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RESEARCH ARTICLE

The effect of experimentally induced energy imbalance on fasting serum obestatin

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Abstract

Background: Obestatin is a recently discovered adipocytokin. Its role has been studied in the fields of obesity, diabetes mellitus, and psychogenic eating disorders.

Objectives: Assessing the effect of energy imbalance on the serum obestatin level and its relation to BMI, blood glucose, insulin level, HOMA -IR and lipid profile.

Design: 54 adult male Wister albino rats were utilized. They were divided into three equal groups according to their diet regimens; **Group1:** average caloric diet (ACD) was fed a standard chow diet (3.84 Kcal/gm); **Group2:** restricted caloric diet (RCD) was fed a reduced caloric diet (2.30 Kcal/gm), and **Group3:** High calorie diet (HCD) was fed a high fat diet (4.89 Kcal/gm). The animals were sacrificed (6 animals from each group) 10 days apart (after 10, 20, & 30 days).

Results: Fasting obestatin level did not show significant changes in the ACD group at different studied time points. While, significant increases in its levels were demonstrated in the RCD group after 20 and 30 days. Inversely, it was significantly decreased after 20 and 30 days in the HCD group. Moreover, obestatin was negatively correlated with BMIs, blood glucose, serum insulin and HOMA-IR in the RCD as well as HCD groups after 20 and 30 days.

Conclusion: Obestatin is involved in the regulation of energy balance .So, disturbance in serum obestatin could be used as an indicator of energy imbalance. This gives a new insight about the anticipation of obestatin agonist/s and antagonist/s as new drugs for treatment of obesity and cachexia.

INTRODUCTION

Disturbance in energy balance can lead to complex disorders that are characterized by abnormal eating behaviors, namely cachexia and obesity. Cachexia is marked by the involuntary loss of skeletal muscle and adipose tissue as a result of greater catabolic than anabolic activity. On the other hand, obesity results from chronic imbalance between energy intake (excess) and energy expenditure **(Castaneda et al., 2010).**

Substantial evidence suggests that a complex network of peripheral and hypothalamic signals contribute to the regulation of food intake, energy homeostasis and nutrient partitioning **(Havel, 2001).**

Obestatin, a novel 23 amino acid amidated peptide encoded by the same gene that encodes ghrelin, appears to function as a part of a complex gut-brain network whereby hormones and substances from the stomach and intestine signal the brain regarding satiety and /or hunger. It is thought to oppose ghrelin's effects on food intake **(Bascietto et al.,2011**).As, it appears to act as an anorexogenic hormone, decreasing food intake, slowing gastric emptying and jejunal motility, and hindering weight gain **(Zhang et al.,2005** ; **Zhang et al.,2008**; **Ren et al.,2009)**

Ghrelin has been suggested to be involved in the pathophysiology of feeding disorders. Indeed, baseline plasma ghrelin concentrations and plasma ghrelin responses to calorie ingestion have been found to be deranged in both underweight and obese subjects (**Favaro et al., 2008**).The changes in basal plasma ghrelin as well as obestatin levels have been reported in patients with Anorexia nervosa (AN) and bulimia nervosa (BN), which are psychosomatic disorders, occurring mainly in young women, characterized by abnormal eating behavior leading to imbalance in energy homeostasis **(Sedlackova et al., 2011)**.

The previous studies brought discordant results concerning basal serum obestatin levels during different conditions of fixed, negative and/or positive energy balances. As, **Zhang et al.,(2005)** found no significant change in plasma obestatin level in the fasted or fed state in rodents. In contrast**, Sedlackova et al.. (2008)** observed a decrease in obestatin and gherlin in the plasma of healthy subjects following high carbohydrate breakfast.

In addition, there are contradictory views with respect to the concentrations of obestatin in obese patients. Some experiments revealed that in obesity, the peripheral blood exhibits significantly lower obestatin levels than in healthy, average weight individuals **(Huda et al., 2007).** However, another study reported that fasting obestatin levels were higher in infants with Prader–Willi syndrome, an obesity syndrome characterized by rapid weight gain and excessive food intake, compared with controls **(Butler MG and Bittel , 2007).**

Based on the aforementioned relations between ghrelin and obestatin, it seems plausible that obestatin could be a further candidate involved in pathophysiology of eating disorders. However, the role of obestatin in the balance of energy homeostasis, body weight control, and insulin sensitivity is still unclear and under debate **(Monteleone et al., 2008).**

