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Impact of weight loss on plasma ghrelin level, clinical, and metabolic features of obese women with or without polycystic ovary syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a common reproductive endocrine co-morbidity of obesity. Ghrelin is a peptide which regulates food intake and body weight. The aim of this study was to measure ghrelin levels in obesity and PCOS and to evaluate the impact of weight loss on plasma ghrelin level, metabolic, and phenotypic features of PCOS. This prospective comparative study enrolled obese women without PCOS ($N = 60$) and obese PCOS women ($n = 50$) and 85 control groups. Body compositions including fat mass (FM) and fat-free mass (FFM) were measured by dual-energy X-ray absorptiometry (DEXA). Plasma ghrelin concentrations were measured using enzyme-linked immunosorbent assay (ELISA).

Results: Our results revealed that plasma ghrelin levels were lower in PCOS patients compared to obese (9.49 ± 5.59 ng/ml) and controls (48.21 ± 21.09 ng/ml). Moreover, it was negatively correlated to anthropometric measures, glycemic, lipid profile, and the phenotype characteristics of PCOS. Interestingly, after 12 weeks of following the Mediterranean diet (MD)-based weight loss program, ghrelin levels were increased in both obese groups.

Conclusion: Successful weight loss leads to increase ghrelin levels in both obese and PCOS groups.

Keywords: Ghrelin, PCOS, Weight loss, Mediterranean diet, Obesity

Background

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease; it is associated with a range of reproductive, obstetric, metabolic, and psychological features. Reproductive and obstetric manifestations include hyperandrogenism, menstrual dysfunction, infertility, and pregnancy complications [1–3]. Approximately 50% of PCOS women are either overweight or obese. Obesity is associated with multiple factors that may influence hypothalamic-pituitary function in particularly insulin resistance [4].

Ghrelin is a 28 amino-residue peptide and secreted mainly by the stomach and its secretion is inhibited by food intake and stimulated during fasting and after weight loss [5, 6]. The Mediterranean diet (MD) is rich with vegetables, legumes, fruits and nuts, cereals, olive oil and fish, however a low-to-moderate intake of dairy products and a low intake of meat and poultry are characteristics for this diet [7].

The first choice for PCOS treatment is diet and lifestyle modification [8]. Obese women with PCOS confront difficulty in achieving weight loss when compared to healthy women. This is postulated to be due to either insulin resistance or eating disorder in women with PCOS [9]. To test this hypothesis, we designed this study to measure ghrelin in patients with PCOS and to evaluate the impact of weight loss after 12 weeks of following a Mediterranean diet-based weight loss program on plasma ghrelin level and clinical phenotype of PCOS. We think that this is the first Egyptian study that demonstrated the impact of weight loss on weight loss

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program on plasma ghrelin level and clinical phenotype of PCOS

Methods

A prospective comparative study will be conducted on 110 obese patients, body mass index (BMI) ˃ 30 kg/m^2, and 85 healthy patients with normal BMI, clinical, and laboratory tests. Both groups of study were matched regarding age. After the study purpose and procedures were clarified to all subjects, they signed an informed consent form. The enrolled obese patients were subdivided to obese women without PCOS (N = 60) and obese PCOS women (n = 50). According to the 2004 revised Rotterdam criteria, the diagnosis of PCOS was done [10]. All patients were subjected to thorough history taking, full clinical assessment, and anthropometric measures of obesity. A stadiometer was used to measure the height to the nearest 0.1 cm. BMI was estimated as the ratio of body weight to height squared and expressed as kg/m^2. Waist circumference (with 0.1 cm sensitivity) was measured at the minimum circumference between the iliac crest and the last rib cage at the end of exhalation. The hip circumference was measured using tape as the maximal circumference over the hip and waist-to-hip ratio (WHR) was calculated. Ovarian volume and antral follicular count (AFC) were evaluated by transvaginal ultrasound (TVS). Body compositions including fat mass (FM) and fat-free mass (FFM) were measured by dual-energy X-ray absorptiometry (DEXA). Exclusion criteria for all women included a history of hyperandrogenic states (such as non-classical congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, 21-hydroxylase deficiency, or hyperprolactinemia), DM, hypertension, liver, kidney, or thyroid diseases.

Ethics approval and consent to participate

A written informed consent was taken from all of the participants after explaining details and benefits as well as risks to them. The ethical committee of Faculties of Medicine, Zagazig University, approved the current study.

Nutrition education intervention

The nutrition intervention was designed based on macronutrients and micronutrients requirements, three 45–60 min training sessions at the beginning of the intervention. Participants were provided a 7-d menu plan. The plan per day included seven meals (breakfast, lunch, dinner, two snacks in the morning, and two more snacks in the afternoon). The macronutrient distribution of 50 % total caloric value (TCV) was from carbohydrates, 30% from lipids, 20% from proteins and a healthy fatty acids 30%. Subjects consumption of cholesterol was less than 300 mg/day and focused on low glycemic index and glycemic load (GL) carbohydrate meals [11, 12].

The total energy was calculated using the Harris-Benedict equations revised by Mifflin and St Jeor in 1990 :[13], women BMR = (10 × weight in kg) + (6.25 × height in cm)—(5 × age in years)—161, after we calculated the total energy intake, we subtracted 500 kcal/day as 500 kcal/day deficit. This is expected to reduce the weight by 1 pound/week. Participants were asked to walk for 150 min a week in divided sessions. At baseline and at the end point of the 12-week study, anthropometrical measurements were estimated and blood samples were collected for biochemical analyses.

