

Ghrelin and Obestatin levels in a sample of obese children: A case control study

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Abstract

Background: Childhood obesity represents a serious multifactorial health problem that usually results when energy intake chronically exceeds energy expenditure. Ghrelin and Obestatin are gut-derived hormones that have been described as important physiological regulators of appetite and energy homeostasis.

Aim of the study: The study aims to assess serum levels of Ghrelin and Obestatin hormones among obese children compared to non-obese children.

Subjects and methods: The study is a case control study that involved 60 obese prepubertal children aged (6- < 12) years old of both sexes with body mass index (BMI) \geq 95th percentile according to the Egyptian growth curves, in addition to 31 healthy age and sex matched controls. All children were subjected to medical history, clinical examination and anthropometric assessment: weight, height and calculating BMI. Fasting serum levels of Ghrelin, Obestatin and Glucose were measured.

Results: The mean age of the obese children in our study was 9.28 years \pm 1.66 SD which showed no statistically significant difference from that of the control group (8.81 \pm 1.74 years) (P= 0.210). The obese group had significantly higher mean values of Weight (52.69 \pm 11.46 kg versus 26.36 \pm 5.34 kg) compared to normal-weight children (P= 0.000). Obese children had significantly lower serum levels of fasting Ghrelin (1167.37 \pm 148.25 ng/l) and Obestatin (390.72 \pm 98.49 ng/l) than the control group (1628.10 \pm 154.40 & 462.72 \pm 109.40 respectively). The mean fasting blood glucose of the obese group was 152.16 \pm 20.40 mg/dl and in the control group was 85.46 \pm 7.82 mg/dl with highly significant difference (P= 0.000).

Conclusion: Fasting serum Ghrelin and Obestatin were found lower in obese children with respect to lean participants. This might be explained by the down regulation of Ghrelin and Obestatin as a result of excess energy intake in obese patients.

Key words: Ghrelin- Obestatin- Obesity.

مستوى الجريلين والأوبستاتين في الأطفال البدناء: دراسة مقارنة

الخلفية: واحدة من العوامل الرئيسية في السمنة هو اختلال التوازن بين الطاقة المتولدة والمبدولة. وقد ركزت الأبحاث العلمية على اثنين من الهرمونات التي تفرز في المعدة، الجريلين والمكتشف حديثاً الأوبستاتين، وبورهما في تنظيم تناول الطعام في الأطفال الذين يعانون من السمنة. يعد هرموني الجريلين والأوبستاتين من أهم هرمونات الجهاز الهضمي المنظمة للشهية، حيث يعتبر الجريلين كمنشط للشهية وله دور في التنظيم طويل الأمد لاستهلاك الطاقة. أما هرمون الأوبستاتين فقد تم اكتشاف تأثيره كمثبط للشهية مما يعارض تأثير الجريلين على الشهية وتناول الطعام.

الهدف من البحث: يهدف البحث الى تقييم مستويات الجريلين والأوبستاتين بين الأطفال الذين يعانون من السمنة المفرطة مقارنة مع الأطفال الأصحاء.

الحالات وطرق البحث: هذه الدراسة هي دراسة مقارنة، اشتملت هذه الدراسة على عدد 60 طفلاً يعانون من السمنة المفرطة من كلا الجنسين (27 ذكور و 33 إناث) مع مؤشر كتلة الجسم فوق 95th، بالإضافة إلى 31 من الأطفال الأصحاء (16 ذكور و 15 إناث) من مؤشر كتلة الجسم لديهم ما بين 15 و 85th وفقاً لمنحنيات النمو المصرية للأطفال مع عدم وجود علامات جسدية للبلوغ في المجموعتين. كما تم الحصول على الموافقة الكتابية من احد الوالدين لكل طفل قيد الدراسة. وقد تم إجراء الفحص الطبى مع قياس ضغط الدم والقياسات الأنثروبومترية: الوزن والطول ومؤشر كتلة الجسم، بالإضافة الى القياس المعملى لمستوى هرموني الجريلين والأوبستاتين فى الدم وتحليل سكر صائم.

النتائج: أظهرت المقارنة بين المجموعتين انه لا توجد فروق معنوية بالنسبة للسكر والجنس بينهم، كما أن الأطفال البدناء لديهم أعلى القيم المعنوية في ضغط الدم وفي القياسات الأنثروبومترية المستخدمة ومستوي السكر صائم، ولكن وجدت مستويات هرموني الجريلين والأوبستاتين اقل من الأطفال الأصحاء وهذا قد يشير إلى عملية تكيفية بين هذه البيبتيدات التي قد تقلل من تناول الطعام لدى الناس يعانون من السمنة المفرطة.