Indeed obestatin colocalizes with ghrelin, probably in ε cells of pancreatic islets, suggesting a role in ß -cell function **(Gronberg et al., 2008**; **Volante et al., 2009)**. It promotes proliferation and survival of ß -cells, through increasing the mRNA of genes involved in ß -cell differentiation **(Granata et al., 2008**). It is believed that the glucose concentrations in the pancreatic ß cells critically contribute to the effects of obestatin on insulin secretion. While it was demonstrated that the changes in obestatin levels with the disturbance in the energy balance of feeding, as in fasting or increased blood glucose, lead to disruption in the effect of obestatin on ß- cell of pancreas, indicating that obestatin may be a marker for different caloric imbalances (**Ackermann and Gannon , 2007; Kaneto et al., 2008**)

Conflicting data about the interrelation between obestatin and insulin resistance are detected. In a study on adult humans, decreasing concentrations of obestatin were associated with diabetes and impaired glucose regulation and the insulin sensitivity surrogate homeostasis model assessment (HOMA) of insulin resistance **Qi et al., (2007)**. Similar results were documented by **Lippl et al., (2008)** and **Abou Fard et al., (2014)**, however, **St-Pierre et al., (2010)** showed that normal and diabetic patients display similar levels of circulating obestatin in fasting condition.

Based on the previous controversies, we aim to assess the role of obestatin in energy homeostasis through measuring fasting serum obestatin levels under different caloric diet regiments, and evaluate its associations with BMI, blood glucose, serum insulin level and HOMA-IR

Materials and Methods

Animals:

54 adult male Wister albino rats weighing 170-195 gm were obtained from the animal house of veterinary medicine faculty at Zagazig University. The animals were kept in steel wire cages in the Physiology department laboratory unit in the faculty of medicine under hygienic conditions .They were kept at room temperature & were maintained on a 12h light/dark cycle. The rats were accommodated to our laboratory conditions for two weeks before experiments were started. The experimental protocols were approved by physiology department and by the local medical ethics committee in faculty of medicine, Zagazig University. During the accommodation period rats were fed a standard regular commercial rat chow, and had free access to water and food.

Experimental study:

- Animal groups:

The animals were divided into three equal groups according to their diet regimens: **1st Group: Average caloric diet (ACD):** Consisted of 18 rats that received regular standard chow diet (3.84 Kcal/gm). The diet consisted of Casein 33.11%, Cystine 0.30%, Starch 25.21%, Dextrose 25.21%, Cellulose 5.00%, Soybean oil 5.00%, Minerals 5.00%, Vitamins 1.00%, Colin 0.17%, and Lard 0%.

2nd Group: Restricted caloric diet (RCD): It included 18 rats that received a reduced caloric diet (2.30 Kcal/gm). It was prepared by mixing regular standard chow diet 1:1 cellulose (**Francesc et al., 1986).**

3rd Group: High caloric diet (HCD): It included 18 rats, that received a high caloric diet (4.89 Kcal/gm). It consisted of Casein 33.11%, Cystine 0.30%, Starch 15.21%, Dextrose 15.21%, Cellulose 5.00%, Soybean oil 50 %, Minerals 5.00%, Vitamins 1.00%, Colin 0.17%, and Lard 20%) (**Vigueras-Villasen et al., 2011).**

Each one of the groups was subdivided into three subgroups. Each subgroup consisted of 6 rats. The animals were sacrificed 10 days apart: Subgroup (a) was sacrificed after 10days; subgroup (b) was sacrificed after 20days; while subgroup (c) was sacrificed after 30days (**Swiergiel A. and Cabanac ,1989; Ble-Castillo et al., 2012)** .

-**Measurement of the body mass index (BMI):** animal weights and lengths were measured in a randomly selected manner (6 rats from each group) for determination of the initial BMIs. Then weights & lengths were measured in all rats of each subgroup at the end of their experiment, and immediately before their sacrificing. BMI was measured according to the method described by **Novelli et al., (2007).**

-Sampling of the blood: Blood samples (6ml/rat), were taken at the time of scarification after fasting overnight and each blood sample was allowed to clot for 2 hours at room temperature before centrifugation for 20 minutes at approximately 5000 rpm. The separated serum was stored at -20C. Repeated freezing and thawing were avoided **(Nishizawa et al., 2007).**

-Determination of fasting serum obestatin: by using rat double- antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit; (EIAR-OBS; Ray biotech .inc., USA) which was purchased from sigma aldrich comp.. The method used for measurement was according to manufacturer's instruction**.**

-Determination of fasting serum glucose: By using the enzymatic colorimetric method for quantitative measurement of glucose in the serum (Biotechnology, Egypt); Glucose (GOD-PAP)-liquizyme Kits according to **Tietz, (1995)**.

-Determination of fasting serum insulin: KAP1251-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) Kits: For the quantitative measurement of Insulin in serum by Immuno-enzymatic Assay (BioSource Europe S.A., Belgium), as described by **Starr et al., (1978)**.

