



The relationship between Adipocytokines & Ghrelin and Obesity associated with Type II Diabetes Mellitus among Jordanians

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General Note



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ABSTRACT

Background: Type II DM and obesity are both chronic diseases of high morbidity and mortality rates. Impaired glucose regulation is contributed to DM. Obesity is under effect of appetite regulating peptides including leptin and adiponectin, ghrelin and NPY. **Aim:** This case control study aimed to evaluate the association between leptin, adiponectin, ghrelin and NPY in a sample of Jordanian patients with obesity associated with type II DM, and to figure out their possible roles in obesity pathogenesis. **Subjects and Materials:** 188 subjects (90 diabetic patients and 98 normal healthy subjects as a control group) participated in the study, BMI was determined for all subjects. Blood glucose, triacylglycerols and cholesterol were assayed chemically. ELISA was used to estimate insulin, leptin, adiponectin, ghrelin and NPY. **Results:** Insulin level in NODs was significantly lower than NONDs, while, the difference was insignificant between ONDs and ODs. There was significant increase in leptin levels ODs versus NONDs. The levels of adiponectin showed significant decrease in ODs compared to ONDs and NODs. Ghrelin was increased significantly in ONDs and ODs versus NONDs and NODs. NPY was higher significantly in ONDs than NONDs as well as ODs than NODs. **Conclusion:** BMI is significantly correlated to leptin, adiponectin, ghrelin and NPY in the ODs; also, the significant correlations between leptin-adiponectin, leptin-ghrelin, leptin-NPY, adiponectin-ghrelin, adiponectin-NPY and ghrelin-NPY in ODs may help to explore the role of these hormones in the pathogenesis of obesity related complications as type II diabetes mellitus.

Keywords: Adipocytokines, type II diabetes mellitus, obesity, neuropeptide Y, adipogenesis, insulin sensitivity

1. INTRODUCTION

Obesity is a global epidemic multifactorial disease, it causes illness in the childhood and adolescent life worldwide, also, contributed in the pathogenesis of chronic diseases such as type II diabetes mellitus (type II DM), hypercholesterolemia, hypertension and heart disease (Nathan and Moran, 2008). Obesity results from the disturbance in regulating energy expenditure resulting in enhancing adipogenesis, fat retention (Mishra et al., 2016) and secretion of hormones like ghrelin which can modulate metabolism (Kojima and Kangawa, 2005). Appetite regulating peptides are among the biological factors which play an important role in the etiology of obesity, including besides ghrelin, neuromedin-beta, leptin, and uncoupling proteins, endocannabinoids and their receptors (Ghalandari et al., 2015).

Diabetes mellitus is a global epidemic metabolic disorder that is characterized by hyperglycaemia, glycosuria, glucose intolerance, insulin resistance and β -cell dysfunction (Guariguata et al., 2014). Its prevalence is expected to affect 592 million of the population of the world by the year 2035; type II diabetes accounts for 90-95% of all diabetics, its pathogenesis can be explained by several mechanisms contributed to impaired glucose regulation including obesity, lipotoxicity, and oxidative stress (Wild et al., 2004). Adipocytokines, the polypeptides secreted mainly by adipocytes and inflammatory cells, they include leptin and adiponectin, inflammatory cytokines like TNF- α , interleukin-6 and other proteins such as angiotensinogen and resistin. Insulin sensitization and appetite regulation are among their physiological roles (Sattar, 2012). The relationship between adipocytokines, insulin resistance, obesity and type II DM was reported in various studies which showed that adiponectin levels were significantly decreased both in obesity and type II DM (Calle and Fernandez, 2012). Also, low adiponectin levels were found to be correlated with high plasma insulin and high insulin resistance (Weyer et al., 2001).

