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Original article

Assessment of biochemical changes among Egyptian women with increased body weight

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Background: Obesity is a condition that results from chronic disruption of energy balance where energy intake continuously exceeds energy expenditure and accumulation of body fat results
Objective: We evaluated the relationships between ghrelin and leptin with the metabolic state of normal weight, overweight, and obese Egyptian women.
Methods: We studied 82 subjects with ages from 43 to 65. They were free of endocrine-related disease and divided into three groups according to their body mass index (BMI), group 1 with BMI less than 25 kg/m2, group 2 with BMI between 25 to 30 kg/m2, and group 3 with BMI more than 30 kg/m2. Ghrelin and leptin were determined by ELISA technique. Insulin resistance was measured by homeostasis model assessment. Lipid profile was determined in all groups.
Results: Fasting plasma levels of ghrelin were lower in overweight and obese groups compared to normal weight control group. There was statistically significant negative correlation of ghrelin levels with leptin, BMI and HOMA. Results showed that higher concentrations of fasting leptin were found in overweight and obese groups compared with the normal weight control group. There was statistically significant positive correlation between leptin and other biochemical parameters, insulin, BMI, and HOMA.
Conclusion: Ghrelin and leptin may be associated with obesity. These markers can be of value when assessing management.

Keywords: Ghrelin, HOMA, leptin, obesity

Obesity is a condition that results from chronic disruption of energy balance where energy intake continuously exceeds energy expenditure and accumulation of body fat results [1]. The prevalence of obesity is on the rise and the obesity pandemic is arguably amongst the most serious public health challenges in the world today. Obesity is strongly associated with type 2 diabetes mellitus, hyperlipidemia, and cardiovascular disease Extensive research into the mechanisms of appetite regulation and energy balance has unveiled complex physiological systems behind energy homeostasis, which may yield targets for therapeutic intervention [2]. Prevalence of obesity in adults is high in Egypt where 35% of the population has a BMI over 30. This is particularly true among women. The prevalence of diabetes and hypertension parallels that of obesity [3].

The hypothalamus is responsible for appetite regulation and energy homeostasis. Afferent signals from peripheral sites such as the gastrointestinal tract and adipose tissues are integrated by complex neuronal networks to produce efferent responses responsible for food intake and energy metabolism. There are numerous hypothalamic appetite regulators. Orexigenic (appetite-stimulating) compounds include neuropeptide Y (NPY), agouti-related peptide (AgRP), ghrelin, orexin, and cannabinoids, and anorexigenic (appetite-suppressing) peptides include pro-opiomelanocortin (POMC) and cocaine- and amphetamine- regulated transcript (CART), thyrotropin-releasing hormone (TRH), and corticotropin-releasing hormone (CRH) [4].

The appetite-stimulating function of ghrelin affects growth hormone (GH) release from somatotroph cells of the anterior pituitary [5]. However, ghrelin is the first known peripheral hormone to display orexigenic

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effects through its action on the hypothalamic appetite-regulating pathways [6]. In addition, ghrelin is amongst the most powerful of the orexigenic peptides [7]. While most orexigenic peptides originate from the brain and are only active when injected into the brain, ghrelin is active even with peripheral administration leading to an increase in appetite in rodents and humans [8, 9]. Ghrelin activates NPY/AgRP neurons of the hypothalamic arcuate nucleus (ARC) through its receptor [10]. Plasma ghrelin levels inversely correlate with body mass index (BMI). Thus, ghrelin levels are reduced in those who are obese compared to those of normal body weight controls [11, 12]. Recent evidence suggests that diet-induced obesity causes ghrelin resistance by reducing NPY/AgRP responsiveness to plasma ghrelin and suppressing the neuroendocrine ghrelin axis, in an attempt to limit further food intake [13].

Leptin, known as the prototypical adipokine, is a 167-amino acid peptide with a four-helix bundle motif similar to that of acytokine [14]. It is produced primarily in adipose tissue but is expressed in a variety of tissues including the placenta, ovaries, mammary epithelium, bone marrow [15], and lymphoid tissues [16]. Leptin levels are pulsatile and follow a circadian rhythm, with highest levels between midnight and early morning and lowest levels in the early- to mid-afternoon. Specifically, the concentration of circulating leptin may be up to 75.6% higher during the night as compared to afternoon trough levels [17]. The pulsatile characteristics of leptin secretion are similar in obese and lean individuals, except the obese have higher pulse amplitudes [18].