-Calculation of The homeostasis model assessment of insulin resistance (HOMA-IR) was measured by the formula: HOMA-IR=insulin (mIU/L)× glucose (mg/dl)/405 **(Sun et al., 2007).**

-Determination of the lipid profile:

***Total cholesterol (TC):** Cholesterol RTU 61218 kits: for enzymatic determination of total cholesterol (bioMerieux S.A., Lyon, France). It was estimated according to the method describe by **Flegg, (1973)**.

***Triglycride (TG):** Triglycerides ESPAS SL kits: for the enzymatic determination (Elttech S.A., Sees, France). It was estimated according to the method described by **Naito, (1989).**

***High density lipoprotein-cholesterol (HDL):** Stanbio HDL-cholesterol Procedure No.0599 kits: for the enzymatic determination of High Density Lipoprotein (HDL) cholesterol (Stanbio laboratory Inc., San Antoni, Texas).). It was estimated according to the method described by **Warnick et al., (1983).**

***Low density lipoprotein-cholesterol (LDL):** LDL was estimated using the Friedewald equation **(Friedwald et al., 1972)**.

Statistical analysis

The data were presented as mean ± SD. Data from the experiments were analyzed using one-way analysis of variance (ANOVA). The relationship between plasma obestatin levels, BMI, serum glucose, insulin, HOMA-IR, or lipid profile was examined by Pearson's correlation coefficient. P-values less than 0.05 were considered statistically significant. All analyses were performed using SPSS for windows (version 18.0) (IBM Corporation, Armonk, New York, USA).

Results

The changes in BMI In each group at different time points are illustrated in figure (1).While, the differences in BMI of different groups at the same time point are shown in figure (2).

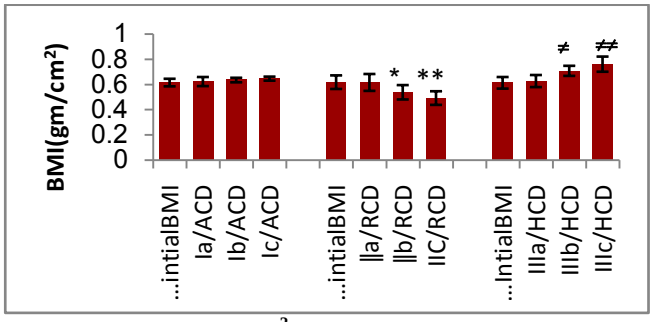

Figure (1): BMIs (g/cm^2) of the control subgroups that received an average caloric diet in 0 (initial/ ACD), 10 (Ia), 20 (Ib) & 30 (Ic) days. Restricted caloric diet (RCD) subgroups at 0 (initial/RCD), 10 (IIa), 20 (IIb) &30 (IIc) days. High caloric diet (HCD) subgroups at 0 (initial/HCD), 10 (IIIa), 20 (IIIb) &30 (IIIc) days. Results are expressed as the mean ± SD (n=6/subgroups). The comparisons between subgroups by using one way ANOVA. *P<0.01 ,&P<0.001 versus initial/RCD. ≠P<0.01, & ≠≠P<0.001 versus initial/HCD**

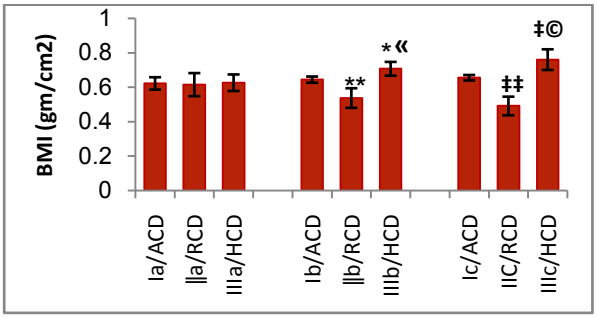

Figure (2): Comparison between BMIs (g/cm2) at 10 days of Ia / ACD, Ib/RCD &Ic/HCD; 20 days of IIa / ACD, IIb/RCD &IIc/HCD; and30 days of IIIa / ACD, IIIb/RCD & IIIc/HCD. Results are expressed as the mean ± SD (n=6/subgroups). Comparing between subgroups by using one way ANOVA. *P<0.05& **P<0.01 versus Ib/ ACD. «P<0.001 versus IIb/RCD. ‡P<0.01, &‡‡P<0.001 versus Ic / ACD. ©P<0.001 versus IIc /RCD.

