ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Evaluation the activity of Neuropeptide Y (NPY), Glucagon like peptide-1 and Ghrelin in controlling appetite in obesity in Erbil City

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A B S T R A C T:
The epidemic of obesity is at previously unheard-of heights. In 2016, it was predicted that 39% and 13% of individuals worldwide were overweight or obese, respectively. Obesity, which is defined as an imbalance between energy intake and expenditure, results from multiple interactions between genetic, environmental, psychological, behavioral, and nutritional variables, making it a difficult condition to study and treat. The present study aimed to approach the understanding of appetite, bodyweight regulation and the causes of obesity through studying gastrointestinal hormones such as (NPY, GLP-1, and ghrelin) hormones and their impact on controlling appetite in obesity. This comparative study included (152) participants (Obese and non-Obese) in the age range (30-66 years), both males and females who visited (The surgical specialty hospital cardiac center, Hawler teaching hospital, and Erbil's court). A self-administered questionnaire was used for data collection. Fasting serum metabolic parameters and hormones were measured included (S.NPY, S.GLP-1, S.Ghrelin) and (S.Total Cholesterol, S.Triglycerides, S.HDL, S.LDL, and S.VLDL). According to the BMI of participants, there was a significant association ($P < 0.001$) between NPY, GLP-1 hormones and BMI, and a significant ($p = 0.033$) association between Ghrelin and BMI. The present study concluded that serum NPY, GLP-1 and Ghrelin hormones significantly impacted controlling appetite in obesity, would be a useful and important marker to evaluation for obesity. And a strong relationship between these hormones and obesity.

KEY WORDS: Obesity, Appetite, Neuropeptide Y (NPY), Glucagon like peptide-1 (GLP-1), Ghrelin.
DOI: http://dx.doi.org/10.21271/ZJPAS.35.1.21
ZJPAS (2023) , 35(1);210-222 .

1. INTRODUCTION:

One of the major health issues of the twenty-first century is the increasing obesity epidemic. It has been linked to an increase in morbidity and is a risk factor for heart disease, type 2 diabetes, hypertension, dyslipidemia, and numerous malignancies. The physiological processes behind food intake behavior and overeating, particularly in humans, are still poorly understood (Hanssen et al., 2021). Neuropeptide Y (NPY) one of the most effective endogenous orexigenic neurotransmitters, as well as one of the most plentiful (Newmyer et al., 2013).

The NPY is involved in the physiological regulation of food intake. Several studies have shown that central administration of NPY increases food intake significantly, resulting in increased fat mass and body weight (Katus et al., 2021). GLP-1 (glucagon like peptide-1) is a cleavage product of the pre-proglucagon gene that acts as a gut hormone. The primary function is to stimulate insulin production while inhibiting glucagon secretion. GLP-1 reduces stomach emptying and limits food intake by increasing satiety (Villanueva-Peñacarrillo et al., 2011). Researchers discovered that participants with overweight and obesity class I, II, and class III had larger GLP-1 peaks than subjects with normal weight (Acosta et al., 2015). Ghrelin is a stomach peptide hormone that regulates hunger, food

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Article History:
Received: 25/07/2022
Accepted: 18/09/2022
Published: 20/02 /2023

intake, and body weight peripherally acting orexigenic manner (Briggs and Andrews, 2011). Obese people had lower fasting ghrelin concentrations than normal-weight persons. However, after meals, ghrelin concentrations decrease to a lesser extent in obese people, resulting in the preservation of relatively high postprandial levels and excessive food consumption (Pardak et al., 2022). This study aimed to assess serum peptide hormones within the context of obesity and BMI among obese and non-obese adult in Erbil City.

2.MATERIALS AND METHODS:

Study population and design of the study:

A comparative study included 152 participants. The subjects of this study were grouped into three categories: Normal group (Group I): Fifty-five non-obese were selected as normal weight subjects and their BMI were between (18.5-24.9). Overweight group (Group II): Forty-two overweight selected their BMI were between (25.0-29.9). Obese group (Group III): Fifty-five obese selected their BMI were between (30.0-40.0). This group was subdivided into three subgroups: Obese Class (I): their BMI were between (30.0-34.9). Obese Class (II): their BMI were between (35.0-39.9). Obese Class (III): their BMI were (≥ 40.0). (BMI) Body mass index was calculated as weight (kg) divided by the square of height (metre^2) for all samples. Medical examinations were performed for each participant.

Collection of blood samples:

A total of 152 blood samples were collected from 55 normal weigh, 42 overweight and 55 obese. The obtained samples underwent a 10-minute centrifugation process at 3500 rpm. The separated serums were used for the measurement of S. NPY, S. GLP-1, S. Ghrelin, S. total cholesterol, S.HDL, S.LDL, and S.VLDL. The serum samples for hormone tests were stored at -70°C until analyzed according to manufacturer guidance kits must kept at 2-8℃ until analysing.

Inclusion and exclusion criteria:

Both genders, adults aged 30-66 years, were obese, normal weight, and overweight samples, ≥30-years old, with cardiovascular disease, hypertension and diabetic were included. Exclusion criteria were underweight samples, less than 30-years, patients taking hormones replacement therapy, taking antioxidant supplements, any history of endocrine diseases, pregnant women, cancer, chronic kidney diseases, individuals who were dieting, taking hormonal supplements, haemolysed blood samples, gastric bypass, and cholecystectomy.

Study timeline:

The present study carried out from 1st September 2021 until 30th March 2022, by a collaboration of surgical specialty hospital cardiac center, Hawler teaching hospital, and Erbil's court in Erbil/ Iraq.

