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Effect of Nutritional Rehabilitation on Osteocalcin and Insulin Resistance in Pediatric Obesity

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ABSTRACT

Background: Children's obesity and overweight are new health issues and are associated with insulin resistance. Osteocalcin level is inversely correlated with obesity and has a metabolic role in insulin resistance.

Aim of work: The study aimed to detect the effect of nutritional intervention on serum osteocalcin and HOMA-IR in obese children. **Patients and Methods:** This interventional study was conducted on 40 obese children and 20 control recruited from Clinical Nutrition Unit, Ain Shams University. The participants were subjected to nutritional analysis, body composition, anthropometric measurements, and laboratory parameters including a full lipid profile, osteocalcin, fasting insulin, fasting glucose, and HOMA-IR calculation at baseline and 3-month intervals after being subjected to a nutritional weight loss and exercise program.

Results: In contrast to the controls, the patients' serum levels of triglycerides, LDL, fasting glucose, fasting insulin, and HOMA-IR were all significantly higher, while HDL and osteocalcin were lower. BMI, waist-hip ratio, and waist-height ratio significantly decreased after the nutritional intervention. Also, fasting insulin and HOMA-IR were dramatically decreased, Osteocalcin increased, and fasting glucose level did not significantly alter. The anthropometric measures, fasting blood glucose, insulin, and HOMA-IR had notable negative relationships with osteocalcin, but these were non-significant. **Conclusion:** Insulin resistance is demonstrated in pediatric obesity and correlates with osteocalcin levels. Early nutritional intervention and exercise programs causing weight loss results in elevation of osteocalcin level coupled with improvement in IR suggesting osteocalcin's role as a prognostic marker.

Keywords: Pediatric Obesity; Osteocalcin; Insulin resistance; Nutritional intervention.

INTRODUCTION

Overweight and obesity, as defined by the World Health Organization (WHO), are conditions where there is an excessive buildup of body fat that has a negative impact on health [(1)]. According to the CDC, BMI at or above the 95th percentile for children and teens is the cutoff threshold for diagnosing obesity [(2)].

In comparison to the Middle East and Sub-Saharan Africa, North Africa is one of the regions of the world where there is a higher prevalence of obesity and overweight. One of the nations reportedly exhibiting this rising occurrence is Egypt [(3)]. Seventeen percent of school children in Sohag were overweight, and 15% were obese, according to a recent survey [(4)].

Childhood obesity and overweight are linked to a higher frequency of various short- and long-term consequences, such as diabetes, impaired insulin sensitivity, respiratory and musculoskeletal issues, elevated blood pressure, stroke, and a higher likelihood of becoming adult obesity [(5)], moreover, causing death and affecting mental health [(6)].

Insulin resistance (IR) is the inability of a given insulin dose to promote the absorption and utilization of glucose [(7)].

In children, increased adiposity and obesity are the main risk factors for impaired insulin sensitivity, and the association between obesity and the development of metabolic and cardiovascular problems is made by insulin resistance/hyperinsulinemia [(8)]. Osteocalcin (OCN), a hormone produced by osteoblasts in the bones, plays a crucial function in bone development and metabolism control. These functions include improving mitochondrial function and proliferation, lowering body fat, and stimulating insulin secretion and sensitivity [(9)]. Obesity and osteocalcin are adversely associated [(10)] and their levels are lower in overweight and obese children. It is also hypothesized that it contributes to insulin resistance in obese children [(11)].

To treat childhood obesity, behavior-changing therapies that aim to boost physical activity, improve nutritional intake, and reduce sedentary behavior are prescribed and advised [(12)]. The recommended dietary strategy consists of calorie restriction along with a decrease in the consumption of carbohydrates with a high glycemic index. Exercise increases calorie expenditure and muscle insulin sensitivity [(13)]. Osteocalcin levels rise after weight loss in overweight people [(14)]

The current study sought to determine the impact of a weight-loss exercise program and nutritional intervention on blood markers of insulin resistance and osteocalcin in obese children.

PATIENTS AND METHODS

At the Clinical Nutrition Clinic, Children's Hospital, Faculty of Medicine, Ain Shams University, an interventional study on obese children aged 5 to 12 years was undertaken from August 2021 to February 2022.

It compared 40 obese children who met the inclusion criteria to 20 controls who were of a similar age and gender.

