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Original Article

Effect of a 24-week weight management program on serum leptin level in correlation to anthropometric measures in obese female: A randomized controlled clinical trial

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ABSTRACT

Background: Obesity is a major contributor to preventable disease and death across the globe. Obesity is complex. Although its risk factors are myriad and compounding, there is an urgent need for a deeper understanding of the way risk factors interact with each other. Leptin is a peptide regulates food intake and body weight. However, the notion of leptin as an anti-obesity hormone was called into question because obesity is typically associated with high leptin levels and not leptin deficiency thus, we aimed to measure leptin levels in obese female in correlation to anthropometric measures and to evaluate the impact of weight loss on its level and metabolic parameters.

Subject and methods: case-control study enrolled 40 control groups, 50 obese women. We measured anthropometric measures BMI, Waist/hip ratio (WHR). Fat mass index (FMI%) and free fat mass index (FFMI%) were assessed by dual energy X-Ray absorptiometry (DEXA) The serum levels of leptin were measured by ELISA.

Results: Our results revealed that serum leptin levels were higher in obese women compared to controls. Moreover, it was positively correlated to anthropometric measures, glycemic and lipid profile. Linear regression analysis revealed that BMI was the main independent studied parameters associated with serum leptin level among other clinical and laboratory biomarkers. Interestingly, after 12 weeks of following the Mediterranean diet (MD)–based weight loss program, serum leptin levels were decreased. Logistic regression analysis was performed to detect the main predictors' biomarkers associated with weight loss among obese women. We found that serum leptin and FMI% were an independent predictor of response with odds ratios of 1.69 and 1.64 respectively ($P < 0.001$), Receiver operating characteristic analyses revealed that the AUC of serum leptin in discriminating obese women from lean ones was 0.893 (95% CI = 0.815–0.917) with sensitivity = 90%, specificity = 96%, and the cutoff values was 36.32 ng/ml.

Conclusion: Serum leptin could be a valuable diagnostic marker of obesity and its comorbidities. Moreover, significant weight loss leads to decrease serum leptin levels and improvement of glycemic and lipid profiles.

1. Introduction

Existing reports suggest that obesity is actually an epidemic problem in the world; it has become truly a global problem affecting countries rich and poor. An estimated 500 million adults worldwide are obese and 1.5 billion are overweight or obese [1]. Particularly the prevalence of obesity in Egypt has increased at an alarming rate during the last three decades affecting 22% of adult males and 48% of adult females [2]. It is associated with several comorbidities including hypertension, dyslipidemia, type 2 diabetes mellitus (T2DM), coronary heart disease, stroke, osteoarthritis, sleep apnea, and respiratory problems, as well as some types of cancers [3,4].

Leptin is a 16-kDa protein hormone made up of 167 amino acids.

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It is mainly produced by adipocytes and leptin is confirmed as the major regulator of body weight since it decreases food intake and increases energy expenditure [5]. Leptin is a pro-inflammatory cytokine. Its synthesis is mostly dependent on the amount of body fat but it is also enhanced during acute infection and inflammation. Secretion of leptin is also regulated by the actions of proinflammatory mediators such as tumor necrosis factor (TNF)-α, IL-6, and IL-1 [6].

Leptin levels fall rapidly in response to fasting and evoke profound changes in energy balance and hormone levels and low leptin levels induce overfeeding and suppress energy expenditure, thyroid and reproductive hormones, and immunity [7]. Leptin replacement reverses these alterations in metabolism, immunity, and levels of hormones and hypothalamic neuropeptides [8,9].

Although several efforts tried to investigate the role of weight loss on obesity related comorbidities, conflicting data have been reported regarding the impact of weight loss on the leptin level. Therefore, to the best of our knowledge, this is the first research in Egypt that evaluates the influence of weight loss after 12 wk of following a Mediterranean diet–based weight loss program on serum leptin level and other metabolic parameters.

