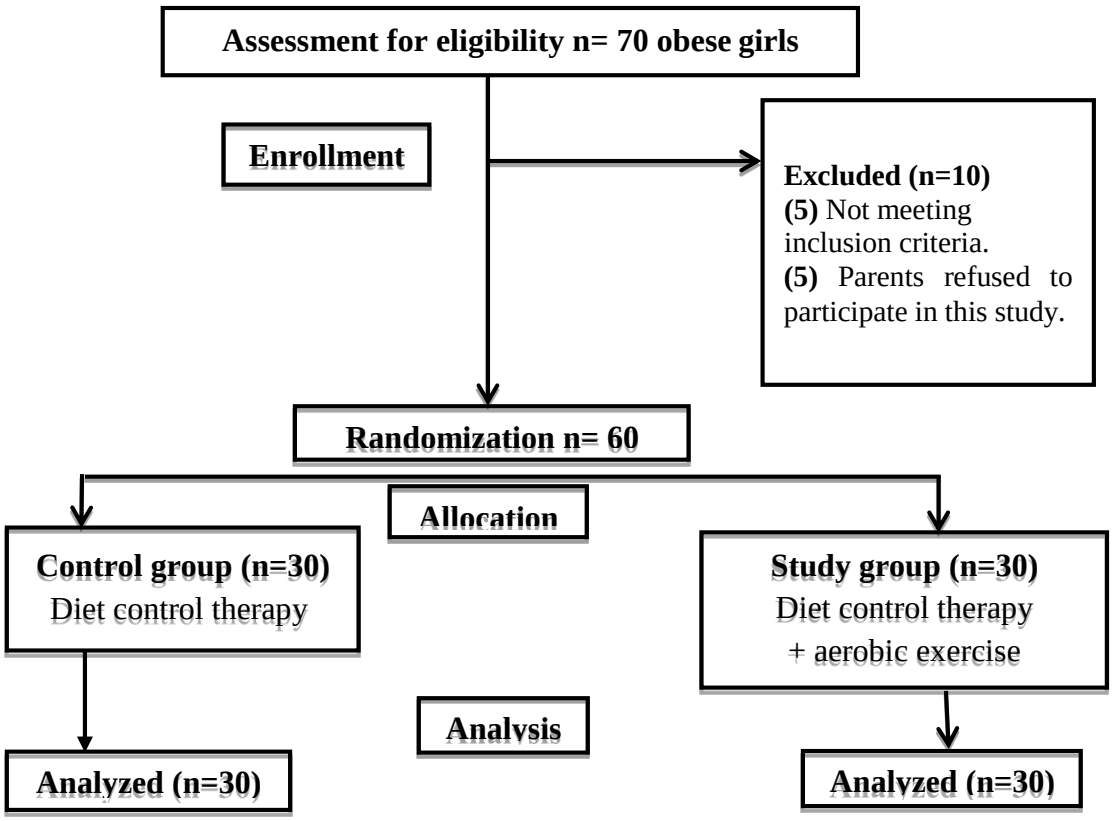

Figure (1): Flow chart of the randomized controlled clinical trial.

Data collection:
Each girl was evaluated (history taken, 24h record) and tested individually following the standard protocol before and after 3 successive months of treatment. Both CRP and IgG were measured using blood analysis. BMI was calculated as body weight in kilograms divided by body height in square meters and waist circumference measured by tape measurement. The obese girls were defined as obese according to CDC growth curves**.**

Procedures for evaluation:
Measurement of anthropometric parameters:
BMI was calculated as body weight in kilograms divided by body height in square meters. The obese girls were defined as obese according to CDC growth curves**.** Waist circumference (WC) was measured to the nearest 0.5 cm at the midpoint between the lower border of the rib cage and the iliac crest at the end of a normal expiration [10].

Measurement of laboratory analysis:
A blood sample (5 ml) was drawn from vein for each girl in study and control groups for measurement of CRP and IgG. Serum was separated and stored at (-20 C) till analysis.