الخلاصة: ان قلة مستويات الجريلين والأوبستاتين بشكل ملحوظ عند الأطفال البدناء مقارنة بالأطفال الأصحاء قد تكشف عن تكيف فسيولوجي محتمل يهدف لتوازن الطاقة في هؤلاء الأطفال.

الكلمات المفتاحية: جريلين - أوبستاتين - أطفال - سمنة.

Introduction:

Childhood obesity represents a serious universal public health problem as overweight children might show early signs of long- lasting disease without being aware of the problem and therefore aggravating the expected disease outcome (Godfrey et.al., 2017).

Obesity is a state of excess body fat or adipose tissue that results when energy intake chronically exceeds energy expenditure. Hormonal studies focused on 2 gastric hormones, Ghrelin and Obestatin and their role in the regulation of food intake in the children with obesity. (Chirico et.al., 2014)

As an orexigenic (appetite stimulant) hormone, endogenous Ghrelin levels increase in the fasting state and decrease postprandially, which refers to its role in energy balance, appetite and weight gain. In the long-term, it decreases fat utilization and increases fat deposition and food intake (Sato et.al., 2015).

Obestatin is a 28 amino acid peptide that is mainly found in the stomach and derived from the same preproghrelin gene as Ghrelin (Gurriarán Rodríguez et.al., 2011); however, Obestatin is an anorexigenic (appetite suppressant) hormone, which decreases food intake and inhibits gastrointestinal motility (Wang et.al., 2014).

Several studies showed that Obestatin has the opposite effect to Ghrelin on food intake and suggested that it may provide new targets for the control of obesity (Granata et.al., 2012; Ren et.al., 2013).

Parallel changes were found in Ghrelin and Obestatin secretion in pathological conditions characterized by energy imbalance as obesity (Zhang et.al., 2011), which suggests that the balance between Ghrelin and Obestatin is essential to adapt the body's response to nutritional challenges (Hassouna et.al., 2010).

Subjects And Methods:

This study is a cross- sectional case control study that comprised 60 prepubertal obese children aged (6-<12) years old of both sexes (27 Males& 33 Females) with body mass index \geq 95th percentile according to the Egyptian growth curves (Ghalli et.al., 2008), in addition to 31 normal weight children serving as controls (16 Males& 15 Females) with body mass index = 15-< 85 percentile. Children with congenital anomalies, chronic diseases or taking medications that can affect their normal growth were excluded. All obese and control children showed no physical signs of puberty according to Tanner's classification (Tanner and Karlberg, 1990). The study was carried out in the Obesity Clinic of the Diabetes, Endocrine and Metabolism Pediatric Unit (DEMPU), Pediatric Hospital, Cairo University and "Management of Visceral Obesity and Growth Disturbances Unit" at the "Medical Research Excellence Center (MERC)", National Research Centre, during the period from January 2016 to November 2016. Ethical consideration and approval of the research ethics committees in the faculty of Postgraduate Childhood studies and National Research Centre was taken (Reg. No. 15:091).

All children were subjected to: medical history, clinical examination including blood pressure, in addition to growth assessment (body weight, height and calculated BMI) following the recommendation of

international Biological program (Hiernaux and Tanner, 1969) and charting on the Egyptian growth curves (Ghalli et.al., 2008). Laboratory assessment of Ghrelin, Obestatin and Glucose serum levels was done: A 5cm³ sample of venous blood was obtained from each child after 12 hours of fasting. The blood samples were centrifuged and the serum was separated and kept at -8 °C for batch assessment. Peptides were extracted and total plasma Ghrelin and Obestatin levels were measured by enzyme immunoassay according to the manufacturer's protocols. The sensitivity of the assay of Ghrelin was (100- 3000 ng/ l) and of Obestatin was (20- 400 ng/l). The commercially available kits were purchased from WKEA Med Supplies Corp, China. Glucose level was assessed using GOD- POD enzymatic colorimetric method. The test kit code no: SU018/ SU019 (Chemelex, S.A., Barcelona) according to the method of (Tietz, 1995).

Statistical Analysis:

SPSS version 16 computer software was used for statistical comparisons of data. Descriptive statistics (mean \pm standard deviation) were calculated. In order to find out whether there are group differences; independent t- test was carried out to compare between 2 groups the parametric data (quantitative). Standards of probability were set to P< 0.01; which considered highly significant; and P< 0.05; which considered statistically significant.

Results:

No sex specific differences were found between obese males and obese females or between control males and control females regarding studied anthropometric or laboratory parameters as shown in table (1) and (2), so data for males and females in were pooled and considered as one group.