2. Subject and methods

A case-control study was conducted on 50 obese women, body mass index (BMI) ˃ 30 kg/m^2, who recruited from Outpatient Clinics of the Endocrinology Unit of Internal Medicine, Faculty of Medicine, Zagazig University, Egypt and 40 healthy lean women BMI < 25 matched for age. After being informed of the purpose and procedures of the study, all subjects signed an informed consent form. All patients were subjected to thorough history taking, full clinical assessment and anthropometric measures of obesity. Height was assessed by using a stadiometer that measured 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). Body compositions including fat mass (FM) and fat-free mass (FFM) were measured by Dual-energy X-ray absorptiometry (DEXA).

Patients with cancer, stroke, or liver, kidney, thyroid, and cardiovascular or any active inflammatory diseases were excluded from this study. None of the participants had a history of abdominal surgery that could have an impact on abdominal fat distribution, as well as receiving medications that affect endocrine parameters, glucose metabolism, and or for weight reduction or participating in a dietary or exercise programs during the preceding 6 months or immediately preceding month (anti-inflammatory drugs). 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.

2.1. 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 with a 7-d menu plan. The plan was composed of seven meals per day, including breakfast, lunch, dinner, two snacks in the morning, and two more snacks in the afternoon, a macronutrient distribution of 50% total caloric value (TCV) from carbohydrates, 20% proteins and 30% lipids, a healthy fatty acids 30% and a cholesterol consumption lower than 300 mg/day, and focused on low glycemic index and glycemic load (GL) carbohydrate meals [10,11].

The total energy was calculated using the Harris-Benedict equations revised by Mifflin and St Jeor in 1990: [12], 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/d as 500 kcal/day deficit (which ideally should produce a 1 pound/week weight loss, Participants were asked to walk for 150 min a week in divided sessions. At baseline and at the end point of the 24-weeks study, anthropometrical measurements were estimated as well as and blood samples were collected for biochemical analyses.

2.2. Sampling of blood

The blood samples of all study's subjects were drawn after an overnight fast and divided into 3 portions: 1 ml of whole blood was collected into EDTA tubes, for HbA1c; 1 ml of whole blood was collected into potassium oxalate and sodium fluoride containing tubes for fasting plasma glucose (FPG). Sera were separated from the remaining sample part and stored at −20 °C until analysis.

2.3. Biochemical analysis

We determined FPG levels using the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol (TC), HDL cholesterol, and triglycerides (TG) levels were measured by routine enzymatic methods (Spinreact, Girona, Spain). The LDL cholesterol level was calculated using the Friedewald formula [13].

2.4. Detection of serum leptin by ELISA method

Serum leptin levels were assessed using commercial ELISA (Enzyme-Linked Immunosorbent Assay) kit (Human Leptin ELISA kit, (Biovendor [Cat No: RD191001100], USA).

2.5. Dual-energy X-ray absorptiometry (DEXA)

The accurate and precise values of the body composition parameters were estimated from the DXA scan of the total body, which included; fat mass (FM), fat-free mass (FFM), additionally, the FM index (FMI; FM/height2), FFM index (FFMI; [BMC + LM]/height2), were calculated.

2.6. 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 the “t” test. Pearson correlation coefficient between serum leptin with clinical, anthropometric and laboratory characteristics of obese groups. Receiver operating characteristic (ROC) analysis was performed to assess the potential accuracy of serum leptin for the diagnosis of obesity, the area under the curve (AUC), and the cutoff values. We considered P to be significant at <0.05.

3. Results

The final total numbers of obese women enrolled in this study were 50, at the beginning of study 64 obese women were included, we excluded all women who did not complete this study.

3.1. Clinical and biochemical characteristics of the studied groups at baseline

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, TC, TG, LDL, FPG, and HbA1c compared to the control group. On the contrary, we detected significantly lower HDL cholesterol in obese patients than in those healthy women. Regarding dietary characteristics, there was a statistically significant difference between studied groups as regards, Dietary characteristics; energy intake (kcal/d), meal frequency (meals/d), proteins (% TCV/d), lipids (% TCV/d), carbohydrates (% TCV/d) P ˂0.001*, (Table 1).

3.2. The impact of weight loss on clinical, anthropometric and laboratory characteristics of obese patients

The most important finding of our study was the impact of weight loss on clinical and phenotype characteristics of obese women. After 12 weeks of following MD weight loss programmed and we found that there were statistically significant decreases of obesity indices measures, glycemic and lipid profile. Interestingly, the dietary component and energy intake of obese women were also decreased after weight loss compared to baseline, P˂0.001*, (Table 2).

