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Detection of Leptin and Ghrelin Hormones and the Expression of their Receptors in Iraqi Obese Individuals

Sura F. Alsaffar[1], Hind M.Jumaa[2], Noor N. Baqer [3]
[1]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq*
[2]*Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq.*
[3]*Directorate of Water and Environment, Ministry of Science and Technology, Baghdad, Iraq*

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Abstract

Obesity is a complex disease and a major worldwide health hazard with adult mortality. Obesity is defined by an increase in the body-mass index of 30 kg m^{-2} or greater. It belongs to the genetic predisposition and more consumption of high-energy foods and decreased requirement for physical activity in modern society. This study was designed to evaluate leptin and ghrelin hormones levels and the gene expression of leptin and ghrelin receptors in obese individuals. Seventy-five obese (45 females and 30 males) and 25 (15 females and 10 male) normal individuals were admitted to the Obesity Research and Therapeutic Unit at Alkindy College of Medicine/ University of Baghdad. All blood samples were pulled from obese and normal weight individuals. Leptin and ghrelin hormones were evaluated by ELISA technique and leptin and ghrelin receptors expressions were estimated by RT-PCR. Leptin level was lower in obese than normal 2.74±0.14 ng/ml, 3.47± 0.38 ng/ml respectively. While ghrelin level was higher in obese than normal (26.3 ± 0.56 ng/ml, 17.3 ± 0.5 ng/ml respectively**).** There was a significant decrease in leptin receptor Ob-Rb mRNA expression in obese individuals ($P<0.05$). Obese individuals had more ghrelin receptor GHS-R mRNA expression than normal people. In conclusion, obese patients were found to have higher ghrelin hormone and GHS-R as well as low leptin and Ob-Rb mRNA expressions.

Keywords: Leptin, Ghrelin, Leptin receptor OB-R, Ghrelin receptor GHS-R, Obesity.

التحري عن هرموني اللبتين والجرلين والتعبير الجيني لمستقبلاتهما في البدناء من العراقيين

سرى الصفار ، هند جمعة ، نور باقر
[1]قسم الأحياء، كلية العلوم، جامعة بغداد، بغداد، العراق
[2] قسم التكنولوجيا الحيوية، كلية العلوم، جامعة بغداد، بغداد، العراق
[3]مديرية المياه والبيئة، وزارة العلوم والتكنولوجيا، بغداد، العراق

الخلاصة

تشكل السمنة خطرًا صحيًا كبيرًا في جميع أنحاء العالم مع وفيات للبالغين ،يتم تعريف السمنة من خلال زيادة مؤشر كتلة الجسم بمقدار 30 كغم / م ^{2} أو أكثر ، عالميا ان وباء السمنة يكون ناتج عن الاستعداد الوراثي ،زيادة الأطعمة عالية الطاقة وانخفاض النشاط البدني في المجتمعات الحديثة ،لقد صُممت هذهالدراسة

*Email: suraa.alsaffar@sc.uobaghdad.edu.iq

لتقييم مستويات هرمونياللبتين والجيرلين بالاضافة الىالتعبير الجيني لمستقبلاتهما في الأفراد الذين يعانون من السمنة المفرطة. شملت الدراسة 75 شخصاً(45اناث و30 ذكور) يعانون من السمنة المفرطة و 25 (15 اناث و 10 ذكور) من الاشخاص الطبيعيين الذين زاروحدة أبحاث السمنة وعلاجها في كلية الطب الكندي / جامعة بغداد. تم سحب عينة الدم من الأشخاص البدناء والأشخاص الطبيعيين ،كما تم تقييم هرمونياللبتين والجريلين بتقنية ELISA .بالاضافة الى تقدير تعبير مستقبلات اللبتين و الجريلين بواسطة RT–PCR. اظهرت النتائج فروق معنوية في تعبير مستقبلات اللبتين Ob–Rb mRNA بين الأفراد الطبيعيين والأفراد الذين يعانون من السمنة المفرطة (P <0.05).فضلا عن ذلك فأن الأفراد الذين يعانون من السمنة المفرطة قد اظهروازيادة في تعبير مستقبلات الجريلين GHS–R أكثر من الأشخاص الطبيعين. كان مستوى اللبتين أقل في حالة السمنة من الحالة الطبيعية (2.74 ± 0.14 نانوغرام / مل ، 3.47 ± 0.38 نانوغرام / مل) على التوالي ، بينما كان مستوى الجريلين في السمنة أكثر من الحالة الطبيعية (26.3 ± 0.56 نانوغرام / مل ، 17.3 ± 0.5 نانوغرام / مل) على التوالي .يُستنتج من ذلك ، ان المرضى الذين يعانون من السمنة المفرطة لديهم تعبير عن GHS–R أعلى مع انخفاض التعبيرعن Ob–Rb mRNA.