Questionnaire form design:

The data collection instrument is a structured direct interviewer-administered face-to-face questionnaire that is pretested with modifications made before its use in the study, along with access to medical records or records which contain intimate personal information, and are individually identifiable and are not publicly available. The questionnaire includes the demographic variables (name, age, gender, home address, time and date), clinical risk factors of the patient, family history, and smoking habitual of the patient.

Ethical considerations:

Ethical approval was obtained from the ethics committee of Hawler Medical University. Verbally Informed consent was taken from each patient. A Complete explanation of the nature and aim of the study was given to each participant, and reassure about the confidentiality of the data and their anonymity.

Hormonal assays:

The fasting serum hormones tests were performed using method Enzyme-linked immunosorbent assay (ELIZA), by using (ELIZA Kit USA), delivered company Al-Shkairate establishment for medical supply, for the determination of tests (NPY), (GLP-1), and (Ghrelin). The assay was performed according to the manufacturer's specifications. Serums were separated by centrifugation at 3500rpm for 10 minutes. NPY levels are considered to be (**15.625-1000pg/ml**) in adults. GLP-1levels are considered to be (**0.313-20ng/ml**) in adults. Ghrelin levels are considered to be (**46.875-3000pg/ml**) in adults.

Biochemical assays:

The fasting serum lipid tests were performed by using colorimetric-enzymatic methods, using a fully automated biochemistry analyzer (Roche/Hitachi COBAS C-311, Germany) for the

determination of total cholesterol, triglycerides, HDL-cholesterol. LDL-cholesterol. The assay was performed according to the manufacturer's specifications. Serums were separated by centrifugation at 3500rpm for 10 minutes. Cholesterol levels are considered to be below 200 mg/dL in adults (Greiling and Gressner, 1995). Triglyceride levels are considered below 150 mg/dL in adults (Greiling and Gressner, 1995). HDL-C levels are considered (45-65 mg/dL) in Female and (33-55 mg/dL) in male (Nauck et al., 1997). LDL-C levels are considered to be below 100 mg/dL in adults (Rifai et al., 1992). (VLDL-C) was determined indirectly using the formula (VLDL-C = triglycerides/5). And (VLDL-C) levels in adults are considered to be between (2-30) mg/dL.

Statically analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 26). Results were expressed as mean± SD and mean ± SE. The difference among mean values of groups was determined by using the chi-square test, and a one-way (ANOVA) test was used to examine the difference between mean values of groups. Pearson correlation coefficient (r) was calculated to assess the strength of correlation between two numerical variables. A (p-value of ≤ 0.05) was considered statistically significant.

3.RESULTS:

The total number of participants in this study was 152. Participants were divided into three groups: Normal group (Group I): 55 (36%) non-obese, who represented of the study population. Overweight group (Group II): 42 (28%) overweight represented of the study population. Obese group (Group III): total 55 (36%) obese represented of the study population, this group was subdivided into three subgroups: Obese class (I): 15 obese, class (II): 20 obese, class (III): 20 obese. The age range was 30-66 years. The majority (73.7%) of the participants were aged less than 50 years, and (71.1%) were males. Other details are presented in Table 1. Table 2 presents medical history. Arithmetic means of biochemical parameters in the study population are presented in Table 3. However, significantly lower serum levels of TG ($p<0.025$), and VLDL ($p <0.025$) with higher serum levels of HDL ($p<0,023$), were estimated in normal compared with overweight and obese patients, and there was no significant association serum level of T.C (p=0.383) and LDL (p=0.645) among individuals represented in table 4. It is evident from Table 5 that the more the BMI, the less the NPY ($p < 0.001$). The mean±SE of GLP-1 was (2.22± 0.16 ng/ml) among people with normal weight which was significantly less than the means of overweight and obese individuals ($p < 0.001$), in contrast the mean±SE of Ghrelin of normal weight individuals (0.97±0.03 pg/ml) was significantly more than the means of the overweight and obese individuals ($p = 0.033$). It is evident in Table 6 that there was inverse significant correlation between NPY and BMI ($r = -0.604$, $p < 0.001$). A positive significant correlation was detected between the GLP-1 and BMI ($r = 0.549$, $p < 0.001$), while inverse significant correlation was found between Ghrelin and BMI ($r = -0.173$, $p = 0.033$). Lower serum NPY and serum Ghrelin levels observed in obese compared to non-obese (Figure 1) and (Figure 3) respectively, and Higher serum GLP-1 levels estimated in (Figure 2). Correlation of hormone parameters with lipid parameters in obesity (Table 7) shows that the result from Pearson correlation coefficient (r) analysis between hormones and lipid parameters in obese individuals. Because BMI and age might mediate the relationship between hormones and lipids. These variables were excluded from multivariate analysis. Serum NPY had no statistically significant associations with serum T.C (p=0.06), TG (p=0.106), HDL (p=0.966), LDL (p=0.303) and VLDL (p=0.106) among obese. While serum GLP-1 showed a significant was found a positive correlation with serum T.C (r=0.207, p=0.011), TG (r=0.279, p=0.001), VLDL (r=0.279, p=0.001). And there was no statistically significant with HDL (p=0.961), and LDL (p=0.714). Serum Ghrelin showed significant negative associations with HDL (r=-0.231, p=0.004), while positive significant correlation for TG (r=0.263, p=0.001), VLDL (r=0.263, p=0.001). And no statistically significant with T.C (p=0.642), and LDL (p=0.268).