3.3. Comparison of serum leptin (ng/ml) in studied groups

In the obese group, we found significantly higher levels of serum leptin (42.43 ± 2.38) compared to controls (9.6 ± 03), P ˂0.001*, Fig. 1.

3.4. Impact of weight loss on serum leptin (ng/ml) level in obese groups

The most important finding of our study was the impact of weight loss on serum leptin levels we detected in obese group significant lower levels of serum leptin (20.24 ± 4.4) compared to baseline (42.43 ± 2.38), P ˂0.001*, Fig. 2.

3.5. Correlation between serum leptin (ng/ml) and clinical and laboratory parameters among obese women

In Obese group, (n = 50), serum leptin levels were significantly positively correlated with BMI, waist/hip ratio, FMI%, FFMI%, TC, TG, LDL, FPG, FSI, and HbA1c (Table 3, P ˂0.001*).

3.6. A stepwise multiple linear regression analysis in obese women

Linear regression analysis revealed that BMI was the main independent studied parameters associated with serum leptin level among other clinical and laboratory biomarkers (Table 4).

3.7. Assessment of the power 12 weeks of following MD weight loss programmed in improving clinical and laboratory features of obese women

Logistic regression analysis was performed to detect the main predictors' biomarkers associated with weight loss among obese women. Our findings revealed that among clinical and laboratory features of obese women, serum leptin and FMI% were an independent predictor of response with odds ratios of 1.69 and 1.64 respectively (P < 0.001), (Table 5).

3.8. The accuracy of circulating serum leptin (ng/ml) for the diagnosis of obesity by ROC analysis

The power of serum leptin to diagnose obese women among the studied group was evaluated using ROC analysis. The AUC was 0.893 (95% CI = 0.815–0.917) with sensitivity = 90%, specificity = 96%, and the cutoff values (36.32), (Fig. 3).

4. Discussion

Accumulating epidemiological evidence supports the concept that obesity is a complex, multifactorial, and largely preventable disease, affecting, along with overweight, over a third of the world's population today [14]. If secular trends continue, by 2030 an estimated 38% of the world's adult population will be overweight and another 20% will be obese [15,16].

A preponderance of evidence suggests that leptin is mainly produced by adipose tissue, more specifically by visceral white adipocytes in rodent and by subcutaneous adipose tissue in human

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

	Control group (mean ± SD), (n = 40mj)	Obese group (mean ± SD), (n = 50)	P
Age (years)	29.07 ± 6.4	30.05 ± 6.473	0.265
Systolic blood pressure (mm Hg)	125.68 ± 7.54	130.25 ± 6.9	˂0.05*
Diastolic blood pressure (mm Hg)	85.18 ± 3.88	87.04 ± 4.320	˂0.05*
Body mass index (kg/m2)	24.55 ± 2.61	36.37 ± 2.26	˂0.001*
Waist/hip ratio	0.96 ± 0.189	1.64 ± 0.258	˂0.001*
FMI%	6.58 ± 0.67	13.2 ± 0.68	˂0.001*
FFMI%	18.56 ± 1.9	23.8 ± 1.96	˂0.001*
Total cholesterol (mg/dL)	165.7 ± 20.64	184.9 ± 11.18	˂0.001*
Triglycerides (mg/dL)	177.6 ± 11.19	269.5 ± 91.46	˂0.001*
LDL cholesterol (mg/dL)	106.8 ± 4.44	129.7 ± 13.77	˂0.001*
HDL cholesterol (mg/dL)	51.16 ± 4.34	36.8 ± 4.4	˂0.001*
Fasting plasma glucose (mg/dL)	83.02 ± 8.95	91.01 ± 6.81	˂0.001*
HbA1c (%)	4.77 ± 0.147	5.9 ± 0.10	˂0.001*
Dietary characteristics Energy intake (kcal/d)	1983.5 ± 426.64	2261.8 ± 443.2	˂0.001*
Meal frequency (meals/d)	3.72 ± 1.01	4.45 ± 1.496	˂0.001*
Proteins (% TCV/d)	21.05 ± 2.9	23.09 ± 6.4	˂0.001*
Lipids (% TCV/d)	26.1 ± 3.74	28.38 ± 9.469	˂0.001*
CHO (% TCV/d)	46.02 ± 6.68	51.04 ± 16.9	˂0.001*

FM; fat mass, FFM; fat free mass, FMI; fat mass index, FFMI; fat free mass index; p; .TCV; total caloric value, CHO; carbohydrates, *p˂0.05.