Procedures for treatment:
Both groups received diet control therapy and behavioral modification techniques:

Diet control therapy:
All of the girls in this study were given a well-balanced diet. A dietitian modified this regimen on a weekly basis with the goal of lowering energy intake. All groups receive a balanced diet that is low in fat (20-25%), high in complex carbohydrates (50-60%), and sufficient in protein (20-30%) to support growth. Calorie intake was calculated using a 24-hour dietary recall (24HR). A detailed, structured interview with each girl and her parents was used [11]. The diet was reduced by 500 kcal until it reached 1200-1600 kcal, according to WHO guidelines [12].

Behavioral modification techniques:
Behavior therapy includes different strategies to change and regulate children's activities and diet. These strategies include removing unhealthy foods from the home, tracking what is eaten, setting goals for energy intake and physical activity, and rewarding. Indoor activities can include dancing, hula hooping, and working out to videos. Reduce sedentary behavior by taking stairs instead of the lift, walking, and playing with your siblings instead of watching television [13].

Treadmill training:
The girls in the study group were given a treadmill gait training programme (3 sessions per week for 12 weeks). According to Brown and Levine [14], who divided the treadmill training programme into three phases, Table 1 summarizes the three phases of the training programme.

Table (1): Treadmill gait tanning phases.

Time periods	Total duration of the session	Warming up phase	Main exercise phase with no inclination	Cooling down phase
First phase (the 1st four weeks)	20 min/ session	Walk on treadmill at 4 km/h for 5 min.	Each child was instructed to begin a brisk walk at a speed of 5.6-6.4 km/h for 2-3 minutes. The speed will increase by.32km/h every 2-3 minutes. This phase lasts 10 minutes.	Walk on treadmill at 4 km/h for 5 min
Second phase (the 2nd four weeks)	22 min for the 5th week. 24 min for the 6th week. 26 min for the 7th week. 28min for the 8th week.	Walk on treadmill at 4 km/h for 5 min	Each child gradually increases the amount of time she can walk on the treadmill. Higher speeds introduced. The treadmill parameters were 6.4-7.2 km/h for a maximum speed of 8km/h at the end of the training session. This phase lasted 12, 14, and 16 minutes for the fifth, sixth, and seventh weeks, respectively.	Walk on treadmill at 4 km/h for 5 min
Third phase (the 3rd four weeks)	28 min for the 9th week. 30 min for the 10th week. 32 min for the 11th week. 34 min for the 12th week.	Walk on treadmill at 4 km/h for 5 min	Each child begins to lengthen the time it takes her to complete a task, and the time and speed increase as follows: The speed was 7.2-8 km/h, increasing to 9.6 km/h by the end of the training session. This phase lasts 18, 20, 22, and 24 minutes for the 9th, 10th, 11th, and 12th weeks, respectively.	Walk on treadmill at 4 km/h for 5 min

Ethical consideration:

This randomized controlled trial was approved by Research Ethical Committee of the Faculty of Physical Therapy, Cairo University (no. P.T.REC/012/001394). The purpose and the protocol of this work were explained to all girls and their parents before conducting the study. Informed consent was obtained from all the participant's caregivers. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis:

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 26 for Windows® (IBM SPSS Inc, Chicago, IL, USA). The mean and standard deviation (SD) were calculated for each variable, for study and control groups before and after 3 months following the application of treatment program. Paired t-test was used to compare the pre and post-treatment results for each group to determine the effect of each treatment. Independent t-test was used to compare the pre and post-treatment results of both groups. Pearson's correlation coefficient was conducted to determine the relationship between the measured variables. P value ≤ 0.05 was considered significant.

RESULTS

There were non-significant differences between both groups regarding pretreatment mean values of age, height, weight and BMI ($p>0.05$) as shown in table 2.

Table (2): Comparison of pre-treatment mean values of demographic characteristics between control group and study group.

