Hormonal levels of leptin, insulin, ghrelin, and neuropeptide Y in lean, overweight, and obese Saudi females

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ABSTRACT

Objectives: To studied the relationship that exists between leptin, ghrelin, insulin, neuropeptide Y (NPY), anthropometric, and metabolic variables in Saudi females.

Methods: The study was conducted at the Department of Genetics, King Faisal Specialist Hospital & Research Center, Riyadh, Kingdom of Saudi Arabia from November 2004 to August 2005. One hundred and twenty-two Saudi females were divided into 3 body mass index (BMI) groups: lean (N=60), overweight (N=17), and obese (N=45). Fasting leptin, ghrelin, insulin, NPY and glucose concentrations were determined.

Results: Leptin levels in overweight and obese groups were significantly higher than those in lean group. Leptin levels showed a positive correlation with BMI in obese (0.81), overweight (0.78), and lean (0.48). In contrast, ghrelin concentration decreased in obese and overweight subjects compared to lean subjects. Ghrelin levels were negatively correlated with BMI in obese (-0.81), overweight (-0.58), and lean subjects (-0.62). Negative correlations were found between serum insulin and ghrelin concentrations in lean and obese subjects. Glucose and insulin levels were significantly higher in the obese group compared to controls. No differences were found in serum NPY between the 3 groups.

Conclusion: Leptin levels increased remarkably with increasing BMI. A leptin resistance state seems to exist in many obese and overweight individuals. Ghrelin concentration was decreased in overweight and obese subjects. These data demonstrate a significant inverse relationship between ghrelin and leptin levels in overweight and obese subjects.

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Obesity has become a major public health problem throughout the world.[1] At least one-third of Arabs are obese, and this figure is rising steadily despite increased interest in fitness.[2,3] In order to understand the pathophysiology of obesity it is necessary to investigate the physiology of normal body weight regulation at the hormonal level. In this regard, energy intake, and body weight are tightly regulated at a remarkably consistent "set-point" by control systems in the hypothalamus.[4] These hypothalamic circuits receive feedback from peripheral signals. Furthermore, leptin and insulin have an established role as "adiposity" signals that regulate long-term body weight homeostasis through actions on the central nervous system (CNS), particularly the hypothalamic arcuate nucleus, where both receptors are highly expressed.[5-9] Central administration of either insulin or leptin reduces food intake and body weight.[10,11] However, neuropeptide (NPY) has been suggested to be an essential conduit for the leptin and insulin signals.[12] It has been demonstrated that leptin directly inhibits NPY expression in the arcuate nucleus and, thus, represses the activity of this potent stimulator of food intake.[13-15] Therefore, insulin along with other factors including leptin, regulates the synthesis, and release of the orexigenic NPY in the hypothalamus.[16,17] Leptin is a small protein produced and released in several tissues in addition to adipocytes.[18] It is secreted into the circulation and crosses the blood-brain barrier into the CNS.[19] It acts at the level of the hypothalamus by binding to its receptors and activating secondary signals that inhibit food intake and increase energy expenditure.[20] Administration of a small amount of leptin into the cerebral ventricles corrects the obesity and metabolic abnormalities present in the leptin-deficient obese mice.[20,21] In the great majority of obese humans, however, leptin levels are increased, indicating that human obesity is leptin resistant.[22,23] However, ghrelin is an acylated 28-amino acid peptide identified in rat stomach.[24]

This novel peptide hormone is a natural ligand of the growth hormone (GH) secretagogue receptor, a G protein-coupled receptor primarily expressed in the pituitary and hypothalamus,[25] and has been shown to be a potent stimulant of GH secretion.[26] Apart from this effect, ghrelin has attracted attention for its involvement in the control of food intake and energy balance. When administered centrally or peripherally to rodents and humans, ghrelin enhances appetite, reduces fat utilization, and causes adiposity.[27-30] In contrast, an intracerebroventricular administration of anti-ghrelin immunoglobulin G robustly suppressed feeding.[28] Based on the fact that ghrelin is mainly produced in the stomach and major binding sites of ghrelin have been identified in hypothalamic areas, ghrelin might represent an important endocrine signal connecting peripheral mechanisms sensing caloric intake with brain centers controlling energy homeostasis.[31] The stimulatory action of ghrelin on food intake appears to be mediated via the orexigenic NPY/agouti-related peptide pathway.[32-35] Gastric expression and circulating levels of ghrelin increase on fasting and decrease following food intake indicating a putative regulation of ghrelin secretion by nutrients or related factors such as insulin.[36,37] Plasma ghrelin concentrations have been shown to be lower in obese patients when compared with normal subjects.[38-40] We aimed in this study to investigate the correlation between obesity and several key hormones involved in the regulation of body weight homeostasis in Saudi females. These include leptin, ghrelin, NPY, and insulin.