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Inclusion criteria: Obese children aged 5 to 12 years with BMI at 95th centile or more according to the CDC growth curve.

Exclusion criteria: Obesity secondary to other diseases or drugs e.g., Cushing syndrome, hypothyroidism, and patients on steroids.

A thorough medical history, including the age at which obesity first appeared, a family history of the condition, a history of all medications, and a thorough dietary history, including a 24-hour recall of all meals and snacks, were obtained from all study participants. Anthropometric measurements were taken including weight, height, BMI calculation, waist circumference, and hip circumference. The National Centre for Health Statistics (NCHS)'s growth chart and Z scores in 2010 were used. The waist-hip ratio was calculated and interpreted following **Table 1**. Waist-to-height ratio (WHtR) was also calculated, and a cutoff of 0.5 was used to distinguish between low and high WHtR.[15] WHtR >- 0.5 denotes central obesity, while <0.5 is considered normal [16]. Weight (kg/lbs), BMI, visceral fat rating, body fat%, metabolic age, total body water, body fat mass, bone mass, fat-free mass, muscle mass, and basal metabolic rate were all measured using a Tanita SC-330P scale. HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was determined using the formula: fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5[17]. Laboratory investigations also included a complete lipid profile, osteocalcin, fasting glucose level, and fasting insulin level (IU/ml).

All enrolled cases were subjected to a tailored integrated program which included a nutritional regimen for diet adjustment and an exercise program for 3 months.

The tailored integrated program entailed:

1. Diet plan that included prescribing a healthy balanced diet. The calories were determined according to a nutrition analysis of the patient`s intake then 500 calories were removed from the total daily caloric intake which is expected to decrease weight by 0.5 kg/week[18]. Advice was given to reduce high-fat food and sugary beverages, increase intake of fruits and vegetables and maintain a balanced diet.
2. Behavioral modification and motivational interviewing on the health and diet of children by avoiding eating while watching TV, must take breakfast, avoid frequent snacking and eating. Moreover, families were advised to ensure regular mealtimes and eat together.
3. Exercise was insisted upon, and cases were instructed to walk 30 minutes daily while wearing the proper shoes.

Follow-up took place monthly for all cases to ensure compliance, and 24 hours recall (of 3 different days) and recalculation of energy and nutrient intake concerning RDA was done. Additionally, full anthropometric measurements and body mass index (BMI) calculations were done.

Laboratory tests were done for all cases at the end of the three months including fasting glucose level (mg/dl), serum osteocalcin (ng/ml), fasting insulin level (IU/ml), and HOMA-IR was calculated.

Ethical Consideration

First, before participants were enrolled in the trial, their legal guardians provided written consent. The Pediatric Department and the Ethics Committee of the School of Medicine at Ain Shams University gave their permission. The Proclamation of Helsinki, the World Medical Association's code of ethics for human subjects research, was followed in the conduct of this study.

Data Analysis

The Statistical Package for Social Science, IBM SPSS version 23, was used to collect, examine, organize, and input the data. The quantitative data were presented as averages, standard deviations, and ranges (IQR) when they were parametric; medians and interquartile ranges if they were non-parametric. Additionally, qualitative characteristics were displayed as proportions and numbers.

Based on the kind and distributions of the data, the groups were compared utilizing Chi-square analysis, independent t-test, Mann-Whitney test, Paired t-test, or Willcoxon test. Depending on the nature and distribution of the data, the recurring measurement ANOVA test or the Friedman test was used to compare two paired sets. Applying Spearman correlation coefficients, the relationship between two numeric variables within the same set was assessed. The confidence interval was established at 95%, whereas the allowed margin of error was established at 5%. Consequently, the p-value was considered significant if it was $p < 0.05$.

