Effect of Omega-3 Polyunsaturated Fatty Acids Combined with Dietary Intervention on Childhood Obesity and Insulin Resistance

Abstract

Background: Obesity is one of the multifactorial diseases associated with numerous cardiometabolic comorbidity conditions and IR. Modifying the lifestyle like following healthy nutrition in addition to physical activity, are considered main strategies for obesity management. Besides various treatment options, omega-3 FAs supplementation is suggested as a potential solution to alleviate several obesity-related issues. Aim of the work: to evaluate the effects of omega-3 polyunsaturated FAs in conjunction with dietary intervention on BMI and IR in children with obesity. Subjects and Methods: it's a prospective randomized controlled longitudinal study that was carried out on sixty children & adolescents suffering obesity with BMI of 95th percentile for age and gender or even higher. They were categorized into 2 groups (thirty children in each group): group A received oral omega-3 PUFAs in addition to a dietary intervention for six months, and group B received only a dietary intervention during the same period. Another thirty healthy children & adolescents having BMI ranged from 5th to 85th percentile for gender and age were matched with the obese children's groups for age and gender and served as controls. All participants included in the study underwent a history taking, thorough clinical assessment, anthropometric measurement, and various investigations such as ALT, AST, CRP, lipid profile, HOMA-IR, serum adiponectin, serum leptin, and ALR. Results: After the therapeutic interventions, group A (omega-3-supplemented) showed significant improvement in waist circumference, serum triglycerides, HDL-C, inflammatory adipokines (that includedleptin, adiponectin, and ALR) as well as markers for insulin sensitivity compared to group B. Conclusion: Combining omega-3 PUFAs with dietary intervention improved various obesity-related parameters, enhancing insulin sensitivity markers and reducing inflammatory adipokines in obese children and adolescents.

Keywords: omega-3 PUFAs, Dietary Intervention, Childhood Obesity, Insulin Resistance

INTRODUCTION:

Obesity is currently considered an important health issue globally with increased prevalence in low as well as middle- income and many high-income countries [1]. In 2019, the World Obesity Federation predicted that by 2030, approximately 254 million children & adolescents of 5–19 y age will suffer obesity, up from 206 million in 2025 [2]. In Egypt, 15% of primary school children were overweight, while 10.5% were obese [3].

Obesity is a complex multifactorial disease associated with different cardiometabolic diseases including dyslipidemia, HTN, DM, metabolic syndrome, and insulin resistance [4].

Insulin resistance (IR), an essential link between obesity and various cardiometabolic consequences, is characterized by a reduction in the tissue response to insulin and elevated serum levels [5].

Adipose tissue releases various inflammatory markers that can lead to IR and beta -cell failure. Leptin and adiponectin are two such markers [6]. Reduced adiponectin levels have been linked to the development of IR and metabolic syndrome due to the absence of its anti-inflammatory, anti-atherogenic, in addition to its insulin-sensitizing criteria [7].

Childhood obesity and its associated insulin resistance can be managed effectively through lifestyle interventions, which include a healthy diet and increased physical activity [8]. Additional adjuvant treatments like metformin treatment or omega-3 polyunsaturated FAs (PUFAs) can also promote insulin resistance [9].

Omega-3 PUFAs like α-linolenic acid,eicosapentanoicacid,and docosahexaenoic acid are essential FAs necessary for human beings. They are primarily found in fatty fish, other seafood, certain nuts, and seeds [10].

Omega-3 PUFAs enhance postprandial satiety along with expression of genes responsible for fat oxidation and reduction of fat deposition in tissues, which can explain weight loss and body fat reduction. They increase insulin sensitivity by activating adipokine secretion, particularly adiponectin. They also improve hepatic glucose uptake and hinder gluconeogenesis. They are involved in the inhibition of inflammatory cytokines release that are proved to be the main factors responsible for the etiopathogenesis of obesity [11].

This study aimed to evaluate the role of Omega-3 PUFAs in conjunction with dietary intervention on body mass index and insulin resistance in children suffering obesity.

Methodology:

This prospective cross-sectional and longitudinal randomized controlled study was carried out on 90 children and adolescents recruited from the Nutrition Outpatient Clinic, Gastroenterology and Clinical Nutrition Unit, Pediatric Department, Tanta University

Hospital. Sixty children & adolescents suffering obesity with a BMI of 95th percentile or even higher for gender & age were categorized to 2 groups (30 children in each). Group A received oral PUFAs in addition to dietary intervention for six months, and Group B received only dietary intervention for the same period. Another thirty healthy children & adolescents having BMI ranged from 5th - 85th percentile for gender and age were matched with the obese cases for age & gender and served as controls.