Methods. The study was conducted at the Department of Genetics, Research Center, King Faisal Specialist Hospital & Research Center (KFSH&RC), Riyadh, Kingdom of Saudi Arabia from November 2004 to August 2005. A total of 122 Saudi females volunteers were recruited, aged 20-30 years (mean ± standard error of mean [SEM]). The subjects were divided into 3 groups according to their body mass index (BMI); lean (n=60, BMI 18.5-24 kg/m^2), overweight (n=17, BMI 25-29 kg/m^2) and obese (n=45, BMI ≥30 kg/m^2). The general characteristics of the subjects are summarized in **Table 1**. All subjects were healthy, free from any medication with regular menstrual cycle, and no history of gastrointestinal or endocrine disorders. Ethical approval was obtained from the KFSH&RC Research Ethical committees, and written informed consent was obtained from all subjects.

Protocol. The hormonal and metabolic assessment was made between day 2 and 4 of the menstrual cycle. After an overnight fast (12 hours) a venous blood sample was obtained from all subjects in the morning between 8:00-9:00 am for the determination of fasting serum leptin, ghrelin, insulin, NPY, and glucose. Serum was aliquoted, and stored at -80°C until required for assay.

Anthropometric measurements. Measurements were performed after an overnight fast. Body mass was measured on calibrated balances or electronic scales to the nearest 0.1 kg. Body height was measured to the nearest centimeter. The BMI was calculated as body mass (kilograms) divided by body height (meters) squared. Using a tape measure, with the subject standing, the waist was measured as the narrowest circumference between the lower costal margin and the iliac crest. The hip was the maximum circumference at the level of the femoral trochanter.

Analytical method. Plasma glucose was measured by a glucose oxidase method using the Roche Glucose HK liquid assay on Roche/Hitachi 917 automatic analyzer. Serum insulin was measured with electro chemiluminescence immunoassay (ECLIA) technique, using Elecsys insulin kit on E 170 immunoassay analyzer (Roche). Serum NPY was measured in duplicate using a double antibody RIA kit from EURO-DIAGNOSTIC AB (Medeon, Sweden). Lower and upper limits of detection for this assay were 6 and 300 pmol/L. Serum leptin was measured in duplicate using human leptin enzyme linked immunoassay (ELISA) kit from Linco Research, Inc. (St. Charles, MO), with a lower limit of detection of 0.5 ng/mL. Serum ghrelin levels were measured in duplicate using a commercial ghrelin (human) enzyme immunoassay kit (EIA) from (Phoenix Pharmaceuticals, Inc (Belmont, California) with a lower limit of detection of 0.06 ng/mL.

Statistical analysis. The descriptive characteristics of the group variables were expressed as mean ± SEM. The comparisons between overweight, obese, and their lean matched control were carried out using the independent t-test with respect to all variables. Pearson correlation coefficients was used to find the correlation between leptin, insulin, NPY, BMI, and other studied variables. Significance was declared when *p*-values are less than 0.05. All statistical analyses were performed using the StatView program for Windows (version 8.0; SAS Institute, Inc., Cary, North California).

Results. The mean age, BMI, anthropometric, mean leptin, ghrelin, NPY, insulin concentrations, and fasting glucose of the groups are shown in **Table 1**. As presented student's t-test was applied, and significant differences were found in the waist, hip, and waist/hip ratio among over weight and obese control subjects compared with the lean control group. Correlation analyses were performed between BMI and all parameters (**Table 2**). In the lean group, BMI correlated positively with age, waist, hip, and leptin and negatively with ghrelin. In overweight group, BMI correlated positively with

Table 1 - Baseline Characteristics of Participants.

