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Effect of Metformin Combined with Dietary Intervention on Childhood Obesity and Insulin Resistance

Abstract

Background: Obesity is a multifactorial disease with multiple comorbidities such as insulin resistance (IR), cardiovascular disease, diabetes mellitus, and hypertension. Puberty is physiologically responsible for IR; during this period of life, insulin sensitivity decreases by 25-50% and then improves. A change in lifestyle that promotes healthy nutritional habits and physical activity, as well as complementary treatments such as metformin therapy, is recommended as the first line of treatment.**Aim of the work:**Toassess the effect of metformin combined with dietary intervention on BMI and IR in obese children and adolescents in the prepubertal and pubertal stages. **Methods:** This study included 90 children and adolescents divided into two groups: 60 obese children and adolescents with BMIs greater than the 95^{th} percentile for their sex and age (30 prepubertal and 30 pubertal), and 30 healthy children and adolescents with BMIs between the 5^{th} and 85^{th} percentile for their sex and age (15 prepubertal and15 pubertal).They underwent a full clinical evaluation, which included anthropometric measurements and investigations such as serum adiponectin, leptin, the adiponectin-leptin ratio (ALR), plasminogen activator inhibitor-1(PAI-1), a lipid profile, and HOMA-IR. Obese children and adolescents received metformin with dietary intervention for 6

months.**Results:**After the therapeutic intervention, anthropometric measures (weights, weight Z-scores, BMIs, BMI Z-scores, waist and hip circumferences, and waist-hip ratios) and the lipid profile (total serum cholesterol, LDL-C, HDL-C, and serum triglycerides) improved significantly in both obese groups (prepubertal and pubertal).Prepubertal obese children significantly improved more than pubertal obese children in terms of inflammatory adipokines (leptin, adiponctin, PAF-1, and ALR) and insulin sensitivity markers. **Conclusion:** Puberty is an important physiologic stage that influences metformin response and should be researched further, particularly in terms of dose-effect relationships.

Keywords: Metformin, Dietary Intervention, Childhood Obesity, Insulin Resistance

Introduction:

The obesity epidemic has been regarded as a global pandemic. The prevalence of overweight and obesity grew by 28% among adults and 47% among children on a global scale. There are approximately 2.1 billion overweight or obese persons in the world [1].

In Egypt, 15.02 percent of elementary school pupils were overweight and 10.55 percent were obese [2].

Obesity is a complex, multifactorial disease that is strongly associated with multiple comorbidities, including insulin resistance (IR), dyslipidemia, certain cancers, cardiovascular disease, disability, diabetes mellitus, gallbladder disease, hypertension, osteoarthritis, sleep apnea, and stroke [3].

Insulin resistance is a decreased tissue response to insulin-mediated cellular actions with elevated its serum level[4]. prevalence of this condition is higher among obese individuals, approximately 50% of obese children are insulin resistant [5].

Puberty is physiologically responsible for IR; in fact, during this period of life, insulin sensitivity undergoes a decline of around 25–50% and improves when puberty ends [6].

There are a growing number of inflammatory markers that could promote insulin resistance and β-cell failure. Several hormones (as leptin and adiponectins) released by adipose tissue which acts as an endocrine organ that may excessively stimulate specific insulin-dependent pathways resulting in acanthosis nigricans, ovarian hyperandrogenism, lipodystrophy [7].

Visceral adipose tissue is associated with the ectopic deposition of fat mainly into the muscle and liver and associated with decreasing adiponectin levels and releases interleukin-6 and plasminogen activator inhibitor-1 (PAI-1) [7].

Adiponectin has anti-inflammatory, anti-atherogenic and insulin-sensitizing properties. Therefore, reduced levels of this adipocytokine have been implicated in the pathogenesis of insulin resistance and metabolic syndrome [8].

PAI-1 is a cytokine that is expressed in adipose tissue and vascular endothelium. PAI-1 levels were displayed a positive correlation with insulin resistance markers and by BMI [9].

The first-line treatment recommended for obese children and adolescents with IR is a change in lifestyle that promotes healthy nutritional habits and physical activity, along with complementary treatments, such as metformin therapy or omega-3 polyunsaturated fatty acids, that help to improve or reverse IR [10].

Metformin is a biguanide used to treat type 2 diabetes in children and adolescents as a result of its ability to lower hepatic glucose synthesis, enhance peripheral insulin sensitivity, and preserve islet cells [11].

Metformin has been suggested as an adjunctive therapy for childhood obesity, particularly in the context of insulin resistance with or without T2D. Metformin also decreased the profile of cardiovascular risk and inflammatory indicators [12].

The purpose of this study was to evaluate the effect of metformin coupled with dietary intervention on the body mass index and insulin sensitivity in obese prepubertal children and adolescents.