Table (1) show non-significant changes in the fasting serum obestatin, serum glucose & insulin levels with HOMA-IR indices after 20 &30 days of receiving average caloric diet compared to their measurements after 10 days in the same group (P > 0.05 for all). On the other hand, there were significant increases in the serum obestatin levels of IIb and IIc subgroups that received restricted caloric diet for 20 and 30 days compared to their levels after 10 days of RCD (P<0.05 & P <0.001 respectively) .These increases were associated with significant decreases in fasting serum blood glucose, fasting serum insulin levels and HOMA- IR indices of the mentioned subgroups compared to their values in the IIa /RCD subgroups (P<0.05 & P<0.001 for glucose), and (P<0.05 & P<0.01 for insulin), and (P<0.05 & P<0.01 for HOMA- IR) respectively.

In addition, the obestatin level was more significantly increased after 30 days in IIc/ RCD when compared with its value after 20 days in IIb/RCD subgroup (P<0.01). This increase in obestatin level was in parallel with further significant decreases in the serum glucose, insulin and HOMA-IR of the same subgroup in comparison with their values in IIb/RCD subgroup (P<0.05 for all parameters).

Table (1): Changes in the fasting serum obestatin, glucose & insulin;and HOMA-IR; in the average caloric diet(ACD),restricted caloric diet(RCD),and high caloric diet(HCD) subgroups at different times :At10 days(Ia/ACD, IIa/RCD & IIIa/HCD); At 20 days (Ib/control, IIb/RCD & IIIb/HCD);and At 30 days (Ic/ACD, IIc/RCD & IIIc/HCD).(n=6/each subgroup)

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Parameters	ACD			Ano-va P	RCD			Anova P	HCD			Anova P
	At 10 days Ia	At 20 days Ib	At 30 days Ic		At 10 days IIa	At 20 days IIb	At 30 days IIc		At 10 days IIIa	At 20 days IIIb	At 30 days IIIc	
Obestatin ng/L	213.50±38	235.2 ±26.3	224 ±18	NS	218 ±19	330 ±45*	450.6 ±66$^{***\text{«\text{«}}}$	P<0.001	210.3 ±42.5	120.1 ±25.4‡	30.0 ±7.48$^{‡‡®}$	P<0.05
Glucose mg/dl	90.7 ±9.5	89.5 ±17.5	90.8 ±14.3	NS	90.5 ±8.5	80.0 ±6.7*	70.0 ±7.5$^{**\text{«}}$	P<0.01	88.5 ±8.2	102.8 ±15.7‡	122.6 ±14.4$^{‡‡®®}$	P<0.01
Insulin uIU/ml	11.6 ±1.7	11.3± 1.9	12.0 ±1.38	NS	11.1 ±1.5	9.03 ±1.4*	6.63 ±1.26$^{**\text{«}}$	P<0.001	12.5 ±3.56	16.3 ± 3.2‡‡	26.7 ± .6$^{‡‡‡®®®}$	P<0.001
HOMA-IR Indices	2.31 ±0.45	2.53 ±0.54	2.46 ±0.28	NS	2.4 ±0.6	1.64 ±0.4*	0.81 ±0.38$^{**\text{«}}$	P<0.01	2.63 ±0.64	4.51 ±1.0‡	8.36 ±2.3$^{‡‡‡®®®}$	P<0.001

Values are means± standard deviation (± SD). Least significant difference (LSD) among values was analyzed by one way ANOVA, When the Interaction was significant (P<0.05),NS(non-significant).*P<0.05, **P<0.01,& ***P<0.001 versus IIa/RCD;«P<0.01 , &««P<0.01 versus IIc/RCD; ‡P<0.05, ‡‡P<0.01 &‡‡P<0.01 versus IIIa /HCD;and ©P<0. 05, ©©P<0.01& ©©©P<0.001versus IIIb /HCD.

Also, The serum obestatin levels of IIIb and IIIc/HCD subgroups that received a high caloric diet for 20 and 30 days, were significantly decreased compared to their levels in the subgroup followed HCD for 10 days (P<0.01& P<0.001 respectively). These decreases in obestatin were accompanied by significant increases in serum glucose, serum insulin and HOMA-IR in the previous subgroups in comparison with their values in the IIIa /HCD subgroups (P<0.01 & P<0.001 for glucose), (P<0.05 & P<0.001 for insulin), and (P<0.05 & P<0.001 for HOMA- IR) respectively. Moreover, There were significant decreases in obestatin as well as glucose, insulin and HOMA-IR in rats fed HCD for 30 days compared with their values in their counterparts fed HCD for 20 days (P<0.05 for obestatin), (P<0.01 for glucose), and (P<0.001 for insulin and HOMA- IR) respectively (Table 1).

Figure (3, 4, 5 & 6) show comparison between obestatin levels, serum glucose, insulin levels and HOMA-IR in the different subgroups that revealed a non –significant changes between their values after 10 days in Ia /ACD, IIa/RCD and IIIa/HCD subgroups (P >0.05 for all) . While, there were significant changes between their values after 20 days in Ib /ACD, IIb/RCD and IIIb/HCD subgroups (P <0.01 for obestatine and P <0.001 for the others). In addition, there were significant differences between their values after 30 days in Ic /control, IIc/RCD and IIIc/HCD subgroups (P <0.001 for all).