4. DISCUSSION:

Public health issues related to obesity and its complications are getting worse and worse. It has long been understood that hormonal dysregulation derived from the gut and obesity are related. More specifically, peptide hormones that control appetite include, PYY, GLP-1, CCK, insulin, leptin, NPY and ghrelin (Koliaki et al., 2020). The body mass index (BMI) represents the most used

tool to assess the degree of obesity (Flehmig et al., 2014). Hunger and satiety signals, activated in response to appetitive stimulation and ingestion, are substantially in control of feeding behavior and body weight management. The study on the control of enter-endocrine hormones in obesity was conducted by (Muñoz et al., 2015). The amygdala's NPY neurons play a significant function in eating (Ip et al., 2019). Evidence suggests that NPY, acting through the NPY Y1 receptor is essential for controlling short-term food intake, such as after fasting and in obesity. Studies have shown that leptin reduces NPY, making their interaction the most significant mechanism in the control of body weight, the appetite by blocking the hypothalamic NPY gene and lowering the serum NPY level (Alkan et al., 2019). This study showed that a negative significant correlation between Neuropeptide and BMI ($r = -0.604$, $p < 0.001$), (Table 6). The levels of NPY in normal weight was (3.08±0.02 pg/ml), (Table 5) which was higher than both of overweight and obese with three classes (I, II, III), (Figure 1). These studies had reported similar results (Milewicz et al., 2000), (Baltazi et al., 2011), (Tyszkiewicz-Nwafor et al., 2021). Studies who showing that negative correlation between BMI and NPY were observed in obese and serum NPY levels were significantly lower in obese. Our results seem to be consistent with recently published research (Shalitin and Gat-Yablonski, 2022), who suggested that Ghrelin was found to increase the activity of proopiomelanocortin (POMC) and corticotropin-releasing hormone (CRH) cells, while decreasing the activity of neuropeptide Y/agouti-related protein (NPY/ AgRP) and hypocretin/orexin cells. Furthermore, the responses of the appetite inhibiting hormone GLP-1 remain significantly increased, whereas the orexigenic hormone ghrelin decrease in obese (Iepsen et al., 2016). GLP-1 effects on satiety may be mediated directly through the hypothalamic GLP-1-receptor (Sauer et al., 2013). In the present study positive significant correlation was detected between the GLP-1 and BMI ($r = 0.549$, $p < 0.001$), (Table 6). The levels of GLP-1 in normal weight was (2.22±0.16 ng/ml) (Table 5), which was less than both overweight and obese with three classes (I, II, III), (Figure 2). The findings in this study correlated with these studies (Purtell et al., 2011), (de Boer et al., 2016). Who reported that high levels of satiety-inducing hormone GLP-1 and self-reported feelings of satiety, significantly greater hunger persistent. In line with other studies, we discovered that obese participants had significantly higher fasting GLP-1 levels. The only independent gut peptide predictor of obesity markers was GLP-1, which is noteworthy (Stinson et al., 2021). Our results seem to be conducted with recently published research (Stinson et al., 2021), (Ali Ahmad et al., 2022). Another possibility is that GLP-1 levels in obese participants are naturally elevated as a homeostatic defense mechanism to balance out energy surplus. Overeating has been shown to raise GLP-1 levels in the fasting state, making this hypothesis plausible (Kubota and Yabe, 2021). Suggesting that fasting GLP-1 may be a marker of cardiometabolic risk. Most importantly, these results point to potential therapeutic applications. GLP-1, an incretin hormone, for example, controls glucose homeostasis and inhibits hunger. Ghrelin levels have been characterized as being lowered in obesity, and ghrelin production has been described as being particularly resistant to the inhibitory impact of food consumption. It has been proposed that decreased ghrelin production and action may play a role in obesity-related neuroendocrine and metabolic changes. Through vagal pathways, ghrelin enhances stomach emptying rate and motility. During obesity, the hypothalamic NPY and Agouti-related protein circuits resist ghrelin-induced food intake. The hypothalamus detects an excess of positive energy balance or calorie intake and responds by inhibiting the neuroendocrine ghrelin axis at the lateral ventricle (Briggs et al., 2010). In this study, significant inverse correlation was found between Ghrelin and BMI ($r = -0.173$, $p = 0.033$), (Table 6). The levels of ghrelin in normal weight was (0.97±0.03 pg/ml), (Table 5), which was higher than both overweight and obese with three classes (I, II, III), (Figure 3). This study agreed with those has been done by (Rosická et al., 2003), (Tassone et al., 2003), (Soriano-Guillén et al., 2004), (Greenman *et al.*, 2004), (Geliebter et al., 2008), (Samy et al., 2014), (Atas et al., 2021). Studies who claimed that obesity causes ghrelin resistance in arcuate NPY/AgRP neurons and confirm earlier findings that obesity disrupts the hypothalamic circuits controlling energy balance. Finally, we discovered that participants who were obese had significantly lower fasting