METHODOLOGY:

This prospective cross-sectional and longitudinal controlled studywas carried out on 90 children and adolescentswho recruited from the Nutrition Outpatient Clinic, Gastroenterology and Clinical Nutrition Unit, Pediatric Department, Tanta University Hospital.Sixty obese children and adolescents with BMI > the 95thpercentile for the sex and age. They were divided according to the pubertal stage into two groups: 30 prepubertal group: with Tanner stage I and 30 pubertal group: with Tanner stage II, III, IV.Thirty apparently healthy children and adolescents with BMI between the 5th and 85th percentile for sex and age. Also, they were divided according to the pubertal stage into two groups, prepubertal and pubertal groups (fifteen children in each group). These groups were matched with the obese children groups for age and sex.

Inclusion criteria:

1. Age ranged from8 to15 years.
2. Obese children and adolescents with body mass index (BMI) equal to or greater than the 95th percentile for the sex and age.

Exclusion criteria:

1. Children received metformin in the last 6 months.
2. Children with syndromic obesity (e.g., Prader Willi, Laurence-Moon Biedle syndrome, etc.)
3. Children with obesity due to endocrinal causes such as Cushing's syndrome and hypothyroidism.
4. Children suffering from any inflammatory or collagen disease or systemic diseases such as liver diseases, malignancy or type 2 diabetes mellitus.

5. The use of medications with metabolic side effects such as diuretics, β-blockers, α-adrenergics, corticosteroids or anti-thyroid drugs or weight loss medications that could modify lipids and glucose levels.

All patients were subjected tofull medical history, clinical examination (Anthropometric measures, blood pressure measurement,laboratory investigations [complete blood count(CBC), blood urea, serum levels of creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), C-reactive protein (CRP), serum lipid profile],research investigations [Serum leptin, serum adiponectin, serum plasminogen activator inhibitor-1(PAI-1), adiponectin–leptin ratio (ALR), fasting blood glucose, fasting serum insulin].

Anthropometric measures:

Body mass index (BMI):Calculation was made according to the following formula:

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

Calculation of Z-score was done using the Egyptian Z score tables for weight, height and BMI[13].

To identify individuals with unhealthy growth, the WHO recommends cut-off values of + 2 Z-scores[14].Waist circumference, hip circumference and waist/hip ratiowere measured.

Medical Intervention:

Oral metformin hydrochloride was given as a single dose per day during meal. The medicine was administered in a starting dose of 500 mg per day for 7 days, then subsequently and gradually increased to a maximum of 1,000 mg as a single daily dose for 6 months if the patients tolerated it.Checking for patient compliance and asking for reported adverse effects.

The dietary intervention[15]:

1. It was focused on basic healthy lifestyle eating and activity habits rather than caloric restriction.

2. Caloric requirement was estimated for children/adolescents by **Schofield equation** to estimate the basal metabolic rate (BMR) in calories[15].

3. BMR was multiplied by stress activity factor.

4. Balanced low glycemic index (GI) healthy diet was described [15].

- The total calories were divided as follows

- 55% from carbohydrates,
- 20% from proteins,
- 25% from lipids (> 7% saturated fat , > 300 mg/d cholesterol, and > 1% trans fat)

- < 3 g salt per day.

5. For obese children up to age 12 years: 125- 250 kcals /day were subtracted for ¼ kg / week weight loss.

6. For obese children > 12 years, 1000 calories /day were subtracted to achieve no more than 1 kg /week weight loss.

7. Daily energy intake should not be less than 900 calories for children aged from 6-12 years and 1200 calories for children aged from 13-18 years.

Statistical analysis:Statistical Package for Social Science (SPSS) version 20.0 software was used to code, edit, tabulate, and analyse the obtained data (Armonk, NY: IBM Corp). For different qualitative and quantitative data describing the study population, descriptive statistics, including percentages (%), arithmetic mean (X), and standard deviation (SD), were produced. At the 5% significance level, the acquired findings were deemed significant. The tests utilised were the Chi-square test, the student t test, the paired t test, the Kruskal Wallis test, the Wilcoxon signed ranks test, the Mann Whitney test, the McNemar's test, and the Marginal Homogeneity Test.

Measurement of the inflammatory adipokines; aprinciple of leptin, adiponectin, and PAI1:

The kits use a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay their levels in samples. Adding them to monoclonal antibody Enzyme well which is

pre-coated with humanmonoclonal antibody for these markers, this followed by incubation; then, adding their antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carrying out incubation and washing again to remove the uncombined enzyme. Then adding Chromogen Solution A, B (the color of the liquid changes into the blue), and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human Substance leptin, adiponectin, and PAI1 of sample are positively correlated.