Table (2) show non-significant differences between the means of the ACD group lipid profiles (P>0.05) at different time points. Also, There were non-significant differences between lipid profiles in both 20 days restricted and high caloric diet subgroups compared to 10 days restricted and high caloric diet subgroups (P>0.05) .

However, there was a significant increase in HDL-cholesterol associated with significant decreases in TC, TG and LDL- cholesterol after 30 days of RCD compared to their levels in the 10 days restricted subgroup (P<0.01 for all). Moreover, there was a significant decrease in HDL-cholesterol as well as significant increases in TC, TG and LDL-

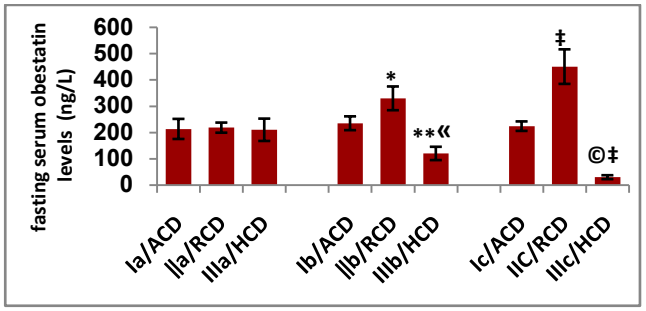

Figure (3): Comparison between fasting obestatin levels (ng/L) at 10 days of Ia / ACD, Ib/RCD &Ic/HCD; 20 days of IIa / ACD, IIb/RCD &IIc/HCD; and30 days of IIIa / ACD, IIIb/RCD & IIIc/HCD. Results are expressed as the mean ± SD (n=6/subgroups). Comparing between subgroups by using one way ANOVA. *P<0.01 & **P<0.001 versus Ib/ ACD. «P<0.001 versus IIb/RCD. ‡P< <0.001 versus Ic / ACD. ©P<0.001 versus IIc /RCD.

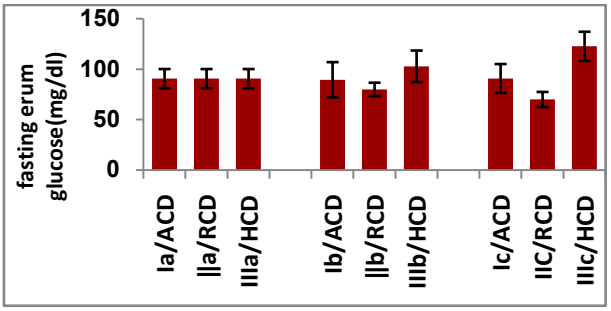

Figure (4): Comparison between fasting serum glucose levels (mg/L) at 10 days of Ia / ACD, Ib/RCD &Ic/HCD; 20 days of IIa / ACD, IIb/RCD &IIc/HCD; and30 days of IIIa / ACD, IIIb/RCD & IIIc/HCD. Results are expressed as the mean ± SD (n=6/subgroups). Comparing between subgroups by using one way ANOVA. *P<0.05 versus Ib/ACD & «P<0.01 versus IIb/RCD. ‡P< <0.01 versus Ic / ACD & ©P<0.001 versus IIc /RCD.

cholesterol after 30 days of RCD compared to their levels in the 10 days restricted subgroup (P<0.05 for HDL-cholesterol), (P<0.001 for TC and TG), and (P<0.01 for LDL-cholesterol) respectively.

Finally, the obestatin levels were negatively correlated with the BMIs, blood glucose, serum insulin and HOMA-IR in IIb & IIc / RCD subgroups. Moreover, they were negatively correlated with the same parameters in IIIb & IIIc / HCD subgroups. Also, data revealed a non-significant correlation between obestatin levels and lipid profile parameters in the different studied subgroups(Table 3).

Table (2): Changes in Lipid profile in the average caloric diet(ACD),restricted caloric diet(RCD),and high caloric diet(HCD) subgroups at different times :At10 days(Ia/ACD, IIa/RCD & IIIa/HCD);At 20 days(Ib/ACD, IIb/RCD & IIIb/HCD);and At 30 days(Ic/ACD ,IIc/RCD & IIIc/HCD).(n=6/each subgroup).