Sampling of blood

After 12 hrs fasting; the blood samples were collected from all participants. One ml of whole blood was added onto EDTA tubes, for HbA1c; another 1 ml of whole blood was collected into potassium oxalate and sodium fluoride containing tubes for fasting plasma glucose (FPG). The remaining samples were subjected to centrifugation and Serum samples were separated and stored at −20 °C until analysis.

Biochemical analysis

FPG levels were measured according to glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol, HDL cholesterol, and triglycerides levels were assessed by routine enzymatic methods (Spinreact, Girona, Spain). Friedewald formula was used to calculate The LDL cholesterol level [14].

Immunochemical assays

Fasting serum insulin (FSI), follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone were evaluated. The insulin resistance (IR) with the homeostatic model assessment-IR (HOMA-IR) index were calculated. The FSI value (lU/mL) × FPG value (mg/dl)/ 405. A HOMA-IR value of 2.5 is taken as an indicator of IR in adults [15]. The β-cell function was calculated using HOMA-β as follows: {20*[FSI (μU/mL)]/ [FPG (mmol/L) - 3.5]}.

Detection of ghrelin level by ELISA method

Ghrelin level was measured by a commercially available ELISA kit supplied by DRG® Ghrelin (Human) ELISA International Inc., USA (Catalog No.: EIA-3706).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 21.0; SPSS Inc., Chicago, IL, USA). Data were expressed using a descriptive statistic (mean ± standard deviation) and were analyzed using paired t test. Pearson correlation coefficient was used to assess the association between. Receiver operating characteristic (ROC) analysis

was performed to assess the potential accuracy of plasma ghrelin for the diagnosis of PCOS, the area under the curve (AUC), and the cutoff values. We considered P to be significant at < 0.05.

Results

At the beginning of the study the total number of obese women who shared in the study was 130 (obese women without PCOS, n = 70 and obese PCOS women, n = 60), but during the study, some of the participants could not tolerate weight loss program so we excluded them from the study. The final total numbers of obese women enrolled in this study were 110 (obese women without PCOS, n = 60 and obese PCOS women, n = 50).

Clinical and biochemical characteristics of the studied groups

In the obese group, we found significantly higher levels of body composition parameters; BMI, waist/hip ratio, FMI%, and FFMI %. Also, systolic blood and diastolic blood pressure, total cholesterol, triglycerides, LDL cholesterol, FPG, FSI, HbA1c (%), and HOMA-IR compared to control group. PCOS phenotype including total testosterone, androstenedione, hirsutism score, ovarian volume FSH, LH, LH/FSH, DHEA-S, and AFC values was significantly high in obese cases compared to controls. On the contrary, we detected significant lower HDL cholesterol and HOMA-β levels in obese patients than in those healthy women. Moreover, we observed significantly lower levels of ghrelin in obese cases compared to controls.

Regarding dietary characteristics, there was a statistically significant difference between studied groups, $P < 0.001^{*}$ (Table 1).

The impact of weight loss on clinical, anthropometric, and laboratory characteristics of obese non-PCOS group

We tested the impact of weight loss in obese non-PCOS group after 12 weeks of following MD weight loss programed, and we found that there was a statistically significant decrease of obesity indices measures; BMI, waist/hip ratio, FMI%, and FFMI% compared to baseline values. In addition, we observed that there was a significant improvement of glycemic and lipid profile after significant weight loss. Regarding dietary characteristics, there were statistically significant improvements in dietary habit compared to the baseline, $P < 0.001^{*}$ (Table 2).

The impact of weight loss on clinical and phenotype characteristics of obese PCOS group

The most important finding of our study was the impact of weight loss on clinical and phenotype characteristics of obese PCOS group. After 12 weeks of following MD weight loss programmed, we found that there were statistically significant decreases of obesity index measures, glycemic, and lipid profile. Interestingly, the phenotype characteristics of PCOS obese women were also improved in particularly, hirsutism score, ovarian volume, AFC, FSH, DHEA-S, T testosterone, and androstenedione. Regarding dietary characteristics, there were statistically significant improvements of dietary habit compared to baseline, $P < 0.001^{*}$ (Table 3).

Comparison of plasma ghrelin (ng/ml) in studied groups

In the obese group, we found significantly lower levels of plasma ghrelin compared to controls, $P < 0.001$*, Fig. 1 and Table 1.

Impact of weight loss on plasma ghrelin (ng/ml) level in obese groups

The most important finding of our study was the impact of weight loss on plasma ghrelin levels we detected in both studied obese groups significant higher levels of plasma ghrelin compared to baseline. In obese non-PCOS group (n = 60), plasma ghrelin levels were (12.89 ± 5.45) at baseline however after weight loss for 12 weeks the level increased (26.9 ± 25.77). Furthermore, in obese PCOS group (n = 60), plasma ghrelin levels were (5.4 ± 1.6) at baseline however after weight loss for 12 weeks the level increased (16.72 ± 23.2), $P < 0.001$*, Fig. 2.