Results:

Demographic Data:

Our study included 60 obese children and adolescents divided into two groups (30 children each) based on tanner staging: prepubescent group: Tanner stage I. Their ages ranged from 8 to 11.5 years, with a mean age of 9.52 ± 0.99.Tanner stages II, III, and IV are included in the pubertal group. Their ages ranged from 10.6 to 15 years old, with a mean of 13.08 ± 1.29 years. Fifty percent (50%) of the prepubertal obese group were males, 50% were females, 36.7% were males, and 63.3% were females. There was a significant difference in age between prepubertal and pubertal obese children, but no significant difference in sex.

The control group included 30 healthy children and adolescents with BMIs ranging from the 5^{th} to the 85^{th} percentile for their sex and age. In addition, the children were divided into two groups based on their pubertal stage: prepubertal and pubertal (15 children in each group). For the prepubertal and pubertal groups, the age ranges and mean age values were (8-11), 9.39 ± 1.05, and (11-15), 13.47 ± 1.20 years, respectively. The prepubertal controls were 46.7% male and 53.5% female, while the pubertal controls were 60% male and 40% female. There was no significant difference in age or sex between obese children and their controls.

Family history of the studied groups:

The percentages of obese prepubertal children with a positive family history of obesity, type 2 diabetes mellitus, or hypertension were 60%, 60%, and 46.7%, respectively, and these

percentages were 60%, 76.7%, and 46.7%, respectively for the pubertal obese group. These percentages were significantly higher than those of their corresponding controls, which were 26.7%, 20%, and 13.3% for both the prepubertal and pubertal control groups. However, these percentages were comparable between both obese groups.

Clinical parameters in studied groups

Table 1 shows: Regarding the anthropometric measures; before therapy, prepubertal and pubertal obese participants in our study had significantly higher weights, BMIs, BMI Z-scores, waist circumferences, hip circumferences, and waist-hip ratios than controls. These measures improved significantly after the therapeutic intervention, but there was still a significant difference between the obese prepubertal or pubertal children and their controls. Meanwhile, there were no significant differences in height Z-score across the studied groups before and after the intervention. Central obesity was found to be more prevalent in the pubertal group than in the prepubertal group. Waist circumference and waist-hip ratio serve as good markers of insulin resistance.

Regarding acanthosis nigricans in the prepubertal and pubertal obese group; the grading and percentage of acanthosis nigricans were significantly higher before therapy than after therapy.

Between the prepubertal and pubertal obese groups;the grading and percentage of acanthosis nigricans were significantly higher in the pubertal than prepubertal obese children before therapy, while no significant difference between both groups after therapy.

Blood pressure was significantly higher in pubertal than prepubertal obese children.In the prepubertal obese group; the systolic and diastolic blood pressure before therapy were significantly higher than those after the intervention and those of controls. While the systolic and diastolic blood pressure after therapy were comparable to those of controls. In the pubertal obese group;the systolic and diastolic blood pressure before therapy were significantly higher than those after the intervention and those of controls. Moreover, the

systolic and diastolic blood pressurewere significantly higher than those of controls despite the therapeutic intervention.**Table 1**

Table 1: Changes in some clinical parameters in studied groups

	Prepubertal group				Pubertal group				P
	Before therapy	**After therapy**	**Controls**	**P**	**Before therapy**	**After therapy**	**Controls**	**P**	P4
Weight (Kg)	62.13±16.29	54.83± 14.98	32.83 ± 10.29	p_1<0.001* p_2<0.001* p_3<0.001*	89.13 ± 12.65	80.58 ± 13.15	59.83 ± 7.94	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
Height (cm)	4.50 (3.8–5.5)	3.60 (3–4.30)	-0.3 (-0.7–0.8)	p_1<0.001* p_2<0.001* p_3<0.001*	3.70 (2.9–5)	3.20 (2.7–3.7)	-0.20 (0–0.98)	p_1<0.001* p_2<0.001* p_3<0.001*	>0.05
BMI	31.26 ± 5.20	26.77 ± 4.81	18.08 ± 3.38	p_1<0.001* p_2<0.001* p_3<0.001*	36.81 ± 3.63	32.38 ±3.62	22.66 ± 2.56	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
BMI Z-Score	4.10 (3.10–4.90)	3.15 (2.70–3.30)	0.00 (-0.20 – 0.25)	p_1<0.001* p_2<0.001* p_3<0.001*	3.80 (3.0 – 4.70)	2.90 (2.3–3.40)	0.05 (-0.35 – 0.29)	p_1<0.001* p_2<0.001* p_3<0.001*	>0.05
WC (cm)	85.67 ± 4.37	76.90 ± 5.71	56.13 ± 3.14	p_1<0.001* p_2<0.001* p_3<0.001*	97.97 ± 7.97	88.73 ± 7.81	61.47 ± 2.0	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
HC (cm)	93.01 ± 2.93	88.70 ± 3.11	67.33 ± 3.99	p_1<0.001* p_2<0.001* p_3<0.001*	104.03 ± 5.51	99.51 ± 5.93	73.0 ± 2.14	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
Waist-Hip Ratio	0.92 ± 0.05	0.85 ± 0.03	0.82 ± 0.02	p_1<0.001* p_2<0.001* p_3<0.001	0.94 ± 0.06	0.89 ± 0.06	0.83 ± 0.02	p_1<0.001* p_2<0.001* p_3<0.001*	0.001*