Parameters	ACD			Ano-va P	RCD			Anova P	HCD			Anova P
	At 10 days Ia	At 20 days Ib	At 30 days Ic		At 10 days IIa	At 20 days IIb	At 30 days IIc		At 10 days IIIa	At 20 days IIIb	At 30 days IIIc	
TC (mg/dl)	96.31 ±11.3	95.2 ±7.12	94.3 ±6.18	NS	94.8 ± 2.5	93.3 ±2.4	82.83$^{**«}$ ±2.31	P<0.05	96.0 ±7.5	106.66 ±6.7‡	160.3 ±11.8$^{‡‡‡®®}$	P<0.01
TG (mg/dl)	49.10 ±6.86	48.2 ±1.94	51.3 ±6.86	NS	48.5 ±2.9	47.5 ±4.9	30.16 ±3.5$^{**««}$	P<0.05	49.73 ±4.2	50.81 ±3.4	72.0 ±2.6$^{‡‡‡®®®}$	P<0.05
HDL (mg/dl)	40.51 ±3.8	39.96 ±6.7	39.40 ±5.4	NS	41.3 ±6.1	42.5 ±5.3	52.90 ±4.81$^{**«}$	P<0.05	39.96 ±3.5	38.80 ±4.63	33.76 ±0.78$^{‡®}$	P<0.05
LDL (mg/dl)	29.11 ±3.76	28.36 ±6.69	27.90 ±5.4	NS	28.8 ±6.4	27.5 ±5.3	21.90$^{*«}$ ±4.81	P<0.05	29.8 ±3.54	31.80 ±4.63	39.76 ±6.78$^{‡‡®}$	P<0.05

Values are means± slandered deviation ($\bar{x}$ ± SD).Least significant difference (LSD) among values was analyzed by one way ANOVA, When the Interaction was significant (P<0.05), NS (non-significant).*P<0.05, **P<0.01,& ***P<0.001 versus IIa/RCD;«P<0.01 , &« «P<0.01 versus IIc/RCD; ‡P<0.05, ‡‡P<0.01 &‡‡P<0.01 versus IIIa /HCD; and ©P<0. 05, ©©P<0.01&©© ©P<0.001versus IIIb /HCD.

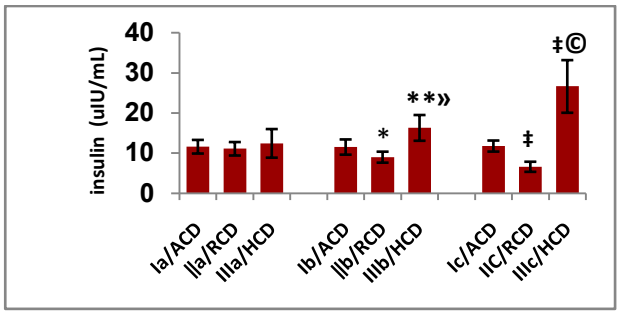

Figure (5): Comparison between fasting serum insulin levels (mlU/L) at 10 days of Ia /ACD, Ib/RCD &Ic/HCD; 20 days of IIa / ACD, IIb/RCD &IIc/HCD; and30 days of IIIa / ACD, IIIb/RCD & IIIc/HCD. Results are expressed as the mean ± SD (n=6/subgroups). Comparing between subgroups by using one way ANOVA. *P<0.05 & **P<0.01 versus Ib/ ACD & «P<0.001 versus IIb/RCD. ‡P< <0.001 versus Ic / ACD ©P<0.001 versus IIc /RCD.

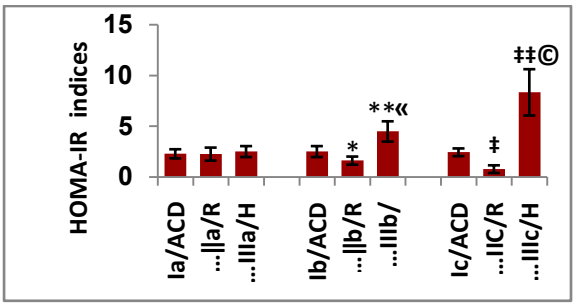

Figure (6): Comparison between fasting HOMA-IR indices at 10 days of Ia / ACD, Ib/RCD &Ic/HCD; 20 days of IIa / ACD, IIb/RCD &IIc/HCD; and30 days of IIIa / ACD, IIIb/RCD & IIIc/HCD. Results are expressed as the mean ± SD (n=6/subgroups). Comparing between subgroups by using one way ANOVA. *P<0.05 & **P<0.001 versus Ib/ ACD. «P<0.001 versus IIb/RCD. ‡P<0.01 & ‡‡P< <0.001 versus Ic /control. ©P<0.001 versus IIc /RCD.

