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Plasma orexin and ghrelin response to the oral glucose tolerance test in obese women

Stężenie w surowicy oreksyny i greliny w teście doustnego obciążenia glukozą u otyłych kobiet

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Abstract

Introduction: The aim of the present study was to examine the response of plasma orexin and ghrelin to the oral glucose tolerance test (OGTT) in obese women without additional disease.

Material and methods: The study group comprised 15 obese women aged 30.4 ± 9.7 years of mean BMI 34.7 ± 3.8 kg/m^2. The measurements were performed after an overnight fast and 30, 60 and 120 minutes after the oral administration of 75 grams of glucose. Serum concentrations of ghrelin and orexin A were measured by an enzyme — linked immunosorbent assay (ELISA) kit. Serum concentrations of insulin were measured by radioimmunoassay (RIA). Plasma glucose was determined by an enzymatic procedure. Body composition was determined by impedance analysis using Bodystat.

Results: We observed no significant differences between serum concentrations of ghrelin and orexin during OGTT. No correlations were found between serum ghrelin and orexin concentrations and serum insulin and glucose concentrations in any of the measurements.

Conclusion: Oral glucose administration did not change serum concentrations of ghrelin and orexin A in obese women without additional disease.

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Key words: *obesity, ghrelin, orexin*

Streszczenie

Wstęp: Celem badania była ocena zmiany stężeń oreksyny i greliny w surowicy podczas testu doustnego obciążenia glukozą (OGTT, *oral glucose tolerance test*) u otyłych kobiet bez dodatkowych chorób towarzyszących.

Materiał i metody: Badana grupa składała się z 15 otyłych kobiet w wieku 30,4 ± 9,7 lat ze średnim BMI wynoszącym 34,7 ± ± 3,8 kg/m^2. Próbki krwi pobrano rano na czczo, a następnie po 30, 60 i 120 minutach po doustnym podaniu 75 gramów glukozy. Stężenie greliny i oreksyny A w surowicy oceniano metodą bioimpedancji (ELISA). Do pomiaru stężenia insuliny w surowicy wykorzystano metodę radioimmunologiczną (RIA). Stężenie glukozy w osoczu określono metodą enzymatyczną. Skład masy ciała określono, stosując analizę impedancji z użyciem aparatu Bodystat.

Wyniki: Nie stwierdzono istotnych różnic między stężeniem greliny i oreksyny w surowicy podczas OGTT. Nie wykazano żadnych korelacji między stężeniem greliny i oreksyny w surowicy a stężeniem insuliny i glukozy.

Wnioski: Doustne podanie glukozy nie spowodowało zmiany stężeń greliny i oreksyny A w osoczu u otyłych kobiet bez chorób towarzyszących.

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Słowa kluczowe: *otyłość, grelina, oreksyna*

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Introduction

A large number of studies have recently been performed in order to elucidate the regulation of appetite and food intake. Currently two peptides, ghrelin and orexin, are known to participate in the regulation of appetite.

The peptide hormone ghrelin is predominantly produced by the stomach, although its expression has also been demonstrated in the bowel, pancreas, kidneys, placenta, gonads, pituitary, hypothalamus and adipose tissue [1, 2, 3].

Ghrelin increases expression of hypothalamic neuropeptide Y (NPY) and stimulates food intake in both rodents and human [4]. Circulating ghrelin levels are increased by fasting and energy restriction and are decreased by food intake, glucose, insulin and somatostatin [5].

The mean normal serum ghrelin level in humans is 117 ± 37 fmol/ml [6]. A previous study revealed that serum concentrations of ghrelin are reduced in obese humans [7] and that weight loss increased serum concentrations of ghrelin [8].

Orexins A and B are pairs of neuropeptides that are expressed in the lateral hypothalamic area [9]. Orexin receptors are present in the hypothalamus, pancreas and gut [10]. Kastin et al. [11] revealed that orexins can pass the blood-brain barrier and are expressed in the peripheral tissues. Centrally administered orexin A stimulates food intake in rodents [12]. Peripherally administered orexin A increases insulin secretion [13].