				*					
Positive AN (>0)	15 (50.0%)	6 (20.0%)	- - - -	p_1=0.002*	20 (66.7%)	11 (36.7%)	- - - - -	p_1=0.002*	>0.05
Grading of AN	1.0 (0.0 – 2.0)	0.0 (0.0 – 0.0)	- - - -	p_1<0.001*	2.0 (1.0 – 3.0)	0.0 (0.0 – 1.0)	- - - -	p_1<0.001*	>0.05
Systolic (mmHg)	116.83 ± 8.56	92.67 ± 5.21	88.67 ± 3.52	p_1<0.001* p_2<0.001* p_3=0.324	132.17 ± 12.64	119.67 ± 10.17	103.87 ± 6.76	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
Diastolic (mmHg)	75.17 ± 8.66	63.67 ± 5.40	62.67 ± 3.72	p_1<0.001* p_2<0.001* p_3=0.524	85.0 ± 9.28	74.50 ± 6.34	68.53 ± 3.98	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*

Data are presented as mean ± SD or median (IQR), **IQR:** Inter quartile range.**BMI:** Body mass index,**WC:** Waist Circumference, **HC:** Hip Circumference,**AN**: acanthosis nigricans.**p1:** p value for comparing between before therapy and after therapy, **p2:** p value for comparing between obese children and controls (before therapy), **p3:** p value for comparing between obese children and controls (after therapy), **p4:** p value for comparing between prepubertal and pubertal, ***:** Statistically significant at $p \leq 0.05$

laboratory and inflammatory markers in the studied groups

Table 2 shows: Regarding CRP and lipid profiles in the current study, the prepubertal and pubertal obese groups had significantly higher serum CRP, total cholesterol, LDL-C, and triglyceride levels than their controls, but lower serum HDL-C levels. Following therapy, both obese groups have shown significant improvements in all of theseparameters with no significant differences observed when compared to their controls. However, pubertal as compared to prepubertal obese children had significantly higher triglycerides. Even after treatment, there was no significant difference in total cholesterol and LDL-C, but pubertal versus prepubertal obese children had significantly higher triglycerides and significantly lower HDL-C.

In the present study, fasting blood glucose, fasting serum insulin levels and HOMA-IR before therapy were significantly higher in the obese groups (prepubertal and pubertal) compared to their controls.In the current study, after therapy, all of these parameters (fasting blood

glucose, fasting serum insulin levels, and HOMA-IR) decreased significantly in both obese groups, and when compared to their controls, we found no significant difference between prepubertal obese children and their controls, but a significant difference between pubertal obese children and their controls.Regarding the fasting blood glucose, fasting serum insulin levels and HOMA-IR between the prepubertal and pubertal obese children before therapy, there were no significant difference but after therapy; these levels were significantly lower in the prepubertal obese group compared to the pubertal obese group. **Table 2**

In our study before therapy, both obese groups (prepubertal and pubertal) had significantly higher serum leptin and PAI-1 levels and significantly lower serum adiponectin level and adiponectin-leptin ratio (ALR) compared to their than their controls.After therapy; all these parameters (serum leptin, PAI-1, adiponectin and ALR) improved significantly in the two obese groups, and on comparing with their controls we observed that, there was no significant difference between prepubertal obese children and their controls, but still a significant difference between pubertal obese children and their controls.A significant improvement in the ALR was observed in the prepubertal children after taking metformin. The ALR is considered a potential surrogate marker for cardiometabolic disease.Comparing between prepubertal and pubertal obese groups before and after therapy; we observed that serum leptin and PAI-1 level were significantly higher and serum adiponectin and ALR significantly lower in the pubertal compared to prepubertal obese children.**Table 2**

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

	Prepubertal group				Pubertal group				
	Before therapy	**After therapy**	**Controls**	**P**	**Before therapy**	**After therapy**	**Controls**	**P**	**P4**
CRP (mg/l)	7 (4 – 10)	3.5 (0 – 6)	3.0 (1.0 – 6.5)	p_1=0.002* p_2=0.005* p_3=0.573	9.0 (5 – 11)	3 (0–5)	5 (0-6)	p1=0.001* p2=0.002* p3=0.865	>0.05
ALT (U/L)	45.0 (31.0 –	26 (28.0 – 30.0)	24.0 (20.0 –	p_1<0.021* p_2<0.011*	43.0 (27.0 –	29 (27 -	25.0 (18 – 27)	p_1<0.001*	>0.05

