The Serum Ghrelin, Obestatin, and Copeptin Levels During Oral Glucose Tolerance Test In Pregnancy

Gebelerde Oral Glukoz Tolerans Testi Esnasında Serum Ghrelin, Obestatin ve Copeptin Düzeyleri

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ABSTRACT

Aim: The aim of this study was (1) to compare the serum insulin, ghrelin, obestatin and, copeptin levels between pregnant and non-pregnant women and (2) investigate the changes in these parameters in pregnant women that measured at before and second hour after 75 gram (g) oral glucose tolerance test (OGTT).

Materials and Method: Thirty pregnant and 27 healthy non-pregnant women were included in the study, and 75 g OGTT was also performed to pregnant women between 24 and 28 weeks of gestation. The venous blood samples of pregnant women were collected 0 and 2 h after a 75 g glucose loading, and also fasting venous blood samples of non-pregnant women were taken for measurement of these parameters. The measurements of ghrelin, obestatin, copeptin, and insulin were performed by ELISA method.

Findings: In pregnant women, glucose and insulin levels measured at 2 h after glucose loading were significantly increased compared to fasting levels of pregnant and non-pregnant women ($p=0.000$). The serum ghrelin, obestatin, and copeptin levels were not also different between the pregnant and non-pregnant groups ($p>0.05$). However, 75 g glucose loading did not cause any change on ghrelin, obestatin, and copeptin levels as compared to before and after 2-h of OGTT ($p>0.05$).

Conclusions: The results of this study concluded that ghrelin, obestatin, and copeptin levels did not differ between those who were pregnant women and those who were not. Another result of this study was that 75 g of glucose loading test in pregnant women could not cause any significant change in these parameters.

Keywords: Ghrelin, obestatin, copeptin, insulin resistance, oral glucose tolerance test.

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ÖZET

Amaç: Bu çalışmada, (1) gebe ve gebe olmayan kadınlarda serum insülin, ghrelin, obestatin ve copeptin düzeylerinin karşılaştırılması ve (2) gebe kadınlara 75 gram (g) oral glukoz tolerans testi (OGTT) öncesi ve ikinci saatinde ölçülen bu parametrelerdeki değişikliklerin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya 30 gebe ve 27 sağlıklı gebe olmayan kadın katıldı ve 75 g OGTT sadece gebeliğinin 24-28. haftaları arasında olan gebe grubuna uygulandı. Gebe kadınlardan açlık ve glukoz yüklenmesinden iki saat sonra ve gebe olmayanlardan sadece açlık venöz kan örnekleri bu parametrelerin ölçülmesi için alındı. Ghrelin, obestatin, copeptin ve insülin ölçümleri ELISA yöntemi ile gerçekleştirildi.

Bulgular: Gebe kadınlarda, glukoz yüklenmesinden sonraki ikinci saatte ölçülen glukoz ve insülin seviyeleri, gebe ve gebe olmayan kadınların açlık seviyelerine göre önemli ölçüde yükseldi ($p=0.000$). Serum ghrelin, obestatin ve copeptin düzeyleri gebe ve gebe olmayan gruplar arasında benzerdi ($p>0.05$). Bununla birlikte, OGTT öncesi ve ikinci saat değerleri karşılaştırıldığında, 75 gram glukoz yüklemesi gebelerde ghrelin, obestatin ve copeptin düzeylerinde anlamlı bir değişikliğe neden olmadı ($p>0.05$).

Sonuç ve öneriler: Bu çalışmanın sonuçları, ghrelin, obestatin ve copeptin düzeylerinin gebe kadınlarda, olmayanlara göre anlamlı değişim göstermediğini ortaya koydu. Yine, gebe kadınlarda 75 g glukoz yükleme sonrası ikinci saatte, yükleme öncesine göre bu parametrelerde önemli bir değişikliğin olmaması çalışmamızın diğer bir sonucuydu.

Anahtar Kelimeler: Ghrelin, obestatin, copeptin, insülin direnci, oral glukoz tolerans testi.