Correlation between plasma ghrelin (ng/ml) and clinical and laboratory parameters among obese women

In obese non-PCOS group (n = 60), plasma ghrelin levels were significantly negatively correlated with BMI, waist/hip ratio, FMI%, FFMI%, TC, TG, LDL, hirsutism score, AFC, ovarian volume, FPG, FSI, HbA1c, and HOMA-IR (Table 4, $P < 0.001$*).

Regarding obese PCOS group (n = 50), ghrelin levels in plasma were significantly negatively correlated with BMI, waist/hip ratio, FMI%, FFMI%, FPG, FSI, HbA1c, and HOMA-IR (Table 4, $P < 0.001$*).

The accuracy of circulating plasma ghrelin (ng/ml) for the discrimination of obesity by ROC analysis

The power of plasma ghrelin to discriminate obese women among the studied group was evaluated using ROC analysis. The AUC was 0.674 (95% CI = 0.599–0.749) with sensitivity = 96.4%, specificity = 96%, and the cutoff values (2.13), (Fig. 3).

The accuracy of circulating plasma ghrelin (ng/ml) for the discrimination of PCOS by ROC analysis

The power of plasma ghrelin to discriminate PCOS among obese women with the AUC was 0.863 (95% CI = 0.794–0.932) with sensitivity = 88%, specificity = 97.5%, and the cutoff values (7.5) (Fig. 4).

Table 1 Clinical, anthropometric, and laboratory characteristics at base line

	Control group (mean ± SD), (*n* = 85)	Obese group (mean ± SD), (*n* = 110)	*P*
Age (years)	29.07 ± 6.4	30.05 ± 6.473	0.265
Body mass index (kg/m^2)	24.55 ± 2.61	36.37 ± 2.26	< 0.001*
Waist/hip ratio	0.96 ± 0.189	1.24 ± 0.258	< 0.001*
Systolic blood pressure (mm Hg)	127.68 ± 6.54	130.95 ± 6.8	< 0.05*
Diastolic blood pressure (mm Hg)	85.18 ± 3.88	87.04 ± 4.320	< 0.05*
Hirsutism score	5.42 ± 0.741	9.84 ± 4.22	< 0.001*
Ovarian volume	5.15 ± 0.89	8.783 ± 3.96	< 0.001*
AFC	6.16 ± 1.319	9.64 ± 4.21	< 0.001*
FMI%	6.38 ± 0.67	9.4 ± 0.68	< 0.001*
FFMI%	18.16 ± 1.9	26.8 ± 1.96	< 0.001*
Total cholesterol (mg/dL)	166.7 ± 20.64	198.9 ± 11.18	< 0.001*
Triglycerides (mg/dL)	149.6 ± 11.19	260.5 ± 91.46	< 0.001*
LDL cholesterol (mg/dL)	105.8 ± 4.44	142.7 ± 13.77	< 0.001*
HDL cholesterol (mg/dL)	51.16 ± 4.34	33.8 ± 4.0	< 0.001*
Fasting blood glucose (mg/dL)	85.02 ± 8.95	94.01 ± 6.81	< 0.001*
Fasting serum insulin (lU/mL)	6.84 ± 1.443	15.1 ± 8.50	< 0.001*
HbA1c (%)	4.77 ± 0.147	5.9 ± 0.10	< 0.001*
HOMA-IR	1.43 ± 0.34	3.53 ± 2.00	< 0.001*
HOMA β	146.5 ± 62.0	96.8 ± 58.91	< 0.001*
FSH (mIU/mL)	4.89 ± 1.01	6.06 ± 1.49	< 0.05*
LH (mIU/mL)	6.54 ± 1.22	7.439 ± 1.5	< 0.001*
LH/FSH	1.52 ± 0.395	1.29 ± 0.38	< 0.001*
DHEA-S (mg/mL)	0.92 ± 0.48	1.45 ± 0.84	< 0.001*
Total testosterone (ng/mL)	0.51 ± 0.15	0.83 ± 0.23	< 0.001*
Androstenedione (ng/mL)	1.20 ± 0.35	1.936 ± 0.496	< 0.001*
Plasma ghrelin (ng/ml)	48.21 ± 21.09	9.49 ± 5.59	< 0.001*
Dietary characteristics			
Energy intake (kcal/day)	1813.5 ± 446.64	2406.8 ± 297.2	< 0.001*
Meal frequency (meals/day)	3.78 ± 1.01	4.9 ± 1.496	< 0.001*
Proteins (% TCV/day)	21.16 ± 3.008	25.21 ± 7.609	< 0.001*
Lipids (% TCV/day)	26.3 ± 3.74	31.38 ± 9.469	< 0.001*
CHO (% TCV/day)	47.02 ± 6.68	56.04 ± 16.9	< 0.001*

AFC antral follicle cells, *FM* fat mass, *FFM* fat-free mass, *FMI* fat mass index, *FFMI* fa-free mass index, *p HOMA-IR* homeostasis model assessments of insulin resistance, *DHEA* dehydroepiandrosteron, *TCV* total caloric value*

Discussion

PCOS is a heterogeneous complex genetic trait of multifactorial nature, and it is one of the most common metabolic and reproductive disorders affecting women during their reproductive period [16]. Obesity is an upcoming health hazard affecting the whole world and it is one of the most important and concerning predisposing factor of development of PCOS, since about 35-80% of PCOS women are overweight or obese [17, 18]. Weight loss and exercise as part of lifestyle modification could be the cornerstone in the management of PCOS [19].

