



# The effect of weight reduction on plasma concentrations of ghrelin and insulin-like growth factor 1 in obese women

Wpływ redukcji masy ciała na stężenie w osoczu greliny i insulinopodobnego czynnika wzrostu 1 u otyłych kobiet

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## Abstract

**Introduction:** The aim of the present study was to examine how weight loss treatment modulates plasma concentrations of ghrelin and insulin-like growth factor 1 (IGF-1) in obese women and to determine whether there is any association with possible changes in plasma concentrations of these hormones after weight loss.

**Material and methods:** The study group consisted of 22 obese women without additional disease (age  $40.6 \pm 12.9$  years; BMI  $37.2 \pm 4.6$  kg/m<sup>2</sup>). All subjects participated in a 3-month weight reduction program. The measurements were performed at baseline and after weight loss. Plasma concentration of ghrelin and IGF-1 were measured by enzyme-linked immunosorbent assay (ELISA) kit. Serum concentrations of insulin were measured by radioimmunoassay (RIA). Body composition was determined by bioelectrical impedance analysis using a Bodystat analyser.

**Results:** The mean weight loss was  $9.3 \pm 4.1$  kg ( $9.7 \pm 4.3\%$ ). Following weight loss, plasma ghrelin and IGF-1 concentrations increased significantly ( $63.5 \pm 13.0$  vs.  $72.8 \pm 15.1$  pg/ml;  $p < 0.01$ ;  $126.9 \pm 67.0$  vs.  $170.5 \pm 83.3$  ng/ml  $p < 0.01$ , respectively) and serum insulin concentrations decreased significantly ( $17.5 \pm 8.5$  vs.  $14.8 \pm 10.4$   $\mu$ IU/ml  $p < 0.05$ ). We observed a significant positive correlation between the increase of ghrelin and decrease of body fat percentage after weight loss ( $r = 0.44$ ,  $p = 0.03$ ). There are no correlations between change of ghrelin and IGF-1 concentrations and between changes of insulin and IGF 1 concentrations.

**Conclusion:** Plasma concentrations of ghrelin and IGF-1 increased after weight loss. However, it seems there is no association between serum concentrations of ghrelin and IGF-1 in obese women. (*Pol J Endocrinol* 2008; 59 (4): 301-304)

**Key words:** obesity, ghrelin, IGF-1, weight loss

## Streszczenie

**Wstęp:** Celem pracy była ocena wpływu redukcji masy ciała na stężenie w osoczu otyłych kobiet greliny i insulinopodobnego czynnika wzrostu 1 (IGF-1) oraz określenie czy występuje związek między ewentualnymi zmianami tych związków podczas redukcji masy ciała.

**Materiał i metody:** Grupa badana składała się z 22 otyłych kobiet bez chorób towarzyszących (wiek  $40,6 \pm 12,9$  lat; BMI  $37,2 \pm 4,6$  kg/m<sup>2</sup>). Wszystkie badane uczestniczyły w 3-miesięcznej kompleksowej grupowej kuracji odchudzającej. Pomiarów dokonano przed i po redukcji masy ciała. Stężenie greliny i IGF-1 w osoczu oznaczono metodą immunoenzymatyczną (ELISA). Stężenie w surowicy insulin oznaczono metodą radioimmunologiczną (RIA). Skład ciała oceniono metodą bioimpedancji z użyciem aparatu Bodystat.

**Wyniki:** Średnia redukcja masy ciała wynosiła  $9,3 \pm 4,1$  kg ( $9,7 \pm 4,3\%$ ). Stężenie greliny i IGF-1 w osoczu istotnie wzrosło (odpowiednio  $63,5 \pm 13,0$  vs.  $72,8 \pm 15,1$  pg/ml;  $p < 0,01$ ;  $126,9 \pm 67,0$  vs.  $170,5 \pm 83,3$  ng/ml;  $p < 0,01$ ), natomiast stężenie insulin obniżyło się istotnie ( $17,5 \pm 8,5$  vs.  $14,8 \pm 10,4$   $\mu$ IU/ml  $p < 0,05$ ). Zaobserwowano istotne dodatnie korelacje między wzrostem stężenia greliny i obniżeniem się procentowej zawartości tłuszczu ( $r = 0,44$ ,  $p = 0,03$ ). Nie występowały korelacje między zmianami stężeń greliny a IGF-1 oraz między zmianami stężeń insulin a IGF-1.

**Wnioski:** Redukcja masy ciała powoduje wzrost stężenia w osoczu greliny i IGF-1. Wydaje się jednak, że nie występuje związek między zmianami stężeń greliny i IGF-1 u otyłych kobiet. (*Endokrynol Pol* 2008; 59 (4): 301-304)

**Słowa kluczowe:** otyłość, grelina, IGF-1, redukcja masy ciała

## Introduction

It has been shown that growth hormone secretion is negatively and independently associated with age and adiposity in humans [1]. Circulating GH levels are also de-

creased in obesity [2]. Therefore, it seems that obesity is a real condition of GH insufficiency [3] despite which (IGF-1) levels are reported as low normal or normal [4-7]. However, some studies revealed decreased IGF-1 levels in obese adults [8] or increased IGF-1 levels in obese chil-



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dren [9]. Kaytor et al. [10] suggested that lack of decrease of IGF-1 production might be an effect of the augmented action of insulin on intracellular GH signalling.