Table 2
The impact of weight loss on clinical, anthropometric and laboratory characteristics of obese patients.

Variables	Obese group		
	Base line	Week 12	P value
Systolic blood pressure (mm Hg)	130.25 ± 6.9	129.06 ± 7.7	0.110
Diastolic blood pressure (mm Hg)	87.04 ± 4.320	86.5 ± 4.25	0.589
Body mass index (kg/m2)	36.37 ± 2.26	26.31 ± 5.8	<0.001*
Waist/hip ratio	1.64 ± 0.258	1.12 ± 0.274	<0.05*
FMI%	13.2 ± 0.68	8.09 ± 1.56	<0.001*
FFMI%	23.8 ± 1.96	23.05 ± 4.45	<0.001*
Total cholesterol (mg/dL)	184.9 ± 11.18	181.4 ± 24.83	<0.001*
Triglycerides (mg/dL)	269.5 ± 91.46	250.9 ± 91.35	<0.001*
LDL cholesterol (mg/dL)	129.7 ± 13.77	103.5 ± 19.6	<0.001*
HDL cholesterol (mg/dL)	36.8 ± 4.4	37.2 ± 5.681	<0.001*
Fasting plasma glucose (mg/dL)	91.01 ± 6.81	89.51 ± 8.51	<0.001*
HbA1c (%)	5.9 ± 0.10	5.87 ± 0.147	<0.001*
Dietary characteristics Energy intake (kcal/d)	2261.8 ± 443.2	1809.3 ± 263.3	<0.001*
Meal frequency (meals/d)	4.45 ± 1.496	7.08 ± 0.76	<0.001*
Proteins (% TCV/d)	23.09 ± 6.4	37.1 ± 7.8	<0.001*
Lipids (% TCV/d)	28.38 ± 9.469	17.4 ± 4.8	<0.001*
CHO (% TCV/d)	51.04 ± 16.9	40.8 ± 5.49	<0.001*

FM; fat mass, FFM; fat free mass, FMI; fat mass index, FFMI; fat free mass index; p; „TCV; total caloric value, *p<0.05.

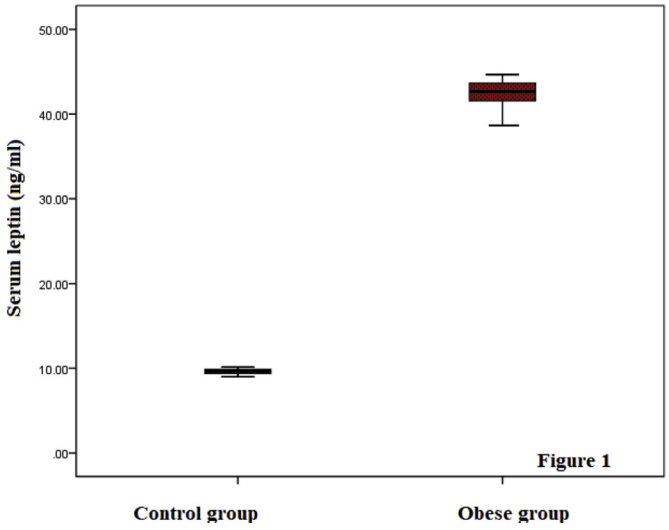

Fig. 1. Comparison of serum leptin level among studied groups.