Table(3): Pearson's correlation coefficient between fasting serum obestatin and BMIs; fasting serum glucose &insulin; and HOMA-IR at 20 and 30 days of restricted and high caloric diet group (n=6)

Parameters	Fasting serum obestatin (ng/L)							
	RCD				HCD			
	At 20 days IIb		At 30 days IIc		At 20 days IIIb		At 30 days IIIc	
	R	P value	r	P value	r	P value	R	P value
BMI(gm/cm^2)	-0.718	P<0.01	-0.810	P<0.01	-0.772	P<0.01	-0.840	P<0.01
Fasting glucose (mg/dl)	-0.615	P<0.05	-0.782	P<0.01	-0.730	P<0.01	-0.790	P<0.01
Fasting Insulin (uIU/ml)	-0.624	P<0.05	-0.711	P<0.01	-0.750	P<0.01	-0.825	P<0.01
HOMA-IR Indices	-0.614	P<0.05	-0.600	P<0.05	-0.790	P<0.01	-0.813	P<0.01

Discussion

Peptides of the gut-brain axis have a pivotal role in the regulation of energy homeostasis. Obestatin is a sibling of ghrelin derived from preproghrelin **(Zhang et al., 2008).** Although it was thought that obestatin opposes ghrelin effects on food intake, and changes in ghrelin levels have been associated with eating disorders, controversies still exist on the definite effects of obestatin on food intake/energy balance as well as on the hormone levels in different conditions of disturbed energy balance **(Gao et al., 2010; Maier et al., 2010; Agnew et al., 2011; Granata et al., 2012)**.

The results of the present study revealed that the serum levels of obestatin did not show significant changes among the studied groups after 10 days of different dietary regimens. While, fasting serum obestatin levels were significantly increased following 20 and 30 days of RCD compared to their serum levels in the ACD group. However, there were significant decreases in fasting serum obestatin levels in rats receiving HCD after 20 and 30 days in comparison with their levels in the serum obtained from the ACD group after the same duration respectively. In addition, fasting serum obestatin levels were negatively correlated with changes in the body mass index in the above mentioned subgroups (RCD and HCD) at 20 and 30 days.

These results are in accordance with other similar data obtained from previous studies on experimental models of disturbed +ve energy balance which reported a significant reduction in basal obestatin levels in obese rodents induced

by high fat diet **(Zhang et al., 2005; Abou Fard et al., 2014).**In addition, another study, demonstrated elevation in the serum obestatin levels after bariatric gastric slieving surgery in obese rats **(Zhou et al., 2014).**

Moreover, these results are in agreement with several human findings in different physiological and pathological status of energy imbalance, reporting a decrease in plasma obestatin levels during +ve energy balance and increase in plasma obestatin levels during -ve energy balance. As, **Zamrazilová et al., (2008**) observed lower plasma obestatin levels in obese women compared with normal weight and anorexic patients. In addition**, Harada et al.,(2008)**, **Nakahara et al.,(2008)** found that underweight anorexia nervosa (AN) patients displayed significantly increased circulating levels of obestatin when compared with the normal control group associated with decreased ghrelin/obestatin ratio, indicating that obestatin secretion is higher than ghrelin secretion in these patients.

Also, in pregnancy, which is a unique metabolic state because it involves a short term rapid weight gain where the possibility of concurrent gestational diabetes may occur, followed by a significant weight loss and euglycemia immediately after birth. The concentration of obestatin was significantly lower in obese and obese diabetic pregnant women **(Lacquaniti et al., 2011)**.

Obestatin has not only been studied in adults, but has also been associated with obesity in childhood. **Zou et al., (2009)** studied the role of obestatin in obese children by measuring both of serum fasting obestatin and the ghrelin / obestatin ratio. Their results showed a reduction in fasting plasma obestatin levels with elevated ghrelin / obestatin ratio in these obese children.

Interestingly obestatin levels appear to change following periods of weight reduction in human**. Haider et al., (2007)** reported that adults undergoing significant weight loss after gastric banding experienced increases in obestatin levels. Also, **Zou et al., (2009)** found that obestatin levels were elevated in obese children subsequent to weight loss, due to "summer camp" interventions.

But in contrary to the present results **Sedlackova et al., (2008**) observed a decrease in obestatin and ghrelin in the circulation of healthy subjects following a high carbohydrate breakfast (383.2 Kcal). In addition, **Zhang et al., (2005)** stated that fasting rodents for 48 h and then re-feeding had no effect on obestatin levels. The discrepancy between the results of our study and the previous studies could be explained by species differences, the type of diet provided (high fat diet versus high carbohydrate diet), the total calories in the diets (certain calorie load may be needed to elicit a hormonal response), and the duration of feeding (chronic vs .acute feeding).