A preponderance of evidence suggests that ghrelin stimulates growth hormone secretion, regulates glucose metabolism, appetite, body weight, endocrine pancreatic, and ovarian functions [20, 21].

Despite many supporting pieces of evidence about the impact of weight loss on obesity and PCOS characteristics, conflicting data have been reported regarding the impact of weight loss on ghrelin level. Therefore, we think that this is the first research that evaluates the influence of weight loss after 12 weeks of following a Mediterranean diet-based weight loss program on plasma ghrelin level and clinical phenotype of PCOS.

Table 2 The impact of weight loss on clinical, anthropometric, and laboratory characteristics of obese non-PCOS group

Variables	Obese non-PCOS group (n = 60)		
	Base line	Week 12	P value
Body mass index (kg/m^2)	35.87 ± 2.74	31.31 ± 5.8	˂ 0.001*
Waist/hip ratio	1.23 ± 0.26	1.12 ± 0.274	˂ 0.05*
Systolic blood pressure (mm Hg)	131.1 ± 6.21	129.06 ± 7.7	0.110
Diastolic blood pressure (mm Hg)	86.9 ± 4.27	86.5 ± 4.25	0.589
Hirsutism score	6.4 ± 1.45	6.31 ± 1.64	0.819
Ovarian volume	6.1 ± 2.12	5.98 ± 2.08	0.754
AFC	6.6 ± 1.737	6.2 ± 1.7	0.439
FMI%	9.28 ± 0.85	8.09 ± 1.56	˂ 0.001*
FFMI%	26.4 ± 2.43	23.05 ± 4.45	˂ 0.001*
Total cholesterol (mg/dL)	197.9 ± 12.49	181.4 ± 24.83	˂ 0.001*
Triglycerides (mg/dL)	308.8 ± 82.96	250.9 ± 91.35	˂ 0.001*
LDL cholesterol (mg/dL)	139.19 ± 15.9	123.5 ± 19.6	˂ 0.001*
HDL cholesterol (mg/dL)	34.6 ± 4.751	37.2 ± 5.681	˂ 0.001*
Fasting plasma glucose (mg/dL)	93.8 ± 6.73	89.51 ± 8.51	˂ 0.001*
Fasting serum insulin (lU/mL)	16.7 ± 9.532	14.5 ± 10.11	˂ 0.001*
HbA1c (%)	5.9 ± 0.118	5.87 ± 0.147	˂ 0.001*
HOMA-IR	3.9 ± 2.25	3.29 ± 2.42	˂ 0.05*
HOMA-β	109.02 ± 70.6	132.1 ± 78.5	0.056
FSH (mIU/mL)	5.77 ± 1.347	5.25 ± 1.31	˂ 0.05*
LH (mIU/mL)	7.37 ± 1.44	7.12 ± 1.68	˂ 0.001*
LH/FSH	1.33 ± 0.38	1.4 ± 0.457	0.397
DHEA-S (mg/mL)	1.07 ± 0.80	1.11 ± 0.71	0.259
Total testosterone (ng/mL)	0.82 ± 0.19	0.71 ± 0.26	˂ 0.05*
Androstenedione (ng/mL)	1.9 ± 0.50	1.63 ± 0.58	0.717
Dietary characteristics			
Energy intake (kcal/day)	2423.6 ± 329.1	1409.3 ± 263.3	˂ 0.001*
Meal frequency (meals/day)	4.6 ± 1.34	7.08 ± 0.76	˂ 0.001*
Proteins (% TCV/day)	23.7 ± 6.84	37.1 ± 7.8	˂ 0.001*
Lipids (% TCV/day)	29.5 ± 8.52	17.4 ± 4.8	˂ 0.001*
CHO (% TCV/day)	52.7 ± 15.22	40.8 ± 5.49	˂ 0.001*

AFC antral follicle cells, *FM* fat mass, *FFM* fat-free mass, *FMI* fat mass index, *FFMI* fat-free mass index, *p HOMA-IR* homeostasis model assessments of insulin resistance, *DHEA* dehydroepiandrosteron, *TCV* total caloric value, *p ˂ 0.05

The results of the current study showed statistically significant elevations of obesity measures: BMI, WHR, FMI%, FFMI%, metabolic characteristics, and phenotype characteristic of PCOS compared to controls. The finding of our present study consistent with our previous studies [22–26].

The main finding of the present study is that, after 12 weeks of the MD weight loss programmed, there was a significant weight reduction within both groups when compared to baseline. In PCOS group, there was a statistically significant improvement of anthropometric measures, glycemic, and lipid profile, and the phenotype characteristics of PCOS obese women were also improved in particularly, hirsutism score, ovarian volume, AFC, FSH, DHEA-S, T testosterone, and androstenedione.

In accordance to our results, a study conducted by Tolino et al. showed after 4 weeks of weight loss program, there was a reduction of free testosterone and fasting insulin levels as well as improvement in menstruation [27].

In agreement with our results, Van Dam et al. observed that after achieving 10% weight loss via a VLCD, the estradiol-dependent negative feedback on LH was normalized and led to the resumption of ovulation [27].