Introduction

Obesity is an abnormal or excessive fat accumulation that leads to adverse effects on health and life expectancy. Obesity was proposed to associate with high expression of many genes like adiponectin [1], TNF- alpha, IL-1, IL-6, TLR-4, FTO, STAT-3…etc., as well as hormonal imbalance, excessive consumption of food and lazy lifestyle. It is characterized by accumulation of adipose mass, high cholesterol, imbalance in metabolic energy, insulin desensitization, lethargy, gallstones; shortness of breath, emotional and social problems.
Obesity is associated with diabetes mellitus, hypertension, coronary heart disease, polycystic ovarian syndrome and certain forms of cancer [2].

Leptin is a protein hormone that is encoded by the obesity (OB) gene. It is produced primarily from white adipose tissue, skeletal muscles, placenta and the stomach [3]. Circulating leptin can cross the blood–brain barrier and bind to leptin receptors in the brain where it acts to reduce food intake and promote energy expenditure [4]. Leptin acts via the leptin receptor (OBR). The OBR gene found on the first chromosome, encodes a protein with 1162 amino acids. The hypothalamus and the cerebellum highly express OB-Rb, a component of the OBR gene. Leptin regulates body weight and energy homeostasis through a feedback mechanism that signals regulatory centers in the brain to inhibit food intake and regulate body weight and energy homeostasis [5].

Leptin has been shown to inhibit orexigenic neuropeptide Y (NPY) and agouti-related protein (Agrp) neurons while activating anorexigenic pro-opiomelanocortin (POMC) neurons in the arcuate nucleus by activating ATP-activated protein kinase (KAMP) channels [6].

Ghrelin (hunger hormone) is secreted by the enteroendocrine cells in the gastrointestinal tract, especially the stomach, before meals to stimulate food intake and secretion of gastric acid. It has many physiological functions, including stimulation of appetite, fat accumulation and growth hormone release[7]. It regulates the satiety signal with leptin and is encoded by the ghrelin gene located on third chromosome. Common genetic variants in ghrelin and its receptor genes may contribute to polygenic obesity susceptibility [8]. It acts via growth hormone secretagogue receptor 1a (GHS-R1a). Many studies have demonstrated the omnipresence of ghrelin and its receptors in many peripheral organs including the brain, pituitary, pancreas, thyroid, intestine, adrenal gland, kidney, heart and blood vessels. Chronically, obese patients have lower circulating ghrelin levels than normal subjects, as well as higher insulin levels (insulin resistance)which is most likely explained by the direct effect of insulin [9].

Ghrelin improves cardiovascular functions by increasing cardiac performance, lowering blood pressure and protecting against cardiac ischemia and heart failure [10].

Material and Methods

Seventy five over obese (BMI ≥30) and 25 normal individuals (BMI<25) were admitted to the Obesity Research and Therapeutic Unit at Alkindy College of Medicine/ University of Baghdad.

The study was approved by the ethic committee in the Department of Biology, College of Science, University of Baghdad Ref. No. CSEC/0122/0024 on January 15, 2022. The blood samples were pulled both from obese and normal individuals. Leptin hormone (mybiosource, USA, CAT No. MBS727499)[11]and ghrelin hormones(mybiosource, USA, Catalog No MBS720885) [12] were evaluated by ELISA technique after 10 hours of fasting. RNA was extracted from whole blood by (RNA Maxwell® RSC simply RNA Blood Kit, Promega, USA, CAT No. AS1380).Real time PCR was used to evaluate the ghrelin and leptin receptors expressions mRNA by the LightCycler®, Roche.

A computer program was used to estimate the mRNA quantities and levels of expression, considering the standard preparations of each gene (concentration obtained by spectrophotometer). The mRNA: cyclophilin (housekeeping gene) ratio of each amplification had to be computed. Primers sequences are presented in Table 1.