Inclusion criteria: Obese children and adolescents between 8 & 15 y old with a BMI $\geq 95^{th}$ percentile for their age and gender

The following were the exclusion criteria for this study:

- Children who received omega-3 PUFAs within the past six months.
- Children suffering syndromic obesity (such as Prader Willi or Laurence-Moon Bidle syndrome).
- Children whose obesity is due to endocrinal causes like Cushing syndrome or decreased thyroid functions.
- Children with inflammatory or collagen disease, systemic diseases (such as liver diseases), neoplasm, or type 1 or 2 diabetes mellitus.
- Female patients who have reached menarche.
- Using medications with metabolic adverse influences like diuretic, β-blocker, β-adrenergic agonists, corticosteroids, or anti-thyroid drugs.
- Using weight loss therapy that might alter lipid and glucose levels.
- Any child who was non-compliant with either the drug or dietary intervention.

During the study, all participants were subjected to a comprehensive assessment that included a detailed medical history, thorough clinical examination including the anthropometric measurements, and various laboratory investigations, such as ALT,AST, CRP, lipid profile, serum leptin, serum adiponectin, ALR, FBG, FSI, and HOMA-IR.

Anthropometric measurements:

BMI:

Calculation was made depending on this formula:

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height}^2\ (\text{m}^2)}$$

Z-scores were calculated using the Egyptian Z-score tables for weight, height, and BMI [12].

To identify individuals with unhealthy growth, the WHO recommended cut-off values of + 2 Z-scores [13]. WC, HC, and waist/hip ratio were assessed.

Medical intervention:

Group A obese patients received oral omega-3 PUFAs 1200 mg in the form of 2 capsules (each capsule contains fish oil 1200 mg, 50% active omega-3 PUFAs containing EPA 360 mg, DHA 240 mg, and vitamin E 19 IU) as a single dose/ day with a meal for six months.

The dietary intervention [14]:

1. Basic healthy lifestyle eating and activity habits included:
    - Restriction of simple carbohydrates and carbonated sugary drinks.
    - Reducing the consumption of saturated fat-containing food.
    - Encouragement of vegetables and fruit intake that are rich in fiber.
2. Estimation of the caloric requirement via the use of Schofield equation to assess the BMR in calories, and the result was multiplied by the stress activity factor [14].
3. A balanced low glycemic index healthy diet was described [14],
    - The total calories were as follows:
        - 55% from carbohydrate
        - 20% from protein
        - 25% from lipid (more than 7% saturated fats, > 300 mg/d cholesterol, and > 1% trans-fat)
    - Salt intake was limited to ≤ 3 g/ day.
4. Obese children <12 years, 125- 250 kcals/day were subtracted for ¼ kg/week weight loss.

5. Obese children >12 years, 1000 kcals/day were subtracted to achieve no more than 1 kg/week weight loss.
6. Daily energy intake should not be < 900 kcals for children aged 6-12 years and 1200 kcals for those aged 13-18 years.

Statistical analysis:

Collection, coding, revision, tabulation of data was done to be analyzed via SPSS version 20.0 software from IBM Corp in Armonk, NY. Descriptive statistics like percentages (%), the arithmetic mean, the SD, the median and the IQR were calculated for both quantitative and qualitative data to describe the study population. The obtained results were analyzed for significance at a level of 5%. The statistical tests utilized are the chi-square test, ANOVA F-test, paired t-test, Kruskal Wallis test, Wilcoxon signed ranks test, Mann Whitney test, McNemar's test, and Marginal Homogeneity Test.

Measurement of the inflammatory adipokines (leptin and adiponectin);

The principle:

The kit for any of these inflammatory markers used a double-antibody sandwich ELISA method. First, addition of the inflammatory marker to a pre-coated MAP enzyme wellwas carried out. The sample was then incubated before the addition of biotin-labeled antibodies to combine with streptavidin-HRP forming immune complex. Then, further incubation and wash for the removal of any uncombined enzymes was done.Addition of the Chromogen solution A & B was done, causing the colour of the liquid to turn bluish. At the last, the colour changed to yellow due to the influence of the acid. The concentration of leptin and adiponectin in the sample was positively correlated with the intensity of colour seen.