Table (1): Waist-to-hip designation

Gender	Average	Good	Excellent	At Risk
Females	0.80-0.86	0.75-0.79	<0.75	>0.86
Males	0.90-0.95	0.85-0.89	<0.85	>0.95

RESULTS

In the current study, there was no significant difference between patients and control as regards age and gender with P-values of 0381 and 0.582 respectively. The mean age in the control group was 7.50 ± 2.37 years with a range of 5 – 12 years, and that of the patients was 8.06 ± 2.30 years with a range of 5 – 12 years. Out of the studied patients, 17 (42.5%) were females and 23 (57.5%) were males while 10 (50.0%) were females and 10 (50.0%) were males in the control group.Comparison between patients and controls as regards anthropometric measurements revealed significantly higher weight, BMI, BMI Z score, WC, HC, WHR, and waist-to-height ratio in patients with P-values of 0.000 for each comparison. Also, there was significantly higher height with a P-value of 0.029 in patients. There was no statistically significant difference

in the height Z score between the studied groups. BMI Z score showed that all patients were obese; while in the control group 18 (90%) were normal and 2 (10%) were overweight. Regarding WHR, it ranged between 8.89-1.06 with mean±SD of 0.97±0.04 in the patient group, while in the control group, it ranged between 0.8-0.91with mean±SD of 0.85±0.02. Concerning WHR groups, 33 (82.5%), 6 (15%), and 1 (2.5%) were at risk, average and good in the patient group, while 2 (10%), 9 (45%), 6 (30%), and 3 (15%) were at risk, average, good, and excellent in the control group respectively. Regarding waist to height, it ranged between 0.6-0.8 with mean±SD of 0.68±0.06 in the patient group, while in the control group, it ranged between 0.43-0.6 with mean±SD of 0.52±0.04. Regarding WHtR groups, all patients had central obesity, compared to 7 (35%) were normal, and 13 (65%) had central obesity in the control group. On comparing patients and controls as regards body composition, there was significantly higher body fat (%), body fat (Kg), fat-free mass (kg), muscle mass (Kg), and body water (Kg) in patients with P-values of 0.000 for each comparison. Also, there was significantly lower muscle mass % and body water % in patients with P-values of 0.000.

Comparison between patients and controls regarding lipid profile showed significantly higher cholesterol, cholesterol group, LDL, LDL group, and triglycerides in patients with P-values of 0.002, 0.000, 0.001, 0.001, and 0.001 respectively. Also, there was a significantly lower HDL and HDL group in patients with P-values of 0.040 and 0.010 respectively.

Regarding laboratory parameters, there was significantly higher fasting glucose in patients with a P-value of 0.007. Fasting glucose was higher in patients ranging between 73-127 mg/dl with mean±SD of 96.90±14.41 mg/dl compared to the control group in which it ranged between 75-105 mg/dl with mean±SD of 87.15±8.79 mg/dl. Also, Regarding the FG group 23 (57.5%) patients were normal, 15 (37.5%) were prediabetics and 2 (5%) were diabetics, while all control group candidates were normal. Insulin was significantly higher in patients with a P-value of 0.000. Insulin was higher in patients ranging between 7.8-37.55 mIU/L, with mean±SD of 17.11±7.22 mIU/L, compared to the control group in which it ranged between 3.03-9.21 mIU/L, with mean±SD of 6.47±1.89 mIU/L. Also, Regarding the FI group, 17 (42.5%) of patients were normal, 23 (57.5%) had abnormal levels, while all the control groups were normal. Also, HOMA IR was significantly higher in patients with a p-value of 0.000. Regarding the HIR group, 13 (32.5%) patients had no IR, 18 (45%) were moderately IR, and 9 (22.5%) were severely IR. While no one had IR in the control group. Although osteocalcin was lower in patients compared to controls, ranging between 1.2-94.5 ng/ml with mean±SD of 57.66±28.43 ng/ml in patients compared to a range of 7.2-91 ng/ml with mean±SD of 66.21±19.71 ng/ml in controls, the difference was non-significant with P-value of 0.233. The effect of nutritional intervention as regards nutrition analysis is shown in **Table (2).** Regarding protein and Mg intake, there were significant increases between the different visits with P-values of 0.005 and 0.000 respectively, while there was no statistically significant difference between the different visits as regards the remaining parameters. **Table (3)** shows a comparison between all visits in the patients as regards anthropometric measurements after the nutritional intervention. There was a significant increase in height between visits in patients with a P-value of 0.000. However, there was a significant decrease in each weight, BMI, WC, and WHtR between visits in patients with a P-value of 0.000 for each. Also, there was a significant decrease in WHR between visits in patients with a P-value of 0.037. On the other hand, there was no significant change in height Z score, BMI Z score, and HC with P-values 1.00, 0.985, and 3.207 respectively.