Previous studies reported a direct proportion relationship between increased leptin levels, leptin resistance and obesity degree; also, there is an association between leptin levels and insulin resistance (Hajer et al., 2008). Ghrelin, the appetite stimulating hormone, is produced as a 117 amino acids preproghrelin molecule by X/A-like cells within gastric oxytic glands, then, cleaved into a small signal peptide, ghrelin and obestatin. Ghrelin exerts its (a G protein-coupled receptor) biological effects through growth hormone secretagogue receptor 1a (GHSR1a) receptor, with a high expression rate in different organs including pituitary, pancreatic islets and adrenals (Gnanapavan et al., 2002). Its receptor can be activated by addition of C8 or C10 fatty acid at third position its N-terminal end of by ghrelin O-acyl-transferase (Gutierrez et al., 2008).

The acylated ghrelin causes insulin resistance and DM progression due to its hyperglycemic effects (Sharifi et al., 2013), while, the enhancement of insulin sensitivity was observed by the unacylated form due to its hyperglycemic counter effects. Regulating the level of plasma ghrelin is inevitable for DM management which can be done by insulin administration (Tong et al., 2010). Ghrelin stimulates food intake and increases BMI in humans considered as one of the regulatory factors for appetite, energy intake and energy expenditure (Miljković et al., 2017). NPY, 36 amino acids, is a widely distributed in CNS of humans. It activates a family of at least six G protein-coupled Y receptors (Y1-Y6) found in multiple tissues including those involved in metabolism such as liver and

adipose tissues. In obesity, NPY is stimulating orexigenic pathways via Y1 receptor activation in CNS and in peripheral tissues via Y2 and Y5 receptors. In adipocytes, NPY enhances lipogenesis and inhibits lipolysis suggesting its significant role in lipid uptake and storage (Larsson et al., 2006; Kos et al., 2009).

Aim

To investigate the association between ghrelin, leptin, adiponectin and NPY in a sample of Jordanian patients with obesity associated with type II DM, also, to explore their possible roles in the pathogenesis of obesity.

2. SUBJECTS AND MATERIALS

Flow Chart of the Methodology

A. Technical Design

1. A case control study
2. Study population: in Al-Karak city, the target population was 188 subjects of Jordanian ethnic origin, whether they were already or newly diagnosed as diabetic patients and checking in the Diabetic Clinic.

Blood samples were collected from the patients and the controls in the laboratory of Al-Karak Governmental Hospital under complete care circumstances to avoid any harm effects for the patients and controls, also, the hemolysis of the blood samples. Validity and reliability of the results were checked by estimating glucose. Triacylglycerols and cholesterol twice, while, the determinations of insulin, leptin, adiponectin, ghrelin and NPY using ELISA method were carried out in triplets to avoid any mistakes during the process.

B. Statistical design: SPSS statistical package, version 20, Student's unpaired *t* test, ANOVA and Multivariate linear regression analyses were applied to the obtained results of the present study.

C. Administrative design: all aspects of the methods of the study were designed, planned and reviewed by all authors.

D. Operational design was subdivided into preparatory phase in which the criteria for selecting the patients and the controls, also the exclusion criteria were settled down, checking the instrumentation for validity and reliability and chemical supplies for the analyses, samples collection phase in the laboratory of Al-Karak Governmental Hospital under complete supervision of most of the authors, then, performing the chemical and the hormonal analyses, statistical analysis phase of the obtained results. Finally, results presentation phase by tabulating the results and preparing the figures to represent the work in the present study.

98 healthy subjects (control group) and 90 diabetic patients group were enrolled in the study. Both groups were subdivided into obese and non-obese according to BMI (BMI > 30 considered is obese). Subjects participated in the study were selected from those attending the Diabetic Clinic, Al-Karak Governmental Hospital. Type II DM. was diagnosed based on American Diabetes Association criteria (2009). Insulin-treated diabetic patients, patients with liver and renal impairment, subjects suffering from chronic disease or taking medications known to have effects on glucose metabolism and patients on diet regimen during the last two weeks before the study were excluded from the study. A fasting blood sample was withdrawn from each participant in a heparinized test tube, centrifuged and plasma was transferred to a plain test tube, then, rapidly frozen (-80°C) for hormonal analyses.