ghrelin levels. Obesity causes a decrease in fasting ghrelin while diet-induced weight loss causes an increase. Although the underlying mechanisms have not been clarified, it has been observed that plasma ghrelin levels significantly drop after eating. The findings in this study correlated with recently published research (Zigman et al., 2016), (Woodward et al., 2022). The level of plasma ghrelin appears to be suppressed by increased caloric intake, and decreased ghrelin may be compensatory rather than causal as a physiological response to the positive caloric balance and excess body weight. This study found lower mean values of TG levels in normal weight compared to overweight and obese with three classes (Table 4) (150.31 ± 130.464mg/dl) and (p=0.025). In addition the result of this study illustrated higher mean values of HDL levels in normal weight compared to overweight and obese with three classes (Table 4), (41.31 ± 11.047mg/dl) and (p=0.023). However, the result of this study showed lower mean values of VLDL levels in normal weight compared to overweight and obese with three classes (Table 4), (30.062 ± 26.0927mg/dl) and (p=0.025). our study conducted with these studies (Bhatti *et al*, 2001), (Garaulet et al., 2001), (Garcés et al., 2005), (Fan et al., 2019). Obesity was linked to a worse lipid profile, according to these investigations obese people had greater TG levels and decreased HDL-C. Small dense LDL cholesterol (sdLDL-c) is a distinct LDL cholesterol subtype that is associated with increased TG and decreased HDL-c levels in obesity and diabetes, and plays a distinct metabolic role in atherosclerosis. A higher level of sdLDL-c could be a key marker associated with Metabolic syndrome progression even before the appearance of central obesity, diabetes, and inflammation (Fan et al., 2019). Obesity is reason for developing risk factors such as cardiovascular, hypertension and diabetes type-2 in this study. A Numerous ideas and methods have been developed to comprehend and avoid, to underline the already present linked complications of type 2 diabetes and obesity, and to generate estimative risk models (Minciună et al., 2021). According to this study, participants who were obese had statistically significant differences in GLP-1, and ghrelin with TC, TG, HDL and VLDL. The outcome of this study noticed that GLP-1 highly significant correlated with serum T.C (r=0.207, p=0.011), TG (r=0.279, p=0.001), VLDL (r=0.279, p=0.001). And serum Ghrelin negatively correlated with HDL (r=-0.231, p=0.004), while positively correlated with TG (r=0.263, p=0.001) and VLDL (r=0.263, p=0.001) according obesity (Table 7). It is verified that a marker of obesity is increased serum levels of TC, TG, and LDL-C and decreased levels of HDL-C (Guo et al., 2022). Our findings highlight the link between obesity and peptide hormone secretion profiles that are dysregulated (Steinert et al., 2017).

5.Strengths and Limitations

To the best of our knowledge, this is the first study examining the relationships between gut peptide hormones and obesity in adults in Erbil City. In addition, we measured total GLP-1, NPY, and ghrelin, which gave us real evidence about the secretion of the hormones and their effects than measurements of intact (or active). However, there were a number of restrictions on this analysis. It began by concentrating only on fasting peptide hormone concentrations, which varied according to food intake and macronutrient composition. On the other hand, this preliminary data revealed (lipid profile) as one of the risk marker in obesity. Unresolved issues in this study may be clarified by investigating postprandial concentrations and how they relate to obesity. Additionally, our sample included people from Erbil city who were 33-66 years old. Future research is required to determine whether the relationships found here apply to other age groups. Finally, the relatively small sample size and comparative nature of the study limit the validity of our findings. Therefore, to validate our findings and identify potential cause-and-effect relationships, prospective studies with powered samples should be conducted.

6. CONCLUSIONS:

In this study, we found that obese and non-obese adults in Erbil City had significantly different serum concentrations of peptide hormones. This is the first study assessing serum (Neuropeptide Y), (Glucagon like peptide-1) and (Ghrelin) among obesity in this region. The present study concluded that serum NPY, GLP-1 and Ghrelin hormones significantly impacted controlling appetite in obesity. Due to low levels of both NPY and Ghrelin with high GLP-1levels in obese compared with non-obese individuals and a strong

relationship between these hormones and BMI, by affecting lipid profile and BMI markers GLP-1 was shown positively related to T.C, TG and VLDL, while Ghrelin negatively related to HDL and positively related with TG and VLDL in obese. GLP-1 and Ghrelin may be used as predictor in obesity. It is highly recommended that the body weight gain and increase in appetite were correlated with the actions of these hormones in obesity. According to this study, these hormones were significant in controlling appetite in obesity.

Acknowledgement
The authors would thank the operators and technics staff of Erbil cardiac center and employees of Erbil's court for their supports and cooperation during the experimental data collection procedures.

Conflict of Interest
The authors declared that they have no conflicts of interest.

Table 1. Habits and Socio-demographic characteristics

Variables	**No. (%)**
Age (years)	
30-39	66 (43.4 %)
40-49	46 (30.3 %)
50-59	28 (18.4 %)
≥ 60	12 (7.9 %)
Gender	
Male	108 (71.1 %)
Female	44 (28.9 %)
Smoking	
Never	79 (52 %)
Ex-smoker	20 (13.2 %)
Current smoker	53(34.9**%**)
Consumption of sweets, soft drinks and Junk foods	
Yes	125(82.2**%**)
No	27(17.8**%**)
Alcohol	
Never	139(91.4**%**)
Occasionally	3(2**%**)

Sometimes	10(6.6%)
Diet	
I eat meat	148(97.4%)
I don't love meat	4(2.6%)
Meals	
Once	8(5.3%)
Twice	30(19.7%)
Three times	103(67.8%)
More than three times	11(7.2%)
Total	**152(100%)**

Table 2. Medical History

Variables	**No. (%)**
Diabetes	
Type 2 DM	20(13.2%)
No DM	132(86.8%)
Chronic heart disease	
None	123(80.9%)
Cardiovascular disease (CVD)	12(7.9%)
Congestive heart failure (CHF)	7(4.6%)
CVD and CHF	5(3.3%)
CVD and previous myocardial infarction	5(3.3%)
Hypertension	
Yes	25(16.4%)
No	127(83.6%)

