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Fasting and postprandial acyl and desacyl ghrelin levels in obese and non-obese subjects

Przedposiłkowe i poposiłkowe zmiany stężeń obu form greliny u osób otyłych i nieotyłych

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Abstract

Introduction: The potentially differential roles of both forms of ghrelin in obesity are undefined, and little is known about desacyl ghrelin's (DAG) regulation by meals. We aimed to assess changes in acyl ghrelin (AG) and DAG in response to mixed-meal consumption in obese and non-obese subjects.

Material and methods: Venous blood for plasma glucose, AG and DAG assays were collected in both groups after an overnight fast and two hours after the consumption of a standard 300 kcal-mixed meal (Nutridrink, Nutricia).

Results: Mean fasting values of both AG and DAG were significantly lower in the obese individuals. On the other hand, among non-obese controls, the mean postprandial DAG levels did not change and AG levels decreased, whereas in obese individuals the mean DAG levels after a mixed-meal diminished and AG levels were unchanged.

Conclusions: It is necessary to distinguish between the desacylated and acylated forms of ghrelin, as we have shown differential postprandial AG and DAG responses in obese and non-obese individuals. Whether targeting changed proportions between AG and DAG could be a successful strategy in obesity treatment remains a question for future studies. **(Endokrynol Pol 2014; 65 (5): 377–381)**

Key words: *desacyl ghrelin; acyl ghrelin; obesity; meals*

Streszczenie

Wstęp: Niewiele wiadomo jak w otyłości zmienia się wydzielanie obu krążących form greliny — acylowanej (AG) i dezacylowanej (DAG) oraz jak posiłek wpływa u otyłych na stężenie DAG. Dlatego autorzy postanowili ocenić zmiany stężeń obu form hormonu przed i po posiłku w dwóch grupach: z BMI ≥ 30 i < 30 kg/m^2.

Materiał i metody: W obu grupach pobrano krew żylną na czczo i 2 godziny po podaniu standardowego posiłku zawierającego 300 kcal (Nutridrink, Nutricia). Oznaczono stężenia obu form greliny.

Wyniki: Stężenia DAG i AG na czczo były niższe w grupie otyłych niż w kontrolnej grupie osób nieotyłych. Po posiłku u osób bez otyłości nie zaobserwowano zmian stężenia DAG, a stężenie AG zmalało, podczas gdy u otyłych stężenie DAG uległo istotnemu obniżeniu, a AG pozostało bez zmian.

Wnioski: Konieczne jest oznaczanie obu form greliny, gdyż — jak wykazano — zmiany ich stężeń po posiłku mogą być zupełnie odmienne u osób otyłych niż w grupie nieotyłych. Odwrócenie zaburzonych proporcji między AG i DAG może okazać się skutecznym sposobem leczenia otyłości. **(Endokrynol Pol 2014; 65 (5): 377–381)**

Słowa kluczowe: *grelina dezacylowana; grelina acylowana; otyłość; posiłek*

Introduction

Ghrelin, mainly derived from the stomach, is the only known orexigenic peptide [1]. It also plays an important role in energy metabolism and storage [2]. Plasma ghrelin levels fluctuate during the day. They rise before meals and fall after ingestion [3]. In normal-weight subjects, the postprandial suppression of ghrelin is proportional to the calories consumed [4]. Obese individuals have lower fasting ghrelin levels than lean controls [5, 6] and it has been demonstrated that they present evidently reduced suppression in postprandial ghrelin levels [7, 8]. Such dysregulation of ghrelin secretion may lead to hyperphagia and reinforce obesity [8, 9].

To date, most studies have assessed total ghrelin levels and have not differentiated between AG (acyl-ghrelin) and DAG (desacyl-ghrelin). As described originally by Kojima, the hormone circulates in those two forms [1]. Desacylated ghrelin (DAG) was long considered to be an inactive degradation product of acylated form. The acylation of a peptide is crucial to activating GHR1a receptor and exerting many known

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biological activities of acylated ghrelin (AG) [1]. But emerging evidence suggests that the desacylated form of the hormone may interact with another uncharacterised receptor and induce different physiological and metabolic effects independently of AG [10, 11]. Moreover, it has been suggested that DAG can either antagonise or support the activities of AG [10]. It has been reported that DAG has an antagonistic effect on AG in relation to appetite stimulation [12]. The potentially differential roles of both forms of ghrelin under different pathophysiological conditions such as obesity are undefined.