The effect of nutritional intervention on body composition is shown in **Table (4).** There was a significant increase between all visits in the patients` group as regards muscle mass (%) with a P-value of 0.000. Also, there was a significant increase in body water (%) in the patient group with a P-value of 0.014. However, there was a statistically significant decrease between all visits in the patients` group as regards body fat (%), body fat (Kg), fat-free mass (kg), and body water (Kg) with a P-value of 0.000. There was no statistically significant difference between all visits in the patients` group as regards muscle mass (kg) with a P-value of 0.522.

The effect of nutritional intervention on laboratory tests is shown in **Table (5).** There was a significant increase between the first and fourth visits as regards osteocalcin with a P-value of 0.000 and a mean change of 97.93 ± 228.11SD. However, there were significant decreases between the first and fourth visits as regards insulin and HOMA IR with a P-value of 0.000 for each. On the other hand, there was no significant difference between the first and fourth visits as regard fasting glucose, fasting insulin group, and HIR group with P-values of 0.985, 0.501, and 0.637 respectively. Although there was a non-statistically significant difference between the first and fourth visits as regards the HIR group, the number of patients with severe IR decreased from 9 (22.5%) in the first visit to 7 (17.5%) in the fourth visit while the number of patients with moderate IR decreased from 18 (45%) in the first visit to 16 (40%) in the fourth visit, and the number of patients without IR increased from 13 (32.5%) in the first visit to 17 (42.5%) in the fourth visit.There were distinct negative correlations between osteocalcin level and weight, BMI, WHR, and waist-height ratio; however, these were statistically non-significant with P-values of 0.521, 0.404, 0.291, and 0.601 respectively and r values of -0.105, -0.136, -0.171 and -0.085 respectively. Also, there were negative correlations with no statistically significant difference between osteocalcin and fasting glucose, insulin, and HOMA-IR with P-values of 0.687, 0.977, and 0.790 and R-values of -0.066, -0.005, and -0.043 respectively.

Table (2): Comparison between the four visits in patients as regards nutrition analysis

		1st visit	2nd visit	3rd visit	4th visit	Test value	P-value	Sig.
		No. = 40	No. = 40	No. = 40	No. = 40			
Energy	Mean ± SD	3753.60± 1262.56	3534.04 ± 998.54	3582.17 ± 875.31	3745.46± 1043.73	1.615•	0.198	NS