INTRODUCTION

Gestational Diabetes Mellitus (GDM), one of the subclasses of Diabetes Mellitus (DM), is a condition of hyperglycemia first detected in pregnancy. The stress caused by pregnancy, with other genetic and/or nutritional factors, are the main causes of GDM due to the increase of insulin counter-regulatory hormones (1). Hyperglycemia can be seen during all periods of pregnancy; however, it is more common during the 24th week. It was suggested that the maximum level secretion of the human placental lactogen hormone (HPL) in these weeks might be one of the important factors (2, 3). There is no universal gold standards for screening of GDM. General consensus suggests that all pregnant women, regardless of the existence of risk factors for the development of GDM, should undergo an oral glucose tolerance test (OGTT) with dosages of fasting plasma glucose (FPG), 1-h and 2-h after ingestion of 75 gram (g) of glucose, between 24-28 weeks of pregnancy. It proposed cut-off points at 92 mg/dL, 180 mg/dL, and 153 mg/dL, respectively According to these criteria, if at least one of these values is equal to or above those limits, GDM will be diagnosed (1). It has been reported that many hormones, especially those secreted from the adipose tissue and gastrointestinal tract, are closely related to obesity and Type 2 DM development. A relation was also reported between GDM and the circulating amounts of peptide hormones (4). Ghrelin and obestatin are gastrointestinal peptides that function in the regulation of metabolic functions. These peptides are thought to play important roles in appetite control, weight gain, and obesity. Although both peptides originate from the common precursor hormone, ghrelin is orexigenic and obestatin is anorexigenic as a result of their posttranslational differentiation (5). It was known that human fat from pregnant women expresses the transcript coding for the proposed obestatin receptor (GPR-39) (6). Ghrelin has also an important function in reproductive functions such as prenatal growth and implantation, embryo development, gonadotropin secretion, and the regulation of gonadal function. In healthy pregnancies, maternal ghrelin concentration has been reported to increase in the first trimester, reaching the highest level in the second trimester, and descending to its lowest level in the third trimester of pregnancy (7).

Copeptin is a glycosylated peptide that the C-terminal part of Arginine's Vasopressin Precursor (AVP) and a leucine-rich core section at a length of 39 amino acids. Although AVP is a fairly short-lived and unstable protein, copeptin is a highly stable molecule in serum or plasma at room temperature (8). Several cross-sectional population studies have shown copeptin to be strongly and positively associated with insulin resistance, obesity and metabolic abnormalities and major risk factors for the development of diabetes (9). It has been shown that normal human pregnancy dramatically affects the hypothalamus-pituitary-adrenal (HPA) axis and copeptin acts as a regulatory factor (10). Also, it has been reported that copeptin could have diagnostic and therapeutic implications in both T2DM and GDM (11).

It was aimed to compare the levels of serum insulin, ghrelin, obestatin,and copeptin between pregnant and non-pregnant women, and also investigate the change in these parameters in pregnant women that measured at before and second hour after 75 g oral glucose tolerance test (OGTT) in this study.

MATERIALS AND METHODS

The study was performed from June to November on 2016. An informed consent was obtained from all participates and approval from the local Ethics Committee was obtained as well (2016/36). The research was carried out following the ethical principles in the Declaration of Helsinki 2018, which was adopted by the World Medical Association. Thirty women with uncomplicated pregnancies and 27 non-pregnant women were recruited. Exclusion criteria were as follows: preeclampsia, pregnancy-induced hypertension, smoking, history of fetal anomalies, previous chronic diseases (e.g. epilepsy, renal insufficiency, and heart disease) or medical treatment, especially glucocorticoids. The diagnosis of pregnancy was based on a positive serum beta human chorionic gonadotrophin test (b-HCG) and the presence of a fetal heart beat in the uterine cavity on ultrasonographic evaluation. Gestational ages were evaluated by last menstrual period and were confirmed by ultrasound performed until 14^{th} gestational week, based on crown rump length (CRL) values of embryos. After an overnigh fasting, a 75-g OGTT was performed to pregnant women (12). Venous blood samples were taken to at 0 and 2-h after a 75- glucose loading from pregnant women and fasting blood samples were taken from the non-pregnant to the gel tubes (BD-Belliver, Industrial Estate, UK) which did not contain anticoagulants for measurement of ghrelin, obestatin, copeptin, insulin, and other biochemical parameters. The blood samples were kept at room temperature for at least 30 min to allow the blood to clot and were then centrifuged at 1800*g for 12 min, and also serum samples were stored immediately at -70°C. The venous blood samples were also taken from all women to the tube (BD-Belliver, Industrial Estate, UK) which contains K_2EDTA as anticoagulants for measurement complete blood count parameters,