This study was therefore aimed at comparing fasting and postprandial AG and DAG levels in obese and non-obese subjects, using standard mixed macronutrient meals.

Material and methods

The study group consisted of 24 out-clinic patients with simple obesity without diabetes mellitus (7 M/17 F), who were at the beginning of the preparation period before bariatric surgery. The control group included 13 non-obese healthy volunteers without any history of chronic disease, and who were not taking any medications (1 M/12 F) Both groups were above 18 years old and did not differ with respect to age (data presented in Table 1). There were smokers in both groups (5/24 among obese and 2/13 among controls). The mean BMI in the obese group was 43.8 kg/m^2 (range: 32.5–56.6 kg/m^2) and among controls 23.0 kg/m^2 (range: 18.1–27.5 kg/m^2). Nine persons from the obese group were diagnosed with metabolic syndrome according to the International Diabetes Federation (IDF) criteria.

The inclusion criteria were BMI > 30 kg/m^2 and informed consent to the study.

The exclusion criteria were as follows: use of diabetic medication, history of cardiovascular incidents, chronic liver or renal disease, and pregnancy. The study protocol was approved by the local university bioethical committee.

After an overnight fast (12 h), waist circumference and body weight and body composition with bioimpedance analysis (TANITA SC 330) were conducted in both groups. The body mass index [BMI: body weight (kilograms)/height (metres)^2] was then calculated.

Venous blood for plasma glucose, acylated and desacylated ghrelin assays were collected in both groups after an overnight fast and two hours after the consumption of a 300 kcal-mixed meal (mixed-meal test, MMT), which consisted of 16% protein, 49% carbohydrates and 35% fat (Nutridrink standard 200 ml, Nutricia).

Table I. *Characteristics of the studied populations*
Tabela I. *Charakterystyka badanej populacji*

Parameters	Obese (n = 24) Mean ± SD	Non-obese (n = 13) Mean ± SD
Age (years)	35.4 ± 9.1	37.2 ± 9.4
M/F	7/17	1/12
Smoking	5/24	2/13
BMI [kg/m^2]	43.8 ± 6.8[a]	23.0 ± 3.5
Waist circumference [cm]	129.9 ± 16.1[a]	76.3 ± 9.3
Fat (%)	46.4 ± 7.6[a]	27.1 ± 10.1
Fat mass content [kg]	59.9 ± 17.2[a]	17.9 ± 7.3
Fasting glucose [mg/dL]	89.5 ± 9.2	87.7 ± 7.8
Glucose 2 h MMT [mg/dL]	91.8 ± 18.1[b]	74.4 ± 6.0
Fasting insulin [μU/mL]	10.7 ± 3.2	–
Insulin 2 h MMT [μU/mL]	23.1 ± 17.4	–
HOMA-IR	2.4 ± 0.9	–
HDL-CH [mg/dL]	44.3 ± 8.8	–
LDL-CH [mg/dL]	124.7 ± 27.9	–
TG [mg/dL]	143.7 ± 50.3	–

[a]$p < 0.00001$; [b]$p < 0.001$; MMT — mixed-meal test

Blood samples were collected in tubes containing EDTA and p-hydroxymercuribenzoic acid to prevent the degradation of acyl-ghrelin by protease. They were immediately centrifuged at 3,500 x g for 10 min at +4°C and kept at –80°C until further analyses were achieved. Plasma desacylated and acylated ghrelin levels were measured with a commercial enzyme immunoassay (Human Acylated Ghrelin EIA Kit, Human Unacylated Ghrelin EIA Kit, Biovendor, Czech Republic) in accordance with the supplier's specifications. DELTA AG was then calculated as the difference between fasting and 2h post mixed-meal AG levels.

Glucose was measured by the hexokinase method (Abbott Laboratories, USA).