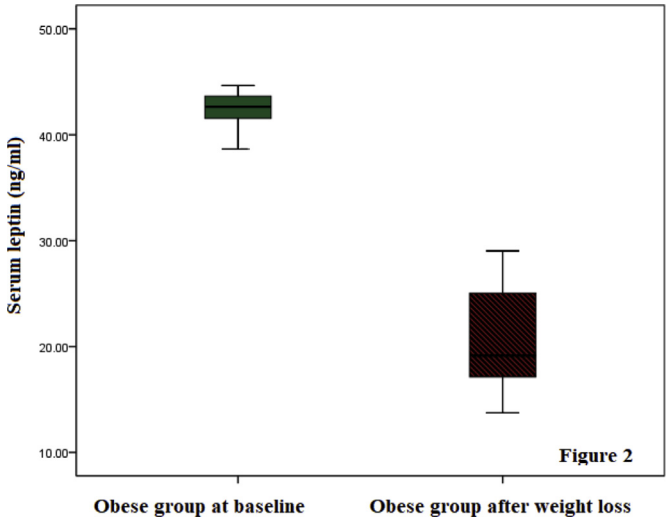

Fig. 2. Impact of weight loss on serum leptin level among obese women.

Table 3
Pearson correlation coefficient between serum leptin (ng/ml) with clinical, anthropometric and laboratory characteristics of obese groups.

	Obese group	
	r	p
Systolic blood pressure (mm Hg)	0.107	0.260
Diastolic blood pressure (mm Hg)	0.024	0.871
Body mass index (kg/m2)	0.262	<0.001*
Waist/hip ratio	0.234	<0.001*
FMI%	0.234	<0.001*
FFMI%	0.248	<0.001*
Total cholesterol (mg/dL)	0.355	<0.001*
Triglycerides (mg/dL)	0.416	<0.001*
LDL cholesterol (mg/dL)	0.571	<0.001*
HDL cholesterol (mg/dL)	−0.051	0.657
Fasting plasma glucose (mg/dL)	0.295	<0.001*
HbA1c (%)	0.375	<0.001*

[17]. However, local expression of leptin mRNA and protein in CNS has been reported [18]. Circulating levels of leptin are dynamic and susceptible to different regulatory factors such as metabolism, body fat mass, a circadian cycle and sexual dimorphisms [19]. Moreover, restoration of leptin in patients lacking fat cells (lipodystrophy) improves reproductive function and reverses abnormal lipid and glucose metabolism [20,21].

Despite many supporting pieces of evidence about the impact of weight loss on obesity and its comorbidities, conflicting data have been reported regarding the impact of weight loss on the leptin level. Therefore, to the best of our knowledge, this is the first research in Egypt that evaluates the influence of weight loss after 12 wk of following a Mediterranean diet–based weight loss program on serum leptin level and other metabolic parameters.

The results of the current study showed statistically significant elevations of obesity measures; BMI, WHR, FMI%, FFMI%, metabolic characteristics as well as FPG of obese compared to controls. The finding of our present study consistent with our previous studies [22,23].

Previous studies demonstrated that body weight is regulated by a complex system, including both peripheral and central factors. Two of the hormones that seem to play an important role in the regulation of food intake and body weight are leptin and ghrelin. Both originate in the periphery and signal through different pathways to the brain, particularly to the hypothalamus [24,25]. Yet a

Table 4
Linear regression analyses in obese women to test the influence of the main independent variables against serum leptin levels (dependent variable).

Model		Unstandardized Coefficients		Standardized Coefficients	t	P value	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	−41.751	5.114		−8.164	<0.001*	−51.866	−31.636
	TC	0.011	0.029	0.020	0.384	0.702	−0.046	0.068
	FPG	0.031	0.062	0.021	0.503	0.616	−0.092	0.155
	Waist/hip ratio	0.166	2.070	0.003	0.080	0.936	−3.928	4.261
	BMI	1.934	00.161	0.843	12.048	<0.001*	1.616	2.251
	FFM%	0.178	.247	00.059	0.721	0.472	−0.311	0.668

Table 5
Logistic regression analysis was used to analyze the effect of 12 weeks of following MD weight loss programmed on serum leptin in association with other studied parameters.

