View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Via Medica Journals

Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 61; Numer/Number 4/2010
ISSN 0423–104X

The relationship between ghrelin levels and insulin resistance in men with idiopathic hypogonadotrophic hypogonadism at diagnosis and after therapy

Zależność między stężeniem greliny i insulinoopornością u mężczyzn z idiopatycznym hipogonadotropowym hipogonadyzmem w momencie rozpoznania i po leczeniu

M. Ilkin Naharci[1], Erol Bolu[2], Nuri Karadurmus[1], Yalcın Basaran[1]

[1]*Departments of Internal Medicine, Gulhane Military Medical Academy, Ankara, Turkey*
[2]*Endocrinology and Metabolism, Gulhane Military Medical Academy, Ankara, Turkey*

Abstract

Introduction: It has recently been shown that ghrelin affects energy balance and reproductive function, but the role of ghrelin in the pathogenesis of insulin resistance is unclear. Firstly to assess the interaction between insulin resistance and ghrelin levels in hypogonadal men, and then to show the effects of testosterone (T) therapy on insulin and ghrelin.

Material and methods: Twenty-four male patients newly diagnosed with idiopathic hypogonadotropic hypogonadism (IHH) and 20 healthy male subjects were enrolled in this study. Ghrelin, insulin, glucose, total and free testosterone levels, HOMA-IR and QUICKI, and percentage of body fat mass were determined at baseline in all subjects and after therapy in hypogonadal men.

Results: When compared with control subjects, hypogonadal men had significantly lower total and free T concentrations, ghrelin levels, and QUICKI whereas they had significantly higher body fat mass and HOMA-IR score. Following T therapy, a significant increase in ghrelin and QUICKI, and a decrease in HOMA-IR score and body fat mass were demonstrated in hypogonadal men. Calculation of the Pearson coefficient showed that ghrelin concentrations in hypogonadal men were positively correlated with free and total testosterone and QUICKI, whereas they were negatively correlated with body fat mass and HOMA-IR. After six months of T therapy, these correlations were still observed.

Conclusions: Our data supports the notion that ghrelin may constitute an important link between the regulation of reproduction and metabolic homeostasis. **(Pol J Endocrinol 2010; 61 (4): 351–358)**

Key words: *testosterone replacement therapy, ghrelin, insulin resistance*

Streszczenie

Wstęp: Ostatnio wykazano, że grelina wpływa na bilans energetyczny i czynności rozrodcze, jednak jej rola w patogenezie insulinooporności nadal nie została wyjaśniona. Celem badania jest przede wszystkim ocena zależności między insulinoopornością i stężeniem greliny u mężczyzn z hipogonadyzmem, a następnie wykazanie wpływu leczenia testosteronem na stężenia insuliny i greliny.

Materiał i metody: Do badania włączono 24 mężczyzn z nowo rozpoznanym idiopatycznym hipogonadotropowym hipogonadyzmem (IHH, *idiopathic hypogonadotropic hypogonadism*) i 20 zdrowych mężczyzn. Stężenie greliny, insuliny, glukozy, testosteronu całkowitego i wolnego, wartości wskaźników HOMA-IR i QUICKI oraz procentową zawartość tłuszczu w organizmie określono u wszystkich osób na początku badania, a u mężczyzn z hipogonadyzmem również po zakończeniu terapii.

Wyniki: U mężczyzn z hipogonadyzmem stwierdzono istotnie niższe stężenia całkowitego i wolnego testosteronu, greliny i mniejszą wartość wskaźnika QUICKI niż w grupie kontrolnej, natomiast procentowa zawartość tłuszczu w organizmie, i wartość wskaźnika HOMA-IR były wyższe u mężczyzn z IHH niż u zdrowych mężczyzn. Po leczeniu testosteronem u mężczyzn z hipogonadyzmem odnotowano istotne zwiększenie stężenia greliny i wskaźnika QUICKI oraz zmniejszenie wartości wskaźnika HOMA-IR i masy tkanki tłuszczowej. Obliczono współczynnik korelacji Pearsona i wykazano, że u mężczyzn z hipogonadyzmem stężenie greliny jest skorelowane dodatnio ze stężeniami całkowitego i wolnego testosteronu i wartością wskaźnika QUICKI oraz ujemnie z zawartością tkanki tłuszczowej i wskaźnikiem HOMA-IR. Po 6 miesiącach terapii testosteronem korelacje te nadal były obecne.