Leptin regulates energy homeostasis and reproductive, neuroendocrine, immune and metabolic profiles. Its concentration reflects the amount of energy stored in body fat. Circulating leptin levels are directly proportional to the amount of body fat [19] and fluctuate with acute changes in caloric intake [20]. Leptin controls energy homeostasis and body weight primarily by activating ObRb in the hypothalamus [21].

The ObRb activate numerous JAK2/STAT3-dependent and –independent signaling pathways that act in coordination as a network to fully mediate leptin action. The activation of individual pathways in the leptin signaling network appears to be differentially regulated in discrete subpopulations of ObRb-expressing neurons. These pathways are also likely to be regulated by various other hormonal, neuronal, and metabolic signals that cross-talk with leptin. Hence, it is important to fully determine whether and how positive and negative regulators of ObRb signaling, metabolic state, and/or neuronal activity regulate leptin signaling networks in a cell/tissue type-specific manner and how activation of these signaling pathways mediates leptin's effects in humans [22]

Material and methods

The study protocol was approved by the Ethical Committee of October 6 University, Cairo, Egypt. This study was conducted on 82 participants, with ages from 43 to 65 and free from malignancies and endocrine-related disease (e.g., diabetes). They were divided into three groups according to their body mass index (BMI). Group 1 with BMI less than 25 kg/m2, group 2 with BMI between 25 to 30 kg/m2, and group 3 with BMI more than 30 kg/m2. BMI was calculated for all groups as body weight (kg) divided by body height squared (m^2). Insulin resistance was assessed by means of the homeostasis model assessment (HOMA), which was measured by multiplying fasting serum insulin (micro-units per milliliter) and fasting plasma glucose (micromoles per liter) divided by 22.5 [23].

Blood samples were obtained from participants in the morning after a 12-hour overnight fast. Within 1 hour of collection, samples were processed and stored at -70°C. Total plasma ghrelin concentrations were measured using a commercial radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals Inc, Belmont, CA) that uses 125I-labeled bioactive ghrelin as a tracer and a polyclonal antibody raised against full-length acylated human ghrelin, as previously described [24].

Total plasma leptin concentrations were measured using a commercial RIA kit (Linco Research Inc, St Charles, MO) [19]. Plasma glucose level was estimated by God-PAP enzymatic colorimetric method [25] using Biomerieux test kit, Cat. no. 5127. Serum insulin was detected by commercially available radio-immunoassay (Abbott IMx Insulin assay) which is a micro-particle Enzyme Immunoassay [MEIA] for the quantitative measurement of human insulin [26].

Measurement of lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides) by commercial enzymatic methods (Aeroset automated analyzer, Abbott Laboratories, Abbott IL). LDL cholesterol was calculated by using Friedewald's formula [27].

Standard descriptive statistics were used to summarize the data such as means and standard

deviations (SD). To assess between group differences, we used non-parametric test (Wilcoxon–Mann–Whitney test). Correlation coefficients reported as Spearman rank correlations.

Results

Clinical features of the women who joined this study are summarized in **Table 1**. By design, there were statistical differences between the three groups as regards the BMI. The incidence of hypertension increased among the obese group compared to the control group.

The Over-weight and obese groups showed significant reductions (p <0.001) in the level of ghrelin with means±SD (11.6±4.1 ng/ml) and (12.9±8.7 ng/ml) respectively compared with the normal weight group (GI) (23.7±9.5 ng/ml). A significantly difference between over-weight group and obese group was observed. The level of leptin hormone in sera of over-weight group were significantly increased with a mean of (25.3±11.6 ng/ml) while the level of leptin hormone in obese group found to be highly significant increased with a mean (35.8±16.4 ng/ml), as compared with normal weight group (12.6±8.2ng/ml). The serum level of leptin showed a significant difference between over-weight group and obese group.

The glucose, insulin, and insulin resistance concentrations in the sera of the over-weight group, obese group, and normal weight group showed no statistically significant difference (p >0.05) between the three groups with means of (86.8±13.9 mg/dl), (84.2±6.2 mg/dl), and (89.3±7.9 ng/ml) for glucose, (16.5±5.1 µlU/ml, (18.8±11.7 µlU/ml), (17.3±6.8 µlU/ml) for insulin, and (3.67±1.3 µlU/ml), (4.08±2.8 µlU/ml), and (3.77±1.4 µlU/ml) for insulin resistance concentrations respectively as shown in **Table 2**.