Variable		Groups			
		Control	Study	t-value	P-value
Age	**Mean ± SD**	14.66 ± 1.817	14.643 ± 1.882	-0.035	0.972
Height	**Mean ± SD**	154 ± 6.782	154.767 ± 7.842	0.405	0.687
Weight	**Mean ± SD**	75.65 ± 12.443	75.863 ± 12.447	0.066	0.947
BMI	**Mean ± SD**	31.422 ± 7.151	31.896 ± 7.315	-0.467	0.642

Data are expressed as mean ± standard deviation, t-value: independent t-test. P-value: Probability value, BMI: Body Mass Index.

The results of this study revealed a statistically significant increase in immunoglobulin G levels in both groups, while CRP showed a statistically significant decrease in both groups. When comparing both groups regarding post treatment means value of immunoglobulin G and CRP. There was more improvement in the study group than in the control group, but it was not statistically significant as shown in tables 3 and 4.

Table (3): Within and between group comparisons of the pre- and post- treatment mean values of immunoglobulin G of control and study groups.

IgG (RU/ml)		Groups			
		Control	Study	t-value	p-value
Pre	**Mean ± SD**	821.55 ± 203.11	748.583 ± 166.526	-0.762	0.449
Post	**Mean ± SD**	935.667 ± 232.427	893.933 ± 213.553	-0.660	0.512
Mean Differences	**MD**	-114.117	-145.350	---	
t-value		-2.680	-3.720		
P-value		0.012*	0.001*		

SD: Standard deviation MD: Mean difference P- value: Probability value
P*: Significant t-value: Independent t-test t-value: paired t-test
IgG: Immunoglobulin G

Table (4): Within and between group comparisons of the pre- and post- treatment mean values of C-reactive protein of control and study groups.

CRP		Groups			
		Control	Study	t-value	p-value
Pre	**Mean ±SD**	5.210 ± 1.121	4.843 ± 1.072	-0.432	0.667
Post	**Mean ±SD**	3.457 ± 0.714	2.860 ± 0.701	-1.225	0.225
Mean Differences	**MD**	1.753	1.983	---	
t-value		4.306	5.510		
P-value		<0.001*	<0.001*		

SD: Standard deviation MD: Mean difference P- value: Probability value
P*: Significant t-value: Independent t-test t-value: paired t-test
CRP: C - reactive protein

The results of this study revealed a statistically significant decrease in BMI and waist circumference in both groups when comparing both groups regarding post treatment means value BMI and waist circumference. There was more improvement in the study group than in the control group, but it was not statistically significant, as shown in tables 5 and 6.

Table (5): Within and between group comparisons of the pre- and post- treatment mean values of body mass index of control and study groups.

BMI		Groups			
		Control	Study	t-value	P-value
Pre	Mean ± SD	31.896 ± 4.19	31.422 ± 3.651	-0.467	0.642
Post	Mean ± SD	30.22 ± 3.784	29.356 ± 3.529	-0.914	0.364
Mean Differences	MD	1.676	2.065	---	
t-value		11.702	12.856		
P-value		<0.001*	<0.001*		

SD: Standard deviation MD: Mean difference P- value: Probability value
P*: Significant t-value: Independent t-test t-value: paired t-test
BMI: Body Mass Index

Table (6): Within and between group comparisons of the pre- and post- treatment mean values of waist circumference of control and study groups.

Waist circumference		Groups			
		Control	Study	t-value	P-value
Pre	Mean ±SD	90.433±8.653	88.800 ± 9.148	-0.710	0.480
Post	Mean ±SD	88.033 ± 7.736	86.150 ± 8.823	-0.879	0.383
Mean Differences	MD	2.400	2.650	---	
t-value		6.077	15.162		
P-value		<0.001*	<0.001*		

SD: Standard deviation MD: Mean difference P- value: Probability value
P*: Significant t-value: Independent t-test t-value: paired t-test

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DISCUSSION

The purpose of this study was to assess the effect of treadmill training on immunoglobulin G, CRP, BMI, and WC in obese adolescents. The results of the present study showed statistically significant improvement in both groups in all measured variables, including IgG, CRP, BMI, and WC in favor of the study group, but it was not statistically significant when comparing both groups and their post-treatment results in all measured variables. It is suggested that the study be repeated with a larger sample size and over a longer period of time, as this may make the difference more defined and statistically significant.