In the obese group of patients, fasting additional serum samples were collected for total cholesterol, high-density lipoprotein cholesterol (HDL-CH), low-density lipoprotein cholesterol (LDL-CH), triglycerides (TG), insulin and HbA1c measurements. They were analysed on the day of collection. Serum lipids concentrations were measured by the oxidase method (Abbott Laboratories, USA) and the Friedewald formula was used to calculate the LDL-CH concentration. Serum insulin levels were determined by EIA (Abbott Laboratories, USA). Serum HbA1c levels were assessed by Tosoh G8 HPLC Analyser (TOSOH, Japan).

Insulin resistance was then estimated using the homeostasis model assessment (HOMA-IR), which was

Table II. *Fasting and postprandial (2 h MMT) AG and DAG concentrations in obese and non-obese group*
Tabela II. *Stężenia AG i DAG na czczo i po posiłku (2 h MMT) u otyłych i grupy bez otyłości*

	Obese (n = 22)			Non-obese (n = 12)		
	Fasting	2 h MMT	p	Fasting	2 h MMT	p
DAG [pg/Ml]	287 ± 219	192 ± 139	0.006	583 ± 369*	532 ± 339*	0.64
AG [pg/mL]	44 ± 26	41 ± 23	0.47	148 ± 56*	122 ± 66*	0.01
AG/DAG	22 ± 22	33 ± 29	0.09	29 ± 12*	30 ± 23	0.95

*p < 0.05 obese *vs.* non-obese; MMT — mixed-meal test

calculated in line with the following formula: fasting insulin (μU/mL) × fasting glucose (mg/dL)/405 [13].

Statistical analysis

The data is expressed as the mean ± SD. The ratio of acylated/desacylated ghrelin was calculated for fasting and 2h after mixed-meal test values. A Kolmogorov-Smirnov test was used to verify whether the variable distribution was normal. Differences between various parameters of obese and non-obese individuals were evaluated by an independent Student's *t* test and U Mann-Whitney test was used when the distribution of the variable was not normal. Fasting and postprandial values were compared with the use of the paired Student's *t* test and Wilcoxon signed-range test was used when the distribution of the variable was not normal. Spearman's rank correlation coefficient (r) was used to evaluate the relationships between the variables.

Statistical analysis was performed using STATISTICA version 10 (StatSoft, Poland). P values < 0.05 were considered as statistically significant.

Results

The general characteristics of both studied populations are presented in Table I. Both groups were comparable for age, percentage of females and smokers, while BMI, fat percentage, fat mass and glucose level 2 h after mixed-meal consumption were significantly higher in the obese group.

The metabolic and endocrine parameters of the obese individuals are included in Table 1. Mean LDL-CH, HDL-CH and triglyceride levels amounted to respectively: 124.7 ± 27.9, 44.3 ± 8.8 and 143.7 ± 50.3 mg/dL. Fasting and postprandial insulin levels amounted to respectively 10.7 ± 3.2 and 23.1 ± 17.4 μU/mL and HOMA-IR – 2.40 ± 0.9.

Average concentrations of AG and DAG before and after a meal in the whole group of patients (n = 37) did not differ between males and females nor between smokers and non-smokers. There were also no differences in the average concentrations of the above hormones in the subgroups with and without metabolic syndrome.

Mean fasting values of both ghrelin forms in the obese and non-obese groups are shown in Table II. As expected, mean fasting values of both acylated and desacylated ghrelin were significantly lower in the obese individuals (287 ± 219 *vs.* 583 ± 369, p = 0.004 for DAG, and 44 ± 26 *vs.* 148 ± 56, p < 0.00001 for AG).

On the other hand, among non-obese controls, the mean postprandial DAG levels did not change (583 ± 369 *vs.* 532 ± 339; p = 0.64) and AG levels decreased (148 ± 56 *vs.*122 ± 66; p = 0.01 for AG), whereas in obese individuals the mean DAG levels after a mixed-meal diminished (287 ± 219 *vs.* 192 ± 139; p = 0.006) and AG levels were unchanged (44 ± 26 *vs.* 41 ± 23; p = 0.47).

At baseline, the ratio of acylated/desacylated ghrelin (AG/DAG) was lower in the obese subjects compared to the non-obese ones (22 ± 22 *vs.* 29 ± 12; p = 0.016). However, after a meal, the ratio was similar in both groups (obese 33 ± 29 *vs.* non-obese 30 ± 23; p = 0.95).