	55.50)		28.0)	p_3=0.105	59.0)	40.5)		p_2<0.001* p_3=0.201	
AST (U/L)	48.0 (35.0 – 54)	26 (20 –39)	21 (18.5 – 28)	p_1<0.001* p_2<0.024* p_3=0.105	45.5 (32– 59)	24 (18.5–40)	23 (16.5 – 25)	p_1<0.001* p_2<0.001* p_3=0.105	>0.05
TC (mg/dl)	186.17 ± 27.87	136.80 ± 13.62	133.67 ± 9.09	p_1<0.001* p_2<0.001* p_3=0.365	199.17 ± 33.16	136.87 ± 11.63	132.07 ± 6.76	p_1<0.001* p_2<0.001* p_3=0.148	>0.05
HDL (mg/dl)	46.93 ± 11.49	71.0 ± 7.82	69.53 ± 7.06	p_1<0.001* p_2<0.001* p_3=0.155	46.30 ± 10.79	64.07 ± 13.77	68.27 ± 6.25	p_1<0.001* p_2<0.001* p_3=0.167	0.021*
LDL (mg/dl)	106.63 ± 18.38	81.23 ± 13.48	80.0 ± 9.17	p_1<0.001* p_2<0.001* p_3=0.752	107.13 ± 15.13	87.03 ± 12.83	84.47 ± 7.06	p_1<0.001* p_2<0.001* p_3=0.392	>0.05
TG (mg/dl)	118.17 ± 26.03	97.57 ± 8.76	97.47 ± 7.85	p_1<0.001* p_2<0.001* p_3=0.970	122.57 ± 27.06	108.13 ± 22.27	104.8 ±12.42	p_1<0.001* p_2<0.001* p_3=0.532	<0.05*
FBG (mg/dl)	97.83 ± 10.26	76.0 ± 4.01	76.13 ± 4.24	p_1<0.001* p_2<0.001* p_3=0.920	102.40 ± 11.49	95.80 ± 9.38	81.20 ± 5.29	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
FSI(mU/ml)	13.43 ± 3.26	6.92 ± 1.77	6.45 ± 1.51	p_1<0.001* p_2<0.001* p_3=0.386	15.23 ± 4.04	11.19 ± 3.56	5.86 ± 1.66	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
HOMA-IR	3.40 ± 0.97	1.93 ± 0.82	1.93 ± 0.42	p_1<0.001* p_2<0.001* p_3=1.000	3.93 ± 1.13	2.72 ± 0.99	1.92 ± 0.49	p_1<0.001* p_2<0.001* p_3=0.001*	0.001*
Serum adiponectin (mg/l)	11.64 ± 1.35	34.37 ± 3.77	34.94 ± 2.69	p_1<0.001* p_2<0.001*	9.11 ± 1.12	11.11 ± 1.42	35.93 ± 1.72	p_1<0.001* p_2<0.001	<0.001*

				p_3=0.603				p_3<0.001*	
Serum leptin (ng/ml)	12.94 ± 1.65	4.28 ± 1.02	4.25 ± 1.03	p_1<0.001* p_2<0.001* p_3=0.935	14.67 ± 1.0	11.99 ± 1.23	4.70 ± 1.09	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
ALR	0.99 ± 0.02	3.83 ± 0.49	3.84 ± 0.45	p_1<0.001* p_2<0.001* p_3=0.947	0.60 ± 0.11	1.05 ± 0.11	3.66 ± 0.42	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*
PAI-1 (ng/ml)	20.77 ± 1.54	8.65 ± 1.79	8.38 ± 1.73	p_1<0.001* p_2<0.001* p_3=0.629	35.32 ± 1.74	22.26 ± 1.88	8.81 ± 1.35	p_1<0.001* p_2<0.001* p_3<0.001*	<0.001*

Data are presented as mean ± SD or Median (IQR),IQR: Inter quartile range. **TC:**Total cholesterol, **TG:** Triglycerides, **FBG:** Fasting Blood Glucose, **FSI:** Fasting Serum Insulin, **HOMA-IR:** Homeostatic Model Assessment-Insulin Resistance, **ALR:**Adiponectin–leptin ratio,**PAI-1:** Plasminogen activator inhibitor-1,p1: p value for comparing between before therapy and after therapy, p2: p value for comparing between obese children and controls (before therapy), p3: p value for comparing between obese children and controls (after therapy), p4: p value for comparing between prepubertal and pubertal, *: Statistically significant at $p \leq 0.05$