Total	**152(100%)**

Table 3. Arithmetic means of biochemical parameters in the study population

Variables	**Mean ±SD**	**p-value**
FBS (mg/dl)	124.86± 43.69	<0.001
BMI (kg/m^2)	29.73± 7.49	<0.001
Total cholesterol (mg/dl)	165.37± 36.75	0.15
Triglycerides (mg/dl)	187.72± 119.03	0.004
HDL (mg/dl)	39.07± 9.30	0.37
LDL (mg/dl)	88.79± 30.67	0.83
VLDL (mg/dl)	37.55± 23.80	0.004

FBS: Fasting blood sugar, BMI: Body mass index, SD: Standard deviation, TC: Total Cholesterol, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very-low-density lipoprotein, p: p-value

Table 4: Arithmetic means of biochemical parameters among groups

Metabolic parameters		**N**	**Mean ± SD**	**95% CI Lower-Upper**	**p-value**
Total Cholesterol (mg/dl)	Normal	55	161.09 ± 40.806	150.06-172.12	0.383
	Overweight	42	166.17 ±33.185	155.83-176.51	
	Obese class I	15	175.33± 44.089	150.92-199.75	
	Obese class II	20	157.90 ± 31.417	143.20-172.60	
	Obese class III	20	175.45± 30.120	161.35-189.55	
	Total	152	165.37 ± 36.751	159.48-171.26	
Triglycerides (mg/dl)	Normal	55	150.31 ± 130.464	115.04-185.58	0.025
	Overweight	42	189.14 ± 83.029	163.27-215.02	

	Obese class I	15	239.60 ± 150.588	156.21-322.99	
	Obese class II	20	220.70 ± 103.690	172.17-269.23	
	Obese class III	20	215.70 ± 117.181	160.86-270.54	
	Total	152	187.72 ± 119.027	168.64-206.79	
HDL (mg/dl)	Normal	55	41.31 ± 11.047	38.32-44.30	0.023
	Overweight	42	36.81 ± 6.876	34.67-38.95	
	Obese class I	15	40.53± 11.167	34.35-46.72	
	Obese class II	20	34.75 ± 7.376	31.30-38.20	
	Obese class III	20	40.85± 6.450	37.83-43.87	
	Total	152	39.07 ± 9.299	37.58-40.56	
LDL (mg/dl)	Normal	55	89.720± 27.3769	82.319-97.121	0.645
	Overweight	42	91.521± 32.1038	81.517-101.526	
	Obese class I	15	86.880 ± 39.6962	64.897-108.863	
	Obese class II	20	79.260 ± 28.4193	65.959-92.561	
	Obese class III	20	91.465 ± 32.0645	76.458-106.472	
	Total	152	88.791 ± 30.6708	83.876-93.706	
VLDL (mg/dl)	Normal	55	30.062 ± 26.0927	23.008-37.116	0.025
	Overweight	42	37.836 ± 16.6016	32.662-43.009	
	Obese class I	15	47.920 ± 30.1176	31.241-64.599	
	Obese class II	20	44.140 ± 20.7380	34.434-53.846	
	Obese class III	20	43.140 ± 23.4363	32.171-54.109	
	Total	152	37.545 ± 23.8045	33.731-41.360	

Table 5. Descriptive statistics of the studied parameters by BMI categories

Hormone Parameters	**BMI**	**Mean ± SE**	**95% CI Lower-Upper**	**p-value**
Neuropeptide Y (pg/ml)	**Normal**	3.08±0.02	3.03-3.12	
	Overweight	2.80±0.06	2.67-2.92	
	Class I*	2.32±0.06	2.19-2.45	
	Class II*	2.22±0.08	2.05-2.39	

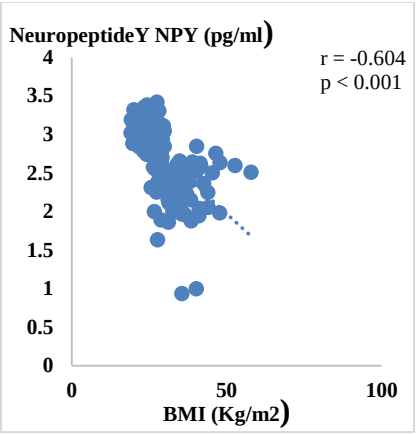
	Class III*	2.37±0.09	2.18-2.57	<0.001
	Total	2.72±0.04	2.65-2.79	
Glucagon likepeptide-1(ng/ml)	**Normal**	2.22±0.16	1.90-2.54	
	Overweight	3.32±0.10	3.11-3.52	
	Class I*	3.71±0.02	3.67-3.76	
	Class II*	3.67±0.08	3.50-3.84	
	Class III*	3.81±0.01	3.79-3.83	<0.001
	Total	3.07±0.08	2.90-3.23	
Ghrelin (pg/ml)	**Normal**	0.97±0.03	0.90-1.04	
	Overweight	0.92±0.05	0.83-1.01	
	Class I*	0.80±0.07	0.64-0.95	
	Class II*	0.79±0.06	0.67-0.91	
	Class III*	0.88±0.05	0.78-0.97	0.033
	Total	0.90±0.02	0.86-0.95	

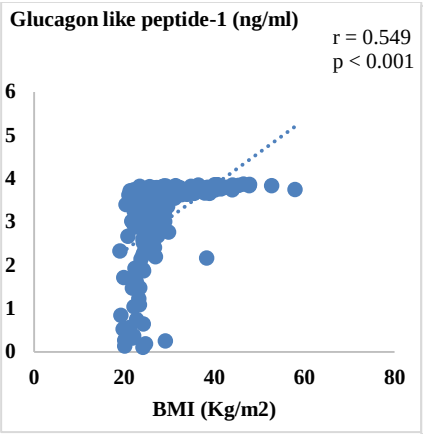
*Refers to classes of obesity. CI: Confidence interval. By ANOVA, p: P-Value for all, SE: Standard Error.