	Range	2066.33 – 9089	2216.67– 6939.33	2179 – 5373	2093 – 6824.67			
CHO	Mean ± SD	642.31 ± 161.21	653.64 ± 156.74	640.31 ± 142.03	631.55 ± 157.25	0.409•	0.738	NS
	Range	266 – 954.23	416.33 – 1002.33	384.33 – 886.87	312.33 – 928.67			
Fat	Mean ± SD	126.25 ± 33.84	126.90 ± 38.57	125.58 ± 28.88	123.29 ± 35.93	0.302•	0.792	NS
	Range	63 – 190	38.03 – 209.33	62.33 – 168.67	46.67 – 184			
Protein	Mean ± SD	203.58 ± 82.30	194.68 ± 83.96	246.81 ± 106.09	214.90 ± 111.62	4.940•	0.005	HS
	Range	95 – 419.33	93.67 – 373	106.67 – 528.2	90 – 548.33			
Water	Mean ± SD	1348.97 ± 509.28	1328.74 ± 520.47	1422.53 ± 557.82	1379.49 ± 456.85	0.816•	0.460	NS
	Range	617.33 – 3148.57	567 – 2994.13	566 – 3203.57	767 – 2901.9			
Na	Mean ± SD	3567.16 ± 832.59	3753.80± 1021.23	3654.37± 1239.14	3556.43 ± 766.81	0.927•	0.421	NS
	Range	1902.67– 5140.83	2021.33– 6635.67	1730.33– 7759.67	1772.67 – 5119			
Fiber	Median (IQR)	105.65 (68.83–142.17)	86.83 (66.33– 132.87)	122.33 (75.1– 161.5)	93.17 (61.17 – 142.5)	2.947‡	0.400	NS
	Range	39.33 – 1112	33.63 – 924.67	45.33 – 900.97	30.67 – 897.67			
Potas-sium	Mean ± SD	5207.73 ± 960.66	5398.32± 1304.92	5091.11± 1252.78	5193.32± 1010.19	1.168•	0.320	NS
	Range	3307.67– 7864.33	2973 – 9613.67	2932.67 – 8087	3262.33– 7511.33			
PO4	Mean ± SD	1505.91 ± 337.26	1463.58 ± 379.29	1469.41 ± 338.63	1511.51 ± 316.45	0.617•	0.594	NS
	Range	819 – 2190.67	816.33 – 2395	795 – 2415.33	728 – 2135			
Ca	Mean ± SD	1107.16 ± 490.44	1155.47 ± 755.03	1290.53± 1163.05	1031.19 ± 413.80	1.330•	0.267	NS
	Range	386 – 2934.33	476.33 – 4550.67	489 – 7296.33	391.67 – 2400			
Mg	Mean ± SD	450.10 ± 121.43	472.93 ± 166.07	555.40 ± 204.37	481.20 ± 169.00	8.160•	0.000	HS
	Range	265.67 – 779	244.45 – 962	284 – 1010.33	256.67 – 1001.34			
Zinc	Mean ± SD	14.36 ± 4.03	14.37 ± 3.90	15.14 ± 4.56	14.71 ± 5.00	0.792•	0.480	NS
	Range	3.44 – 21.89	5.19 – 21.65	7.22 – 27.01	2.67 – 25.91			
Iron	Mean ± SD	122.17 ± 36.71	119.18 ± 35.62	127.58 ± 31.23	122.15 ± 33.13	1.421•	0.244	NS
	Range	64 – 187.33	44.67 – 182.33	74.67 – 221.67	57.68 – 208.33			
Copper	Mean ± SD	1.70 ± 0.96	1.65 ± 0.92	1.91 ± 1.07	1.64 ± 0.76	1.533•	0.220	NS
	Range	0.63 – 5.52	0.76 – 5.52	0.91 – 5.33	0.57 – 4.95			
Vit A	Mean ± SD	8503.63± 2518.85	8682.78± 2962.21	8204.41± 2352.82	8072.27± 2598.91	1.103•	0.344	NS
	Range	3945 – 13637.33	4872 – 16941	3141 – 16088.33	2806.33 – 16363			
Fructose	Mean ± SD	1.85 ± 4.33	1.91 ± 4.29	2.75 ± 5.97	2.62 ± 8.62	0.467•	0.544	NS
	Range	0.58 – 28.47	0.55 – 28.14	0.66 – 28.4	0.71 – 55.74			