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

Table 3 shows: According to our study's analysis of the 24-hour dietary recall, the prepubertal and pubertal obese groups' daily total calorie intakes were significantly higher than those of their controls before the intervention. After a 6-month dietary intervention, each group's caloric intake decreased, with no significant difference between prepubertal obese children and their controls, but still a significant difference between pubertal obese children and their controls. Before the dietary intervention, we discovered that the energy intake from carbohydrate, fat, and saturated fat, the proportion of children with high trans fat intake, as well as the consumption of sugary drinks and salt, were significantly higher in the prepubertal and pubertal obese children than in controls. On the other hand, they consumed much less dairy and had a lower energy intake from protein. After six months of the dietary intervention

we found that, in the prepubertal obese children, all of these intakes (carbohydrate, fat, and protein) significantly improved and were on par with their controls. While in the pubertal obese group, none of these intakes improved, and they remained significantly different from those of controls, except for high trans fat consumption, which improved as more obese patients consumed the recommended amount.

Comparing the prepubertal and pubertal obese groups' 24-hour dietary recall, we observed that, before the intervention, the energy intake from carbohydrate and salt consumption were significantly higher, while the energy intake from protein and dairy product consumption were significantly lower in the pubertal obese group than in the prepubertal obese group. Meanwhile, there was no significant difference between the two groups regarding the energy intake from saturated fat, the number of children with high trans fat intake, or sugary beverage consumption. After the dietary intervention, we found that energy intakes from carbohydrate, fat, and saturated fat, as well as sugary beverages and salt consumption, were significantly higher in the pubertal than the prepubertal obese children, while the energy intake from protein and dairy products consumption were significantly lower. Meanwhile, number of obese patients consuming the healthy amount of trans fat increased in both groups, with no significant difference between them. Therefore, our study showed a statistically significant difference between prepubertal and pubertal obese children in response to 6 months of dietary intervention regarding the varieties of food consumption and nutrient intakes.**Table 3**

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

	Prepubertal group				Pubertal group				
	Before	**After**	**Controls**	**P**	**Before**	**After**	**Controls**	**P**	**P4**
Caloric intake (kcal/day)	2148.33 ± 187.31	1561.67 ± 56.76	1596.67 ± 76.69	$p_1<0.001^*$ $p_2<0.001^*$ $p_3=0.132$	2803.33 ± 248.07	1858.33 ± 182.74	1743.33 ±127.99	p1=0.001* p2=0.002* p3=0.019*	<0.001*

Percentage of CHO in calories /day	62.43 ± 4.07	51.63 ± 3.08	52.60 ± 3.87	p_1<0.001* p_2<0.001* p_3=0.408	65.57 ± 4.36	57.33 ± 4.51	53.20 ± 3.38	p_1<0.001* p_2<0.001* p_3=0.003*	<0.05*
Sugary beverages (serving/d)	4.0 (3.0 – 5.0)	1.0 (1.0 – 2.0)	1.0 (1.0 – 2.0)	p_1<0.001* p_2<0.001* p_3=0.572	3.0(3.0 – 42.0)	2.0 (2.0 – 3.0)	1.0 (0.0 – 2.0)	p_1<0.001* p_2<0.001* p_3=0.014*	<0.05*
Percentage of fat in calories /day	28.00 ± 3.79	24.80 ± 2.54	24.80 ± 2.54	p_1<0.001* p_2=0.001* p_3=0.319	29.67 ± 4.33	27.13 ± 2.92	25.53 ± 2.67	p_1=0.004* p_2=0.002* p_3=0.006*	<0.001*
Saturated fat (%/d)	10.50(10.0 – 14.0)	5.50(4.0 – 8.0)	5.5 (3.0 – 6.50)	p_1<0.001* p_2<0.001* p_3=0.480	12.0(10.0 – 15.0)	6.50 (5.0 – 11.0)	5.0 (4.0 – 7.50)	p_1<0.001* p_2<0.001* p_3=0.007*	<0.001*
Trans fat (< 1%)	9 (30.0%)	25 (83.3%)	13 (86.7%)	p_1<0.001* p_2<0.001* p_3=1.000	4 (13.3%)	20 (66.7%)	11 (73.3%)	p_1<0.001* p_2<0.001* p_3=0.743	0.136
percentage of protein in calories	13.90 ± 2.23	16.37 ± 2.18	16.87 ± 1.88	p_1<0.001* p_2<0.001* p_3=0.808	12.17 ± 2.68	15.27 ± 1.88	17.20 ± 1.86	p_1<0.001* p_2<0.001* p_3=0.049*	<0.05*
Dairy products (serving/d)	1.00 (0.0 – 1.30)	2.0 (1.0 – 3.0)	2.0 (1.0 – 3.0)	p_1<0.001* p_2<0.001* p_3=0.631	0.5 (0.0 – 1.0)	1.0 (1.0 – 2.0)	2.0(1.0 – 3.0)	p_1<0.001* p_2<0.001* p_3=0.046*	<0.05*
Salt intake (gm/d)	3.41 ± 0.69	2.35 ± 0.39	2.25 ± 0.39	p_1<0.001* p_2<0.001* p_3=0.533	4.24 ± 1.0	3.07 ± 0.73	2.44 ± 0.79	p_1<0.001* p_2<0.001* p_3=0.016*	<0.001*