Results obtained by Adam et al. [14] revealed that plasma orexin A levels are decreased in obese individuals.

The aim of the present study was to examine the response of plasma orexin and ghrelin to the oral glucose tolerance test (OGTT) in obese women without additional disease.

Material and methods

The study group consisted of 15 obese women with a mean age of 30.4 ± 5.7 years and a mean body mass index (BMI) of 34.7 ± 3.8 kg/m^2. The study group is characterised in Table I.

All the obese subjects included in the study were diagnosed as having simple obesity without additional disease. All patients had serum concentrations of glucose and insulin within the reference ranges. The exclusion criteria were evidence of present or recent (during the preceding three months) infectious disease, fever and drug therapy. The study was conducted after obtaining the informed consent of all the subjects and was approved by the local ethics committee.

Body weights and heights were measured, and BMI calculated as weight in kilograms divided by the square of the height in metres. Body composition was determined by impedance analysis using a Bodystat analyser.

Table I
Characteristics of study subjects

Tabela I
Charakterystyka badanych

	N = 15
Weight [kg]	91.4 ± 13.1
BMI [kg/m^2]	34.7 ± 3.8
Body fat [kg]	39.7 ± 9.7
Body fat (%)	42.9 ± 4.5
Fat-free mass [kg]	51.7 ± 4.7
Fat-free mass (%)	57.1 ± 4.5

After an overnight fast 6–8 ml samples of venous blood were collected 30, 60 and 120 minutes after oral administration of 75 grams of glucose. The blood samples were collected according to the recommendation of the kit manufacturer. The blood for both ghrelin and orexin A measurements was collected into Lavender Vacutainer tubes containing EDTA. The Lavender Vacutainer tubes were then gently rocked several times immediately after the collection of blood for anti-coagulant. Next the blood was transferred from the Lavender Vacutainer tubes to centrifugal tubes containing aprotinin (0.6 TIU/ml of blood) and gently rocked several times to inhibit the activity of proteinases. The plasma was collected after centrifugation of the blood at 1,600 × g for 15 minutes at 4°C. The plasma obtained was drawn into plastic vials, and stored at –80°C until the time of assay.

Plasma glucose was determined by an enzymatic procedure using a commercially available test kit (Cormay). Insulin was determined by radioimmunoassay (RIA) (Diagnostic Products Corporation, USA) with a lower limit of sensitivity of 1.2 mIU/ml and intra-assay and inter-assay coefficients with variations of 5.2% and 5.8% respectively.

The blood plasma of ghrelin was measured using a commercially available highly sensitive enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals USA). The sensitivity of the ghrelin assay is typically less than 6.0 pg/ml. The mean intra-assay coefficient of variance was < 6.0% and the mean inter-assay coefficient of variance was < 9.0%.

The blood plasma of orexin A was measured using a commercially available highly sensitive enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals USA). The sensitivity of the orexin assay is typically less than 37.0 pg/ml. The mean intra-assay coefficient of variance was < 5.0% and the mean inter-assay coefficient of variance was < 14.0%.

Table II
Changes in the study parameters during OGTT

Tabela II
Zmiany badanych parametrów w czasie OGTT

	Minutes after oral administration of 75 grams of glucose			
	0′	30′	60′	120′
Ghrelin [pg/ml]	77.4 ± 49.5	80.6 ± 62.7	69.8 ± 48.7	73.7 ± 39.2
Orexin A [pg/ml]	372.3 ± 84.1	405.6 ± 81.8	397.6 ± 109.9	435.5 ± 121.1
Insulin [μIU/ml]	16.0 ± 10.5	115.1 ± 49.1***	111.2 ± 59.0***	117.5 ± 49.1***
Glucose [mg/dl]	94.8 ± 8.5	132.0 ± 22.9***	132.7 ± 33.7***	121.9 ± 25.1**

p < 0.005;*p < 0.001

Statistical analysis

All text and table values are expressed as means ± SD. Changes in the study parameters between baseline and 30, 60 and 120 minutes were evaluated using a paired *t* test. The relationships between the study parameters were examined by Pearson's correlation analysis. A p value < 0.05 was considered statistically significant.