Variables	Control lean (n=60)	Overweight (n=17)	*P*	Obese (n=45)	*P*
Age (year)	23.95 ± 0.60	21.59 ± 0.94	NS	26.49 ± 0.96	0.03
BMI (kg/m²)	20.85 ± 0.25	27.38 ± 0.37	<.0001	35.90 ± 0.92	<.0001
Waist (cm)	66.85 ± 0.70	81.59 ± 1.82	<.0001	100.29 ± 2.14	<.0001
Hip (cm)	94.59 ± 0.91	105.29 ± 1.78	<.0001	121.96 ± 2.14	<.0001
WH Ratio	0.71 ± 0.01	0.78 ± 0.01	<.0001	0.82 ± 0.01	<.0001
NPY (pmol/L)	70.07 ± 1.34	76.47 ± 4.66	NS	74.04 ± 3.35	NS
Fasting Leptin (ng/ml)	11.70 ± 0.46	23.79 ± 1.55	<.0001	46.04 ± 3.07	<.0001
Fasting Ghrelin (ng/ml)	0.57 ± 0.02	0.44 ± 0.02	<.0001	0.28 ± 0.01	<.0001
Fasting Insulin (pmol/L)	52.57 ± 2.29	63.93 ± 9.95	NS	104.69 ± 5.58	<.0001
Fasting Glucose (mmol/L)	4.54 ± 0.05	4.66 ± 0.15	NS	4.99 ± 0.07	<.0001

values are expressed as mean ± SE, BMI - body mass index,
WH ratio - waist hip ratio, NPY - neuropeptide Y, NS - non significant
P level by student's t-test

Table 2 - Correlation between BMI and different studied variables in lean, overweight and obese groups.

Variables	Control-Lean (n=60)		Control-Overweight (n=17)		Control-Obese (n=45)	
	r	*p*	r	*p*	r	*p*
Age (year)	0.57	<0.001	-0.073	0.390	-0.08	0.31
Waist (cm)	0.62	<0.001	0.29	0.132	0.9	<0.001
Hip (cm)	0.72	<0.001	0.532	0.014	0.9	<0.001
WH Ratio	-0.073	0.29	-0.039	0.440	0.4	0.009
NPY (pmol/L)	0.02	0.9	-0.52	0.033	0.36	0.02
Leptin (ng/ml)	0.48	<0.001	0.78	0.0002	0.81	<0.0001
Fasting Ghrelin (ng/ml)	-0.62	<.0001	-0.58	0.014	-0.81	<.0001
Fasting Insulin (pmol/L)	0.087	0.51	0.6	0.012	0.37	0.013
Fasting Glucose (mmol/L)	0.235	0.037	0.5	0.029	0.009	0.477

WH ratio - waist hip ratio, NPY - neuropeptide Y,
BMI - body mass index, *P* level by student's t-test

Table 3 - Correlation analysis of serum leptin, ghrelin, insulin, and NPY levels with other studied variables in lean subjects.

Variables	Fasting leptin		Fasting ghrelin		Fasting insulin		Fasting NPY	
	r	*p*	r	*p*	r	*p*	r	*p*
Age (year)	0.120	0.360	-0.31	0.012	0.24	0.071	-0.05	0.73
BMI (kg/m²)	0.48	<.0001	-0.62	<.0001	0.087	0.51	0.02	0.9
Waist (cm)	0.45	0.0003	-0.48	<.0001	0.095	0.47	-0.15	0.27
Hip (cm)	0.49	<.0001	-0.49	<.0001	0.069	0.60	-0.14	0.28
WH Ratio	-0.038	0.78	-0.04	0.76	0.047	0.72	-0.04	0.79
NPY (pmol/L)	0.219	0.094	-0.005	0.97	-0.023	0.86	-	-
Leptin (ng/ml)	-	-	-0.373	0.0034	-0.048	0.71	0.22	0.1
Fasting Ghrelin (ng/ml)	-0.37	0.003	-	-	-0.26	0.043	-0.01	0.97
Fasting Insulin (pmol/L)	-0.048	0.71	-0.26	0.043	-	-	-0.023	0.86
Fasting Glucose (mmol/L)	0.48	0.031	0.021	0.93	0.32	0.18	0.55	0.013

WH ratio - waist hip ratio, NPY - neuropeptide Y,
BMI - body mass index, *P* level by student's t-test

many variables as shown in **Table 2**, and the strongest correlation was found between BMI and both leptin and insulin. A negative correlation was found between BMI and both ghrelin and NPY. In the obese group, a strong correlation was found between BMI and waist, hip, and leptin and a weak correlation with NPY. A negative correlation was found between BMI and ghrelin. Serum concentrations of leptin were markedly and significantly higher in obese and overweight subjects compared with lean controls. Correlation analyses were performed between leptin levels and the other parameters (**Tables 3-5**). Leptin levels correlated strongly and positively with BMI in obese, overweight, and lean groups. Waist circumference and hip were strongly and positively correlated with leptin concentration in obese and lean groups. Leptin levels were found to be negatively correlated with fasting ghrelin levels in the obese and lean groups. A weak correlation was found between leptin and NPY in the obese group. Fasting ghrelin concentration was decreased in overweight and obese subjects as compared with lean subjects (**Table 1**). The mean fasting serum ghrelin concentration in these 3 groups was negatively correlated with BMI (**Tables 2-5**). In the lean group, ghrelin levels negatively correlated with waist, hip, leptin, and insulin (**Table 3**). In the overweight group, the negative correlation was found only between ghrelin levels and both BMI and hip. In the obese group, ghrelin levels negatively correlated with