Table 1 : Sequence of primers

Gene		Sequence
GHS-R	Forward	5¢-ATC TTC ATG CTG GTC GGA GTG-3¢
	Reverse	5¢-TGT TGT AGA GAA TAG GGT TGA T-3¢
OB-Rb	Forward	5¢-GGC CCT CTT CTT TTG GAG-3¢
	Reverse	5¢-GAC AGG CCT TTC ATT ATT ATT TT-3¢
Cyclophilin House Keeping	Forward	5¢-GGT GAC TTC ACA CGC CAT AA-3¢
	Reverse	5¢-GGT GAT CTT CTT GCT GGT CT-3¢

The cycle threshold (CT) of each sample and housekeeping gene (Cyclophilin), the quantitative real-time PCR of GHS-R and OBRb expression ratio was calculated according to Livak equation ΔCT= CT of target gene – CT of reference gene. The previous equation was applied both for obese and normal individuals as the following:

ΔΔCT = ΔCT (obese) -ΔCT (non-obese).

$$\text{Fold expression} = 2^{-}\Delta\Delta CT$$

Statistical Analysis

A statistical analysis was performedusing anunpaired t test andthe results were expressed as mean ± standard deviation.All analysis was carried out with graph prism 9.3 software. P-value less than 0.05 was considered statistically significant.

Results and Discussion

The current study included seventy-five obese (45 female and 30 male) whose mean BMI was 35.61± 0.60 and 25 normal (15 female and 10 male) whose BMI mean was 24.03± 0.96.Their ages ranged between 15-44 years., obesity was identified by high BMI and increased waist/ hip ratio [13].

Leptin hormone was significantly less in the obeseand the expression of leptin receptor (OBR) showed a significant decrease (P<0.01) in obese (2.74±0.14 ng/ml and 32.62±0.62 ng/ml, respectively) as compared with non-obese (3.47 ±0.38 ng/ml and 67.4±0.65 ng/ml respectively) (**Table 2 and Figure 1)**.

Leptin is an adipokine that is secreted from adipose tissue and transported to the brain through blood brain barrier by leptin transporters called obesity receptors which stimulate the brain to reduce food intake, appetite body and regulate energy homeostasis.Leptin increases in obese persons [14].Also leptin receptor OBR expression increases to translocate the leptin to the brain to inform the brain about the status of body fat stores and induce anorexia that functions as an afferent signal in a negative feedback loop which maintains homeostatic control of adipose tissue mass[15].

Obradovic and his colleagues indicated that cerebrospinal leptin concentration was 4-5 times more in the serum of obese individuals [16].

The present results showed a decrease in leptin level.Also the expression of OBR in obese may be related to the condition of leptin resistance in which obese demonstrate low leptin level and hyperphagia and increase appetite and body weight which explains why obese have more appetite to eat that leads to put on weight [17].Another study mentioned that leptin receptor variants were associated with leptin resistance that was characterized with low leptin, high body weight and high BMI [18].

These findings, however, contradict a study conducted in Babylon University which found that leptin levels were elevated in obese patients as well as ghrelin (the hunger hormone), was not strongly [1] associated with obesity, and increase in insulin hormone level, thus a disruption in their actions could lead to insulin resistance, obesity, and type II diabetes mellitus[19].Whereas another study found that the leptin hormone level was significantly higher (P<0.01) in obese women [20]in comparison with the control.

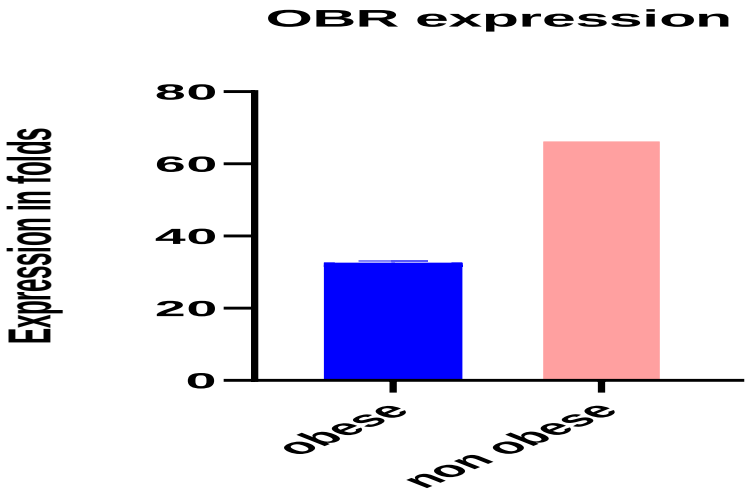

Figure1: Ob-Rb mRNA expression in obese and normal individuals.