Table 1. Demographic data in the different studied groups

	Normal weight group (n = 25)	Over weight group (n = 28)	Obese group (n = 29)
Age (years)	43-65	44-64	43-65
	49±1.5	50±1.9	49±1.2
BMI (Kg/m^2)	22.15±0.91	28.3±1.43*	33.43±1.65*
Hypertension	2/25	5/28	11/29*

*Statistical significant compared to the control group p <0.05

Table 2. Serum levels of human ghrelin, leptin, blood glucose, insulin, and insulin resistance levels in three groups

Groups	Ghrelin (ng/ml)	Leptin (ng/ml)	Blood glucose (mg/dl)	Insulin (ng/ml)	Insulin resistance (ng/ml)
Group 1					
Range	8.6-48.7	1.3-34.7	74-106	2.1-28	0.52-6.42
Mean±SD	23.7±9.5	12.6±8.2	89.3±7.9	17.3±6.8	3.77±1.4
Group 2					
Range	4.3-21.1	2.4-44.1	50-120	5.6-25.1	2.03-7.43
Mean±SD	11.6±4.1	25.3±11.6	86.8±13.9	16.5±5.1	3.67±1.3
Group 3					
Range	3.4-48.9	7.4-69.7	75-99	3.3-49	0.67-11.23
Mean±SD	12.9±8.7	35.8±16.4	84.2±6.2	18.8±11.7	4.08±2.8
***p* value**					
G1:G2	<0.001*	<0.05*	>0.05	>0.05	>0.05
G2:G3	<0.05*	<0.05*	>0.05	>0.05	>0.05
G1:G3	<0.001*	<0.001*	>0.05	>0.05	>0.05

*Significant $p \leq 0.05$, Group 1 = normal weight, Group 2 = overweight, and Group 3 = obese

The levels of cholesterol concentrations in the sera of the studied groups showed no statistically significant difference (p >0.05) between three groups with means of (181.2±17.5 mg/dl) and (179.5±18.6 mg/dl) and (170.6±14.5 mg/dl) respectively (**Table 3**). Whereas, the results revealed significant decrease of HDL cholesterol level in over-weight group and obese group with a mean of (31.6±4.5 mg/dl) and (30.7±5.9 mg/dl) respectively as compared to normal weight group (39.5±3.7 mg/dl). There was no significant difference between over-weight and obese groups. The levels of LDL cholesterol concentration in the sera of the over-weight group, obese group, and normal weight group showed that no statistical significant difference among three groups with means(112.8±12.8 mg/dl), (105±18.9 mg/dl), and (108.6±13.8 mg/dl) respectively.

As regards to triglyceride levels, they were highly significantly increased (p <0.05) in both over-weight group and obese group with means±SD of (141.9±39.3 mg/dl) and (141.1±57.8 mg/dl) respectively as compared with normal weight group (93.7±25.6 mg/dl). There was no statistically significant difference between over-weight and obese groups.

Overall, statistical analysis clarified that the blood urea concentration was increased significantly in the obese group with a mean (29.1±6.8 mg/dl), whereas in the over-weight group, a slight increase was observed with a mean (24.6±5.5 mg/dl) as compared to the normal weight group with a mean (23.9±4.4 mg/dl). Creatinine levels revealed no difference between over-weight group with a mean (0.77±0.17 mg/dl) and normal weight group with a mean (0.75±0.16 mg/dl). However, in the obese group, we noted a significantly increased (0.88±0.17 mg/dl), as compared with the normal weight group.

Table 4 shows the correlation between ghrelin with the other studied parameters in the normal weight group. There was a statistically significant negative correlation between ghrelin, leptin, BMI and HOMA i.e. an increase in ghrelin level is associated with a decrease in leptin, BMI, and HOMA levels. There was no statistically significant correlation between ghrelin, FBS and Insulin.

In regards to leptin, there was a statistically significant positive correlation between leptin, Insulin, BMI, and HOMA i.e. an increase in leptin is associated with an increase in Insulin, BMI, and HOMA. There was no statistically significant correlation between leptin and FBS.