Serum total ghrelin, obestatin, and copeptin were determined by Enzyme-Linked Immunosorbent Assay (ELISA) Method with a suitable kit (Elabscience Biotechnology Co., Ltd. USA) and also serum insulin was determined by ELISA method with a suitable kit (Human insulin, R&D Products, Czech Republic). Results were read at a 450 nm wavelength on an ELISA reader (BioTek ELX800 reader, BioTek ELX50 washer, Winooski, Vermont, United States).

Serum glucose, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH), Blood Urea Nitrogen (BUN), Creatinine (Cr), Calcium (Ca), Phosphate (PO_4) measurements were performed colorimetrically on an autoanalyzer (Architect C8000, Abbott Diagnostics, USA) by using commercial kits (Architect, Abbott Diagnostics, USA). The measurements of hemoglobin (Hb), white blood cell (WBC), and platelet (PLT) were performed by Sysmex XN-1000

model Hematology analyzer produced with the commercial kit and (Sysmex Corporation Ltd., Japan). The insulin resistance assessment was made by the homeostatic assay (HOMA-IR) by using the following formula: HOMA-IR=[glucose(mg/dL)*insulin (μIU/mL)/405], using fasting values (13).

Statistical analysis

The normality assumption of the variables was tested with Kolmogorov-Simirnov Test. The homogeneity control of the group variances was done with the Levene test. While comparing the average of two dependent groups, variables that provide assumptions were compared with the Paired t-test, and variables that did not meet the assumptions were compared with the Wilcoxon Signed Rank Test. If the group variances were not homogeneous, the Mann-Whitney U test was used to compare the groups. Descriptive statistics for the variables were presented as mean ± standard deviation (SD), median, minimum and maximum values For the determination of the linear relations among the variables, Spearman Correlation Analysis was carried out. The statistical significance level was considered as 5% and SPSS statistical program was used for all statistical computations.

RESULTS

The descriptive and biochemical characteristics of pregnant and non-pregnant women are presented in Table 1. The average gestation period of pregnant women is 25.20±0.17 weeks and the number of pregnancies is 2.03±0.17. There was no statistically significant difference between the pregnant and non-pregnant women in age, body mass index (BMI), diastolic blood pressure (DBP), systolic blood pressure (SBP), and also activities of AST, ALT, LDH ($p>0.05$). The pregnant women had significantly increased HOMA-IR ($p=0.020$), WBC ($p=0.000$) and decreased Ca ($p=0.001$), BUN ($p=0.000$), Cr ($p=0.000$), PLT ($p=0.013$), and also Hb ($p=0.001$) levels as compared to non-pregnant women.

Table 1. The descriptive and biochemical characteristics of pregnant and non-pregnant women