Wnioski: Powyższe dane potwierdzają tezę, że grelina może stanowić wspólne ogniwo dla mechanizmów regulujących funkcje rozrodcze i homeostazę metaboliczną. **(Endokrynol Pol 2010; 61 (4): 351–358)**

Słowa kluczowe: *testosteronowa terapia zastępcza, grelina, insulinooporność*

Nuri Karadurmus M.D., Department of Internal Medicine, Gulhane Military Medical Academy, Etlik 06018, Ankara, Turkey, tel.: +90 312 304 40 02, fax: +90 312 304 40 00, e-mail: drnkaradurmus@yahoo.com

Introduction

Resistance to insulin is defined as an impaired biological response to both endogenous and exogenous insulin. In other words, it is a disorder of insufficient insulin efficacy to tissues such as liver, skeletal muscle, and adipose [1]. Although the pathophysiology of peripheral resistance to the action of insulin has not been fully characterized in male hypogonadism, measurable knowledge has been reported in several studies. In healthy male individuals, serum testosterone (T) concentrations are inversely correlated with fasting insulin levels, and high insulin levels and increased insulin resistance (IR) have been detected in decreased serum T concentrations [2–7]. Several studies have consistently demonstrated that androgen treatment improves impaired glucose tolerance and high insulin levels [8–10]. A significant percentage of women with polycystic ovary syndrome (PCOS) display IR [11]. The association between sex steroids and IR that differs depending on gender is not yet clear. Based on currently available data, T replacement therapy in male hypogonads has been revealed to improve resistance to the action of insulin, but the patient populations that were studied have differed dramatically in these studies [12].

Ghrelin is the natural ligand for the growth hormone secretagogue receptor and has a powerful growth hormone (GH) stimulating activity [13]. It is mainly secreted from the stomach enteroendocrine cells, but it is also expressed widely in different tissues such as hypothalamus, pituitary gland, duodenum, jejunum, and lungs [14]. Ghrelin stimulates appetite and induces a positive energy balance that can lead to weight gain and plays an important role in IR [14, 15]. Ghrelin levels are lower in male individuals, in obesity, in type II diabetes mellitus (DM), in states of IR, and in hypertension, and administration of ghrelin in heart failure has been found to improve cardiac function [15–19].

The aim of this study is firstly to investigate the relationship between IR and ghrelin levels in male patients with idiopathic hypogonadotropic hypogonadism (IHH) and secondly to show the influence of T replacement therapy on IR and plasma ghrelin levels.

Material and methods

The investigation was designed as a case-control study. Twenty-four patients with IHH, and 20 eugonadal males as the control group, were enrolled in this study between May 2004 and October 2005 at the Department of Endocrinology, Gulhane Military Medical School, Ankara, Turkey.

The ages of the patients with IHH that were recruited in the study ranged from 20 to 22 years (mean age: 20.75 ± 0.74 years [mean ± SD]), the body weight ranged from 50 to 86 kg (mean body weight: 62.9 ± ± 8.81 kg [mean ± SD]), and the body mass index (BMI) was calculated as 15.61 to 25.68 kg/m^2 (mean BMI: 20.93 ± ± 2.12 kg/m^2 [mean ± SD]).

The ages of eugonadal men ranged from 20 to 23 years (mean age: 21.05 ± 0.83 years [mean ± SD]), the body weight ranged from 52 to 75 kg (mean body weight: 61.70 ± 5.90 kg [mean ± SD]), and the BMI was calculated as 17.37 to 24.77 kg/m^2 (mean BMI: 20.81 ± ± 2.08 kg/m^2).