Results:

Demographic Data:

Our study included 60 obese children and adolescents, categorized into 2 groups (30 patients each): group A had ages ranging from 8 - 15 y, with a mean age of 11.52 ± 2.28. Group B had ages ranging from 8 to 15 years, with a mean of 11.81 ± 2.31 years. 43.3% of group A obese children were males, and 56.7% were females, while in group B, 43.3% were males, and 56.7% were females. 46.66% of group A obese children were prepubertal, and 53.33% were pubertal, while in group B, 50% were prepubertal and 50% were pubertal. Non-significant difference was demonstrated in the age, gender, and Tanner staging between group A and B obese children.

The control group included thirty healthy children along with adolescents with BMI ranging between the 5th to the 85th percentile for the gender & age. The age range and mean age value were (8-15), 11.77 ± 2.47 years, respectively. They included 50% males and 50% females. 46.66% were prepubertal, and 53.33% were pubertal. non- significant differences were observed in the age, sex, or Tanner staging among obese children and their controls.

Family history of the studied groups:

The percentages of obese children in group A with a positive family history of T2DM, HTN, or obesity were 60%, 60%, and 70%, respectively, and these percentages were 53.3%, 50%, and 63.3%, respectively for group B obese children. These percentages were significantly higher than those in controls (23.3%, 33.3%, and 33.3%, respectively). However, these percentages were comparable between both obese groups.

Clinical parameters in studied groups

As regards the anthropometric measurements before therapy, group A and group B obese participants in our study had significantly higher weight, weight Z score, BMI, BMI Z-score, WC, HC, and waist-hip ratio than controls. These measurements improved significantly after the therapeutic interventions, but there were still significant differences between the obese

groups and controls. Meanwhile, non- significant differences in height Z-scores across the studied groups before and after the intervention. Non-significant differences were demonstrated between the 2 obese children's groups as concernng these measurements before and after therapy. However, waist circumference showed significant decrease in group A in comparison with group B obese children.

Regarding acanthosis nigricans before therapy, non-significant difference was demonstrated between the two obese groups in the percentage of affected cases and grading of AN. After the therapeutic interventions, only group A obese children exhibited a significant reduction in the percentsage of the affected patients. However, both obese groups (A and B) improved significantly in AN grading after the interventions. Following the interventions, group A had a significantly lower proportion of obese children with AN and a lower AN grading than group B.

Before therapy, SBP and DBP showed a significant increase in groups A and B obese children than in controls. After the therapeutic intervention, the SBP and DBP decreased in both groups and became significantly lower than before the intervention but were still significantly higher than in controls. Before therapy, non- significant difference was reported between group A and group B obese children regarding SBP and DBP, while after therapeutic interventions, the systolic blood pressure exhibited significant reduction in group A in comparison with group B, and non- significant differences were documented between both groups as regard the diastolic blood pressure**.** Table 1

Table 1: Changes in some clinical parameters between groups

	Group A			**Controls**	**Group B**			**P value bet. groups A&B**
	Before therapy	**After therapy**	**P**		**Before therapy**	**After therapy**	**P**	
Weight (Kg)	89.03 ± 9.39	82.55 ± 8.55	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	66.23 ± 6.43	92.03 ± 11.94	88.10 ± 11.27	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.956$ $P_5=0.598$
Weight	2.20	1.60 (1.40 –	$p_1<0.001^*$	0.0 (-0.40 –	2.25 (1.90 – 2.60)	1.95 (1.70 –	$p_1<0.001^*$	$P_4=0.793$