		1st visit	2nd visit	3rd visit	4th visit	Test value	P-value	Sig.
		No. = 40	No. = 40	No. = 40	No. = 40			
Vit C	Mean ± SD	484.59 ± 130.79	512.38 ± 143.71	531.42 ± 132.03	506.69 ± 125.10	1.750•	0.170	NS
	Range	221.97 – 752	290.47 – 858.33	291.67 – 874.67	220.33 – 833.8			

Table (3): Comparison between all visits in the patients as regards anthropometric measurements.

		1st visit	2nd visit	3rd visit	4th visit	Test value	P-value	Sig.
		No. = 40	No. = 40	No. = 40	No. = 40			
Height (CM)	Mean ± SD	131.60 ± 14.64	131.95 ± 14.65	132.23 ± 14.42	132.58 ± 14.48	40.074•	0.000	HS
	Range	106 – 160	106 – 160	107 – 161	107 – 162			
Height Z Score	Normal	37 (92.5%)	37 (92.5%)	37 (92.5%)	37 (92.5%)	0.000*	1.000	NS
	marginal stunted	3 (7.5%)	3 (7.5%)	3 (7.5%)	3 (7.5%)			
Weight (Kg)	Mean ± SD	57.70 ± 14.77	57.22 ± 15.18	56.36 ± 14.76	55.68 ± 14.68	34.203•	0.000	HS
	Range	31.4 – 90	30 – 91	29 – 89	28 – 87.4			
BMI (Kg/m2)	Mean ± SD	32.95 ± 4.60	32.45 ± 4.66	31.82 ± 4.45	31.27 ± 4.51	59.366•	0.000	HS
	Range	24.16 – 42.27	23.1 – 41	21.93 – 40.97	21.17 – 40.65			
BMI Z Score	Normal	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.019*	0.985	NS
	overweight	0 (0.0%)	1 (2.5%)	1 (2.5%)	1 (2.5%)			
	Obese	40 (100.0%)	39 (97.5%)	39 (97.5%)	39 (97.5%)			
WC (cm)	Mean ± SD	89.35 ± 11.32	88.80 ± 11.21	87.43 ± 11.42	87.85 ± 11.24	48.700•	0.000	HS
	Range	72 – 115	72 – 114	70 – 112	70 – 112			
HC (cm)	Mean ± SD	91.90 ± 11.60	89.93 ± 14.81	89.83 ± 11.42	89.70 ± 11.22	3.207•	0.076	NS
	Range	74 – 115	33 – 114	72 – 113	71 – 113			
WHR	Mean ± SD	0.97 ± 0.04	1.01 ± 0.23	0.92 ± 0.04	0.98 ± 0.05	4.414•	0.037	S
	Range	0.89 – 1.06	0.88 – 2.36	0.86 – 0.99	0.87 – 1.08			
WHR \Group	At Risk	33 (82.5%)	33 (82.5%)	21 (52.5%)	31 (77.5%)	15.659	0.016	S
	Average	6 (15.0%)	6 (15.0%)	13 (32.5%)	8 (20.0%)			
	Good	1 (2.5%)	1 (2.5%)	6 (15.0%)	1 (2.5%)			
	Excellent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
Waist to height	Mean ± SD	0.68 ± 0.06	0.67 ± 0.06	0.60 ± 0.06	0.66 ± 0.05	35.465•	0.000	HS
	Range	0.6 – 0.8	0.59 – 0.81	0.51 – 0.77	0.59 – 0.78			
Waist-to-height group (WHtR)	Normal	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.000*	1.000	NS
	Central Obesity	40 (100.0%)	40 (100.0%)	40 (100.0%)	40 (100.0%)			

Table (4): Comparison between all visits in the patients' group as regards body composition.

		1st visit	2nd visit	3rd visit	4th visit	Test value•	P-value	Sig.
		No. = 40	No. = 40	No. = 40	No. = 40			
Body fat (%)	Mean ± SD	45.29 ± 5.37	44.13 ± 5.14	46.08 ± 5.23	43.75 ± 5.58	16.931	0.000	HS
	Range	34.2 – 54.8	33.4 – 53.2	35.4 – 55.2	31.4 – 52.6			
Body fat (Kg)	Mean ± SD	26.42 ± 8.29	25.56 ± 8.20	26.27 ± 8.34	24.69 ± 8.14	21.865	0.000	HS
	Range	11.34 – 43.18	10.35 – 41.93	11 – 45	10.5 – 41.66			
Fat-free mass (Kg)	Mean ± SD	31.28 ± 7.43	31.66 ± 7.81	30.10 ± 7.27	30.99 ± 7.50	14.714	0.000	HS
	Range	19.23 – 51.3	19.65 – 53.42	18.21 – 48.77	17.5 – 51.22			
Muscle mass (%)	Mean ± SD	52.17 ± 5.79	52.60 ± 5.81	53.29 ± 6.28	54.50 ± 5.88	15.277	0.000	HS
	Range	40.3 – 62.2	39.3 – 61.2	39.4 – 65.8	43.5 – 66.2			
Muscle mass (Kg)	Mean ± SD	29.96 ± 7.96	29.88 ± 7.89	29.85 ± 8.15	30.16 ± 8.10	0.723	0.522	NS
	Range	15.76 – 55.8	16.61 – 53.96	16.06 – 58.56	16.56 – 55.32			
Body water (%)	Mean ± SD	41.02 ± 4.18	44.82 ± 9.11	45.92 ± 9.43	47.76 ± 10.24	4.313	0.014	S
	Range	32.6 – 49.5	33.4 – 64.3	31.5 – 62.4	32.4 – 67.3			
Body water (Kg)	Mean ± SD	23.44 ± 5.58	25.65 ± 8.74	25.86 ± 8.39	17.66 ± 4.73	18.348	0.000	HS
	Range	13.88 – 34.78	10.05 – 55.87	9.14 – 41.4	9.5 – 30.2			