Table 6. Correlation between Hormones with BMI

Variables	**Pearson correlation and P-Value**	**BMI(Kg/m^2)**
Neuropeptide Y (pg/mL)	r	-0.604
	p-Value	< 0.001
Glucagonlikepeptide-1(ng/mL)	r	0.549
	p-Value	< 0.001
Ghrelin (pg/mL)	r	-0.173
	p-Value	0.033

BMI: Body mass index, p: P-Value, r: Pearson correlation





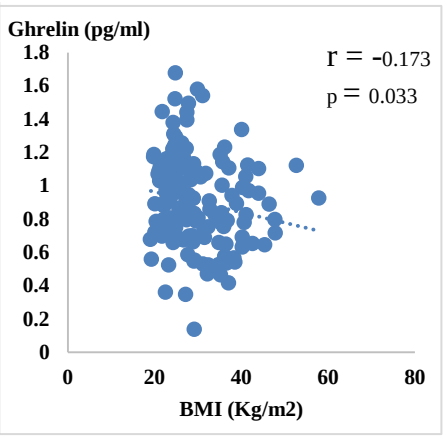


Figure 1: Correlation between BMI and Neuropeptide Y (NPY) **Figure 2: Correlation between BMI and Glucagon like Peptide-1** **Figure 3: Correlation between BMI and Ghrelin**

Table 7: Correlation between hormone parameters with lipid parameters in obesity

Hormone parameters	**Pearson correlation and p-value**	**T.C mg/dl**	**TGs mg/dl**	**HDL mg/dl**	**LDL mg/dl**	**VLDL mg/dl**
Neuropeptide Y (NPY) (pg/mL)	r	-0.152	-0.131	0.003	-0.083	-0.131
	p-value	0.06	0.106	0.966	0.303	0.106
Glucagon likepeptide-1(ng/mL)	r	0.207	0.279	0.004	0.029	0.279
	p-value	0.011	0.001	0.961	0.714	0.001
Ghrelin (pg/mL)	r	0.038	0.263	-0.231	-0.09	0.263
	p-value	0.642	0.001	0.004	0.268	0.001

References:

Acosta, A., Camilleri, M., Shin, A., Vazquez-Roque, M.I., Iturrino, J., Burton, D., O'Neill, J., Eckert, D., Zinsmeister, A.R., 2015. Quantitative Gastrointestinal and Psychological Traits Associated With Obesity and Response to Weight-Loss Therapy. Gastroenterology 148, 537-546.e4. https://doi.org/10.1053/j.gastro.2014.11.020

Ali Ahmad, M., Karavetian, M., Moubareck, C.A., Wazz, G., Mahdy, T., Venema, K., 2022. The Association between Peptide Hormones with Obesity and Insulin Resistance Markers in Lean and Obese Individuals in the United Arab Emirates. Nutrients 14, 1271. https://doi.org/10.3390/nu14061271

Alkan, I., Altunkaynak, B.Z., Altun, G., Erener, E., 2019. The investigation of the effects of topiramate on the hypothalamic levels of fat mass/obesity-associated protein and neuropeptide Y in obese female rats. Nutr. Neurosci. 22, 243–252. https://doi.org/10.1080/1028415X.2017.1374033

Atas, U., Erin, N., Tazegul, G., Elpek, G.O., Yildirim, B., 2021. Changes in ghrelin, substance P and vasoactive intestinal peptide levels in the gastroduodenal mucosa of patients with morbid obesity. Neuropeptides 89, 102164. https://doi.org/10.1016/j.npep.2021.102164

Baltazi, M., Katsiki, N., Savopoulos, C., Iliadis, F., Koliakos, G., Hatzitolios, A.I., 2011. Plasma neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (a-MSH) levels in patients

with or without hypertension and/or obesity: a pilot study. Am. J. Cardiovasc. Dis. 1, 48–59.

Bhatti, M.S., Akbri, M.Z., Shakoor, M., 2001. Lipid profile in obesity. J. Ayub Med. Coll. Abbottabad JAMC 13, 31–33.

Briggs, D.I., Andrews, Z.B., 2011. Metabolic Status Regulates Ghrelin Function on Energy Homeostasis. Neuroendocrinology 93, 48–57. https://doi.org/10.1159/000322589

Briggs, D.I., Enriori, P.J., Lemus, M.B., Cowley, M.A., Andrews, Z.B., 2010. Diet-Induced Obesity Causes Ghrelin Resistance in Arcuate NPY/AgRP Neurons. Endocrinology 151, 4745–4755. https://doi.org/10.1210/en.2010-0556

de Boer, S.A., Lefrandt, J.D., Petersen, J.F., Boersma, H.H., Mulder, D.J., Hoogenberg, K., 2016. The effects of GLP-1 analogues in obese, insulin-using type 2 diabetes in relation to eating behaviour. Int. J. Clin. Pharm. 38, 144–151. https://doi.org/10.1007/s11096-015-0219-8

Fan, J., Liu, Y., Yin, S., Chen, N., Bai, X., Ke, Q., Shen, J., Xia, M., 2019. Small dense LDL cholesterol is associated with metabolic syndrome traits independently of obesity and inflammation. Nutr. Metab. 16, 7. https://doi.org/10.1186/s12986-019-0334-y

Flehmig, G., Scholz, M., Klöting, N., Fasshauer, M., Tönjes, A., Stumvoll, M., Youn, B.-S., Blüher, M., 2014. Identification of Adipokine Clusters Related to Parameters of Fat Mass, Insulin Sensitivity and Inflammation. PLOS ONE 9, e99785. https://doi.org/10.1371/journal.pone.0099785