The peptide hormone ghrelin is predominantly produced by the stomach. However, its expression has also been demonstrated in other tissues such as bowels, pancreas, kidneys, placenta, gonads, pituitary, hypothalamus and adipose tissue [11–13].

The mechanism of ghrelin action on GH secretion is mainly dependent on the interaction between GH-releasing hormone, ghrelin and somatostatin [14–16].

The circulating ghrelin level is increased in anorexia and cachexia but is reduced in obesity [17–19]. Previously we observed an increase in plasma concentrations of ghrelin after weight loss in obese women [20].

Our previous observations [21] and data from literature seem to suggest that ghrelin may be regarded as a new factor influencing GH secretion [22]. On the basis of data from literature [23] and on our previous studies [20, 21], we hypothesised that weight loss may modulate the secretion of IGF-1, and that ghrelin may be one of the factors participating in this process.

The aim of the present study was to examine how weight loss modulates plasma concentrations of ghrelin and IGF-1 in obese women and to determine whether there is any association with possible changes in plasma concentrations of these hormones after weight loss.

## Material and methods

The study group consisted of 22 obese women, age  $40.6 \pm 12.9$  years, weight  $97.0 \pm 15.5$  kg, body mass index (BMI)  $37.2 \pm 4.6$  kg/m<sup>2</sup>. The characteristics of the study group are listed in Table I.

All obese subjects included in the study were diagnosed as having simple obesity without additional diseases. All patients had serum concentrations of glucose and insulin within the reference range. The exclusion criteria included: evidence of present or recent (preceding 3 months) infectious disease, fever or drug therapy.

The study was conducted after obtaining informed consent from all the subjects. The study was approved by the Local Ethical Committee.

All obese patients participated in a 3-month weight reduction program that consisted of 1) a group instruction in behavioural and dietary methods of weight control carried out every two weeks, 2) 1000–1400 kcal/day balanced diet, and 3) physical exercises 30–40 min/day.

The measurements were performed at the baseline and after the 3-month program. Body weight and height were measured, and body mass index (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. Patient characteristics and the effect of weight reduction treatment**

**Tabela I. Charakterystyka chorych i efekty terapii odchudzającej**

	Baseline (mean $\pm$ SD)	After weight loss (mean $\pm$ SD)	p
Weight [kg]	97.0 $\pm$ 15.5	87.4 $\pm$ 15.0	0.001
BMI [kg/m <sup>2</sup> ]	37.2 $\pm$ 4.6	33.7 $\pm$ 4.6	0.001
Body fat [kg]	42.4 $\pm$ 13.8	36.5 $\pm$ 10.0	0.01
Body fat (%)	42.8 $\pm$ 9.6	41.3 $\pm$ 6.4	NS
Fat-free mass [kg]	54.6 $\pm$ 8.0	50.9 $\pm$ 6.6	0.05
Fat-free mass (%)	57.2 $\pm$ 9.4	58.7 $\pm$ 6.7	NS

Six to eight ml samples of venous blood were collected in the morning, following an overnight fast. The blood samples were collected according to the recommendations of the kit manufacturers. The blood for measurements both of ghrelin and IGF was collected into Lavender Vacutainer tubes containing EDTA. Then the Lavender Vacutainer tubes were gently rocked several times immediately after collection of blood for anti-coagulation. Next the blood was transferred from the Lavender Vacutainer tubes to centrifuge tubes containing aprotinin (0.6 TIU/ml of blood) and was gently rocked several times to inhibit the activity of proteinases. The plasma was collected after centrifugation of the blood at  $1600 \times g$  for 15 minutes at 4°C.

The obtained plasma was drawn into plastic vials and stored at  $-80^{\circ}\text{C}$  until the time of assay.

Insulin was determined by radioimmunoassay (RIA); (DPC Diagnostic Products Corporation, USA).

The plasma 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, according to the manufacturer's range, less than 6.0 pg/ml. Mean intra-assay coefficient of variance was  $< 6.0\%$ , and mean interassay coefficient of variance was  $< 9.0\%$ .

Plasma IGF-1 was measured using a commercially available, highly sensitive, enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, USA).

The sensitivity of the IGF 1 assay is typically less than 0.026 ng/ml. Mean intra-assay coefficient of variance was  $< 4.5\%$ , and mean interassay coefficient of variance was  $< 8.5\%$ .