Table 4 - Correlation analysis of serum leptin, ghrelin, insulin, and NPY levels with other studied variables in overweight subjects.

Variables	Fasting leptin		Fasting ghrelin		Fasting insulin		Fasting NPY	
	r	*p*	r	*p*	r	*p*	r	*p*
Age (year)	-0.12	0.65	-0.032	0.90	0.26	0.31	0.076	0.8
BMI (kg/m^2)	0.78	0.0002	-0.58	0.014	0.6	0.012	-0.52	0.033
Waist (cm)	0.09	0.72	-0.35	0.12	0.103	0.7	0.013	0.96
Hip (cm)	0.31	0.23	-0.55	0.02	0.28	0.27	-0.080	0.76
WH Ratio	-0.1	0.73	0.020	0.94	-0.12	0.650	-0.013	0.96
NPY (pmol/L)	-0.44	0.08	0.09	0.72	-0.2	0.47	-	-
Leptin (ng/ml)	-	-	-0.09	0.72	0.35	0.170	-0.44	0.080
Fasting Ghrelin (ng/ml)	-0.1	0.72	-	-	-0.41	0.11	0.09	0.72
Fasting Insulin (pmol/L)	0.5	0.17	-0.41	0.12	-	-	-0.189	0.47
Fasting Glucose (mmol/L)	-0.05	0.84	-0.05	0.841	0.08	0.740	-0.191	0.42

WH ratio - waist hip ratio, NPY - neuropeptide Y,
BMI - body mass index, *P* level by student's t-test

Table 5 - Correlation analysis of serum leptin, ghrelin, insulin, and NPY levels with other studied variables in obese subjects.

Variables	Fasting leptin		Fasting ghrelin		Fasting insulin		Fasting NPY	
	r	*p*	r	*p*	r	*p*	r	*p*
Age (year)	-0.16	0.28	-0.02	0.92	-0.186	0.221	-0.01	0.96
BMI (kg/m^2)	0.81	<.0001	-0.81	<.0001	0.37	0.013	0.36	0.02
Waist (cm)	0.7	<.0001	-0.7	<.0001	0.38	0.01	0.14	0.38
Hip (cm)	0.71	<.0001	-0.72	<.0001	0.3	0.07	0.2	0.311
WH Ratio	0.25	0.097	-0.21	0.162	0.34	0.03	0.02	0.9
NPY (pmol/L)	0.32	0.034	-0.31	0.042	0.2	0.20	-	-
Leptin (ng/ml)	-	-	-0.67	<.0001	0.3	0.058	0.32	0.034
Fasting Ghrelin (ng/ml)	-0.67	<.0001	-	-	-0.33	0.03	-0.31	0.042
Fasting Insulin (pmol/L)	0.3	0.058	-0.33	0.03	-	-	0.19	0.21
Fasting Glucose (mmol/L)	0.22	0.13	0.14	0.33	0.32	0.022	0.4	0.012

WH ratio - waist hip ratio, NPY - neuropeptide Y,
BMI - body mass index, *P* level by student's t-test

waist, hip, NPY, leptin, and insulin (**Table 5**). Serum fasting glucose and insulin levels were significantly higher in the obese group compared to controls, with no significant difference between overweight and lean groups (**Table 1**). Correlation studies were performed between insulin levels and the other parameters, and the results obtained in lean, overweight, and obese controls are presented in **Tables 3-5**. In the lean control group no correlation was observed between insulin level and other parameters, except insulin correlated weakly with ghrelin (**Table 3**). In the overweight control group, insulin demonstrated a moderate positive correlation with BMI. In the obese control group, fasting insulin showed positive and significant, but weak correlation with BMI, waist, waist/hip ratio, and fasting glucose. No differences were found in serum NPY between the 3 groups (**Table 1**). No correlation between serum NPY and ghrelin, insulin, or anthropometric measurements was found in the lean control group, the only correlation was found between NPY and glucose (**Table 3**). A weak positive correlation was observed in obese control subjects between NPY and BMI, leptin, and glucose. However, a negative correlation was found between NPY and ghrelin (**Table 5**).