Table 3 The impact of weight loss on clinical and phenotype characteristics of obese PCOS group

Variables	Obese PCOS group (n = 50)		
	Base line	Week 12	P value
Body mass index (kg/m^2)	36.97 ± 1.29021	31.1 ± 6.51	˂ 0.001*
Waist/hip ratio	1.25 ± 0.25107	1.08 ± 0.25	˂ 0.001*
Systolic blood pressure (mm Hg)	130.8 ± 7.51	127.89 ± 8.4	˂ 0.05*
Diastolic blood pressure (mm Hg)	87.25 ± 4.38	86.32 ± 4.51	0.273
Hirsutism score	13.93 ± 2.36	10.2 ± 4.96	˂ 0.001*
Ovarian volume	11.92 ± 3.24	8.84 ± 4.4	˂ 0.001*
AFC	13.17 ± 3.48	10.19 ± 4.83	˂ 0.001*
FMI%	9.61 ± 0.335	8.092 ± 1.69	˂ 0.001*
FFMI%	27.36 ± 0.95	23.03 ± 4.81	˂ 0.001*
Total cholesterol (mg/dL)	200.03 ± 9.2	180.06 ± 26.2	˂ 0.001*
Triglycerides (mg/dL)	340.8 ± 62.35	259.08 ± 93.1	˂ 0.001*
LDL cholesterol (mg/dL)	146.96 ± 8.8	126.9 ± 21.69	˂ 0.001*
HDL cholesterol (mg/dL)	32.86 ± 2.65	36.25 ± 5.48	˂ 0.001*
Fasting plasma glucose (mg/dL)	94.05 ± 7.01	89.6 ± 9.14	˂ 0.001*
Fasting serum insulin (lU/mL)	13.45 ± 6.72	8.97 ± 4.61	˂ 0.001*
HbA1c (%)	5.98 ± 0.074	5.87 ± 0.166	˂ 0.001*
HOMA-IR	3.1 ± 1.55	2.01 ± 1.15	˂ 0.001*
HOMA-β	81.9 ± 35.9	122.4 ± 71.5	˂ 0.001*
FSH (mIU/mL)	6.44 ± 1.6	5.7 ± 1.704	˂ 0.05*
LH (mIU/mL)	7.6 ± 1.8	7.01 ± 01.80	0.059
LH/FSH	1.24 ± 0.378	1.3 ± 0.483	0.457
DHEA-S (mg/mL)	1.92 ± 0.628	1.36 ± 0.62	˂ 0.001*
Total testosterone (ng/mL)	.85 ± 0.266	.68 ± 0.29	˂ 0.001*
Androstenedione (ng/mL)	1.9 ± 0.486	1.56 ± 0.577	˂ 0.001*
Dietary characteristics			
Energy intake (kcal/day)	2384.1 ± 253.8	1384.1 ± 289.5	˂ 0.001*
Meal frequency (meals/day)	5.3 ± 1.6	7.16 ± 0.51	˂ 0.001*
Proteins (% TCV/day)	26.9 ± 8.07	35.9 ± 8.85	˂ 0.001*
Lipids (% TCV/day)	33.5 ± 10.05	16.9 ± 4.28	˂ 0.001*
CHO (% TCV/day) l	59.8 ± 17.94	40.4 ± 5.19	˂ 0.001*

*p ˂ 0.05

We in this study attempted to pierce out the levels of plasma ghrelin in both obesity and PCOS, and we found that there were significantly lower levels of plasma ghrelin in PCOS women compared to obese women without PCOS and controls.

Our findings are in concordance with Houjeghani et al. meta-analysis study, and they observed that ghrelin levels were significantly lower in PCOS patients than in controls [28].

Our results are in consistence with those reporting the low levels of ghrelin in PCOS patients compared to the control group [29, 30]. However, an interesting study by Orio et al. identified has shown no difference in ghrelin levels among PCOS and healthy controls [31].

Against our results, Wasko and colleagues demonstrated that high ghrelin levels in PCOS patients compared to the control group [32].

Considering the association between plasma ghrelin and clinical and laboratory findings among obese women. In the obese non-PCOS group, plasma ghrelin levels were significantly negatively correlated with anthropometric measures, glycemic, lipid profile, and the phenotype characteristics of PCOS.

These results are parallel to those of Kamal et al. who stated that obese PCOS patients confirmed significantly lower ghrelin levels than controls and was negatively correlated with cardiometabolic risk factors [29].