## Statistical analysis

All text and table values are expressed as mean  $\pm$  SD. Changes from baseline in anthropometrics values and hormone levels were evaluated using paired t-test. The

**Table II.** Plasma concentrations of ghrelin, growth hormone and insulin**Tabela II.** Osoczowe stężenie greliny, hormonu wzrostu i insuliny

	Baseline (mean $\pm$ SD)	After weight loss (mean $\pm$ SD)	p
Ghrelin [pg/ml]	63.5 $\pm$ 13.0	72.8 $\pm$ 15.1	0.01
IGF-1 [ng/ml]	126.9 $\pm$ 67.0	170.5 $\pm$ 83.3	0.01
Insulin [ $\mu$ IU/ml]	17.7 $\pm$ 8.5	14.8 $\pm$ 10.4	0.05

relationships between changes in fasting plasma ghrelin levels and changes in IGF-1 levels and body composition following weight loss were examined by Pearson's correlation analysis. Stepwise multivariate analysis was performed with plasma levels of ghrelin,  $\Delta$ ghrelin, IGF-1 and  $\Delta$ IGF-1 as the dependent variables. A value  $p < 0.05$  was considered statistically significant.

## Results

The effects of weight loss treatment are presented in Table I. Mean weight loss was  $9.3 \pm 4.1$  kg. BMI decreased from  $37.2 \pm 4.6$  at the baseline to  $33.7 \pm 4.6$  after treatment. Some significant differences in body composition were also found. The body weight reduction treatment led to a significant decrease of body fat absolute ( $p = 0.01$ ), which was accompanied by a moderate decrease of fat-free mass absolute ( $p = 0.05$ ).

In obese subjects, ghrelin levels ( $p < 0.01$ ) and IGF-1 levels ( $p < 0.01$ ) increased significantly following weight loss (Table II). Serum concentrations of insulin decreased significantly ( $p < 0.05$ ) after treatment.

Ghrelin and IGF-1 did not show any correlation with age, weight, BMI and percentage of body fat, or serum concentrations of insulin. However, a significant positive correlation was found between serum concentrations of insulin and BMI ( $r = 0.54$ ;  $p = 0.01$ ) and a negative correlation was found between serum concentrations of insulin and fat-free mass ( $r = -0.47$ ;  $p = 0.03$ ) before treatment.

We did not observe any correlation between  $\Delta$ ghrelin and  $\Delta$ IGF-1 or between  $\Delta$ IGF-1 and  $\Delta$ BMI,  $\Delta$  body mass,  $\Delta$ body fat absolute and percentage, and serum concentration of insulin. However, there was a positive correlation between  $\Delta$ ghrelin and  $\Delta$ body fat (%) ( $r = 0.44$ ,  $p = 0.03$ ).

We performed stepwise multivariate regression analysis using ghrelin,  $\Delta$ ghrelin, GH and  $\Delta$ GH as dependent variables. The  $\Delta$ ghrelin was related negatively to  $\Delta$ body fat (kg) ( $r = 0.26$ ;  $F = 11.2$ ;  $p = 0.001$ ) and  $\Delta$ body fat (%) ( $r = 0.23$ ;  $F = 14.2$ ;  $p = 0.002$ ). Models were fit-

ted to assess the role of age, BMI, body fat (kg and %),  $\Delta$ body fat (kg and %), fat-free mass (kg and %), and  $\Delta$ fat-free mass (kg and %). Analysis of the remaining regression coefficients did not reveal any significant differences.

## Discussion

As described above, the effect of obesity on the production and serum concentrations of insulin-like growth factor 1 is unclear [4–9]; the IGF-1 serum concentrations increased after weight loss [23]. Experimental studies suggest that endogenous ghrelin may constitute part of a pathway involved in modulating the GH/IGF-1 pathway in response to changes in nutrient availability, such as those induced by a high-fat diet [24]. Some recent studies have shown that ghrelin, the endogenous ligand of the GH secretagogue receptors, is down-regulated in human obesity [17, 25].

In our previous study [20, 21] and in other authors' studies [26], increased circulating levels of ghrelin after weight loss were observed; therefore, we speculate that ghrelin may be one of the factors which increases IGF-1 release after weight reduction.

In the present study, as previously reported in our studies [20, 21], we observed increased plasma concentrations of ghrelin after weight loss and negative correlations between changes of body fat and changes of ghrelin. This result is also in accordance with other authors' observations [27]. We also observed a significant increase of IGF-1 after weight loss. These findings are in accordance with results obtained by Rasmussen et al. [23] and by Engström et al [28]. Additionally, Rasmussen et al. [23] suggested, on the basis of their findings, that changes in plasma concentrations of IGF-1 are independent of changes in growth hormone after weight reduction. However, we did not observe correlations between plasma concentrations of ghrelin and IGF-1 before and after weight loss, or between changes of these parameters after weight loss. Therefore, it seems that ghrelin is not the factor employed in the increase of circulating IGF-1 levels after weight loss. Previous studies revealed that insulin might be a factor participating in stimulation of production of IGF-1 in obese women [10]. However, we did not observe correlations between serum concentrations of insulin and plasma concentrations of IGF-1, or between changes of these parameters. It seems that there are some limitations in the interpretation of the obtained results; we measured total plasma ghrelin concentrations, as it is already known that pro-ghrelin cleavage may generate another peptide obestatin with the opposite physiological action to ghrelin [29]. Further studies are necessary to clarify which factors participate in changes of IGF-1 production after weight loss.

## Conclusions

Plasma concentrations of ghrelin and IGF-1 increased after weight loss. However, it seems that there is no association between plasma concentrations of ghrelin and IGF-1 in obese women.

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