	Pregnant Women (Fasting) n=30	**Non-pregnant Women (Fasting) n=27**
Age (Year) [1]	30.5 (23.0-40.0)	32.0 (20.0-47.0)
SBP (mmHg) [2]	111.13 (9.43)	112.48 (1.27)
DBP (mmHg) [2]	72.2 (8.28)	75.92 (7.14)
BMI (kg/m^2) [1]	30.34 (0.86)	29.5 (1.27)
HOMA-IR [1] [a*]	2.82 (1.19-10.7)	1.85 (0.5-11.23)
AST (U/L) [1]	16.0 (11-35)	16.0 (13-39)
ALT(U/L) [1]	13.0 (6.0-24.0)	13.0 (7.0-57.0)
LDH (U/L) [1]	160.0 (112.0-203.0)	165.0 (121.0-208.0)
BUN (mmol/L) [1]	4.46 (2.14-9.63) [a***]	7.14 (5.35-11.78)
Cr (μmol/L) [1]	44.2 (26.52-61.88) [a***]	53.04 (44.2-70.72)
Ca (mmol/L) [1]	2.17 (2.0-2.37) [a**]	2.30 (2.12-2.47)
PO_4 (mmol/L) [1]	1.15 (0.87-1.42)	1.13 (0.80-1.32)
Hb(g/dL) [1]	11.7 (7.9-13.3) [a*]	12.4 (8.6-14.6)
WBC ($10^3/\mu L$) [1]	9.39 (5.29-13.53) [a***]	5.57 (3.48-8.42)
PLT ($10^3/\mu L$) [1]	205.0 (150.0-270.0) [a*]	217.0 (140.0-417.0)

AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, BMI; Body mass index, BUN; Blood urea nitrogen, Ca; Calcium, Cr; Creatinine, Hb Hemoglobin; DBP; Diastolic blood pressure, HOMA-IR; Homeostatic assay of insulin resistance, LDH; Lactate dehydrogenase, PLT; Platelets, PO_4; Phosphate, SBP; Systolic blood pressure, WBC; White blood cell.
[1]; Mann-Whitney U test, [2]; Student t test, [a] compared to non-pregnant women, *$p<0.05$, ** $p<0.01$, *** $p<0.001$

The insulin and glucose concentrations in the pregnant women obtained at before and 2-h after 75-g glucose loading were markedly higher than the fasting value of pregnant and non-pregnant women (p=0.000). However, ghrelin, obestatin, and copeptin concentrations obtained from pregnant women both the fasting and 2-h after 75-g glucose load and non-pregnant women fasting samples did not change among the groups (p>0.05) (Table 2).

The correlation matrix between variables for pregnant and non-pregnant women are presented in Table 3. In non-pregnant women; significantly positive correlation was found between the ghrelin and copeptin (r = 0.87, p =0.000), ghrelin and obestatin (r = 0.66, p =0.000), obestatin and copeptin (r = 0.59, p <0.01), while the relation between other parameters were not statistically significant (p> 0.05). In the pregnant women, significantly positive correlation was found in the fasting samples between the ghrelin and copeptin (r = 0.95, p =0.000), ghrelin and obestatin (r = 0.70, p =0.001), obestatin and copeptin (r = 0.68, p =0.001), ghrelin and insulin (r = 0.38, p =0.038), while the relation between other parameters were not statistically significant (p>0.05).

Table 2. The comparison of glucose, insulin, ghrelin, obestatin, and also copeptin levels in the pregnant and non-pregnat women according to fasting and 2 h after a 75-g glucose loading.

	Pregnant Women (Fasting) n=30	**Pregnant Women (2-h) n=30**	**Non-pregnant Women (Fasting) n=27**
Glucose (mmol/L) [1]	4.86 (3.88-6.32)	6.57 (5.32-12.82) ^{a***b***}	4.93 (4.10-5.66)
Insulin (mIU/L) [1]	12.71 (5.81-46.59)	113.32 (33.53-238.57) ^{a***b***}	9.14 (2.57-27.53)
Ghrelin (μg/L) [1]	1.74 (0.71-7.26)	1.51 (0.68-8.61)	1.52 (0.80-2.71)
Obestatin (μg/L) [2]	23.41 (10.59)	22.65 (10.48)	21.32 (9.74)
Copeptin (ng/L) [1]	270.78 (104.96-1294.54)	234.02 (102.78-990.57)	250.44 (100.87-530.07)

[1]; Mann-Whitney U test, [2]; Student t test, [a;] pregnant women 2-h value is compared to non-pregnant women fasting value, [b]; pregnant women 2-h value is compared to pregnant women fasting value, *** p<0.001.

Table 3. The correlation matrix between variables for pregnant and non-pregnant women.