Table 3. Serum concentrations of cholesterol, HDL, LDL cholesterol, triglycerides BUN, and creatinine levels in three groups

Groups	Triglycerides (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)	Cholesterol (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)
Group 1 (no = 25)						
Range	57.4-144.5	86.9-169.1	33-47	149.8-210	0.4-1.1	17-34
Mean±SD	93.7±25.6	108.6±13.8	39.5±3.7	170.6±14.5	0.75±0.15	23.7±4.1
Group 2 (no = 28)						
Range	150.6-232	73.2-150	32.1-44.3	152.1-217	0.5-1.2	15-36
Mean±SD	141.9±39.3	112.8±12.8	31.6±4.5	181.2±17.5	0.77±0.16	25.6±4.7
Group 3 (no = 29)						
Range	79.1-250.2	54.2-143.2	28-43.2	145.1-213	0.6-1.3	18-42
Mean±SD	141.1±57.8	105.8±18.9	30.7±5.9	179.5±18.6	0.86±0.17	29.5±5.9
***p* value**						
G1:G2	<0.05*	>0.05	<0.05*	>0.05	>0.05	<0.05*
G2:G3	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
G1:G3	<0.05*	>0.05	<0.001*	>0.05	>0.05	<0.001*

*Significant $p \leq 0.05$, Group 1 = normal weight, Group 2 = overweight, and Group 3 = obese

Table 4. Correlation between ghrelin and other parameters in three groups

	Ghrelin		Leptin	
	(r) value	***p*-value**	**(r) value**	***p*-value**
Ghrelin				
Group 1	1.000	—	-0.548	0.010*
Group 2	1.000	—	-0.883	<0.001*
Group 3	1.000	—	-0.449	0.008*
Leptin				
Group 1	-0.548	0.010*	1.000	—
Group 2	-0.883	<0.001*	1.000	—
Group 3	-0.449	0.008*	1.000	—
FBS				
Group 1	0.348	0.122	-0.143	0.535
Group 2	-0.337	0.220	0.249	0.372
Group 3	-0.114	0.520	0.095	0.593
Insulin				
Group 1	-0.379	0.090	0.918	<0.001*
Group 2	-0.689	0.005*	0.806	<0.001*
Group 3	-0.564	0.001*	0.510	0.002*
BMI				
Group 1	-0.547	0.010*	0.983	<0.001*
Group 2	-0.487	0.050*	0.540	0.038*
Group 3	-0.413	0.015*	0.340	0.049*
HOMA				
Group 1	-0. 313	0. 167	0.883	<0.001*
Group 2	-0.748	<0.001*	0.840	<0.001*
Group 3	-0.542	0.001*	0.517	<0.002*

*Significant $p \leq 0.05$, Group 1 = normal weight, Group 2 = overweight, and Group 3 = obese

In the overweight group, there was a statistically significant negative (inverse) correlation between ghrelin, leptin, insulin, BMI and HOMA i.e. an increase in ghrelin is associated with a decrease in leptin, insulin, BMI, and HOMA.There was no statistically significant correlation between ghrelin and FBS.

While as regards to leptin, there was a statistically significant positive (direct) correlation between Leptin, Insulin, BMI and HOMA i.e. an increase in Leptin is associated with an increase in Insulin, BMI, and HOMA.There was no statistically significant correlation between Leptin and FBS.

In the obese group, there was a statistically significant negative correlation between Ghrelin, Leptin, Insulin, BMI and HOMA i.e. an increase in Ghrelin is associated with a decrease in Leptin, Insulin, BMI, and HOMA. There was no statistically significant correlation between Ghrelin and FBS. While as regards to leptin, there was a statistically significant positive correlation between leptin, insulin, BMI, and HOMA i.e. an increase in leptin is associated with an increase in Insulin, BMI, and HOMA. There was no statistically significant correlation between leptin and FBS.

Discussion

We hypothesized that metabolic profile of overweight individuals would consist of relatively lower concentrations of fasting ghrelin and this is what we found in our study comparing overweight and obese groups with the normal weight control group. There was statistically significant negative correlation between ghrelin levels with leptin, BMI, and HOMA.