Discussion. This study aimed to investigate the correlation between some of the common orexigenic agents and obesity. We focused on serum leptin, ghrelin, insulin, and NPY in lean, overweight, and obese Saudi females. Leptin plays an important role in the regulation of food intake, energy expenditure, and body weight. The results showed that serum leptin concentration was strongly associated with BMI. These results are in line with the reports of previous investigations in which, a positive correlation was demonstrated between leptin and BMI.[41-43] The relation of leptin concentrations with measures of body fat distribution was examined in other population studies. A study in American populations and Mexican Americans found high correlations of leptin concentrations with BMI and waist and hip circumferences and concluded that leptin concentrations are associated with overall adiposity instead of with a specific fat depot.[44,45] In contrast, several authors found an association of leptin concentrations with waist circumference, independent of BMI or percentage body fat, and concluded that body fat distribution may also be an important determinant of leptin concentrations.[46-48] In another study, leptin concentrations were unrelated to waist circumference after adjustment for fat mass, but were associated with hip circumference in women.[49] In our study, leptin concentrations were associated positively and significantly with both waist and hip circumferences in lean and obese groups. Our data shows clearly that obesity in Saudis female is not a leptin deficient state and none of the obese or overweight individuals had leptin deficiency. A leptin resistance state seems to exist in many obese and overweight individuals which is similar to reports by some other studies.[50-52]

Both historical and more recent studies, using various model systems, and experimental approaches, strongly supports the idea that insulin, with leptin, has an important role in the hypothalamic control of energy homeostasis.[53] In our study, a significant positive correlation between BMI and insulin was seen in overweight and obese individuals. In addition, insulin levels were significantly high in obese subjects, confirming that obesity in Saudi females is associated with hyperinsulinemia and insulin resistance. Several recent studies have reported that fasting plasma ghrelin is reduced in obese subjects as compared to lean controls and is negatively correlated to the subjects' BMI and body fat mass.[38-40,54,56] Our data demonstrates a significant decrease in ghrelin serum levels in obese subjects, comparable to that seen in healthy lean control subjects. These alterations are in some respect the counterpart to the previously reported changes in leptin serum levels, which are increased in obesity.[57] The biological effects of the 2 peripheral hormones on the hypothalamus are reciprocal. Both peptides are thought to act at least in part through the changes in NPY.[58] However, peripheral interactions of the 2 hormones are still ambiguous. Kamegai et al[54] demonstrated that leptin directly decreases the secretion of ghrelin. These results are consistent with the recent report that moderate hyperleptinemia prevents an increase of plasma ghrelin during moderate short-term caloric restriction.[59] These results indicated that the anorexic effect of leptin may occur through an action on the stomach by decreasing ghrelin secretion as well as through a hypothalamic action. Alternatively, since ghrelin receptors are expressed in adipose tissue, the principal site of leptin synthesis,[24] it is conceivable that ghrelin might affect leptin secretion. Our study confirmed that there is a significant inverse relationship between ghrelin and leptin serum levels, in lean and obese groups, but not in the overweight group.

Ghrelin and its receptors are expressed in pancreatic beta cells,[60,61] suggesting that it could have effects on insulin secretion. However, the influence of ghrelin on glucose metabolism and insulin is still controversial.[62-65] Under physiological conditions, negative correlations have been reported between plasma insulin and ghrelin concentrations.[37,38,40] In this study, we found a significant negative correlation between ghrelin and insulin in lean and obese groups. In agreement with Malik et al,[64] we observed a negative correlation between ghrelin and BMI, and anthropometric measures in obese and lean subjects.

In the present study, we found that serum NPY levels were similar between the 3 groups. Present results are in line with previous reports having shown neither the baseline plasma level nor the baseline cerebrospinal fluid level of NPY were different between normal weight subjects and obese subjects.[65] These findings contrast with results of a previous report, in which NPY levels were significantly lower in the obese persons compared with the control group.[66] Moreover, we detected that circulating NPY was significantly and negatively correlated to BMI in the overweight group, and a significant weak positive correlation was found between NPY, BMI, and leptin in the obese group, considered as unusual results. It is not known why an expected physiologic relationship was not observed among obese subjects, the control that was exerted by leptin on hypothalamic NPY cannot be seen in peripheral blood. The data may suggest that factors other than BMI may be involved in the modulation of NPY release in obesity.

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