Hypogonadism was defined by T levels less than 3.5 ng/ml associated with low or normal levels of FSH (Follicle stimulating hormone) and LH (Luteinizing hormone) in blood samples, collected at around 08:00 after an overnight fast. Kallmann syndrome was excluded by a normal sense of smell and by normal anatomical view of hypothalamic-pituitary axis on magnetic resonance imaging.

Body height (m) and weight (kg) were measured, and BMI was calculated in all subjects at the start of the study [21]. Percentage of body fat mass and percentage of lean body mass were estimated by bioelectric impedance analyzer (50 kHz model SFB2 SEAC, Brisbane, Australia) [22] was used for bioelectric impedance analysis. Fasting blood glucose, fasting blood insulin, total and free T levels, FSH, LH, estradiol, and sex hormone binding globulin (SHBG) concentrations were measured in all subjects. All blood samples drawn for determination of ghrelin concentrations were frozen at –80°C until the laboratory investigations were performed.

After the comparison of anthropometric data, and biochemical and hormonal parameters, the hypogonadal men were assigned to replacement therapy with intramuscular injection of Sustanon 250 ampoule (Organon®, Oss, The Netherlands) administered in the course of a three-week periods in duration of six months. Each Sustanon 250 ampoule contained 30 mg T propionate, 60 mg T phenylpropionate, 60 mg T isocaproate, and 100 mg T decanoate. Compliance with the therapy was checked by regular assessment of T levels and clinical monitoring. Following this period, in the fourteenth day after the last administration of T, a final examination was conducted including anthropometrical, biochemical, and hormonal parameters.

Fasting blood glucose (mg/dL) was measured using enzymatic colorimetric methods by Olympus AU 600 (Olympus Diagnostics, GmbH — Hamburg, Germany) apparatus. IR was estimated by HOMA-IR (Homeostasis Model Assessment-Insulin Resistance) index, "HOMA-IR = fasting glucose (FG) (mg/dL) × immunoreactive insulin (IRI) (μU/mL)/405" (23). QUICKI index (Quantitative Insulin Sensitivity Check Index), the other indicator of IR, was calculated using the following

Table I. *Anthropometric, hormonal, and metabolic parameters at baseline in patients with IHH and in controls*

Tabela I. *Antropometryczne, hormonalne i metaboliczne parametry wyjściowe u mężczyzn z IHH oraz u mężczyzn z grupy kontrolnej*

Parameters	IHH	Controls	P
Age (years)	20.75 ± 0.74	21.05 ± 0.83	NS
Weight (kg)	62.94 ± 8.81	61.70 ± 5.90	NS
Body Mass Index [kg/m^2]	20.93 ± 2.12	20.81 ± 2.08	NS
Body Fat Mass (%)	26.55 ± 6.51	12.75 ± 4.44	< 0.0001
Lean Body Mass (%)	73.45 ± 6.51	87.25 ± 4.44	< 0.0001
Total T [ng/mL] (2.88–8.00)	1.66 ± 0.37	5.07 ± 1.16	< 0.0001
Free T [pg/mL] (8.6–54.6)	2.83 ± 1.24	22.42 ± 4.09	< 0.0001
Oestradiol [pg/mL] (7.63–42.6)	4.52 ± 2.45	30.26 ± 2.55	< 0.0001
SHBG [nmol/mL] (14.5–48.4)	30.22 ± 6.42	20.53 ± 4.60	< 0.0001
Fasting Glucose [mg/dL] (65–107)	85.17 ± 6.91	75.30 ± 4.99	< 0.0001
Fasting Insulin [μU/ml] (2.6–24.9)	25.61 ± 8.36	13.44 ± 4.27	< 0.0001
HOMA-IR	5.41 ± 1.96	2.54 ± 0.95	< 0.0001
QUICKI-index	0.30 ± 0.01	0.34 ± 0.02	< 0.0001
Ghrelin [fmol/mL] (2.5–160)	2.80 ± 1.07	5.64 ± 1.12	< 0.0001