Garaulet, M., Pérez-Llamas, F., Pérez-Ayala, M., Martínez, P., de Medina, F.S., Tebar, F.J., Zamora, S., 2001. Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. Am. J. Clin. Nutr. 74, 585–591. https://doi.org/10.1093/ajcn/74.5.585

Garcés, C., Gutierrez-Guisado, J., Benavente, M., Cano, B., Viturro, E., Ortega, H., de Oya, M., 2005. Obesity in Spanish Schoolchildren: Relationship with Lipid Profile and Insulin Resistance. Obes. Res. 13, 959–963. https://doi.org/10.1038/oby.2005.111

Geliebter, A., Hashim, S.A., Gluck, M.E., 2008. Appetite-related gut peptides, ghrelin, PYY, and GLP-1 in obese women with and without binge eating disorder (BED). Physiol. Behav. 94, 696–699. https://doi.org/10.1016/j.physbeh.2008.04.013

Greenman, Y., Golani, N., Gilad, S., Yaron, M., Limor, R., Stern, N., 2004. Ghrelin secretion is modulated in a nutrient- and gender-specific manner. Clin. Endocrinol. (Oxf.) 60, 382–388. https://doi.org/10.1111/j.1365-2265.2004.01993.x

Greiling, H., Gressner, A.M., 1995. Lehrbuch der Klinischen Chemie und der Pathobiochemie. Schattauer, F.K. Verlag, Stuttgart New York.

Guo, X., Zhou, Z., Lyu, X., Xu, H., Zhu, H., Pan, H., Wang, L., Yang, H., Gong, F., 2022. The Antiobesity Effect and Safety of GLP-1 Receptor Agonist in Overweight/Obese Patients Without Diabetes: A Systematic Review and Meta-Analysis. Horm. Metab. Res. 54, 458–471. https://doi.org/10.1055/a-1844-1176

Hanssen, R., Kretschmer, A.C., Rigoux, L., Albus, K., Edwin Thanarajah, S., Sitnikow, T., Melzer, C., Cornely, O.A., Brüning, J.C., Tittgemeyer, M., 2021. GLP-1 and hunger modulate incentive motivation depending on insulin sensitivity in humans. Mol. Metab. 45, 101163. https://doi.org/10.1016/j.molmet.2021.101163

Iepsen, E.W., Lundgren, J., Holst, J.J., Madsbad, S., Torekov, S.S., 2016. Successful weight loss maintenance includes long-term increased meal responses of GLP-1 and PYY3–36. Eur. J. Endocrinol. 174, 775–784. https://doi.org/10.1530/EJE-15-1116

Ip, C.K., Zhang, L., Farzi, A., Qi, Y., Clarke, I., Reed, F., Shi, Y.-C., Enriquez, R., Dayas, C., Graham, B., Begg, D., Brüning, J.C., Lee, N.J., Hernandez-Sanchez, D., Gopalasingam, G., Koller, J., Tasan, R., Sperk, G., Herzog, H., 2019. Amygdala NPY Circuits Promote the Development of Accelerated Obesity under Chronic Stress Conditions. Cell Metab. 30, 111-128.e6. https://doi.org/10.1016/j.cmet.2019.04.001

Katus, U., Villa, I., Ringmets, I., Veidebaum, T., Harro, J., 2021. Neuropeptide Y gene variants in obesity, dietary intake, blood pressure, lipid and glucose metabolism: A longitudinal birth cohort study. Peptides 139, 170524. https://doi.org/10.1016/j.peptides.2021.170524

Koliaki, C., Liatis, S., Dalamaga, M., Kokkinos, A., 2020. The Implication of Gut Hormones in the Regulation of Energy Homeostasis and Their Role in the Pathophysiology of Obesity. Curr. Obes. Rep. 9, 255–271. https://doi.org/10.1007/s13679-020-00396-9

Kubota, S., Yabe, D., 2021. Elevation of Fasting GLP-1 Levels in Child and Adolescent Obesity: Friend or Foe? J. Clin. Endocrinol. Metab. 106, e3778–e3780. https://doi.org/10.1210/clinem/dgab301

Milewicz, A., Bidzinska, B., Mikulski, E., Demissie, M., Tworowska, U., 2000. Influence of obesity and menopausal status on serum leptin, cholecystokinin, galanin and neuropeptide Y levels. Gynecol. Endocrinol. 14, 196–203. https://doi.org/10.3109/09513590009167682

Minciună, I.-A., Hilda Orășan, O., Minciună, I., Lazar, A.-L., Sitar-Tăut, A.V., Oltean, M., Tomoaia, R., Puiu, M., Sitar-Tăut, D.-A., Pop, D., Cozma, A., 2021. Assessment of subclinical diabetic cardiomyopathy by speckle-tracking imaging. Eur. J. Clin. Invest. 51, e13475. https://doi.org/10.1111/eci.13475

Muñoz, J.S.G., Rodríguez, D.J., Morante, J.J.H., 2015. Diurnal rhythms of plasma GLP-1 levels in normal and overweight/obese subjects: lack of effect of weight loss. J. Physiol. Biochem. 71, 17–28. https://doi.org/10.1007/s13105-014-0375-7

Nauck, M., März, W., Jarausch, J., Cobbaert, C., Sägers, A., Bernard, D., Delanghe, J., Honauer, G., Lehmann, P., Oestrich, E., von Eckardstein, A., Walch, S.,

Wieland, H., Assmann, G., 1997. Multicenter evaluation of a homogeneous assay for HDL-cholesterol without sample pretreatment. Clin. Chem. 43, 1622–1629.