		B	S.E.	t	P value	Odds ratio	95% C·I.	
							Lower	Upper
1	leptin	0.530	0.126	17.595	<0.001*	1.699	1.326	2.176
	FMI%	0.495	0.110	20.326	<0.001*	1.641	1.323	2.035
	FPG	0.10	0.092	1.316	0.25	1.112	0.928	1.333
	Constant	−12.181	2.840	18.395	<0.001*	.000		

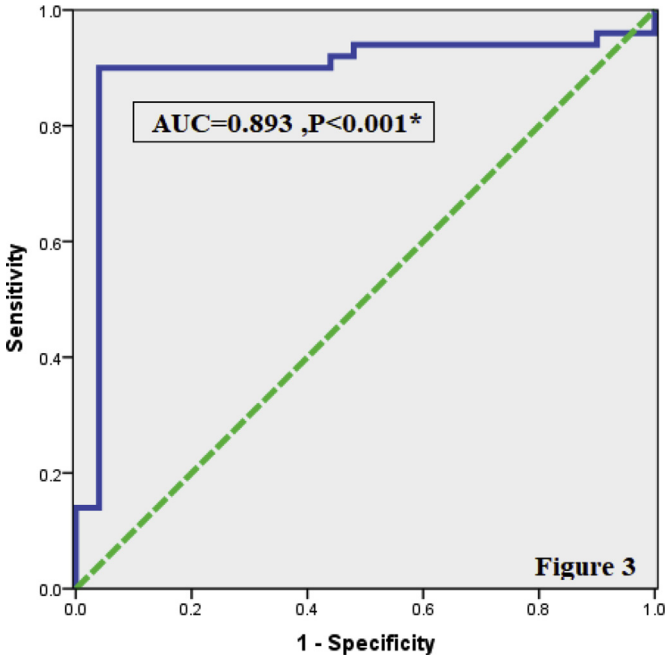

Fig. 3. Receiver operating characteristic (ROC) curve serum leptin (ng/ml) as diagnostic biomarkers of obesity among studied subjects.

seminal experimental study revealed that in the hypothalamus, activation of the leptin or ghrelin receptor initiates different signaling cascades leading to changes in food intake [26].

The interesting finding of the present study is that serum leptin levels were higher in obese women compared to controls. Likewise, it was positively correlated to anthropometric measures, glycemic and lipid profile. Notably, linear regression analysis revealed that BMI was the main independent studied parameters associated with serum leptin level among other clinical and laboratory biomarkers.

These findings are bolstered by studies demonstrating that obese patients had elevated levels of leptin in serum and adipocytes, however, treatment of obesity with leptin had limited response and this could be owing to leptin resistant [27,28]. Mounting evidence indicates that the development of leptin resistance most likely involves a period of over-eating, resulting in the leptin system getting so disturbed that it leads to sustained defects [29,30].

The main finding of the present study is that, after 12 weeks of the MD weight loss programmed, there was a significant reduction of serum leptin compared to baseline as well as there was a statistically significant improvement of anthropometric measures, glycemic, and the lipid profile of obese women were also improved.

Current evidence indicates that not only the size and frequency of meals have an effect on circulating leptin, but also the composition of a meal is a determinant of leptin levels in humans In this issue as expected our results revealed that dietary characteristics; energy intake, meal frequency as well as proteins, lipids and carbohydrates content of food decreased after 12 weeks of weight loss program. In this program, there was a balance in diet component so we could not evaluate the relation between leptin level and type of diet; low fat or high carbohydrates as mention.

There are intriguing reports suggesting that low-fat/high carbohydrate meals result in an increase in circulating leptin concentrations, which is larger, compared with high-fat/low-carbohydrate meals [31].

An interesting study of Havel found that high-fat meals lower 24-h circulating leptin levels relative to high carbohydrate meals [32].

Although there are many laboratory markers that could be used in the assessment of obesity and its comorbidities we noticed neither specific nor sensitive markers of obesity. Accordingly, we analyzed our data by ROC to estimate the sensitivity and specificity of serum leptin, the sensitivity was 96.4% and the specificity was 96%. At cutoff values of serum leptin 36.32 ng/ml, the AUC was 0.893 (95% CI = 0.815–0.917).

In conclusion, we found that circulating serum leptin levels were higher in obese women compared to controls. Moreover, it was positively correlated to anthropometric measures, glycemic and lipid profile. Interestingly, after12 weeks of following MD weight loss program circulating serum leptin levels were decreased in obese women.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be

construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.05.027.

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