IHH — idiopathic hypogonadotropic hypogonadism; NS — Not significant; T — testosterone; SHBG — sex hormone binding globulin; HOMA-IR — Homeostasis Model Assessment-Insulin Resistance; QUICKI-index — Quantitative Insulin Sensitivity Check Index. Data are shown as mean values ± SD

formula: "QUICKI = 1/ (log FG [mg/dL] × log IRI [μU/ /mL])". Plasma insulin, T, FSH, LH, and oestradiol concentrations were assessed by Immulyte 2000, and SHBG concentrations by Immulyte 1000 devices. Free T levels were assessed by radioimmunoassay. Plasma ghrelin concentrations were measured by ELISA (Active Ghrelin Elisa Kit, Linco research, Inc., St. Charles, MO).

All patients and control subjects were informed about the aim and procedure of the study and gave their written consent. The study was approved by the Ethics Committee of the Gulhane School of Medicine.

Statistical analysis

Statistical evaluations were performed by running the SPSS 11.0 (SPSS Inc., Chicago, IL, USA) software package. Results are reported as the mean values ± SD. Comparisons between parameters were analyzed using paired samples t-test and independent samples t-test. The relationship between variables was tested using correlation analysis. Statistical significance was set at $p \leq 0.05$

Results

Arithmetic means of anthropometric, biochemical, and hormonal parameters in both patients with IHH and in the control group before the treatment are reported in Table I. There was no significant difference in age, body weight, or BMI between the two groups. By contrast, the percentage of body fat mass was significantly higher and the percentage of lean body mass was significantly lower in hypogonadal men, compared with the control group ($p < 0.0001$ and $p < 0.0001$, respectively). As expected, serum total T, free T, and oestradiol concentrations in hypogonadal men were found to be significantly lower than those detected in healthy subjects ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$, respectively) whereas SHBG concentrations were found to be higher ($p < 0.0001$). Higher fasting glucose, fasting insulin levels, and HOMA index and lower QUICKI index were detected in hypogonadal men, compared with the control group ($p < 0.0001$ in all comparisons) (Table I). Plasma ghrelin concentrations were statistically lower

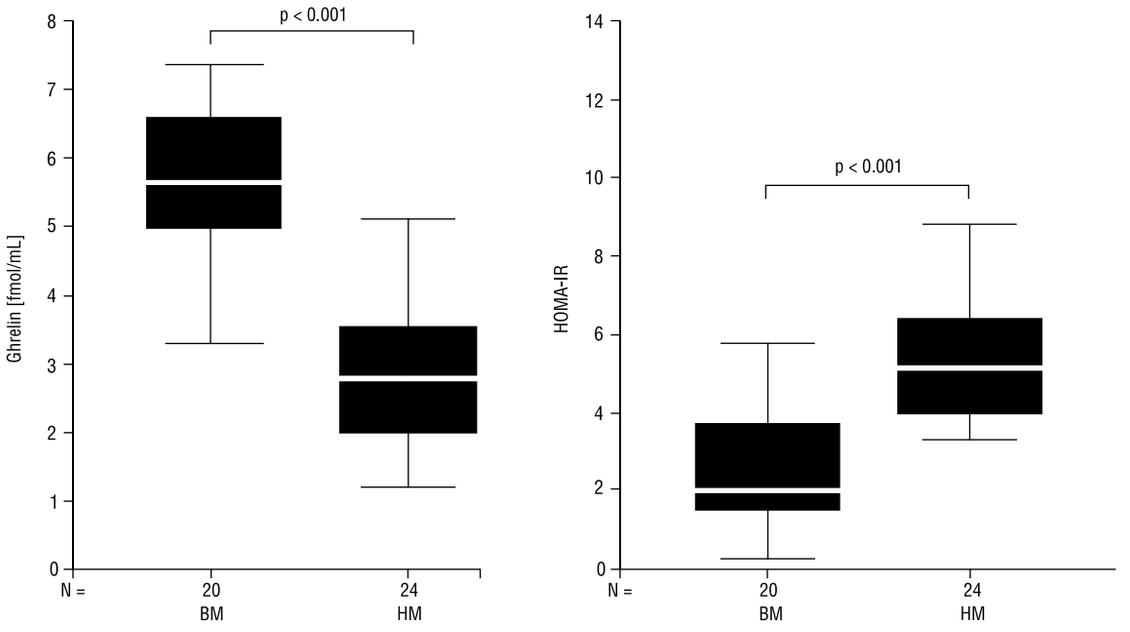

Figure 1. *Mean plasma ghrelin concentrations and HOMA-IR in patients with IHH compared with control group (EM — eugonadal men; HM — hypogonadal men)*