Chemical analyses

Plasma glucose level was determined using by glucose oxidase method (Trinder, 1969). Triacylglycerols and cholesterol were assayed by commercial kits provided by Boehringer Mannheim, Germany according to the methods of Richmond, (1973), Jacobs and Vandemark, (1960), respectively.

Hormonal analyses

Insulin, leptin, adiponectin, ghrelin and NPY were determined by ELISA kits supplied by MyBiosource/USA/Canada using the method of Engvall et al., (1971).

Statistical analyses: was made using the SPSS statistical package, version 20. All values were expressed as mean±SD. When comparing two groups, a Student's unpaired *t* test was applied. To analyze data among groups of three or more, a one way ANOVA was used and secondary analysis was performed using Student's *t*. P value <0.05 was considered statistically significant. Multivariate linear regression analysis was carried out to evaluate the relationship between the parameters. Universal correlations were performed using Pearson correlation and p value <0.05 was considered statistically significant.

3. RESULTS

Our obtained results (table 1) revealed that BMI are 24.8 ± 1.8 , 30.3 ± 3.0 , 27.8 ± 1.9 and 34.1 ± 3.7 Kg/m^2 for NOND, OND, NOD and OD groups, respectively. There is a statistically significant difference ($p < 0.05$) regarding BMI of ONDs versus NONDs, also, BMI of ODs was significantly higher ($p < 0.05$) than NODs. There was insignificant difference of BMI of NONDs when compared to NODs, also, BMI of ONDs versus ODs.

There is an increase in blood glucose level in diabetic patients especially the obese ones. Triacylglycerols and cholesterol levels are higher in diabetic patients when compared to the relevant non-diabetic subjects ($p < 0.05$). Insulin level in NODs (4.79 ± 0.37 ng/ml) was significantly lower than NONDs (5.6 ± 0.9 ng/ml). There is statistically insignificant difference between NODs and ODs regarding the level of insulin. The mean levels of leptin were elevated in obese subjects versus the non-obese ones ($p < 0.05$) especially the diabetic patients. Also, there is a significant increase leptin levels in diabetic patients when compared to the non-diabetic subjects ($p < 0.05$). Adiponectin levels were significantly lower in ODs versus the ONDs and NODs ($p < 0.05$). The results of the study showed higher levels of ghrelin ($p < 0.05$) in ONDs and ODs when compared to ONDs and NODs, respectively. NPY levels are increased significantly in ONDs and ODs versus NONDs and NODs ($p < 0.05$), also, it is significantly higher in diabetic patients than the non-diabetic ones ($p < 0.05$) either obese or non-obese.

Table 1 Demographic and laboratory data

Variables	Non-diabetic		Diabetic		F (between groups)	P
	NONDs	ONDs	NODs	ODs		
N	55	43	49	41		
Age (years)	46.3 ± 2.9	47.7 ± 2.3	49.4 ± 2.4	50.8 ± 2.6		
BMI (Kg/m^2)	24.8 ± 1.8	$30.3 \pm 3.0^*$	27.8 ± 1.9	$34.1 \pm 3.7^{**}$	9.73	0.000
Glucose (mg/dl)	90.07 ± 3.17	98.61 ± 4.48	$158.1 \pm 7.13^*$	$279.0 \pm 16.76^{**}$	94.62	0.000
Triacylglycerols (mg/dl)	133.67 ± 7.23	$155.72 \pm 11.58^*$	$220.86 \pm 8.75^*$	$253.83 \pm 11.9^{**}$	20.60	0.000
Cholesterol (mg/dl)	179.35 ± 5.92	$178.74 \pm 8.15^*$	$199.49 \pm 8.52^*$	$217.73 \pm 12.30^{**}$	4.40	0.005
Insulin (ng/ml)	5.61 ± 0.12	4.51 ± 0.14	$4.79 \pm 0.05^*$	4.56 ± 0.08	0.000	25.46
Leptin (ng/ml)	14.58 ± 0.2	$16.72 \pm 0.36^*$	28.59 ± 1.33	$35.86 \pm 2.92^{***}$	5.92	0.001
Adiponectin ($\mu\text{g/ml}$)	4.54 ± 0.16	4.03 ± 0.11	3.97 ± 0.09	$3.49 \pm 0.11^{***}$	24.75	0.000
Ghrelin (fmol/l)	81.92 ± 3.32	$161.54 \pm 10.3^*$	$156.87 \pm 9.3^*$	$222.14 \pm 14.03^{***}$	24.64	0.000
Neuropeptide Y (ng/ml)	7.0 ± 0.33	9.93 ± 0.4	11.51 ± 1.1	$13.98 \pm 0.63^{***}$	14.24	0.000