	Groups	**Copeptin**	**Ghrelin**	**Obestatin**	**Insulin**
Ghrelin	Non-pregnant women (n=27)	r=0.87 ***			
	Pregnant women fasting (n=30)	r=0.95***			
	Pregnant women 2-h (n=30)	r=0.78***			
Obestatin	Non-pregnant women (n=27)	r=0.59**	r=0.66***		
	Pregnant women fasting (n=30)	r=0.68***	r=0.70***		
	Pregnant women 2-h (n=30)	r=0.83***	r=0.62***		
Insulin	Non-pregnant women (n=27)	r=0.13	r=0.23	r=-0.06	
	Pregnant women fasting (n=30)	r=0.33	r=0.38*	r=-0.04	
	Pregnant women 2-h (n=30)	r=-0.24	r=-0.32	r=-0.04	
Glucose	Non-pregnant women (n=27)	r=0.20	r=0.01	r=0.043	r=0.21
	Pregnant women fasting (n=30)	r=0.06	r=0.17	r=-0.20	r=0.34
	Pregnant women 2-h (n=30)	r=-0.11	r=-0.30	r=0.048	r=0.76***

* p<0.05, ** p<0.01, *** p<0.001.

DISCUSSION

There are many endogenous peptides in the human serum that provide valuable information regarding diagnoses and disease states and act as biomarkers (14). There is some information that relatively few of these peptides may be important in the pathogenesis of gestational diabetes mellitus.

In the present study, it was found that pregnancy and glucose loading did not cause a significant change in the levels of ghrelin, obestatin, and also copeptin. However, a significant and positive relationship was found between ghrelin and copeptin, and also obestatin in both pregnant and non-pregnant women. The effect of glucose loading on ghrelin and obestatin levels in pregnancy is still unclear. The total serum ghrelin consists of a combination of acyl ghrelin and desacyl-ghrelin. It is known that ghrelin also has an important function in glucose hemostasis (15). Riedl et al. also reported that glucose loading did not cause changes in plasma ghrelin levels in pregnant women (16). Baykus et al. reported that the level of desacyl ghrelin decreased significantly in pregnant women with GDM between 24 and 28 weeks of gestation compared to healthy pregnant, but the level of ghrelin did not change between the groups (3). In contrast, Gibson et al. reported that glucose and insulin caused a decrease in the concentration of desacyl ghrelin during pregnancy, but it did not affect the level of acyl ghrelin (17). In our study, the total ghrelin level was measured using double antibody. Therefore, we think that the differences between study results might be due to the type of ghrelin.

Similar to our results, Baykus et al. reported that obestatin did not differ in pregnant women at 24-28 weeks of pregnancy with GDM compared to healthy pregnant women (4). In the experimental studies investigating the relationship between ghrelin and obestatin, it has been observed that administering ghrelin to rats leads to an increase in adipose tissue, while obestatin administration causes weight loss (18, 19). We found significantly positive correlations between obestatin and copeptin in pregnant women. In another study, it was found that plasma obestatin concentration decreased similarly to ghrelin after a high carbohydrate breakfast given to healthy women (20). The results of studies investigating the effects of obestatin on insulin secretion are controversial. Studies show that obestatin has a stimulating effect on insulin secretion (21), as well as an inhibitory effect (22). Some studies even reported that it showed no effects (23). In our study, we could not observe a significant relationship between obestatin and insulin. Supporting our results, Kiewiet et al. reported that obestatin given intravenously in rats did not affect glucose and insulin concentrations in either portal or systemic circulation (24). Sedlackova et al. reported that plasma obestatin concentration decreased similarly to ghrelin after a high carbohydrate breakfast in healthy women, which is another finding that supports the high and positive relationship between ghrelin and obestatin obtained in this study (25).