Our results were in agreement with Yada et al. [28] who stated that ghrelin is important in short-term regulation of appetite and energy balance. The clear pre-prandial rise and post-prandial fall in plasma ghrelin levels support the hypothesis that ghrelin acts as an initiator signal for meal consumption in humans. The pre-prandial increase of ghrelin levels was found to

initiate meal consumption voluntarily, without time- or food-related cues [29], while the post-prandial ghrelin suppression is proportional to the ingested calorie load [30]. Ghrelin also appears to be involved in the regulation of long-term energy homeostasis. Ghrelin shows orexigenic effect through its action on the hypothalamic appetite-regulating pathways, while in the periphery ghrelin increases adipose tissue accumulation and has a diabetogenic effect on the liver and pancreas. Adenosine monophosphate-activated protein kinase (AMPK) has been suggested as one of the mediators of ghrelin's effects. Plasma ghrelin levels are dependent on body mass index as well as food intake patterns. Ghrelin levels are in general reduced in obese individuals and in subjects with insulin resistance [31].

Circulating ghrelin induces abdominal obesity, independently of its central orexigenic activity, via GHS-R-dependent lipid retention [32].In agreement with our results Williams et al. [12] stated that ghrelin levels inversely correlate with body mass index (BMI). Thus, ghrelin levels are reduced in those who are obese compared to normal body weight controls. Evidence suggests that diet-induced obesity causes ghrelin resistance by reducing NPY/AgRP responsiveness to plasma ghrelin and suppressing the neuroendocrine ghrelin axis, in an attempt to limit further food intake [13]. Ghrelin levels have been shown to negatively correlate with factors that are raised in obesity namely, percentage body fat, insulin and leptin levels [11]. In one study, ghrelin levels were not related to fat mass or intra-abdominal fat content, but showed strong negative correlation with insulin levels and insulin resistance [33]. However, in an MRI study of non-obese and obese adults, ghrelin was negatively correlated with visceral adiposity, fasting insulin, and homeostasis model insulin resistance index. Visceral adiposity showed stronger inverse correlation with ghrelin than subcutaneous fat possibly through hyperinsulinemia, as the negative correlations with insulin resistance were even stronger [34]. Abnormal glucose homeostasis inversely correlated with, and was found to be an independent determinant of, plasma ghrelin levels in obese children and adolescents [35].

Our results showed that higher concentrations of fasting leptin were found in overweight and obese groups compared with normal weight control group. In addition, there was statistically significant positive correlation between leptin, insulin, BMI and HOMA.

These results were in agreement with Considine et al. [36] who declared that most obese individuals have higher leptin levels than lean individuals and are resistant or tolerant to the effects of leptin. Leptin resistance was first thought to be due to mutations of the leptin receptor and other rare monogenic obesity syndromes. Mutations of other genes downstream of leptin, including POMC and MC4R, also result in an obese phenotype with associated neuroendocrine dysfunction [37]. However, only a few cases of human obesity are due to monogenic syndromes; instead, most instances appear to be multifactorial [38]. First, leptin transport across the blood–brain barrier that is impaired in obesity. This is partially due to saturation of the transporter by hyperleptinemia, which is associated with obesity, and subsequent decrease in transport activity [39]. Targeting these mechanisms of leptin resistance will be important in the treatment for obesity and has led to development of insulin sensitizers, such as the chemical chaperones [40].

In a study done by Remsberg et al. [41] comparable associations were noted with leptin and most measures of body composition, suggesting that leptin levels reflect total body fat and are less affected by fat distribution. Their results parallel those reported by other investigators in which leptin levels exhibit relatively stable associations across differing types of localized fat deposits [42]. Reports in the literature differ, however, on the relationships, and these associations may reflect variations in groups studied. For example, associations between leptin, body composition, and fat distribution tend to be stronger among men and weaker in the obese. Their findings suggest that subcutaneous or white adipose tissue in the abdominal region may be the best predictor of circulating leptin [43].

Mueller et al. [44] suggested that insulin has been shown to influence circulating leptin concentrations. Specifically, insulin increases leptin concentrations in rodents, both in cultured adipocytes as well as in vivo [45]. These associations have also been found in humans, at the level of the adipocyte and in the peripheral circulation wherein experimental hyperinsulinemia using clamp techniques results in increased leptin concentrations [46]. The relationship between leptin or ghrelin and insulin is potentially more complex because of the possibility of insulin resistance, wherein comparable concentrations in obese individuals would not have the same effect as in leaner persons [47].

Conclusion

Our results suggest coordinated roles of ghrelin and leptin in the modulation of the obesity and these markers can be of value in assessment of treatment of such cases.

The authors declare no conflict of interest.

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