* $p < 0.05$ significant when NOND are compared to NOD individuals

** $p < 0.05$ significant when OND are compared to OD individuals

$p < 0.05$ significant when NOND are compared to OND individuals

$p < 0.05$ significant when NOD are compared to OD individuals

Table 2 and figure 1 show the correlation between BMI and the other studied parameters. There is positive significant correlation between BMI and glucose, triacylglycerols, cholesterol, leptin, ghrelin and neuropeptide Y ($r = 0.48$, $p = 0.043$, $r = 0.67$, $p < 0.01$, $r = 0.50$, $p = 0.031$, $r = 0.92$, $p < 0.001$, $r = 0.91$, $p < 0.001$ and $r = 0.87$, $p < 0.001$, respectively). There is a negative significant correlation between BMI and adiponectin ($r = -0.79$, $p < 0.001$), while, no significant correlation between BMI and insulin levels ($r = 0.13$, $p > 0.05$).

Table 2 Correlation between BMI and the other studied parameters

Variables	BMI	
	Correlation coefficient (r)	p
Glucose	0.48	0.043
Insulin	0.13	>0.05
Triacylglycerols	0.67	<0.01
Cholesterol	0.50	0.031
Leptin	0.92	<0.001
Adiponectin	-0.79	<0.001

Ghrelin	0.91	<0.001