Newmyer, B.A., Nandar, W., Webster, R.I., Gilbert, E., Siegel, P.B., Cline, M.A., 2013. Neuropeptide Y is associated with changes in appetite-associated hypothalamic nuclei but not food intake in a hypophagic avian model. Behav. Brain Res. 236, 327–331. https://doi.org/10.1016/j.bbr.2012.08.015

Pardak, P., Filip, R., Woliński, J., 2022. The Impact of Sleep-Disordered Breathing on Ghrelin, Obestatin, and Leptin Profiles in Patients with Obesity or Overweight. J. Clin. Med. 11, 2032. https://doi.org/10.3390/jcm11072032

Purtell, L., Sze, L., Loughnan, G., Smith, E., Herzog, H., Sainsbury, A., Steinbeck, K., Campbell, L.V., Viardot, A., 2011. In adults with Prader–Willi syndrome, elevated ghrelin levels are more consistent with hyperphagia than high PYY and GLP-1 levels. Neuropeptides 45, 301–307. https://doi.org/10.1016/j.npep.2011.06.001

Rifai, N., Warnick, G.R., McNamara, J.R., Belcher, J.D., Grinstead, G.F., Frantz, I.D., 1992. Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clin. Chem. 38, 150–160.

Rosická, M., Kršek, M., Matoulek, M., Jarkovská, Z., Marek, J., Justová, V., Lacinová, Z., 2003. Serum ghrelin levels in obese patients: the relationship to serum leptin levels and soluble leptin receptors levels. Physiol Res 61–66.

Samy, W., Hassanien, M., El, K., Khosheim, K., 2014. Role of Ghrelin, Leptin and Insulin Resistance in Development of Metabolic Syndrome in Obese Patients 3, 1–1000122.

Sauer, N., Rösch, T., Pezold, J., Reining, F., Anders, M., Groth, S., Schachschal, G., Mann, O., Aberle, J., 2013. A New Endoscopically Implantable Device (SatiSphere) for Treatment of Obesity—Efficacy, Safety, and Metabolic Effects on Glucose, Insulin, and GLP-1 Levels. Obes. Surg. 23, 1727–1733. https://doi.org/10.1007/s11695-013-1005-0

Shalitin, S., Gat-Yablonski, G., 2022. Associations of Obesity with Linear Growth and Puberty. Horm. Res. Paediatr. 95, 120–136. https://doi.org/10.1159/000516171

Soriano-Guillén, L., Barrios, V., Campos-Barros, Á., Argente, J., 2004. Ghrelin levels in obesity and anorexia nervosa: effect of weight reduction or recuperation. J. Pediatr. 144, 36–42. https://doi.org/10.1016/j.jpeds.2003.10.036

Steinert, R.E., Feinle-Bisset, C., Asarian, L., Horowitz, M., Beglinger, C., Geary, N., 2017. Ghrelin, CCK, GLP-1, and PYY(3–36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. Physiol. Rev. 97, 411–463. https://doi.org/10.1152/physrev.00031.2014

Stinson, S.E., Jonsson, A.E., Lund, M.A.V., Frithioff-Bøjsøe, C., Aas Holm, L., Pedersen, O., Ängquist, L., Sørensen, T.I.A., Holst, J.J., Christiansen, M., Holm, J.-C., Hartmann, B., Hansen, T., 2021. Fasting Plasma GLP-1 Is Associated With Overweight/Obesity and Cardiometabolic Risk Factors in Children and Adolescents. J. Clin. Endocrinol. Metab. 106, 1718–1727. https://doi.org/10.1210/clinem/dgab098

Tassone, F., Broglio, F., Destefanis, S., Rovere, S., Benso, A., Gottero, C., Prodam, F., Rossetto, R., Gauna, C., van der Lely, A.J., Ghigo, E., Maccario, M., 2003. Neuroendocrine and Metabolic Effects of Acute Ghrelin Administration in Human Obesity. J. Clin. Endocrinol. Metab. 88, 5478–5483. https://doi.org/10.1210/jc.2003-030564

Tyszkiewicz-Nwafor, M., Jowik, K., Dutkiewicz, A., Krasinska, A., Pytlinska, N., Dmitrzak-Weglarz, M., Suminska, M., Pruciak, A., Skowronska, B., Slopien, A., 2021. Neuropeptide Y and Peptide YY in Association with Depressive Symptoms and Eating Behaviours in Adolescents across the Weight Spectrum: From Anorexia Nervosa to Obesity. Nutrients 13, 598. https://doi.org/10.3390/nu13020598

Villanueva-Peñacarrillo, M.L., Martín-Duce, A., Ramos-Álvarez, I., Gutiérrez-Rojas, I., Moreno, P., Nuche-Berenguer, B., Acitores, A., Sancho, V., Valverde, I., González, N., 2011. Characteristic of GLP-1 effects on glucose metabolism in human skeletal muscle from obese patients. Regul. Pept. 168, 39–44. https://doi.org/10.1016/j.regpep.2011.03.002

Woodward, O.R.M., Gribble, F.M., Reimann, F., Lewis, J.E., 2022. Gut peptide regulation of food intake – evidence for the modulation of hedonic feeding. J. Physiol. 600, 1053–1078. https://doi.org/10.1113/JP280581

Zigman, J.M., Bouret, S.G., Andrews, Z.B., 2016. Obesity Impairs the Action of the Neuroendocrine Ghrelin System. Trends Endocrinol. Metab. 27, 54–63. https://doi.org/10.1016/j.tem.2015.09.010