Table (5): Effect of nutritional intervention on laboratory tests of patients

		1st	4th	% of change	Test value•	P-value	Sig.
		No. = 20	No. = 40	Mean±SD			
F Glucose (mg/dl)	Mean ± SD	96.90 ± 14.41	95.25 ± 15.10	-1.56 ± 8.21	-1.373•	0.178	NS
	Range	73 – 127	70 – 128				
FG Group	Normal 70-100	23 (57.5%)	24 (60.0%)	--	1.019*	0.985	NS
	Pre-diabetes 101-125	15 (37.5%)	15 (37.5%)				
	Diabetes >125	2 (5.0%)	1 (2.5%)				
Insulin (mIU/L)	Mean ± SD	17.11 ± 4.22	15.29 ± 3.63	-10.49 ± 9.71	-6.264•	0.001	HS
FI Group for	Normal 1-15	17 (42.5%)	20 (50.0%)	--	0.453*	0.501	NS
	>15 Abnormal	23 (57.5%)	20 (50.0%)				
HOMA IR	Mean ± SD	4.19 ± 1.12	3.65 ± 0.82	-11.76 ± 13.87	-5.157•	0.001	HS
HIR Group	No IR <3	13 (32.5%)	17 (42.5%)	--	0.901*	0.637	NS
	Moderate IR 3-5	18 (45.0%)	16 (40.0%)				
	Severe >5	9 (22.5%)	7 (17.5%)				
Osteocalcin (ng/mL)	Mean ± SD	57.66±8.43	72.41±2.84	97.93 ± 228.11	4.639•	0.000	HS

DISCUSSION

In the current study, obesity was higher among males (57.5%). These results come in agreement with **Karki *et al.*** who also reported that among children, males were found to be 2 times more likely to be overweight or obese than females[19]. Contrary to these results, **Badawi *et al.*** reported that the frequency of obesity among females was 14% compared to 13% among males[20].

There was significantly higher body fat, body fat percent, fat-free mass, muscle mass, and body water in obese children. Similarly, **Wells *et al.*** reported that obese children were found to have significant excess fat mass, fat-free mass, and body water compared to the control group[21].

In the present study cholesterol, LDL and triglycerides were significantly higher in patients compared to controls, while HDL was significantly lower in patients. Similar results were found in a study done by **Saad *et al.***[22]. Additionally, an investigation that took place in the Bogalusa Heart Study outlined that total cholesterol, LDL cholesterol, and triglycerides were 2.4 to 7.1 times more likely to be elevated in overweight children. [23]. Also, another study revealed that abnormal changes compatible with an atherogenic lipid profile were present in obese subjects[24].

This study showed significantly higher fasting glucose and fasting insulin and HOMA IR in patients. Similarly, **Shashaj *et al.*** reported that children with obesity have approximately double HOMA-IR levels compared to normal-weight children[25]**.** On the other hand, **Mayerhofer *et al.*** reported that, although most of the subjects with extreme obesity had signs of IR implied by elevated HOMA-IR, fasting glucose, HbA1c, and OGTT were normal indicating that overweight and obese children may be suffering from subclinical IR even if they are non-diabetic[26].

A considerable percentage of the variation in the levels of triglycerides, LDL cholesterol, and HDL cholesterol was previously shown to be explained by the degree of insulin resistance, which is connected with dyslipidemia in obese children**.** [24]

In the current study, osteocalcin was lower in patients compared to controls, but with nonsignificant differences. This comes in agreement with **Reinehr and Roth,** who reported that sixty obese children had significantly lower osteocalcin levels (26.8±0.8 ng/ ml) compared with 19 normal weight controls (32.2±2.3 ng/ ml)[14] and **Seok *et al.*** who also stated that serum osteocalcin levels were significantly lower in the overweight group (64.00±20.44 ng/ml vs. 89.56±28.63 ng/ml, P<0.001)[10].

After the nutritional intervention, there was a significant increase in height, and a significant decrease in each weight, BMI, waist circumference, hip circumference, WHtR, and WHR between visits in patients. On the other hand, there was no significant change in height Z score and BMI Z score. Similarly, **Mayerhofer *et al.*** reported that although most of the participants were still obese at the end of a 5 months intervention program, 80% showed a decrease of BMI-SDS by 0.21. [26] This also compares favorably to other studies that saw BMI-SDS decrease from 0.12 to 0.4. [27] and significant decreases in weight, waist circumference, and WHR after dietary treatment[28]. Several studies showed a decrease in mean BMI-SDS with longer intervention periods. **Al-Khudairy *et al.*** demonstrated mean BMI-SDS decreases of 0.02 and 0.13, respectively, for interventions lasting less than and more than six months. [38]. Moreover, **Reinehr *et al.*** reported reductions in BMI z-score between 0.17 and 0.24 in children under the age of 12 and between 0.08 and 0.21 in children over the age of 12; the mean reduction in BMI-SDS was 0.36**.** [30].