NPY	0.87	<0.001

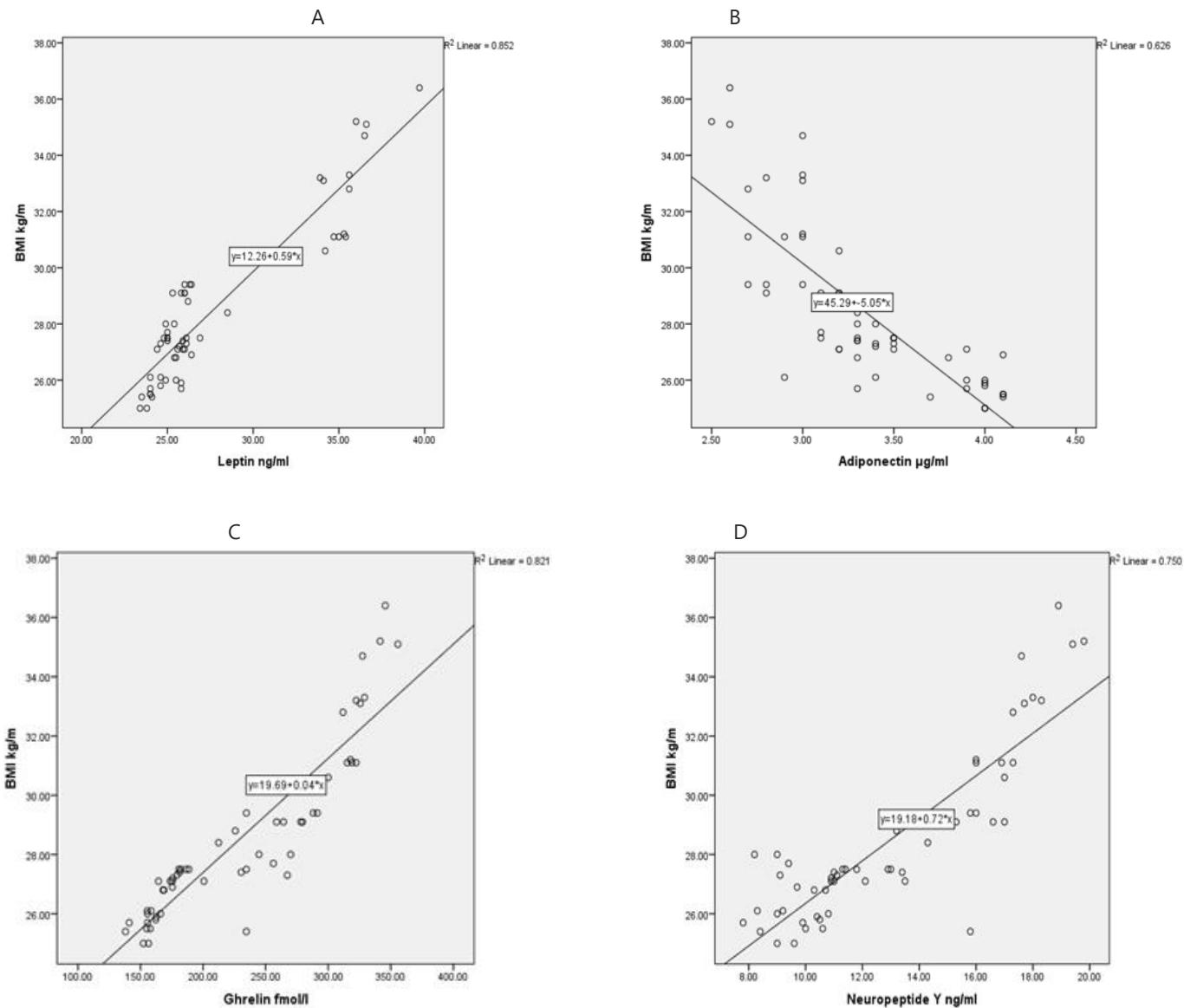


Figure 1 Correlation between BMI and leptin (A), adiponectin (B), ghrelin (C) and NPY (D)

The results revealed a negative significant correlation between leptin and adiponectin ($r=-0.66$, $p<0.001$) and positively significantly correlated with neuropeptide Y and ghrelin ($r=0.82$, $p<0.001$, $r=0.79$, $p<0.001$, respectively) in OD patients. Adiponectin shows a negative correlation with ghrelin ($r=-0.76$, $p<0.001$). The results showed negatively significant correlation between adiponectin and both ghrelin ($r=-0.76$, $p<0.001$), and neuropeptide Y ($r=-0.66$, $p<0.001$) in OD patients, also, ghrelin shows positive correlation with neuropeptide Y in OD patients ($r=0.86$, $p<0.001$) (table 3 and figure 2).

Table 3 The correlation between leptin, adiponectin, ghrelin and neuropeptide Y in the OD group of patients

	Leptin		Adiponectin		Ghrelin	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Adiponectin	- 0.890	<0.001				
Ghrelin	0.820	<0.001	- 0.760	<0.001		
Neuropeptide Y	0.790	<0.001	- 0.793	<0.001	0.897	<0.001