Copeptin has been reported to be associated with disorders in glucose and insulin metabolism (26). Similar to the results of our study, various studies reported that the concentration of copeptin did not change in pregnant women (27, 28). But, Ebert et al. reported decreased serum copeptin levels in pregnancy (11). In another study conducted in China, copeptin concentrations measured as a result of the single-stage loading test with 75 g glucose were divided into four quarters, and higher levels of GDM were observed in the copeptin levels corresponding to the fourth quarter (25.1%) (29). According to our study results, it is thought that pregnancy between 24 and 28 weeks of gestation could not effect the obestatin, ghrelin, and also copeptin levels. However, when the study results mentioned above are examined in the literature, it is seen that further research is needed to reveal the importance of these parameters in pregnancy.

CONCLUSION

As a result, as compared to non-pregnant women, the ghrelin, obestatin, and also copeptin levels did not differ in pregnant women at 24-28 weeks of pregnancy. Beides, serum levels of these parameters did not change significantly after two hours of 75-g glucose loading in pregnant women. We think that the total number of pregnant women included in the study is one of the important factors that may affect the results of the study. Therefore, it was concluded that there was a need for support from more participants.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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REFERENCES

1. Nunes RD, Flôres, ME, Mayara S, Eliane T, Jefferson T. [Two criteria of oral glucose tolerance test to diagnose gestational diabetes mellitus]. Revista da Associação Médica Brasileira. 2020; 66(2): 139-145.
2. Karakulak M, Saygili U, Temur M, Yilmaz Ö, Özün Özbay P, Calan M, Coşar H. [Comparison of umbilical cord ghrelin concentrations in full-term pregnant women with or without gestational diabetes]. Endocr Res. 2017; 42(2):79-85.
3. Harris MI, Eastman RC, Cowie CC, Flegal KM, Eberhardt MS. [Comparison of diabetes diagnostic categories in the U.S. population according to the 1997 American Diabetes Association and 1980-1985 World Health Organization diagnostic criteria]. Diabetes Care. 1997; 20 (12): 1859-1862.
4. Baykus Y, Gurates B, Aydın S, Celik H, Kavak B, Aksoy A, et al. [Changes in serum obestatin, preptin and ghrelins in patients with Gestational Diabetes Mellitus]. Clin Biochem. 2012; 45 (3): 198-202.
5. Slupecka M, Romanowicz K, Wolinski J. [Maternal high-fat diet during pregnancy and lactation influences obestatin and ghrelin concentrations in milk and plasma of wistar rat dams and their offspring]. Int J Endocrinol. 2016; 2016: 5739763.
6. Fontenot E, DeVente JE, Seidel ER. [Obestatin and ghrelin in obese and in pregnant women]. Peptides. 2007;28 (10): 1937-1944.
7. Gualillo O, Caminos J, Blanco M, Garcia-Caballero T, Kojima M, Kangawa K, et al. [Ghrelin, a novel placental-derived hormone]. Endocrinology. 2001; 142 (2): 788-794.
8. Montero S, Mendoza H, Valles V, Lemus M, Alvarez-Buylla R, de Alvarez-Buylla ER. [Arginine-vasopressin mediates central and peripheral glucose regulation in response to carotid body receptor stimulation with Na-cyanide]. J Appl Physiol. 2006; 100 (6): 1902-1909.
9. Wannamethee SG, Welsh P, Papacosta O, Lennon L, Whincup PH, Sattar N. [Copeptin, insulin resistance, and risk of incident diabetes in older men]. J Clin Endocrinol Metab. 2015; 100(9): 3332-3339.
10. Lindsay JR, Nieman LK. [The hypothalamic-pituitary-adrenal axis in pregnancy: challenges in disease detection and treatment]. Endocr Rev. 2005; 26 (6): 775-799.
11. Ebert T, Platz M, Kralisch S, Lossner U, Jessnitzer B, Richter J, et al. [Serum levels of copeptin are decreased in gestational diabetes mellitus]. Exp Clin Endocrinol Diabetes. 2016; 124 (4): 257-260.

12. American Diabetes Association 2. [Classification and Diagnosis of Diabetes. Diabetes Care]. 2016; 39(1), 13-22.

13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. [Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man]. Diabetologia. 1985; 28: 412-9.