Z-Score	(2.0 – 2.70)	1.90)	$p_2<0.001^*$ $p_3<0.001^*$	0.30)		2.30)	$p_2<0.001^*$ $p_3<0.001^*$	$P_5=0.061$
Height (cm)	144.2 ± 12.76	145.6 ± 12.29	$p_1=0.689$ $p_2>0.05$ $p_3>0.05$	146.3 ± 14.40	146.0 ± 12.34	147.1 ± 11.98	$p_1= 0.544$ $p_2>0.05$ $p_3>0.05$	$P_4>0.05$ $P_5>0.05$
Height Z-Score	-0.35(-0.70–0.20)	-0.10 (-0.40–0.30)	$p_1=0.075$ $p_2>0.05$ $p_3>0.05$	-0.10 (-0.40–0.20)	0.0(-0.40 – 0.20)	-0.10 (-0.50–0.30)	$p_10.227$ $p_2>0.05$ $p_3>0.05$	$P_4>0.05$ $p_5>0.05$
BMI Kg/m^2	32.10 ± 5.07	28.47 ± 5.03	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	20.86 ± 2.77	32.35 ± 5.13	30.71 ± 4.80	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.974$ $P_5=0.116$
BMI Z-Score	2.80 (2.60 – 3.10)	2.40(2.10 – 2.80)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	0.20 (-0.30 – 0.40)	2.80 (2.60 – 3.40)	2.50 (2.20 – 2.90)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.961$ $P_5=0.510$
WC (cm)	89.03 ± 9.39	82.55 ± 8.55	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	66.23 ± 6.43	92.03 ± 11.94	88.10 ± 11.27	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.445$ $P_5=0.049^*$
HC (cm)	95.43 ± 6.42	92.0 ± 6.25	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	70.23 ± 5.67	95.30 ± 5.32	91.33 ± 5.67	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.996$ $P_5=0.899$
Waist-Hip Ratio	0.94 ± 0.06	0.86 ± 0.04	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.025^*$	0.84 ± 0.03	0.93 ± 0.04	0.87 ± 0.04	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.029^*$	$P_4=0.409$ $P_5=0.998$
Positive AN (>0)	22 (73.3%)	11 (36.7%)	$p_1=0.01$*	-----	23 (76.6%)	17 (56.6%)	$p_1=0.579$	------
Grading of AN	1.0 (0.0 – 2.0)	0.0 (0.0 – 1.0)	$p_1<0.001^*$	----	1.0 (0.0 – 2.0)	1.0(0.0 – 2.0)	$p_1=0.039$*	------
Systolic (mmHg)	112.0 ± 8.77	102.0 ± 9.52	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.026^*$	96.67 ± 7.11	113.0 ± 13.04	107.50 ± 6.40	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.920$ $P_5=0.021^*$
Diastolic (mmHg)	68.83 ± 5.83	64.67 ± 3.46	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.007^*$	61.0 ± 4.62	67.67 ± 7.51	64.50 ± 5.31	$p_1=0.002^*$ $p_2<0.001^*$ $p_3=0.010^*$	$P_4=0.740$ $P_5=0.989$

(IQR), IQR: Inter quartile range. BMI: Body mass index, WC: Waist Circumference, HC: Hip Circumference, AN: acanthosis nigricans. p_1: p value to compare between before therapy and after therapy, p_2: p value to compare between obese children and controls (before therapy), p_3: p value for comparing between obese children and controls (after therapy), p_4, p_5: p value to compare between group A and group B before and after therapy respectively, *: Statistically significant at p equal or less than 0.05.

Laboratory and inflammatory markers in the studied groups

As regards CRP and lipid profiles before therapy, groups A and B obese children had significant increase in serum CRP, TC, LDL-C, and TGs level than controls, but decreased serum HDL-C levels. Following therapy, both obese groups showed significant improvements in all of these parameters. Regarding CRP, triglyceride, and HDL-C levels, no significant differences was observed when compared to controls in group A. Moreover, CRP, and TGs level showed significant reduction and HDL showed significant increase in group A compared to group B obese children. Table 2.

HOMA-IR FBG, and FSI levels showed significant in obese groups compared to the control subject before therapy. However, after therapeutic interventions, these parameters showed significant reduction in the obese groups yet were still higher in comparison with the controls. Furthermore, the levels showed significant decrease in group A obese children compared to group B. Table 2

Serum leptin, adiponectin and adiponectin-leptin ratio (ALR)

Before interventions, the two obese groups had significant increase in serum leptin and decrease serum adiponectin and ALR in comparison with the control subjects. However, after therapeutic interventions, a significant improvement was detected in these parameters in the 2 obese groups. Despite this improvement, significant differences were present between the 2 obese groups and the control group. Moreover, following therapy, group A obese children had significantly lower serum leptin and higher serum adiponectin and ALR than group B. Table 2

Table 2: Changes in some laboratory and inflammatory markers in the studied groups