In the whole studied group, we noted a strong negative correlation between fasting AG levels and waist circumference (r = –0.65, p < 0.05), BMI (r = –0.64, p < 0.05) and fat mass content (r = –0.58, p < 0.05).

We found that fasting DAG levels were also negatively correlated with waist circumference, BMI and fat mass content, but correlations coefficients (r) were lower (respectively –0.55; –0.56 and –0.44; p < 0.05). All correlations between both forms of ghrelin and other variables in the whole studied group are shown in Table III.

Furthermore, we observed a negative correlation between DELTA AG (calculated as the differential between fasting and 2 h post mixed-meal AG levels) and HOMA-IR (r = –0.49, p < 0.05) (data presented in Fig. 1).

Discussion

Since ghrelin was identified in 1999 as the natural ligand of growth hormone secretagoque receptor, the desacylated form of this peptide has been considered to be only an inactive degradation product of acylated ghrelin [1]. Therefore many important studies on

Table III. *Correlations between both form of ghrelin and other variables in the whole studied group (n = 37)*

Tabela III. *Korelacje między obiema postaciami greliny i innych zmiennych w całej badanej grupie (n = 37)*

(n = 37)	Fasting AG r	Fasting DAG r
& waist circumference	–0.65*	–0.55*
& BMI	–0.64*	–0.56*
& fat mass	–0.58*	–0.44*
& fat percentage	–0.41*	–0.29
& HOMA-IR	0.03	–0.20

*p < 0.05; R — Spearman's rank coefficient

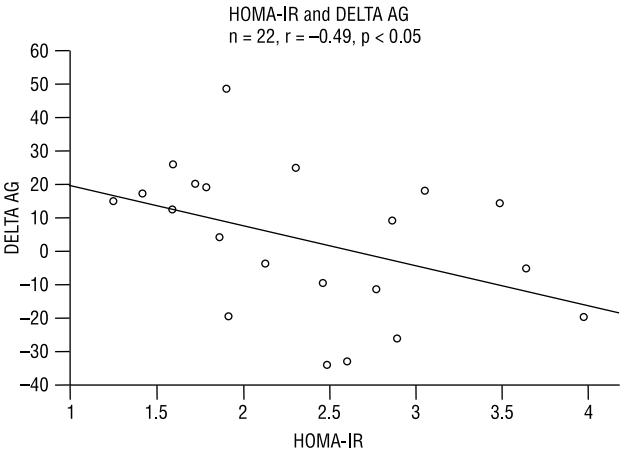

Figure 1. *Correlation between DELTA AG (fasting AG — 2 h post mixed-meal AG) and HOMA-IR in obese individuals*

Rycina 1. *Korelacja między DELTA AG (AG — na czczo 2 godziny po posiłku mieszanym AG) i HOMA-IR u osób otyłych*

ghrelin and energy balance and metabolism have been performed with the use of total ghrelin assays measuring total acylated and desacylated ghrelin. McLaughlin et al. reported that fasting total ghrelin concentrations are higher in insulin-sensitive individuals than in insulin resistant obese subjects [14]. Callahan observed that in normal-weight subjects postprandial suppression of total ghrelin is proportional to calories consumed [4]. The study by le Roux et al. confirmed that total ghrelin levels decrease in normal weight individuals after mixed meals. However, obese subjects demonstrated a much reduced total ghrelin postprandial suppression [7].

It was Broglio et al. who first demonstrated that desacylated ghrelin is metabolically active and probably counteracts the influence of acylated ghrelin on the human metabolism. They suggested that DAG should be considered as a separate hormone [15]. Soon, Askawa et al. reported that DAG has an antagonistic effect on ghrelin in relation to appetite stimulation [12]. More and more studies suggest that DAG can induce different physiological and metabolic effects independently of AG [10, 11]. It can also act as an antagonist of acylated ghrelin, and therefore probably plays an important role in the regulation of food intake and insulin sensitivity. Although the inhibition of total ghrelin has been shown in the insulin resistance state, specific modulations of desacylated and acylated ghrelin remain undefined. There are some reports of a differential acute influence of AG and DAG on glucose metabolism [16, 17]. It is still intriguing as to how the AG/DAG balance could contribute to the development of obesity. Moreover, changes in proportions between AG and DAG induced by meal consumption are not fully defined. The mixed-meal test (MMT) is an interesting model to examine the changes in acylated and desacylated ghrelin profiles. However, to the best of our knowledge, no report has assessed the changes in plasma AG or DAG levels during a mixed-meal test in obese and non-obese individuals.