Also, **Butler and Bittel, (2007)** measured plasma obestatin levels in Prader-Willi syndrome (PWS), an obesity syndrome characterized by rapid weight gain and excessive food intake, establishing that obestatin was higher in infants with PWS compared to control infants. Moreover, **Sedlackova et al., (2011)** found that fasting obestatin were increased in bulimia nervosa (BN) patients. This is a psychosomatic disease characterized by consuming a large amount of food in a short duration, followed by an attempt to rid oneself of the food consumed. The discordance between the present results and these studies may be explained by that the changes in the serum obestatin levels in this pathological condition could not be related to the disturbance in the energy balance, but may be a part of the pathophysiology of these diseases (like increase gene transcription and/or decrease sensitivity of obestatin receptors) which mandates further investigation in the previous conditions.

The present study detected a negative correlation between fasting obestatin levels and serum levels of blood glucose& insulin as well as insulin resistance after 20 and 30 days of restricted and high caloric diets.

These results denoted that the metabolic parameters alteration during energy imbalance may be involved in the changes of the obestatin level and /or the changes in the obestatin levels associated with energy imbalance could be involved in the metabolic parameters alterations (cause and /or effect inter-relationship).

A study conducted by **Anderwald-Stadler et al., (2007)** demonstrated that non- diabetic humans who were markedly insulin resistant, as measured by the hyperinsulinemic clamp test, had lower fasting plasma obestatin concentrations than did matching insulin-sensitive subjects. In addition, several studies reported a negative correlation between plasma obestatin levels and blood glucose, serum insulin & HOMA-IR in diabetic rodent and humans who had glucose intolerance, hyperinsulinemia and increased insulin resistance; when compared with lean non-diabetic controls. **(Zizzari et al., 2007 and Abou Fard et al., 2014)**.

Also, **Baykus et al., (2012)** demonstrated a remarkable increase in postpartum serum obestatin and desacylated ghrelin levels in comparison to their levels in the gestational diabetic patients. They suggested that the changes in obestatin and desacylated ghrelin levels may be implicated in the pathophysiology of gestational diabetes.

Although , obestatin has been initially reported to elicit anorexigenic effects through a central mechanism by most of the authors (**Green et al.,2007; Lagaud et al.,2007; Zhang et al.,2007)**.Unexpectedly the present study revealed a decrease in the serum obestatin level during the +ve energy imbalance and a decrease in the serum obestatin level during -ve energy imbalance.

The changes in the blood glucose and serum insulin levels during energy imbalance and subsequently insulin sensitivity may be the main causes responsible for dysregulation in obestatin level in these conditions.

A study in obese humans by **Guo et al., (2007)** showed a postprandial suppression of both plasma obestatin and ghrelin compared with the fasting state. After the ingestion of a meal, the combined increase in plasma glucose and insulin could account for the decrease in plasma ghrelin and obestatin levels.

Pervious literatures have indicated parallel changes in ghrelin and obestatin secretions in pathological conditions characterized by energy imbalance, suggesting that dysregulated metabolic states may potentially affect the preproghrelin gene expression and/or the splicing of its products **(Anderwald-Stadler et al., 2007).**

The insulin-signaling cascade has been identified in the gastrointestinal tract, which may be regarded as insulin-sensitive tissue. Therefore, the insulin mediated decrease in plasma obestatin and ghrelin might result from insulin-dependent inhibition of the production of the common precursor peptide preproghrelin or the release of the two hormones from gastric cells. This suggests that basal secretions of obestatin and ghrelin may be regulated in a similar manner, being influenced together by adiposity and insulin sensitivity **(Qi et al., 2007) .**

Also, **Lagaud et al., (2007)** reported that obestatin suppressed food intake and body weight gain in rodents with an unusual dose–response relationship. The dose–response relationship was U-shaped such that both low and high doses were without effect on either species.

This result was further explained by **Guo et al., (2007)** who reported that lower level of preprandial obestatin in obese patients might be related to the disturbed satiety perception in obesity and anorexigenic effect of obestatin.

So, from our and others findings a vicious circle affecting the level of obestatin could be established during energy imbalance , leading to aggravation of the condition .Hence the changes in the serum levels of insulin / and or the disturbance in the satiety perception associated with this condition lead to dysregulation in obestatin level and subsequent disturbance in the appetite, which leads to further disturbance in the metabolic parameters and body weights(either weight gain or weight loss).

As regards the correlation between obestatin and different parameters of lipid profile, the present study showed non-significant correlation between the changes in obestatin and the changes in TC, TG, LDL and HDL at different time points in the different studied groups. These findings denote that obestatin homeostasis is more obviously related to the disturbances in insulin and glucose metabolism rather than the changes in the lipid profile.

Conclusion

Obestatin could be involved in the regulation of energy balance and the disturbance in the serum obestatin levels may be used as a marker for energy imbalance. This gives a new insight about the anticipation of obestatin agonist/s and antagonist/s as new drugs for treatment of obesity and cachexia.

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