The mean age of the obese children in our study was 9.28 years \pm 1.66 SD which showed no statistically significant difference from that of the control group (8.81 \pm 1.74) (P= 0.210). The obese children had significantly higher mean systolic (108.17 \pm 11.39) and mean diastolic (65.33 \pm 8.12) BP than in children of the control group (mean systolic = 95.65 \pm 9.20), (mean diastolic 58.87 \pm 6.80) (P= 0.000). The obese group had significantly higher mean values of Weight (52.69 \pm 11.46 kg versus 26.36 \pm 5.34 kg), Height (138.75 \pm 10.08 cm versus 128.58 \pm 8.36 cm) and BMI (27.11 \pm 3.29 Kg/m² versus 15.69 \pm 2.21 Kg/m²) compared to normal- weight children (P= 0.000). The mean fasting serum level of Ghrelin hormone in the obese group was significantly lower than the control group (1167.37 \pm 148.25 ng/l versus 1628.10 \pm 154.40 ng/l respectively, P=0.000). Obestatin mean fasting level was also significantly lower in the obese group than the control group (390.72 \pm 98.49 ng/l versus 462.72 \pm 109.40 ng/l respectively, P= 0.002). The mean fasting blood glucose of the obese group was 152.16 \pm 20.40 mg/dl which was above the desirable range carrying the risk of diabetes (if persistent on two separate occasions). The mean fasting blood glucose of the control group was 85.46 \pm 7.82 mg/dl which was within the desirable range with highly significant difference (P= 0.000) in comparison to obese group. Comparison between obese children and controls are represented in tables (3) and (4).

Table (1) Comparison between obese males and females regarding age, blood pressure, studied anthropometric parameters and Laboratory results.

		Obese Males (N= 27)		Obese Females (N= 33)		t	P- Value
		mean	±Sd	mean	±Sd		
Age (Years)		9.76	± 1.63	8.89	± 1.61	2.08	0.70
Blood Pressure	Systolic Bp (Mmhg)	110.37	10.82	106.36	± 11.68	1.37	0.17
	Diastolic Bp(Mmhg)	67.04	5.42	63.94	± 9.66	1.57	0.12
Anthropometry:	Weight (Kg)	55.13	11.42	50.69	11.27	1.51	0.13
	Height (Cm)	141.11	9.20	136.82	10.50	1.67	0.10
	BMI (Kg/m ²)	27.37	3.06	26.90	3.50	0.54	0.58
Laboratory Results	Ghrelin(ng/l) (100- 3000 ng/l)	1190.52	117.32	1148.42	168.86	1.14	0.26
	Obestatin(ng/l) (20- 400 ng/l)	417.10	115.45	369.13	77.39	1.85	0.07
	Glucose (mg/dl)	149.53	15.04	154.32	23.93	- 0.90	0.37

* Significant at (P< 0.05) ** Highly significant at (P< 0.01)

Table (2) Comparison between control males and females regarding age, blood pressure, studied anthropometric parameters and Laboratory results.

		Control Males (N= 16)		Control Females (N= 15)		t	P- Value
		mean	±Sd	mean	±Sd		
Age (Years)		9.08	1.66	8.51	1.82	0.91	0.37
Blood Pressure:	Systolic Bp (Mmhg)	97.50	7.75	93.67	10.43	1.17	0.25
	Diastolic Bp (Mmhg)	58.75	6.19	59.00	7.61	- 0.10	0.92
Anthropometry:	Weight (Kg)	27.33	5.66	25.33	4.96	1.04	0.30
	Height (Cm)	131.06	8.10	125.93	8.05	1.77	0.08
	BMI (Kg/m ²)	15.76	2.04	15.61	2.45	0.19	0.85
Laboratory Results	Ghrelin (ng/l)	1627.94	140.53	1628.27	172.99	- 0.01	0.99
	Obestatin (ng/l)	462.69	121.88	462.74	98.63	0.00	0.99
	Glucose (mg/dl)	84.70	7.50	86.27	8.33	- 0.55	0.58

* Significant at (P< 0.05) ** Highly significant at (P< 0.01)

Table (3) Comparison between obese and control children regarding age, Blood pressure and the studied anthropometric parameters:

		Obese (N= 60)		Control (N= 31)		t	P- Value
		mean	±Sd	mean	±Sd		
Age (Years)		9.28	1.66	8.81	1.74	1.26	0.210
Blood Pressure:	Systolic Bp (Mmhg)	108.17	11.39	95.65	9.20	5.29	0.000**
	Diastolic Bp(Mmhg)	65.33	8.12	58.87	6.80	4.02	0.000**
Anthropometry:	Weight (Kg)	52.69	11.46	26.36	5.34	14.93	0.000**
	Height (Cm)	138.75	10.08	128.58	8.36	4.82	0.000**
	BMI (Kg/m ²)	27.11	3.29	15.69	2.21	19.64	0.000**