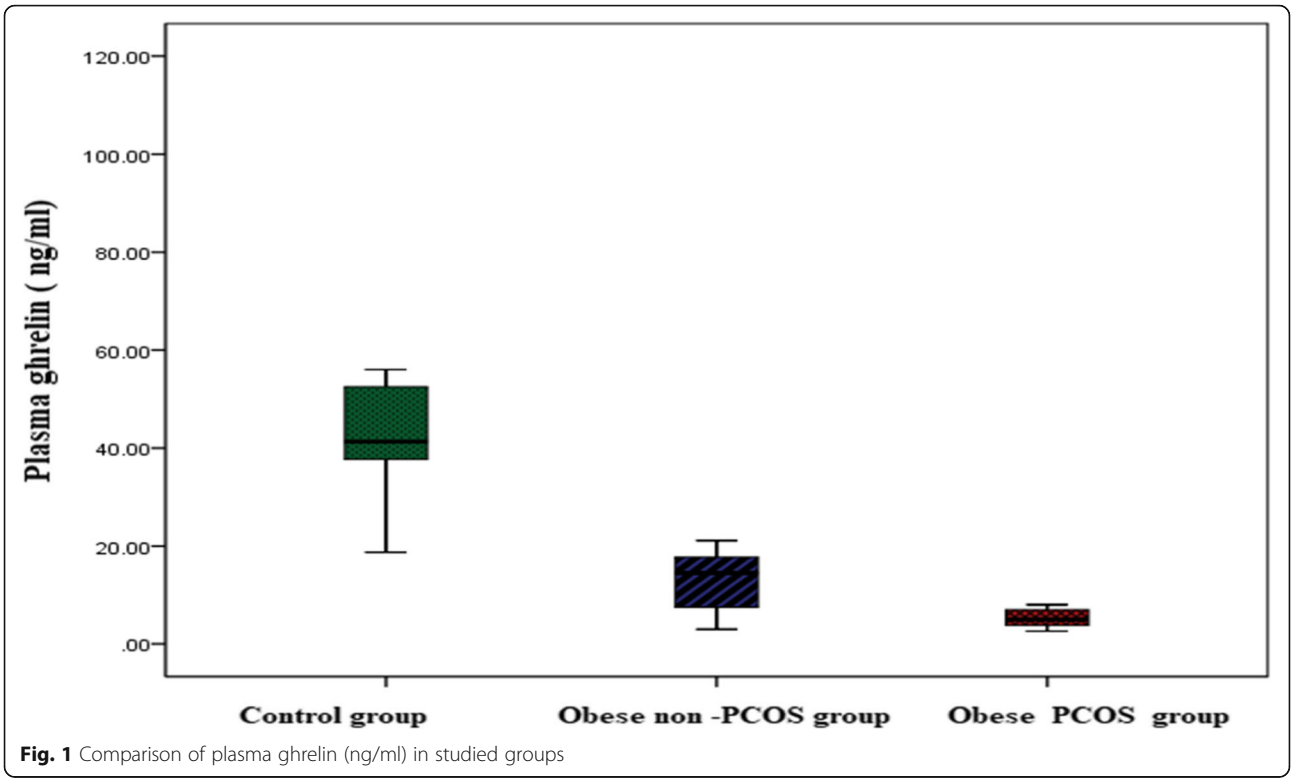

Fig. 1 Comparison of plasma ghrelin (ng/ml) in studied groups

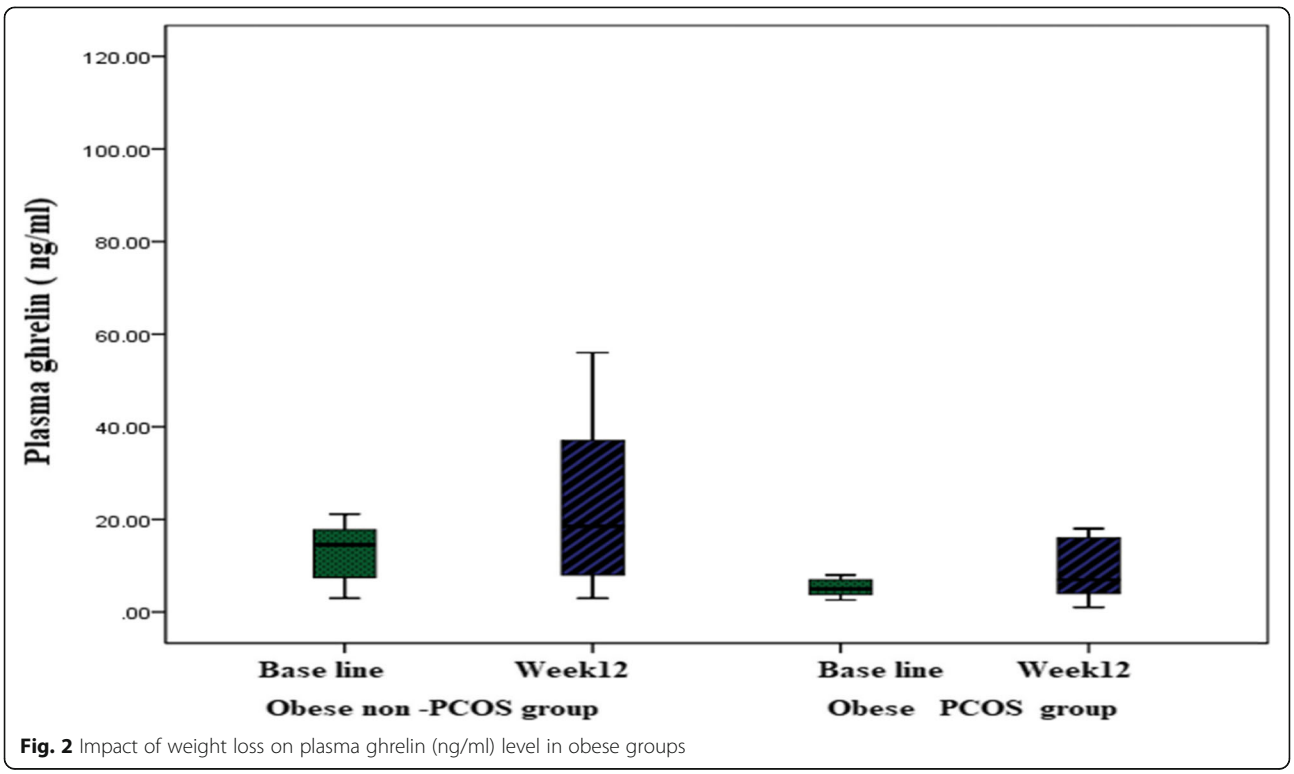

Fig. 2 Impact of weight loss on plasma ghrelin (ng/ml) level in obese groups

Table 4 Pearson correlation coefficient between plasma ghrelin (ng/ml) with clinical, anthropometric, laboratory, and phenotype characteristics of obese groups