Rycina 1. *Średnie stężenie greliny w osoczu i HOMA-IR u pacjentów z IHH w porównaniu z grupą kontrolną (EM — mężczyzna eugonadalny; HM — mężczyzna hipogonadalny)*

in hypogonadal men (2.80 ± 1.07 fmol/ml) when compared with the control group (5.64 ± 1.12) ($p < 0.0001$) (Fig. 1).

Before T replacement therapy, plasma ghrelin concentrations in hypogonadal subjects were positively correlated with lean body mass, total T, free T, and QUICKI index and negatively correlated with body weight, body fat mass, fasting glucose, fasting plasma insulin, and HOMA index, but not with oestradiol and SHBG (Table II) (Fig. 2, 3).

Total T concentrations in the subjects with IHH were not correlated with SHBG, fasting glucose, or oestradiol, whereas they were positively correlated with plasma ghrelin, free T, lean body mass, and QUICKI index, and negatively correlated with body weight, BMI, body fat mass, fasting insulin, and HOMA index.

Plasma ghrelin concentrations in the control group were positively correlated with total T, free T, and fasting glucose concentrations, and negatively correlated with body weight, BMI, body fat mass, fasting insulin, and HOMA index, but not with oestradiol, SHBG, and QUICKI index . Total T concentrations in the control group were not correlated with fasting glucose and oestradiol, whereas they were positively correlated with plasma ghrelin, free T, SHBG, and lean body mass, and negatively correlated with body weight, BMI, body fat mass, fasting insulin, and HOMA index (Table II).

Using stepwise multiple regression analysis at baseline in hypogonadal subjects, free T concentrations were found to affect HOMA independently (Beta: 0.798; t: 6.219; $p < 0.0001$) ($R2 = 0.621$). In control subjects, body fat mass (Beta: 0.538; t: 3.133; $p < 0.05$) and BMI (Beta: 0.425; t: 2.479; $p < 0.05$) were found to effect HOMA index as independent variables ($R2 = 0.838$).

Anthropometric, hormonal, and metabolic variables in the hypogonadal men after six months of T replacement therapy are reported in Table III. Following T replacement therapy, a significant increase in BMI and lean body mass, and a decrease in body fat mass ($p < 0.0001$ in all comparisons) was demonstrated in hypogonadal men. Additionally, an increase in oestradiol, total and free T concentrations, and a decrease in SHBG, fasting glucose, and fasting insulin levels ($p < 0.0001$ in all comparisons) was conducted by T replacement therapy. Moreover, HOMA index decreased, and the QUICKI index increased by the administration of T, indicating a reduction of insulin resistance ($p < 0.0001$ and $p < 0.0001$, respectively).

After six months of T replacement therapy, plasma ghrelin concentrations, which were low at baseline in hypogonadal subjects, significantly increased ($p < 0.0001$). Plasma ghrelin concentrations were not correlated with body weight, BMI, SHBG, oestradiol, or fasting glucose levels, whereas they were positively correlated with lean body mass, and total and free T concentrations, and negatively correlated with body fat mass, HOMA index, and fasting insulin levels (Table II).