	Group A			**Controls**	**Group B**			**P value bet. groups A&B**
	Before therapy	**After therapy**	**P**		**Before therapy**	**After therapy**	**P**	
CRP (mg/l)	6.0 (4.0 – 9.0)	0.0(0.0 – 2.0)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.121$	0.0 (0.0 – 3.0)	8.0 (5.0 – 9.0)	1.50 (0.0 – 6.0)	$p1<0.001^*$ $p2<0.001^*$ $p3=0.370$	$P_4=0.684$ $P_5=0.014^*$
ALT (U/L)	41.67 ± 17.60	26.13 ± 6.10	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.804$	24.73 ± 6.20	41.70 ± 14.72	35.80 ± 12.10	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=1.000$ $P_5<0.001^*$
AST (U/L)	44.13 ± 12.36	27.93 ± 9.21	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.217$	24.0 ± 5.58	42.0 ± 13.90	35.80 ± 11.37	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.742$ $P_5=0.003^*$
TC (mg/dl)	164.2 ± 25.42	149.1 ± 20.12	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	129.7 ± 8.41	172.7 ± 31.89	162.4 ± 27.53	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.497$ $P_5=0.121$
HDL-C (mg/dl)	55.0 ± 8.02	68.33 ± 7.01	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.313$	65.90 ± 5.50	58.70 ± 8.33	61.53 ± 7.01	$p_1=0.013^*$ $p_2=0.001^*$ $p_30.027^*$	$P_4=0.134$ $P_5<0.001^*$
LDL-C (mg/dl)	99.73 ± 23.56	79.30 ± 23.64	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.028^*$	63.17 ± 10.87	104.8 ± 31.62	83.87 ± 32.04	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.003^*$	$P_4=0.681$ $P_5=0.739$
TG (mg/dl)	139.87 ± 28.12	106.4 ± 18.14	$p_1<0.001^*$ $p_2<0.001^*$ $p_3==0.788$	103.3 ± 9.40	128.97 ± 27.15	122.0 ± 23.59	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.169$ $P_5=0.003^*$

FBG (mg/dl)	97.83 ± 10.26	76.0 ± 4.01	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.002^*$	84.43 ± 5.70	102.40 ± 11.49	95.80 ± 9.38	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.541$ $P_5=0.001^*$
FSI (µU/ml)	15.56 ± 3.08	12.83 ± 2.36	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	8.74 ± 1.64	15.74 ± 3.25	14.55 ± 3.07	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.964$ $P_5=0.020^*$
HOMA–IR	3.83 ± 0.75	2.83 ± 0.52	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	1.85 ± 0.44	3.86 ± 0.87	3.41 ± 0.75	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.991$ $P_5<0.001^*$
Serum adiponectin (mg/l)	10.25 ± 1.61	14.22 ± 1.47	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	24.16 ± 3.45	9.45 ± 1.85	10.51 ± 1.96	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.423$ $P_5<0.001^*$
Serum leptin (ng/ml)	18.11 ± 4.14	13.09 ± 2.50	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=<0.001^*$	6.47 ± 0.87	16.29 ± 4.96	15.59 ± 4.90	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.153$ $P_5=0.010^*$
ALR	0.68 ± 0.19	1.06 ± 0.17	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	3.66 ± 0.54	0.66 ± 0.21	0.86 ± 0.14	$p_1<0.001^*$ $p_2<0.001^*$ $p_3<0.001^*$	$P_4=0.949$ $P_5=0.049^*$

IQR: Inter quartile range. **TC:**Total cholesterol, **HDL:** High density lipoprotein**, LDL:** Low density lipoprotein**, TG:** Triglycerides, **FBG:** Fasting Blood Glucose,**FSI:** Fasting Serum Insulin, **HOMA–IR:** Homeostatic Model Assessment-IR, **ALR:**Adiponectin–leptin ratio,**p_1:** p to compare between before therapy and after therapy, **p_2:** p value for comparing between obese children and controls (before therapy), **p_3:** p value for comparing between obese children and controls (after therapy), **p_4, p_5:** p value to compare between group A and B before and after therapy respectively, ***:** Statistically significant at p equal to or less than 0.05.

Effects of dietary intervention on 24-hour-dietary recall analysis in the studied groups

Before the dietary intervention, analysis of the 24-hour dietary recall revealed that the total caloric intake, the energy intake from carbohydrate and fat sources, the saturated fat intake, the percentage of children with high trans-fat intake, and the consumption of sugary beverages and salt showed significant increase in both obese children groups in comparison with controls. Conversely, both obese children groups consumed significantly less dairy and had a lower energy intake from protein sources than controls. After six months of the dietary intervention, the obese groups showed significant improvement in all nutrient intakes. Moreover, non- significant difference was observed in the 24-hour dietary recall between the two obese groups before and after the intervention. Table 3

Table 3: Effect of dietary intervention on 24-hour dietary recall analysis in the studied groups