The results of study showed that treadmill training improve immunity by increase immunoglobulin G level. This finding come in agreement with **Ki-Hyeon *et al.*** [15] who studied the effects of aquatic exercise on immune functions of children with disabilities. They found that Aquatic exercise had a significant effect only on IgG, the type that remains in the greatest quantity and has the longest life span.

In addition to improving immune functions, aerobic training leads to a reduction in BMI and waist circumference. These findings are consistent with those of **Mohamed *et al.*** [16], who compared moderate-intensity aerobic training to resistive training in obese women and measured immunoglobulin (Ig) M and IgG. They found a significant increase in IgM and IgG in response to aerobic training, whereas no significant changes occurred in the resistive training group. There were significant differences in IgM and IgG between the two groups after training in favour of the aerobic training group, so regular moderate aerobic training seems to improve immunity compared with resisted training in obese women.

The results of this study come in agreement with the findings of **Martins *et al.*** [17] who performed aerobic ex on 43 independently living men and women, aged between 65 and 96 years They found that the IgG and IgM increased in both groups, however, In the final follow-up evaluation, only the exercise group maintained higher values. Regular aerobic exercise may assist in increasing immunity.

One of the explanations for the increase in special antibodies after exercise is the flushing of non-systemic immunoglobulins out of the secondary lymphatic sites and going into the circulation due to increased lymphatic flow. **Nieman *et al.*** [18] the significant results of IgG obtained after a study of 3 months of moderate-intensity treadmill training may be because of the increasing capacity to generate interferon γ, catecholamine and related to the stimulation of the central nervous system **Drela *et al.*** [19].

The study's findings contradict those of **Hejazi *et al.*** [20] who conducted a study on semi-endurance elite male runners. The runners participated in the selected exercise for 5 weeks, 12 sessions a week in the morning and afternoon. They measured serum levels of IgG, IgM, and IgA. They found that the levels of IgM were reduced significantly, while the levels of IgG and IgA did not change significantly.

The result of the study group concerning the significant reduction of CRP after aerobic training comes in agreement with the work of **Norizam et al.** [21] who conducted a literature search to identify articles which explain the effects of exercises on CRP and vascular markers among obese children and adolescents. They found a significant decrease in CRP following exercise interventions in obese children and adolescents, studies with significant CRP improvement showed a reduction in body weight and percentage of fat after exercise. These findings may imply that improved CRP is not linked to exercise interventions only but is dependent on the amount of weight loss achieved as well.

The significant reduction of CRP in the study group after aerobic training comes in agreement with the findings of Han *et al.* [22] who conducted a systematic review about the randomized controlled trials that evaluated the ability of exercise training to increase the following factors: CRP, tumor necrosis factor (TNF)-α and interleukin (IL)-6 in obese children and adolescents. According to the results of the meta-analysis, CRP levels were significantly lowered with physical exercise**.**

The results of the study group concerning the significant reduction of CRP disagree with the findings of **Wong *et al.*** [23] who studied the effects of 40 minutes of aerobic and resistive exercise twice a week for 12 weeks on serum CRP in adolescent 13 to 14-year-olds. CRP concentrations were found to be elevated at baseline, and training had no effect on CRP levels.

The results of the study group concerning the significant reduction of CRP dis agree with the findings of **Antonio *et al.*** [24] who conducted a meta-analysis to evaluate the evidence for the effectiveness of physical exercise interventions on CRP levels in obese children and adolescents. They found a non-significant reduction in CRP levels. They concluded that exercise programs for children and adolescents did not reduce the inflammatory effects of obesity, despite a tendency toward reduction.