Table II. *Correlations between anthropometric data, hormonal and metabolic parameters, and plasma ghrelin levels at baseline and after treatment in patients with IHH and in controls*

Tabela II. *Korelacje między cechami antropometrycznymi, parametrami hormonalnymi i metabolicznymi a stężeniami greliny w osoczu na początku badania i po leczeniu u mężczyzn z IHH oraz u mężczyzn z grupy kontrolnej*

Parameters	IHH (n = 24) Plasma ghrelin				Control (n=20) Plasma ghrelin	
	Baseline		After 6 months			
	r	p	*r*	p	*r*	p
Weight [kg]	–0.364	0.08	0.009	0.966	–0.487*	< 0.05
Body Mass Index [kg/m²]	–0.439*	< 0.05	–0.054	0.801	–0.684**	< 0.01
Body Fat Mass (%)	–0.681**	< 0.0001	–0.483*	< 0.05	–0.681**	< 0.01
Lean Body Mass (%)	0.681**	< 0.0001	0.483*	< 0.05	0.681**	< 0.01
Total T	0.745**	< 0.0001	0.436*	< 0.05	0.535*	< 0.05
Free T	0.947**	< 0.0001	0.902**	< 0.0001	0.483*	< 0.05
Oestradiol	0.172	0.421	–0.046	0.831	–0.09	0.705
SHBG	0.238	0.264	0.052	0.811	0.168	0.478
Fasting Glucose	–0.471*	< 0.05	–0.207	0.331	0.511*	< 0.05
Fasting Insulin	–0.738**	< 0.0001	–0.698**	< 0.0001	–0.547*	< 0.05
HOMA-IR	–0.778**	< 0.0001	–0.712**	< 0.0001	–0.537*	< 0.05
QUICKI index	0.829**	< 0.0001	0.762**	< 0.0001	0.137	0.564

*Correlation is statistically significant at 0.05, **Correlation is statistically significant at 0.01 (Pearson, 2-tailed); IHH — idiopathic hypogonadotropic hypogonadism; T — Testosterone; SHBG — sex hormone binding globulin; HOMA-IR — Homeostasis Model Assessment-Insulin Resistance; QUICKI-index — Quantitative Insulin Sensitivity Check Index

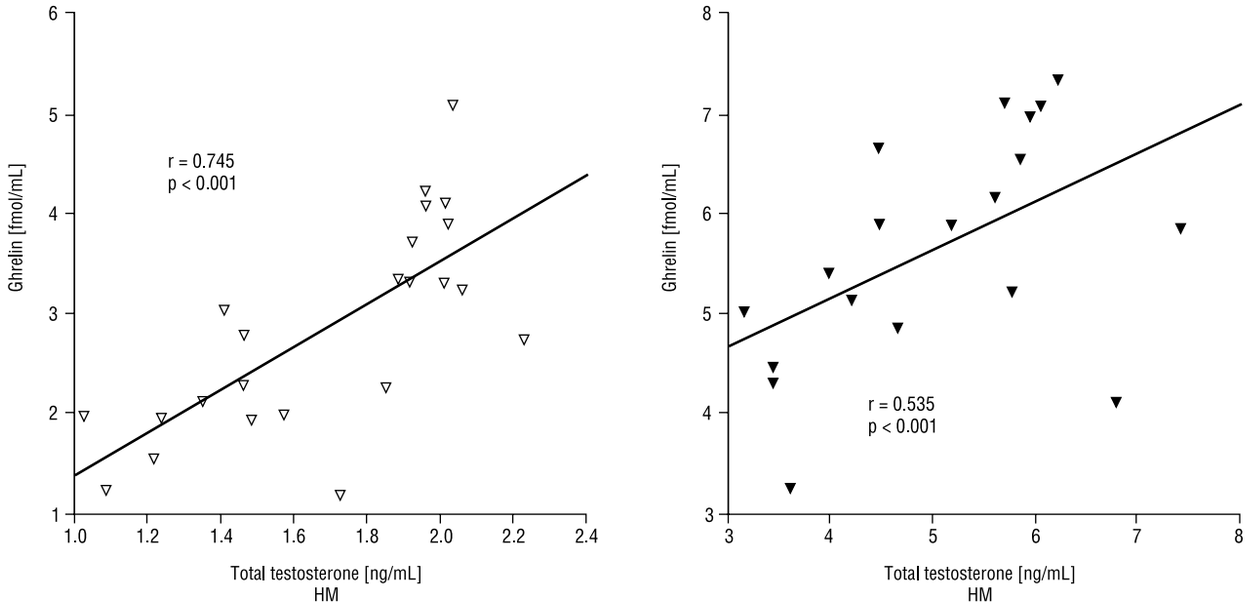

Figure 2. *Correlation analysis of fasting plasma ghrelin levels with serum total testosterone concentrations at baseline in patients with IHH and in the control group (EM — eugonadal men; HM — hypogonadal men)*