The latter findings of the current study especially the decrease in BMI are crucial as it is reported in different studies to be associated with improvement in lipid profile. **De Luis *et al.*** reported that after dietary treatment, systolic pressure, glucose, triglycerides, total cholesterol, leptin, and LDL cholesterol significantly decreased[28]. Similarly, **Kolsgaard *et al.*** found no significant improvements in triglycerides or HDL cholesterol but did find a tiny, but significant improvement in total cholesterol, total cholesterol/HDL cholesterol ratio, and LDL cholesterol concentrations in the complete intervention group. [31].

Upon nutrition intervention of the studied patients, there was a significant decrease in body fat (%), body fat (Kg), and body water (Kg), while there was a significant increase in muscle mass (%) and body water (%). However, there was no significant difference in fat-free mass (kg) and muscle mass (kg). Similarly, **Skelton and Beech,** reported that by enhancing body composition, the nutritional regimen was effective in the short-term treatment of pediatric obesity. [32].

The current study revealed significant decreases in insulin and HOMA IR after the nutritional intervention; however, there was no significant difference between fasting glucose, fasting glucose group, insulin group, and HOMA-IR group. Similarly, **Mayerhofer *et al.*** stated that, during the 5-month intervention period, two-thirds of their obese youngsters improved with therapy, and that the HOMA-IR, a marker for IR, dropped by 2.03 within that time. [26]. Additionally, **Reinehr *et al.*** noted that reducing BMI-SDS and IR with conservative treatment of obesity has been demonstrated. [33]. According to **Kolsgaard *et al.*** who sought to determine the degree of BMI z-score reduction linked with a reduction in cardiometabolic risk variables in overweight and obese children and adolescents, found that a very little reduction in BMI z-score (≥ 0.00-< 0.10) reduced insulin and IR. The rapid change in IR, even before substantial BMI-SDS change, signifies the importance of treatment and the effect of the intervention on metabolism[31].

There was a significant elevation in osteocalcin levels following the nutritional intervention. Similar findings were made by **Reinehr and Roth**, who noted that significant weight reduction was linked to an increase in osteocalcin. [(14)]. Additionally, **Albadah *et al.*** study showed a significant reduction in waist circumference, and insulin resistance following a dietary control program, which was associated with a significant increase in serum adiponectin, uncarboxylated osteocalcin (uOC), and uncarboxylated osteocalcin/ total osteocalcin (uOC/TOC) ratio[(34)]. Contrary to our results **de Luis *et al.*** reported that after dietary treatment, osteocalcin levels have a significant decrease after weight loss (9.76 ± 5.3 vs 9.31 ± 4.1 ng/ml with $p < 0.05$)[(37)]. Also, another study showed no increase in osteocalcin after weight loss[(28)]**.**

In the present study, osteocalcin level was negatively correlated with fasting glucose, insulin, and HOMA-IR, but these associations were not statistically significant. In agreement with our results is **Reinehr and Roth's** results, who reported that osteocalcin was associated negatively with IR index HOMA[(14)]. Additionally, **De Luis *et al.*** revealed that a negative link between osteocalcin and hyperglycemia was found using correlation analysis. [(28)]. Moreover, it was discovered by **Albadah *et al.*** that increased levels of circulating adiponectin and uOC are linked to an improvement in insulin sensitivity[(34)]. On the other hand, **Fernandez-Real *et al.*** found in their study that there is a relationship between osteocalcin and insulin sensitivity only in lean but not obese subjects; however, this is probably explained by the small number of obese participants [(35)]**.**

Conclusion

Insulin resistance is demonstrated in pediatric obesity and correlates with osteocalcin levels. Early nutritional intervention and exercise programs causing weight loss results in the elevation of osteocalcin levels coupled with improvement in IR. Larger scale studies are thus recommended to further study osteocalcin as a valuable prognostic marker in obese children reflecting improvements in associated co-morbidities such as IR status and lipid profile.

STUDY LIMITATIONS

This study has its limitations. The small sample size is one point, and the short nutritional rehabilitation period is another. A third limitation is the fact that the patient's lipid profile wasn`t done simultaneously for further correlations.

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