	Group A			**Controls**	**Group B**			**P value bet. groups A&B**
	Before therapy	**After therapy**	**P**		**Before therapy**	**After therapy**	**P**	
Caloric	2430.0 ±	1703.3 ±	$p_1<0.001^*$	1715.0 ±	2336.7 ±	1665.0 ±	$p_1<0.001^*$	$P_4=0.192$

intake (kcal/day)	257.8	179.5	$p_2<0.001^*$ $p_3>0.05^*$	153.8	193.4	180.6	$p2<0.001^*$ p3>0.05	$P_5>0.05$
Percentage of CHO in calories /day	64.07 ± 3.76	56.27 ± 3.33	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.025^*$	54.07 ± 3.63	62.80 ± 3.59	55.0 ± 2.82	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.003^*$	$P_4=0.377$ $P_5=0.446$
Sugary beverages (servings/d)	3.0 (3.0 – 4.0)	2.0 (1.0 – 2.0)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.029^*$	1.0 (1.0 – 2.0)	4.0 (3.0 – 4.0)	2.0 (2.0 – 3.0)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.03^*$	$P_4=0.549$ $P_5=0.297$
Percentage of fat in calories /day	29.93 ± 4.16	25.0 ± 2.72	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.908$	24.73 ± 2.46	29.13 ± 3.18	24.07 ± 2.21	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.551$*	$P_4=0.625$ $P_5=0.314$
Saturated fat (%/d)	12.0 (10.0 – 15.0)	6.0 (5.0 – 10.0)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.007^*$	5.0 (4.0 – 6.0)	10.50(10.0 – 14.0)	6.0 (4.0 – 8.0)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.184$	$P_4=0.414$ $P_5=0.169$
Trans fat (< 1%)	4 (13.3%)	20 (66.7%)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.146$	25 (83.3%)	8 (26.7%)	25 (83.3%)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3>0.05$	$P_4=0.197$ $P_5=0.136$
Percentage of protein in calories/day	13.87 ± 2.24	17.30 ± 2.31	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.056$	18.60 ± 2.31	15.03 ± 2.27	17.93 ± 1.53	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.433$	$P_4=0.121$ $P_5=0.470$
Dairy products (servings/d)	1.0 (0.0 – 1.0)	2.0 (1.0 – 2.0)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.025^*$	2.0 (2.0 – 3.0)	1.0 (0.0 – 1.0)	1.0 (1.0 – 2.0)	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.003^*$	$P_4=0.329$ $P_5=0.446$
Salt intake (gm/d)	3.82 ± 0.68	2.75 ± 0.52	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.011^*$	2.21 ± 0.63	3.47 ± 0.75	2.28 ± 0.47	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.001^*$	$P_4=0.131$ $P_5=0.470$

Data are presented as mean ± SD or Median (IQR). **IQR**: Inter quartile range, **p_1:** to compare between before therapy and after therapy, **p_2:** to compare between obese children and controls (before therapy), **p_3:** p value for comparing between obese children and controls (after therapy), **p_4, p_5:** to compare between group A and group B before and after therapy respectively, ***:** Statistically significant at $p \leq 0.05$.

Side effects of omega-3 PUFAs in group A obese children

The most common side effects of omega-3 PUFAs were fishy burping followed by abdominal distention, diarrhea, nausea, and vomiting. Table 4

Table 4: adverse effects of omega- 3 PUFAs in the group A obese children

Side effects	Group A (N=30)	
	N	%
Total cases with side effects	10	33.33
Fishy burping	8	26.67
Distention	5	16.67
Diarrhea	3	10.00
Nausea and vomiting	2	6.67
Bleeding	0	0.00

Discussion:

Obesity as a global health problemis associated with increased multiple co-morbidities, including insulin resistance,diabetes mellitus, dyslipidemia, and hypertension [5]. Insulin resistance can result in elevated inflammatory markers, endothelial dysfunction, and a prothrombic state [15].

In the current study, both groups of obese children received a dietary intervention. Group A only received 1200 mg omega-3 PUFAs in the form of two oral capsules (each capsule contains 1200 mg fish oil and 50% omega-3 PUFAs containing 360 mg EPA and 240 mg DHA) once daily with a meal for six months as an additional supplement.

Our analysis of the 24-hour dietary recall showed that before the dietary intervention, the obese groups had significantly higher total caloric intake, energy intake from carbohydrate and fat sources, saturated fat intake, percentage of children with high trans-fat intake, and consumption of sugary beverages and salt in comparison with the controls. In contrast, they had significantly lower dairy intake and energy from protein sources in comparison with the controls. Meanwhile, non- significant differences were documented in the 24-hour dietary recall between the two obese groups. Our findings aligned with those of El-Gazzar et al., Poorolajal et al., Jia et al., and Makri et al., who concluded that children with obesity tend to consume a high number of calories, mainly from energy-dense foods that have high levels of saturated fats, carbohydrates, and sugary beverages. These foods lack whole grains, milk, legumes, fish, fruits, and vegetables, which are essential for a balanced diet [16-19].

After six months of being on the dietary intervention, the obese groups in our study showed significant improvement in their intake of all nutrients. Meanwhile, there was no significant difference regarding all nutrient intakes between the two obese groups after the intervention.