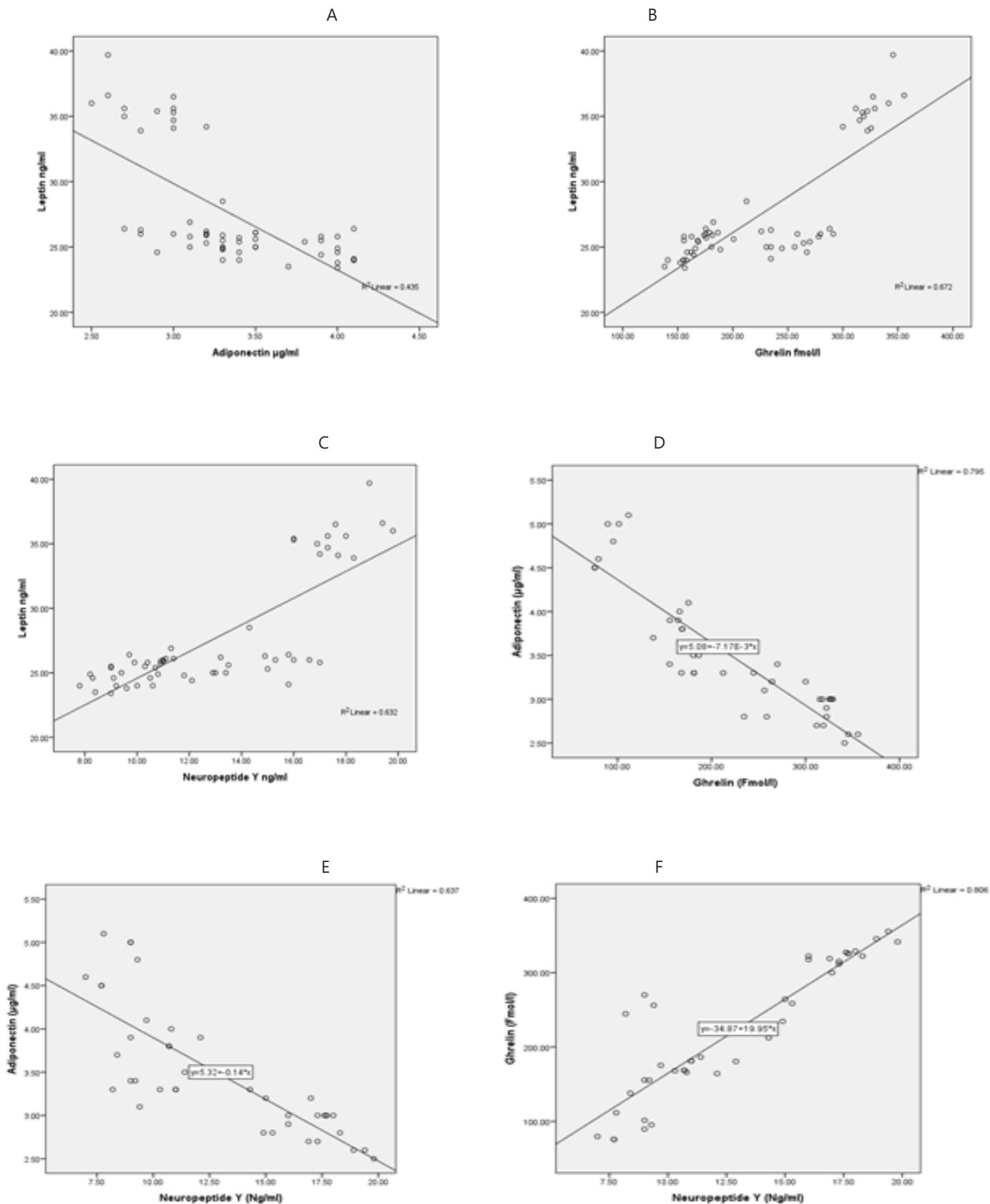


Figure 2 Correlation between leptin and adiponectin (A), ghrelin (B), and NPY (C), correlation adiponectin and ghrelin (D), and NPY (E), and correlation between ghrelin and NPY (F)

BMI was selected as a single dependent variable and the other variables were the independent predictors to obtain the biggest model in the stepwise multiple linear regressions (table 4). In OD group of patients, leptin, triacylglycerols and insulin were significantly inversely associated with BMI ($p=0.037$, .001 and .000, respectively). Ghrelin and glucose had significant positive associations with BMI ($p=0.005$ and .000, respectively). Also, the results showed no multicollinearity. Insulin (standardized coefficient $\beta = -2.633$, $p= .000$), glucose ($\beta = .015$, $p= .000$), triacylglycerols ($\beta = -.011$, $p= .001$), ghrelin ($\beta = .013$, $p= .005$), and leptin ($\beta = -.020$, $p= .037$) had significant association with BMI.