	Obese group (n = 110)			
	Obese non-PCOS group, N = 60		Obese PCOS group, N = 50	
	r	P	r	P
Body mass index (kg/m^2)	− 0.252	˂ 0.001*	− 0.282	˂ 0.0 1*
Waist/hip ratio	− 0.244*	˂ 0.001*	− 0.307	˂ 0.001*
Systolic blood pressure (mm Hg)	− 0.108	0.261	− 0.080	0.579
Diastolic blood pressure (mm Hg)	− 0.014	0.881	− 0.149	0.302
Hirsutism score	− 0.125	0.192	− 0.099	0.494
Ovarian volume	− 0.109	0.257	− 0.133	0.358
AFC	− 0.122	0.203	− 0.491	˂ 0.001*
FMI%	− 0.204	˂ 0.001*	− 0.307	˂ 0.001*
FFMI%	− 0.238	˂ 0.001*	− 0.282	˂ 0.001*
Total cholesterol (mg/dL)	− 0.345	˂ 0.001*	− 0.169	0.241
Triglycerides (mg/dL)	− 0.406	˂ 0.001*	− 0.246	0.085
LDL cholesterol (mg/dL)	− 0.570	˂ 0.001*	− 0.015	0.917
HDL cholesterol (mg/dL)	0.041	0.667	0.118	0.413
Fasting plasma glucose (mg/dL)	− 0.296	˂ 0.001*	− 0.443	˂ 0.001*
Fasting serum insulin (IU/mL)	− 0.266	˂ 0.001*	− 0.396	˂ 0.001*
HbA1c (%)	− 0.365	˂ 0.001*	− 0.521	˂ 0.001*
HOMA-IR	− 0.345	˂ 0.001*	− 0.285	˂ 0.001*
HOMA B	0.111	0.248	− 0.061	0.674
FSH (mIU/mL)	− 0.187	0.051	0.143	0.320
LH (mIU/mL)	− 0.119	0.214	− 0.022	0.878
LH/FSH	− 0.028	0.772	− 0.131	0.364
DHEA-S (mg/mL)	− 0.00	0.967	− 0.733	˂ 0.001*
Total testosterone (ng/mL)	− 0.061	0.527	− 0.454	˂ 0.001*
Androstenedione (ng/mL)	− 0.151	0.114	− 0.462	˂ 0.001*

*p ˂ 0.05

Although there are many laboratory markers that could be used in the diagnosis of PCOS, we noticed neither specific nor sensitive markers of PCOS. Accordingly, we analyzed our data by ROC to estimate the sensitivity and specificity of plasma ghrelin for diagnosis of obesity by ROC analysis, the sensitivity was 96.4%, and the specificity was 96%. The power of plasma ghrelin to diagnose PCOS among obese women, the sensitivity was 88%, and specificity was 97.5%. Regarding the finding of the ROC curve, Kamal et al. detected that the sensitivity of plasma ghrelin to diagnose PCOS was 70% and specificity was 86% [29].

The results presented herein are innovative; as this study performs a robust evaluation of the role of weight loss on plasma ghrelin levels, we detected in both studied obese groups significant higher levels of plasma ghrelin after weight loss for 12 weeks compared to baseline levels.

Similarly, to our finding, different studies confirmed the higher levels of plasma ghrelin after weight reduction [33].

In addition, studies based on intensive energy restriction (very low caloric diet) showed increasing ghrelin concentrations were reported [34, 35], while other studies found that the levels of plasma ghrelin were stable after weight loss [36].

By contrast to our results, Cummings et al. detected decreasing ghrelin levels after weight reduction [37]. The contrast in results may be due to differences in study design, follow-up periods, measurement techniques, surgical intervention, and circadian rhythm. Meanwhile, ghrelin is produced predominately in the stomach, and it is difficult to distinguish the effect of surgery and reduction of overweight in these studies, while the results reported by previous studies using dietary approaches for weight loss observed different results. A study conducted by Reinehr et al. found that there was no significant difference in ghrelin levels after weight loss