The present study was therefore designed to assess potential differences in fasting and meal-induced levels of both circulating ghrelin forms in obese and non-obese individuals.

We found that mean postprandial DAG levels did not change in the control non-obese group, whereas AG levels decreased significantly 2 h after a meal. In contrast, in obese individuals DAG levels 2 h after a meal diminished and AG levels did not change (data presented in Table II). To the best of our knowledge, this is the first report assessing DAG changes after a mixed-meal in obese individuals compared to non-obese subjects. Zwirska et al. studied basal and postprandial plasma levels of total and acylated, but not desacylated, ghrelin in obese and lean women [18]. They found that acylated ghrelin levels dropped significantly after meal ingestion and remain decreased 2 h later in lean controls, whereas in obese individuals no changes of AG were observed at 30, 60 and 120 minutes after meal ingestion. The present study confirms these observations and previous reports that obesity can alter postprandial ghrelin response [7, 8]. We also observed that the proportion between acylated and desacylated ghrelin are modulated by meal consumption in a different manner in obese and non-obese individuals. In the fasting state, the ratio of acylated/desacylated ghrelin (AG/DAG) was lower in obese subjects compared to non-obese ones, but it was similar 2 h after the meal. The meal consumption did not change the AG/DAG ratio in the non-obese group, while AG/DAG tended to be higher after the meal in obese individuals, but the p value was not significant (p = 0.09). We did not assess the postprandial level of satiety and we can only speculate that such postprandial responses of both ghrelin forms in obese individuals may promote hyperphagia. Our results therefore can

partially confirm the hypothesis that the dysregulation of ghrelin secretion may reinforce obesity [8, 9] and that the modulation of altered proportions of both ghrelin forms may be a new treatment strategy for obese individuals. Recently some studies have concentrated on the AG/DAG ratio and their impact on the pathogenesis of obesity [19–21].

St-Pierre et al. evaluated changes of AG and DAG during a euglycemic/hyperinsulinemic clamp in obese menopausal women. They found a significant reduction of AG levels only in an insulin-sensitive subgroup and hypothesised that sustained elevation of circulating acylated ghrelin levels could contribute to the deterioration of insulin sensitivity in obese individuals [19]. Our study found a significant correlation (r = –0.49, data presented in Fig. 1) between HOMA-IR and post-meal AG changes (DELTA AG). This result is in agreement with those of St-Pierre [19] and with the earlier observation that postprandial acylated ghrelin levels are correlated with insulin sensitivity in obese children suffering from Prader-Willi syndrome [22].

Our study also confirmed strong negative correlations between fasting AG levels and waist circumference, BMI, fat mass amount and percentage (data presented in Table III). The relationships between desacylated and acylated ghrelin in obesity and insulin resistance state have also been studied by Barazzoni et al. [20]. They analysed associations of both hormone fasting levels with insulin resistance indices (HOMA-IR) in individuals with metabolic syndrome. They found that obese patients with metabolic syndrome displayed relative AG excess compared to non-obese metabolic syndrome patients [20]. Obesity then could be a state of a relative DAG deficiency, as recently proposed by Delhanty et al. [23].

Furthermore, our results underline the importance of distinguishing between desacylated and acylated forms of ghrelin, as their profile does not have to change in the same pattern, especially under different pathophysiological conditions such as obesity [22]. We have also confirmed the previous authors' conclusion that measuring only the fasting ghrelin concentration is unlikely to provide conclusive facts about the physiology of ghrelin regulation in humans [19].

The present study's results are limited by a small sample size. Secondly, we assessed ghrelin concentrations only at two time points: fasting and 2 h after a meal. However, our results are in agreement with those who have studied ghrelin levels at three or more time points [18, 19].

Conclusions

Taken together, we have shown differential postprandial acylated and desacylated ghrelin responses in obese and non-obese individuals. Whether targeting those changed proportions between AG and DAG could be a successful strategy in obesity treatment remains a question for future studies.

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