That was on par with studies by Smith et al., Ojeda-Rodriguez et al., and Gallardo-Escribano et al., who demonstrated the positive impact of dietary interventions in improving carbohydrates, fat, saturated fat, and sugar intake [20-22].

Our study showed significant improvements in weight Z score, body mass index Z score, waist & hip circumferences, and waist/hip after therapeutic interventions. Nevertheless, non-significant difference was observed between both obese groups regarding these measurements. These results were consistent with several studies, including García-López et al., Sidiartha et al., as well as López-Alarcón et al., which exhibited no significant influence of omega-3 PUFAs supplements on weight along with BMI Z-scores in children with obesity [23-25]. These studies suggested that further extensive research is required on omega-3 PUFAs supplementation in children as well as adolescents with bigger sample size to determine its effects, appropriate dosage, and treatment duration. On the other hand, Juárez-López et al. exhibited a noticeable decrease in BMI of more than 0.5 kg/m^2 after omega-3 PUFAs supplementation [26].

However, in our study, waist circumference showed significant decrease in group A in comparison with group B following the interventions. Our finding is in harmony with previous studies by Pacifico et al. on obese children and Du et al., and Zhang et al.'s research on obese adults [27-29]. However, our results disagree with Ahmedi et al., and de Ferranti et al.'s studies [30, 31]. Jazayeri et al. reported that the reason behind the lack of impact of omega-3 fatty acid supplements on WC in some obese children & adolescents with obesity is still unclear due to limited research on the topic [32]. Additionally, children and adults differ in lipid metabolism and body composition, which may also contribute to differences in results [32].

In our study, the level of ALT and AST before therapy showed a significant increase in the obese groups in comparison with the controls. Following the intervention, these levels decreased significantly, and non-significant difference was determined between group A and control subjects. Yet, a significant difference was observed between group B and control subjects. Before therapy, no significant difference was determined between the 2 obese groups. However, after the intervention, the levels of these enzymes reduced markedly in the omega-3-supplemented group in comparison with the other obese group. Our findings aligned with other studies by Koutny et al., Warnakulasuriya et al., and Hartman et al., which observed increased liver enzymes among overweight children and adolescents [33-35]. However, Valle-Martos et al. discovered elevated ALT levels without a corresponding increase in AST levels and confirmed that high ALT levels are a reliable marker for liver inflammation [36]. Boyraz et al., and Yan et al. concluded that omega-3 PUFAs supplements combined with dietary intervention was accompanied by a marked decrease in ALT and AST levels, which agreed with our results [37, 38].

Regarding the lipid profile in our work before therapy, in both obese groups A and B, total cholesterol, LDL, and triglyceride level was significantly increased, and the HDL level was significantly reduced than those of controls. Our results agreed with Nogueira-de-Almeida and Mello, Milyani and Al-Agha, and Mohamed et al. [39-41]. After therapy in groups A and B, the levels of total cholesterol, LDL-C, and triglycerides decreased significantly and became lower than before the intervention, while the level of HDL-C increased and became higher than before the therapy. Compared to the control group, group A had significantly higher total cholesterol and LDL, but their HDL and TGs level was comparable to the control subjects. On the other hand, group B had significantly higher levels of total cholesterol, LDL, and TGs in comparison with the controls, and their serum HDL levels were still significantly reduced in comparison with that of the controls group.

On comparing groups, A and B before treatment, no significant difference was detected between them regarding plasma cholesterol, LDL, HDL, or TGs level. After treatment, both groups had comparable total cholesterol and LDL level, but group A had significant decrease in TGs and increased HDL level than group B.Our results were parallel with Juárez-López et al., and Boyraz et al. [26, 37]. The systematic review by Khorshidi et al. illustrated that omega-3 FA supplements had a significant decrease in triglyceride levels, especially in those suffering hypertriglyceridemia, and a significant effect on HDL-C status on longer duration but showed lack of significant impact on total cholesterol as along with LDL level [42]. Meanwhile, Janczyk et al. concluded non-significant differences between the omega-3 FAs supplemented as well as the placebo groups as regards the lipid profile [43].

Our study revealed that FBG, FSI levels, and HOMA-IR before therapy showed significant elevation in the obese groups than in controls. Our results were consistent with Oritz-Segura et al., Huang et al., and Sajja et al. [44- 46]. In contrast, Perez et al., and Sadeghabadi et al. revealed non-significant differences regarding fasting blood glucose between obese children and adolescents and normal weightcontrols [47, 48]. It is possible that this finding may be because the studied patients may be metabolically healthy obese [49].