Table 2: Results of obese and non-obese individuals

Parameters	Obese (Mean± SD)	Non-obese (Mean± SD)	P-value
BMI (Kg/m2)	35.61± 0.60	24.03 ± 0.96	P<0.0001
Leptin (ng/ml)	2.74± 0.14	3.47± 0.38	P<0.0388
OBR expression (folds)	32.62 ± 0.62	67.40± 0.65	p<0.0001
Ghrelin (ng/ml)	26.63± 0.56	17.3± 0.5	p<0.0001
GHSR expression (Folds)	70.63±1.19	29.86±0.95	P <0.0001

Ghrelin hormone and receptor (GHS-R) levels showed a highly significant (P< 0.01) increase in obese (26.63 ±0.56 ng/ml and 70.63±1.19 ng/ml respectively) as compared with non-obese (17.3 ±0.5 ng/ml and 29.86±0.95 ng/ml respectively) (**Table 2** and **Figure2)**.

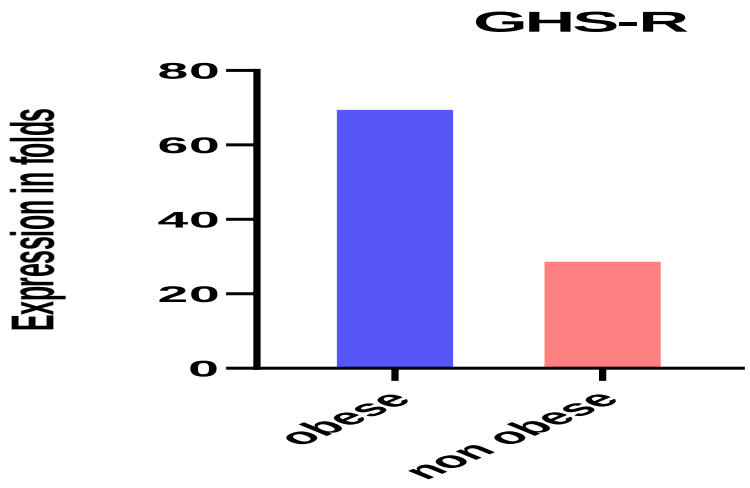

Figure 2: Real-time PCR for GHS-R expression in obese and normal individuals.

Ghrelin hormone is secreted mainly from the stomach and pancreas to the blood and then to the brain to induce food intake (appetite),although high-fat content in obese lead increase in its secretion that stimulates more food intake and increased fat accumulation. Hence, it may be hypothesized that an increased ghrelin level as well as higher expression of GHS-R which may be responsible for the increased secretion of GH in a diet-induced thin state [21]GHS-R, is primarily expressed in the brain; the brain is a key ghrelin targeting site[22].

While Marzullo *et al.* mentioned that total ghrelin and plasma active ghrelin were significantly low in obese when compared with lean subjects [23]

Ghani *et al.*(2015) found that circulating ghrelin levels are decreased in human obesity and plasma ghrelin level showing a negative correlation with BMI [24].

Ghrelin through its receptor GHS-R1a may promote adipose tissue inflammation, increase macrophage infiltration and promote macrophage polarization to M1 and increase of adipocyte diameter, immunohistochemical expression of the ghrelin receptors in atrial adipose tissue is associated with obesity [25].However, deletion of GHS-R1a would decrease the macrophage infiltration in adipose tissue [26]. Different studies have illustrated the relationship of ghrelin

and obesity.It seems that ethnicity has a role in the level of ghrelin, as it was found that ghrelin concentration in obese Caucasians was significantly lower compared to those in their normal weight counterpart compared to Pima Indians [27].While another study explained ghrelin relation with the degree of insulin resistance or diabetes mellitus between obese individuals. It was also found that obese individuals with diabetes mellitus had a significantly lower ghrelin level compared to those without diabetes mellitus [28].

Conclusion:

The obese patients have higher ghrelin, GHS-R as well as low leptin and lower expression of Ob-Rb mRNA.

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Conflict of Interests

The authors report no conflict of interest in this work.

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