* Significant at (P< 0.05) ** Highly significant at (P< 0.01)

Table (4) Comparison between obese and control children regarding Laboratory results:

Laboratory Results		Obese (N= 60)		Control (N= 31)		t	P- Value
		mean	±Sd	mean	±Sd		
Ghrelin (ng/l) (100- 3000 ng/l)		1167.37	148.25	1628.10	154.40	- 13.85	0.000**
Obestatin (ng/l) (20- 400 ng/l)		390.72	98.49	462.72	109.40	- 3.18	0.002**
Glucose (mg/dl)	Non Diabetic (FPG< 110 mg/dL)	152.16	20.40	85.46	7.82	22.36	0.000**
	Prediabetic Or Diabetic (FPG≥ 126 mg/dl)						

ificant at (P< 0.05) ** Highly significant at (P< 0.01)

Discussion:

Understanding the changes that occur in the hormonal regulators of body energy homeostasis and their roles in weight gain is essential to understand the contributing mechanisms of obesity (Lizia and Hemamalini, 2016).

Ghrelin as an orexigenic peptide and Obestatin as anorexigenic peptide are believed to have a major role in long- term regulation of energy homeostasis (Al Massadi et.al., 2018).

Since Ghrelin and Obestatin hormones are considered as products of a single gene, their levels may be under specific regulation and the balance between them may play an important role in obesity (Wali et.al., 2014). So, we conducted this study to assess serum Ghrelin, Obestatin and in obese children compared to healthy non- obese children.

No sex- specific differences were found between obese males and obese females or between control males and control females regarding studied anthropometric or laboratory parameters.

In agreement with our results, two Italian studies with involving children with almost similar age range (7- 11) years old as our study, reported no difference in Ghrelin plasma levels between girls and boys (Bellone et.al., 2002; Bellone et.al., 2004). Also median concentrations of Ghrelin were not different between males and females in a study involving 121 healthy children and adolescents aged (5- 18) years by Whatmore et.al. (2003) in UK.

Similarly, also no differences were found by Reinehr et.al. (2008) between German girls and boys with regards to Obestatin levels.

Opposite to us, studies in Spain and Korea showed that Ghrelin

plasma levels were significantly higher in girls compared with boys (Gil-Campos et.al. (2010); Park et.al. (2005). Also in a study by Zou et.al. (2009), there was a significant difference between Chinese boys and girls as boys had higher levels of Obestatin.

When we compared between obese and control subjects regarding blood pressure. The mean Systolic and diastolic BP are significantly higher in the obese children than in children of the control group. Many studies showed similar associations between childhood obesity and hypertension (Stabouli et.al., 2011; Guo et.al., 2012).

The mean fasting blood glucose of the obese group was 152.16 ± 20.40 mg/dl which was above the desirable range carrying the risk of diabetes (if persistent on two separate occasions). Hyperglycemia in obese children was similarly noticed by other studies (O'Malley et.al., 2010; Eehalt et.al., 2017).

Obese children in our current study had significantly lower serum levels of Ghrelin and Obestatin than the healthy children. In agreement with these results, a meta-analysis done by Zhang et.al. (2011) stated that Ghrelin and Obestatin in normal weight groups were significantly higher than those of obese groups. Guo et.al. (2012) and Zou et.al. (2009) in China also found that Ghrelin and Obestatin levels were significantly lower in the obese group compared to the lean groups. This suggested an adaptive process between these peptides that might decrease food intake in obese people.

In contrast to our results, the total Ghrelin levels were significantly lower ($P= 0.0003$) and the Obestatin levels were significantly higher in obese children ($P= 0.029$) compared with the controls in a study done by (Wali et.al., 2014). This may be due to different genetics or race causing different physiological adaptation response or may be due to difference in sensitivity of laboratory kits.

To the best of our knowledge, the current study is the first study evaluating Ghrelin and Obestatin as appetite regulating hormones among obese Egyptian children. We measured morning fasting serum hormone levels, however multiple further postprandial measurements to include diurnal discrepancy that can create extra information, which may be considered a limitation of our study. However, evaluation of morning fasting Ghrelin concentrations was previously testified to be a trustworthy method to evaluate Ghrelin status even if Ghrelin is secreted periodically (Akamizu et.al., 2005).

In conclusion, fasting serum Ghrelin and Obestatin were found lower in obese children with respect to lean participants. This might be explained by the down regulation of Ghrelin and Obestatin as a result of energy excess intake in obese patients. Ghrelin and Obestatin levels are expected to increase after diet induced weight as an adaptive response to prevent further weight loss by up regulating hunger levels and energy intake.

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