After therapy in our study, FBG, FSI levels, and HOMA-IR decreased significantly in both obese groups, but they showed significant elevation than in controls. Moreover, in comparing group A and group B obese children before therapy, no significant difference was determined between the 2 groups regarding the previous investigations. After therapy, all these parameters showed significant decrease in group A in comparison with group B obese children. In parallel with our results, Boyraz et al., García-López et al., and Huang et al. reported that omega-3 FA with lifestyle intervention (LI) led to an important reduction in insulin and HOMA-IR in comparison with lifestyle intervention only [23,27,45].Hou et al. concluded that fish oil supplementation gave an important effect on insulin sensitivity in

obese cases even with short-term (six months or less) and low doses (EPA+ DHA <1.5 gm/d) [50]. Contrary to these results, Janczyk et al., and López-Alarcón found no significant difference between both groups after omega-3 supplementation regarding these parameters [25, 43].

Huang et al. explained that obesity can be considered as chronic low-grade inflammatory condition in which lipotoxicity contributes to the production of inflammatory mediators like CRP, PAI-1, TNF-alpha, resistin, and adipocytokines such as adiponectin and leptin [45]. The CRP in the current study showed significant elevation in the obese groups in comparison with the controls. Our findings agreed with those of Emam et al., Mohamed et al., Zou et al., and Cura–Esquivel et al. [41,51-53]. After therapy, the CRP decreased significantly in both groups and showed significant decrease in group A in comparison with group B. These results were in parallel with Sidiartha et al. [54]. In contrast to our findings, the earlier work by Machado et al., and Janczyk et al. they suggested that the low adherence to the supplement may have affected the results [43, 55].

In our study before therapy, obese groups A and B had significant elevation in the plasma leptin and significantly decreased plasma adiponectin level and ALR than controls. Meanwhile, there were no significant differences between the two obese groups regarding all these parameters. The results of Ding et al., Mira et al., and Cura–Esquivel et al. agreed with our results [53,56,57]. Following the therapeutic interventions for groups A and B obese children, there were significant improvements in serum adiponectin, leptin, and ALR. However, compared to the controls, serum leptin remained significantly higher, while serum adiponectin and ALR remained significantly lower.

In comparing both groups of obese children, group A had significantly lower plasma leptin levels and markedly elevated blood adiponectin and ALR level than group B. López-Alarcón

et al. and Spahis et al. found significantly lower serum leptin, higher serum adiponectin, and higher ALR in the omega-3-supplemented group, which were in parallel with our results [58,59]. Janczyk et al. study agreed with our results regarding serum adiponectin levels but was against those regarding serum leptin [43]. However, other studies, such as those carried out by Machado et al., and Huang et al. revealed no effect of omega-3 on adiponectin, while leptin improved over baseline [45, 55]. It is worth noting that many of the studies with differing results had relatively small groups of participants.

As regards the adverse effects experienced by our patients who received omega-3 PUFAs supplementation, 33.33% complained of side effects. Fishy burping was the most frequent, followed by distention, diarrhea, nausea, and vomiting. None of them had bleeding as a side effect. These results were consistent with the results of by Del-Río-Navarro et al., Janczyk et al., and Yan et al. in which patients who received omega-3 fatty acids supplementation experienced mainly gastrointestinal side effects [38, 43, 60]. Belching was the most frequently occurring. Apart from the gastrointestinal symptoms and fishy taste, Gidding et al. reported frequent nosebleeds, which resolved spontaneously and did not lead to the discontinuation of the study medication [61].

Our study emphasized the significant role of omega-3 as one of the adjuvant therapies to the dietary intervention in treating obesity. It played a role in the improving insulin resistance markers such as serum FBG, FSI, and HOMA-IR. Moreover, it markedly helped improvement of inflammatory markers such as CRP, serum leptin, serum adiponectin, and ALR with fewer and tolerable side effects.

Our study had a limited number of participants, and further testing with a bigger sample size may be necessary to validate the current findings. We did not examine the impact of puberty on the study outcomes, which could be a relevant factor to consider. Additionally, we didn't

assess physical activity, which could be a determining factor in the success of the lifestyle intervention.

Conclusion:

Combining omega-3 PUFAs with dietary intervention improved many obesity-related parameters. The supplementation enhanced insulin sensitivity markers and reduced inflammatory adipokines in children as well as adolescents suffering obesity.

CONSENT: Written informed consent was signed by the parents of the included cases or the caregivers.

ETHICAL APPROVAL: approvalof this work was obtained from the Ethical Committee of the Research Center, Faculty of Medicine, Tanta University.

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