14. Yin L,Huai Y,Zhao C, Ding H, Jiang T, Shi Z. [Early second-trimester peptidomic identification of serum peptides for potential prediction of gestational diabetes mellitus]. Cell Physiol Biochem. 2018;51(3):1264-1275.

15. Van der Lely AJ, Tschop M, Heiman ML, Ghigo E. [Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin]. Endocr Rev. 2004; 25 (3): 426-457.

16. Riedl M, Maier C, Handisurya A, Luger A, Kautzky-Willer A. [Insulin resistance has no impact on ghrelin suppression in pregnancy]. J Intern Med. 2007; 262 (4): 458-465.

17. Gibson W, Liu J, Gaylinn B, Thorner MO, Meneilly GS, Babich, SL, et al. [Effects of glucose and insulin on acyl ghrelin and desacyl ghrelin, leptin, and adiponectin in pregnant women with diabetes]. Metabolism. 2010; 59 (6): 841-847.

18. Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, et al. [Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans]. J Clin Endocrinol Metab. 2001; 86 (10): 5083-5086.

19. Zhang JV, Ren PG, Avsian-Kretchmer O, Luo C W, Rauch R, Klein C, et al. [Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intak]e. Science. 2005; 310 (5750): 996-999.

20. Sedlackova D, Dostalova I, Hainer V, Beranová L, Kvasnicková H, Hill M, et al. [Simultaneous decrease of plasma obestatin and ghrelin levels after a high-carbohydrate breakfast in healthy women]. Physiol Res. 2008; 57(1): 29-37.

21. Egido EM, Hernandez R, Marco J, Silvestre RA. [Effect of obestatin on insulin, glucagon and somatostatin secretion in the perfused rat pancreas]. Regul Pept. 2009; 152 (1-3): 61-66.

22. Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V, et al. [Obestatin promotes survival of pancreatic beta-cells and human islets and induces expression of genes involved in the regulation of beta-cell mass and function]. Diabetes. 2008; 57 (4): 967-979.

23. Qader SS, Hakanson R, Rehfeld JF, Lundquist I, Salehi A. [Proghrelin-derived peptides influence the secretion of insulin, glucagon, pancreatic polypeptide and somatostatin: a study on isolated islets from mouse and rat pancreas]. Regul Pept. 2008; 146 (1-3): 230-237.

24. Kiewiet RM, Gauna C, Van Aken MO, van de Zande B, van der Lely AJ. [Bolus administration of obestatin does not change glucose and insulin levels neither in the systemic nor in the portal circulation of the rat]. Peptides. 2008; 29 (12): 2144-2149.

25. Sedlackova D, Dostalova I, Hainer V, Beranová L, Kvasnicková H, Hill M, et al. [Simultaneous decrease of plasma obestatin and ghrelin levels after a high-carbohydrate breakfast in healthy women]. Physiol Res. 2008; 57(1): 29-37.

26. Asferg CL, Andersen UB, Linneberg A, Linneberg A, Goetze JP, Jeppesen JL. [Copeptin, a surrogate marker for arginine vasopressin secretion, is associated with higher glucose and insulin concentrations but not higher blood pressure in obese men]. Diabet Med. 2014; 31 (6), 728-732.

27. Oncul M, Tuten A, Kucur M, Imamoglu M, Ekmekci OB, Acıkgoz AS, et al. [Copeptin concentrations are not elevated in gestational diabetes mellitus]. Arch Gynecol Obstet. 2013; 288 (5): 1045-1049.

28. Dabrowski FA, Jarmuzek P, Gondek A Cudnoch-Jędrzejewska A, Bomba-Opoń D, Wielgoś M. [First and third trimester serum concentrations of adropin and copeptin in gestational diabetes mellitus and normal pregnancy]. Ginekol Pol. 2016; 87 (9): 629-634.

29. Ma HH, Yang SY, Wang P, Zhang JF. [Evaluation of the value of plasma concentration of copeptin in the first prenatal visit to diagnose gestational diabetes mellitus]. Acta Diabetol. 2017; 54 (12): 1123-1129.