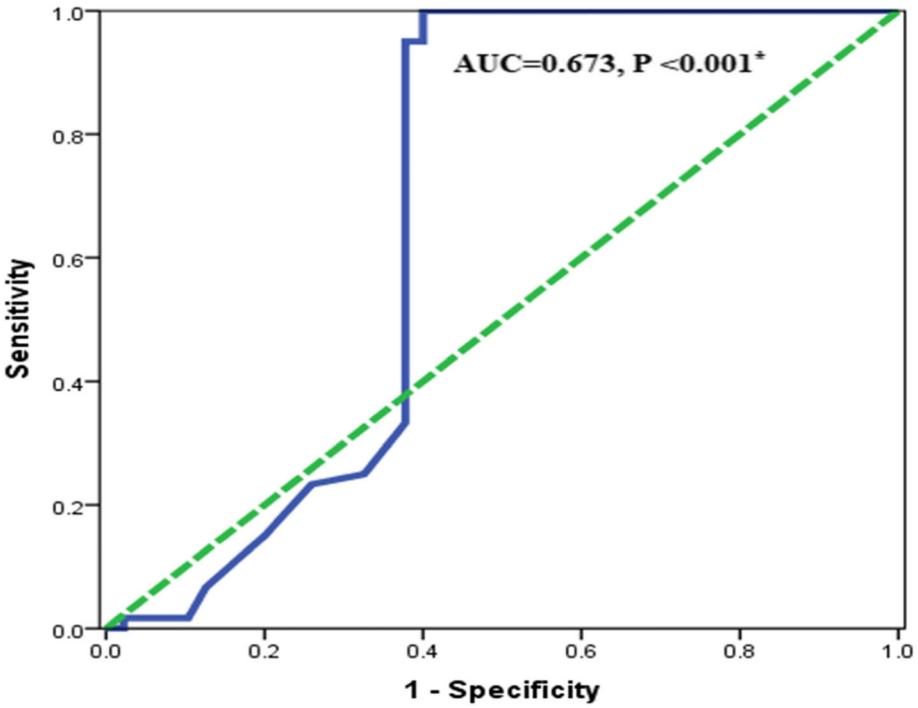

Fig. 3 Receiver operating characteristic (ROC) curve for on plasma ghrelin (ng/ml) as biomarkers for differentiating obese group among studied subjects

program for a long-term period of 1 year due to a high-carbohydrate low-fat diet [38].

The weight loss program we used in our study was guided by MD which is characterized by a high consumption of vegetables, legumes, fruits and nuts, cereals, and olive oil with a moderate high uptake of fish, a low-to-moderate intake of dairy products, and a low intake of meat and poultry [7]. Thus, our findings were different from Reinehr et al. results [38], as diet components greatly affect the gastrointestinal hormones like ghrelin,

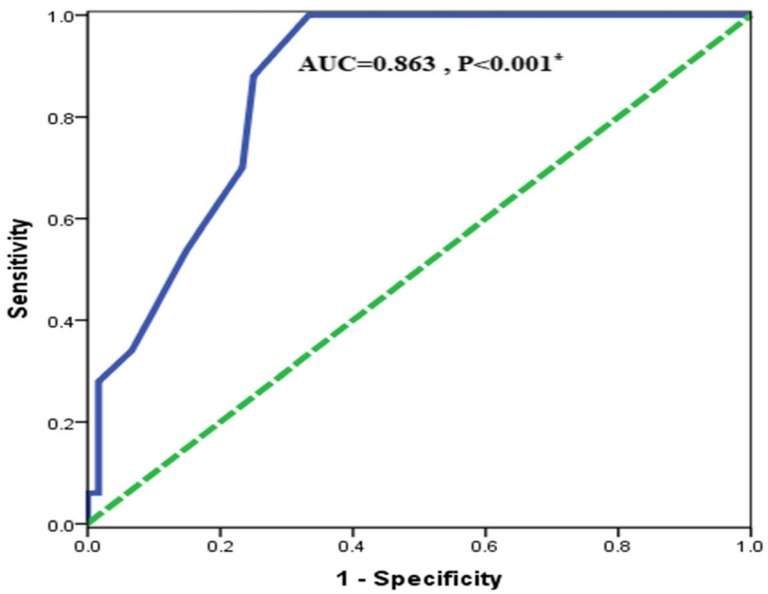

Fig. 4 Receiver operating characteristic (ROC) curve for on plasma ghrelin (ng/ml) as diagnostic biomarkers for diagnosis of PCOS group among obese women

while the other hormones are not directly influenced by diet.

Some limitations should be considered. The sample size was small and non-randomization of the study, and further larger studies should be performed in the future to validate the results and take into consideration the impact of weight loss on fertility

Conclusion

We found that circulating plasma ghrelin levels were lower in PCOS patients compared to obese and controls. Moreover, it was negatively correlated to anthropometric measures, glycemic, lipid profile, and the phenotype characteristics of PCOS. Interestingly, after 12 weeks of following MD weight loss program circulating plasma ghrelin levels were increased in both obese groups. Thus, plasma ghrelin could be used as a useful diagnostic biomarker of obesity and PCOS.

Abbreviations

AFC: Antral follicular count; BMI: Body mass index; DEXA: Dual-energy X-ray absorptiometry.; FFM: Fat-free mass; FM: Fat mass; FPG: Fasting plasma glucose; FSI: Fasting serum insulin; HOMA-IR: The homeostatic model assessment-IR; hs-CRP: Serum high-sensitivity C-reactive protein; MD: The Mediterranean diet; PCOS: Polycystic ovary syndrome; SHBG: Sex hormone-binding globulin; TVS: Transvaginal ultrasound

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Authors' contributions

NMR, YSS, SAA, and AEA collected patients' samples and clinical data. RMA prepared sample for laboratory investigations. NMR wrote the paper. Statistical analysis, interpretation of data, and preparation the paper for submitting international was done by NMR. Critical revision of the manuscript was performed by all of the authors. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

A written informed consent was taken from all of the participants after explaining details and benefits as well as risks to them. The ethical committee of Faculty of Medicine, Zagazig University, approved this study. The ethics committee's reference number is ZU-IRB#5624/1-6-2019.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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