Data are presented as mean ± SD or Median (IQR). IQR: Inter quartile range, p1: p value for comparing between before therapy and after therapy, p2: p value for comparing between obese children and controls (before therapy), p3: p value for comparing between obese children and controls (after therapy), p4: p value for comparing between prepubertal and pubertal, *: Statistically significant at $p \leq 0.05$

Table 4 shows:The most common side effect of metformin hydrochloride was diarrhea (35%) of obese children followed by nausea and vomiting and abdominal pain and flatulence. None of the obese children who received metformin hydrochloride experienced hypoglycemia or lactic acidosis as side effect.

Table 4: The adverse effects of metformin hydrochloride in the studied obese children

Side effects	Obese therapy group (n=60)	
	N	%
Total cases with side effects	24	40
Diarrhea	21	35
Nausea and vomiting	17	28.3
Abdominal pain and flatulence	14	23.3
Metallic taste	9	15
Lactic acidosis	0	0
Hypoglycemia	0	0

Discussion:

Insulin resistance represents the most common associated metabolic disorder in obese children and adolescents [16]. The metabolic consequences of IR can result in hyperglycemia, hypertension, dyslipidemia, visceral adiposity, hyperuricemia, elevated inflammatory markers, endothelial dysfunction, and a prothrombic state [17].

In the current study, the prepubertal and pubertal obese groups received, in addition to the dietary intervention, oral metformin hydrochloride 1000 mg once daily for six months.

Metformin was given at a dose of 1000 mg once daily for six months by Pastor-Villaescusa et al.,[18]and they noticed a significant difference in BMI change between the metformin and placebo groups. On the other hand, Warnakulasuriya et al., [19]noticed the same effect on prescribing metformin in a dose of 1500–2000 mg per day for 6 months.

According to our study's analysis of the 24-hour dietary recall, the prepubertal and pubertal obese groups' daily total calorie intakes were significantly higher than their controls. After a 6-month dietary intervention, each group's caloric intake decreased, but still a significant difference in pubertal obese children with their controls.

In the present study, comparing the prepubertal and pubertal obese groups' 24-hour dietary recall, we observed that, before the intervention, the energy intake from carbohydrate and salt

consumption were significantly higher, while the energy intake from protein and dairy product consumption were significantly lower in the pubertal obese group than in the prepubertal obese group. Meanwhile, there was no significant difference between the two groups regarding the energy intake from saturated fat, the number of children with high trans fat intake, or sugary beverage consumption.

After six months of the dietary intervention, we found that, in the prepubertal obese children, all of these intakes (carbohydrate, fat, protein, dairy products consumption and saturated fat, as well as sugary beverages and salt consumption) significantly improved and were on par with their controls. While in the pubertal obese group, none of these intakes improved, and they remained significantly different from those of controls, except for high trans fat consumption, which improved. Meanwhile, the number of obese patients consuming the healthy amount of trans fat increased in both groups, with no significant difference between them. Therefore, our study showed a statistically significant difference between prepubertal and pubertal obese children in response to the6 months of dietary intervention regarding the varieties of food consumption and nutrient intakes.

Arecent study by Pala et al.[20]reported that consumption of cereal, meat, fat, and sugar groups of nutrients was higher in obese children and adolescents than in non-obese peers.

In our study, there were a significant improvement in weight, weight Z score, BMI, BMI Z score, waist circumference, hip circumference and waist-hip ratio. In the current study, comparing prepubertal and pubertal obese groups, pubertal obese children had significantly waist and hip circumferences than prepubertal obese children both before and after therapy. The waist-hip ratio did not vary significantly between the two groups, but following the intervention, it was significantly higher in obese adolescents than in prepubertal children.This agrees with Pastor-Villaescusa et al., [18], and Sal et al., [21].

Guzzetti et al., 2019[22] observed a reduction in the number of patients with abnormal WC with the progression of puberty. The authors explained that, the physiological changes of puberty driven by the rising secretion of sex hormones are accompanied by changes in body composition.Waist Hip Ratio has been used to describe body fat distribution in adults, however, it is influenced by other body factors and it is a poor measure of body fat distribution and risk of related diseases in children. However, it is associated with high fasting insulin, blood pressure and insulin resistance index in obese children [23].

As regards blood pressure, In the current study, compared to prepubertal obese group, pubertal obese group had significantly higher systolic and diastolic blood pressure. Despite improvements in blood pressure following treatment in both groups, obese adolescents' systolic and diastolic blood pressures were still significantly higher than those of the prepubertal obese children**.** This agreed with Varma et al., [24] andWeihrauch-Blüher et al [25].