The results of the study group concerning the significant reduction of CRP agree with the findings of **Fedewa *et al.*** [25] who conducted meta-analysis to provide a quantitative measure of the changes in CRP following physical exercise interventions they concluded that participating in an exercise program, regardless of age or gender, is associated with a decrease in CRP levels. Moreover, a decrease in BMI or fat percentage results in greater improvements in CRP levels.

The significant reduction of CRP in the study group could be explained by the work of **Camhi *et al.*** [26] who evaluate CRP changed with diet and exercise in women and men with and without metabolic syndrome. There are several physiological mechanisms that can explain the changes in CRP caused by a healthy diet and physical activity. A well-balanced diet may alter nutrient intake and quality, increasing intake of anti-inflammatory foods like fruits and vegetables, which may finally lower CRP. Another possible mechanism is that low-fat foods limit the postprandial glucose response and subsequent endothelial CRP release. Physical activity causes the release of IL-6 from muscle tissue, which results in the release of anti-inflammatory markers (interleukin-1 receptor antagonist and interleukin-10) that inhibit TNF's pro-inflammatory effects [27]. This negative feedback loop implies that regular physical activity lowers CRP levels.

The results of the study group concerning the significant reduction of BMI after aerobic training come in agreement with the results of **Kelley *et al.*** [28] who conducted a meta-analysis of randomized controlled trials to assess the effects of exercise on BMI in over-weight and obese children and adolescents They came to the conclusion that exercise reduces BMI in overweight and obese children and adolescents.

Also the finding come to agree with the results of **Regaieg *et al.*** [29] who studied the effects of a 16-week training programme on the body composition and aerobic capacity of obese children in addition to school physical education. In addition to school physical education, the exercise group completed a 16-week aerobic exercise programme (four 60-minute sessions per week at 70–85% of maximum heart rate). They observed that the exercise group significantly reduced their BMI and waist circumference. They came to the conclusion that aerobic training programmes benefit body composition and aerobic capacity parameters in obese children.

The significant reduction in BMI in the study group could be explained by the work of **Gholamreza *et al.*** [30] who studied the effects of 8 weeks of aerobic exercise and 8 weeks of green tea for weight loss on 82 overweight men Aerobic exercise appears to be important in reducing body weight. One of the possible mechanisms is an increase in the cost of energy. An increase in fat recall due to increased fat tissue activity; a slight increase in relaxed metabolism following exercise; and a possible increase in thermogenic reaction toward food if exercise performed close to the time of eating food.

The significant reduction of BMI in the control group could be explained by the work of **Smethers *et al.*** [31] who studied the efficacy of lowering energy density for weight loss and weight loss maintenance a fiber-rich diet is thought to increase feelings of fullness by increasing chewing time, encouraging stomach expansion, and reducing absorption efficiency.

The results of the study group concerning the significant reduction of waist circumference after aerobic exercise come to agree with the work of **Lee *et al.*** [32] who studied the effects of aerobic exercise versus resistance exercise on abdominal adiposity in 45 obese adolescent boys. They found that 180 minutes of aerobic and resistance exercise per week without caloric restriction resulted in significant reductions in total fat, visceral adiposity, and WC.

The study group's findings about a significant reduction in waist circumference after diet control agree with the findings of **Normayanti and Prayitno [33],** who studied the effect of diet control on WC in 60 adolescent girls for four weeks. They discovered a significant decrease in WC.

In conclusion, aerobic exercises and diet control have a significant effect on improving immune functions, reducing serum CRP and BMI in obese adolescent girls. Aerobic exercise combined with diet control had more improvement than diet control alone, so treadmill gait training is an effective modality that can be used in the rehabilitation program of obese adolescents to improve their immune functions and reduce inflammatory reactions.

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