Table 4 The multivariate linear regression analysis of BMI with insulin, glucose, triacylglycerols, ghrelin and leptin in OD group of patients

Model	SE	t	β	(95% CI)	P value	Collinearity Statistics	
						Tolerance	VIF
Constant	2.642	15.045	39.755	(34.54, 44.969)	.000		
Insulin (ng/ml)	.396	-6.644	-2.633	(-3.415, -1.851)	.000	.524	1.907
Glucose (mg/dl)	.003	5.199	.015	(.009, .021)	.000	.805	1.243
Triacylglycerols (mg/dl)	.003	-3.255	-.011	(-.018, -.004)	.001	.867	1.153
Ghrelin (fmol/l)	.005	2.814	.013	(.004, .022)	.005	.514	1.947
Leptin (ng/ml)	.009	-2.096	-.020	(-.038, -.001)	.037	.795	1.259
R	R square	Adjusted R square		SE	R Square Change	F Change	Sig. F change
0.698	0.487	0.473		3.337	0.012	4.395	0.037

4. DISCUSSION

A large and rapidly growing body of research has been directed at improving the understanding of the role of obesity-related hormones such as leptin and adiponectin in the pathogenesis of type II DM. Leptin acts directly on hypothalamus, thereby regulating food intake and energy expenditure (Morales et al., 2004). It was reported that hyperleptinemia may play a role in the pathogenesis of the complications related to obesity (Matsuzawa et al., 2004). On the other hand, adiponectin increases tissue fat oxidation, leading to reduced levels of fatty acids and triacylglycerols content, thus increasing insulin sensitivity. Low levels of plasma adiponectin were reported in obese individuals suggesting the interrelationship between hypoadiponectinemia and obesity pathophysiology (Arita et al., 1999).

Our results showed that plasma leptin levels were correlated positively to BMI in ODs, while, the correlation was negative with adiponectin levels. Those results are not in accordance with the study of Imagawa et al., (2002) who reported elevation in serum levels of adiponectin in patients with type I diabetes mellitus; the difference could be due to the age at time of disease onset, genetic and ethnic factors. The increase in plasma levels of leptin due the increase in body fat content is attributed to the induction of the gene encoding for *ob* gene. Considine et al., (1996) reported high transcription rate of the *ob* gene in adipocytes in obese subjects when compared to normal weight ones. That finding explained the hypertrophy of adipose tissues leading to two folds increase in the production of leptin in the adipocytes of obese individuals. So, levels of plasma leptin are regulated on genetic level by direct changes in the rate of expression of the gene encoding for *ob* gene due to body fat content changes (Hamilton et al., 1995).

In the present study, the levels of plasma ghrelin were elevated significantly in ODs, also, negatively correlated to the fasting levels of plasma insulin suggesting that insulin is affecting the release of ghrelin by direct inhibition of gastric cells. So, the high levels of plasma ghrelin in our study in ODs can be explained by the decrease in plasma levels of insulin in type II diabetic patients due to decreased secretory capacity of β cells of pancreas. Our results revealed significantly high levels of plasma NPY in ONDs and ODs, also, there is a positive significant correlation between NPY levels and BMI, leptin and ghrelin, while, it was a negative significant correlation with the levels of adiponectin. NPY role in energy regulation is evidenced by the effect of energy balance on its production and release in arcuate nucleus-paraventricular nucleus pathway. So, in the cases of decrease food intake and uncontrolled DM associated with insulin deficiency lead to increase the production and release of NPY which may be due to the polyphagia status in these conditions. It was shown that the activation of hypothalamic NPY production and release is associated with the decrease in signaling cascade of insulin and leptin (Crosset et al., 1995).

Schwartz et al., (1999) reported that the defects in leptin signaling cascade can result in unfavorable activation of hypothalamic NPY arcuate nucleus-paraventricular nucleus pathway. Such a finding is supported by reporting high concentrations mRNA of the

gene encoding for hypothalamic NPY in experimental rat (fa/fa obese) and mice (ob/ob) because the observed genetic obesity in these animals may be due to mutations in the genes encoding for leptin receptor and/or the leptin itself (Sipols et al., 1995). It was found that the inhibition of production and release of NPY by applying antisense oligonucleotides results in impaired the increase in food intake in response to fasting status in rats suggesting that the changes in NPY signaling is participating in regulating normal food intake (Strack et al., 1995).