The liver seems to be significantly impacted by fat deposition in the presence of obesity[26]. Our study also found significant difference between obese groups and lean controls as regards ALT and AST, while after therapy no significant difference was detected. Peng et al.,[27] who found that liver function tests showed high SGPT and SGOT in obese patients.

As regards lipid profiles in the present study, prior to treatment, both prepubertal and pubertal groups had abnormal lipid profiles, but there was no significant difference between them regarding serum total cholesterol, LDL-C, or HDL-C. However, pubertal as compared to prepubertal obese children had significantly higher triglycerides. Even after treatment, there was no significant difference in total cholesterol and LDL-C, but pubertal versus prepubertal obese children had significantly higher triglycerides and significantly lower HDL-C.This agreed with Pastor-Villaescusa et al[18], and Peng et al.[27], but this disagreed with Sadeghabadi et al.,[28] who found that there was no significant difference.

In the current study, HOMA-IR considered a method of determining insulin resistance in subjects who are obese.Fasting blood glucose, fasting serum insulin levels and HOMA-IR were significantly higher in the obese groups which decreased after therapy in both obese groups, and when compared to their controls, we found no significant difference with prepubertal group only.This was agreed with Pastor-Villaescusa et al.,[18]that showed a significant reduction in HOMA-IR in the prepubertal children only.

As regards CRP in the present study, it was significantly higher in both obese groups than controls. And after treatment, itwas no significantly difference. Warnakulasuriya et al., [19]had found a statistically significant reduction was observed in the metformin group compared with placebo in C-reactive protein at 6 months.

In our study, obese groups had significantly higher serum leptin and PAI-1 level and significantly lower serum adiponectin and ALR than controls; serum leptin and PAI-1 level were significantly higher and serum adiponectin and ALR were significantly lower in the pubertal than prepubertal obese children with more improvement in the prepubertal obese children after 6 months therapy.

Pastor-Villaescusa et al. [18] shown that the prepubertal group had lower IFN- and tPAI-1 concentrations in the metformin group in comparison to the placebo group. The concentrations of leptin and adiponectin did not alter over time in either group; however, the ALR rose in prepubescent children treated with metformin vs placebo. In addition, adolescents in the metformin group did not differ from those in the placebo group at the conclusion of the study. Metformin did not affect ALR, tPAI-1, or IFN- differently depending on the pubertal stage.

In our study, prepubescent children who received metformin shown a considerable improvement in their ALR. Considered a possible surrogate marker for cardiometabolic

illness is the ALR. In obese children and adolescents, Frühbeck et al. [29] and Pastor-Villaescusa et al. [18] have shown comparable outcomes.

In our study, the obese children and adolescent group (included all the prepubertal and pubertal obese participants) showed that there was a significant negative correlation between serum adiponectin and weight, BMI, WC, hip circumference, and WHR and a significant positive correlation between serum leptin and all these parameters. Also, it demonstrated a significant positive correlation between serum PAI-1 and weight, BMI, WC, and hip circumference. In agreement with our results, El-Den Mohammed et al., 2020[30] found a statistically significant positive correlation between leptin receptor expression and BMI. Meanwhile, Ambad et al., 2020, [31] reported a correlation between serum leptin level and WHR. Also, the earlier study by Hijjawi et al., 2018[32] found a significant positive correlation between serum leptin level and BMI and WC.

As regards adverse effects experienced by our patients, twenty-four (40%) obese children had side effects with metformin hydrochloride therapy. The most common side effect of metformin hydrochloride was diarrhea (35%) of obese children followed by nausea and vomiting (28.3%) then abdominal pain and flatulence (23.3%). None of the obese children who received metformin hydrochloride experienced hypoglycemia or lactic acidosis as side effect.

Pastor-Villaescusa et al., [18] also reported that gastrointestinal adverse events occurred more frequently with metformin without Hypoglycemia or lactic acidosis. A meta-analysis by Tarry-Adkins et al., 2021[33] and Sadeghi et al., 2020[34] reported fewer rates of epigastric discomfort, vomiting and Diarrhea in patients treated with metformin. No severe adverse events, such as vomiting or lactic acidosis were experienced.

Conclusions:

Metformin combined with the dietary intervention significantly improvedseveral obesity-related parameters in both obese groups (prepubertal and pubertal). Prepubertal obese children showed significantly greater improvements in inflammatory adipokines and insulin sensitivity markers after the therapeutic intervention than pubertal obese children. As a result, puberty is a critical physiologic stage that influences metformin response and should be studied further, particularly in terms of dose-effect relationships.

CONSENT: Written informed consent was signed by the parents/caregivers.

ETHICAL APPROVAL: This study was approved by the local ethics committee of the Faculty of Medicine of Tanta University.

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