Results

The responses of plasma orexin, ghrelin, insulin and glucose to the OGTT are presented in Table II.

We did not observe any significant difference between plasma concentrations of ghrelin and orexin during OGTT.

No correlations were found between body mass, BMI and body composition and serum ghrelin and orexin A concentrations. We did not observe any correlation between plasma ghrelin and orexin A, serum insulin and glucose concentrations in any of the measurements.

Discussion

As described above, recent studies have shown that circulating ghrelin levels are decreased by glucose, insulin, and somatostatin [5]. In our study we observed a tendency to a decrease in ghrelin concentration 60 minutes after oral administration of glucose. This is in agreement with previous observations, which revealed that ghrelin is expressed in the oxyntic mucosal cells of an empty stomach and that its concentration rises sharply before a meal and decreases one hour after a meal [15–17]. Because it was glucose and not a meal that was administered, it seems that the inhibiting effect of glucose on ghrelin release may be weaker than the inhibiting effect of a meal. This is in agreement with the findings reported by Foster-Schubert et al. [18] that the carbohydrate beverage was the most effective in ghrelin inhibition, whereas the fat beverage was less effective. Experimental study in rats has also shown a decrease in ghrelin after nutrient infusion into either the proximal duodenum or proximal jejunum [19]. The effect was more pronounced after infusion of amino acids and glucose than after infusion of lipids [19]. On the other hand, Broglio et al. [20] revealed a significant decrease in ghrelin levels in obese subjects 60 minutes after the glucose load during OGTT. However, they performed OGTT with 100 g of glucose and we used 75 g of glucose.

We did not observe any significant changes in serum concentrations of ghrelin during OGTT. This agrees with the findings of Callahan et al. [21] that both the depth and duration of postprandial ghrelin suppression were directly related to energy load, with more energy as a percentage of total daily energy requirements suppressing ghrelin to a greater degree. Contradictory results were obtained by Cappiello et al. [22]. A significant decrease in ghrelin (25–40%) during OGTT and significant negative correlations between serum ghrelin levels and BMI and insulin levels were observed in both the obese and lean groups. It seems that differences between our results and the observations of Cappiello et al. are caused by differences in the characteristics of the study groups. Cappiello et al. studied a small group of obese subjects (one man and three women) and a small control group (six women) and both groups were older than our study groups. Moreover, Greenman et al [23] showed that ghrelin response to nutrients is modulated by gender.

A correlation between fasting ghrelin level and BMI was also observed by Stock et al. [16]. However, their study group consisted of adolescents. On the other hand, body fat mass was not determined in either of the studies cited and our previous study [8] revealed that body fat rather than BMI correlated with ghrelin

levels. However, Bacha et al. [24] observed negative correlations between ghrelin levels and both BMI and body fat mass in obese children. They also showed a significant decrease in ghrelin levels 60 min. after glucose load during OGTT (24%).

Nowak et al. [13] revealed that peripherally administered orexin A increases insulin secretion in rats. On the other hand, Chang et al. [25] observed that circulating triglycerides stimulate hypothalamic neurons to synthesis of specific feeding stimulatory peptides, including orexin A. In our study we observed a tendency to an increase in orexin A concentration 120 minutes after oral administration of glucose. Therefore it seems that high concentrations of carbohydrate as well as lipids may stimulate hypothalamic synthesis of orexin A, but that the stimulation is weaker than that exerted by lipids.

We did not observe any correlations between serum ghrelin and orexin A concentrations in any of the measurements, which is interesting, as both ghrelin and orexin A inducing food intake involve the neuropeptide Y pathway [4, 26].

Conclusions

Oral glucose administration did not change serum concentrations of ghrelin and orexin in obese women without additional disease.

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