The multiple linear regression analysis of the obtained model of the studied variables to investigate the influence of the independent variables including insulin, glucose, triacylglycerols, leptin and ghrelin on the dependent variable BMI revealed that the independent variables taken together are contributing significantly in predicting BMI where glucose and ghrelin are positively associated with BMI (synergistic interaction), while, insulin, triacylglycerols and leptin are in inverse association with BMI (antagonistic interaction). Our results showed that there is no multicollinearity, so, the interdependent variables are so strongly intercorrelated where they are indistinguishable from each other. Sharifi et al., (2013) stated the inverse relationship between ghrelin and leptin concentrations due to obesity, but, after eliminating the obesity effect, no significant association was observed which confirms the effect of obesity on insulin resistance and those mediators, those findings are in agreement with the obtained results in the present study. Zheng et al., (2013), reported that leptin was significantly inversely associated with BMI in the tuberculosis + type 2 DM patients (B coefficient= -0.164, $p=0.012$), while, ghrelin had a significant positive association (B coefficient= 0.531, $p=0.049$) with BMI in the same group of patients which is also consistent with our results. Those results confirm that ghrelin is the major factor affecting BMI in type 2 DM patients. Possible abnormalities in leptin and ghrelin regulation may be due to other factors that may be associated with nutrition disturbances as inflammatory response in Type 2 DM, which may have more complex and different pathogenesis when associated with obesity. The adjusted R square value is 0.473. So, with the four predictors, 47.3% of the variation of BMI can be explained by this model.

5. CONCLUSION

Our results showed statistically significant increase in BMI of ONDs and ODs versus NONDs and NODs, also, significant positive correlation between BMI and leptin as well as ghrelin and NPY, while, it was negatively correlated with adiponectin. The levels of leptin, ghrelin and NPY were significantly higher in ODs when compared to the other studied groups, but for adiponectin, the levels were significantly lower in ODs compared to the other groups. Those findings are suggesting the interrelationship between insulin and these hormones in energy homeostasis. Also, the results revealed significantly positive correlation between leptin and ghrelin as well as NPY, ghrelin and NPY, and significantly negative correlation between adiponectin and leptin as well as ghrelin and NPY suggesting the association between the studied hormones and the role that may be played by them in the pathogenesis of obesity and its related complications including type II DM.

Author contributions

Al- Sarayreh S - Concepts or Ideas, Design, Definition of intellectual content, Literature search, Experimental studies, Data acquisition, Manuscript preparation, Manuscript editing, Manuscript review

Al-Shuneigat J - Concepts or Ideas, Design, Literature search, Experimental studies, Manuscript preparation, Manuscript review

Al-Saraireh Y - Design, Data analysis, Statistical analysis, Manuscript review

Alsaraireh F - Data acquisition, Data analysis, Statistical analysis, Manuscript editing, Manuscript review

Rawashdeh A - Experimental studies, Data analysis, Manuscript review

Mahgoub S – Concepts or Ideas, Design, Literature search, Data acquisition, Manuscript preparation, Manuscript editing, Manuscript review

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Informed consent

Written & Oral informed consent was obtained from all individual participants included in the study.

Ethics approval and consent to participate

The study has been approved by the Scientific and Ethics Committees [the reference number of the ethical approval: 201239, Faculty of Medicine, Mutah University, Jordan].

Availability of data and materials

The analyzed data are available from the corresponding author on reasonable request.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

Abbreviations

Type II DM:	type II diabetes mellitus
NPY:	neuropeptide Y
ELISA:	enzyme-linked immunosorbent assay
NODs:	non-obese diabetics
NONDs:	non-obese non-diabetics
ONDs:	obese non-diabetics
ODs:	obese diabetics
BMI:	body mass index
SPSS:	Statistical Package for Social Sciences

Peer-review

External peer-review was done through double-blind method.

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