Rycina 2. *Analiza korelacji stężenia greliny w osoczu na czczo ze stężeniem całkowitego testosteronu w surowicy krwi u pacjentów z IHH i w grupie kontrolnej (EM — mężczyzna eugonadalny; HM — mężczyzna hipogonadalny)*

Using stepwise multiple regression analysis, after six months of T replacement therapy in hypogonadal subjects, free T concentrations (Beta: 0.612; t: 5.319; p < 0.0001) and body fat mass (Beta: 0.399; t: 3.467; p < 0.05) were found to effect HOMA independently (R2: 0.794).

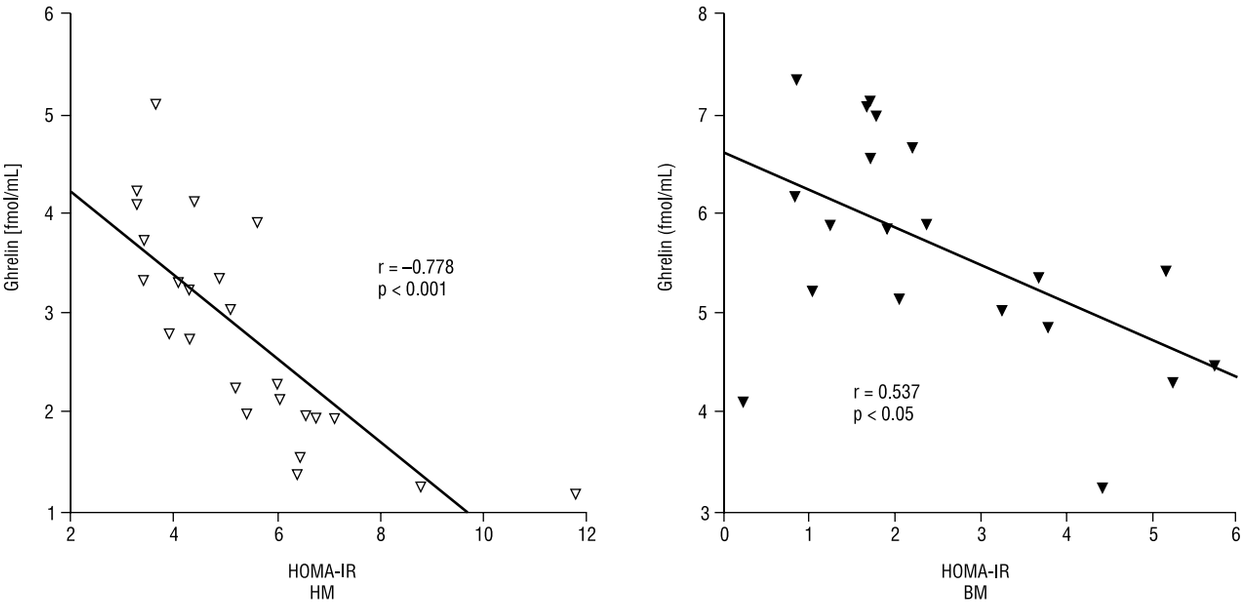

Figure 3. *Correlation analysis of fasting plasma ghrelin levels with HOMA-IR at baseline in patients with IHH and in the control group (EM — eugonadal men; HM — hypogonadal men)*

Rycina 3. *Analiza korelacji stężenia greliny w osoczu z wartością wskaźnika HOMA-IR u pacjentów z IHM i w grupie kontrolnej (EM — mężczyzna eugonadalny; HM — mężczyzna hipogonadalny)*

Table III. ***Comparison of anthropometric data, and hormonal and metabolic parameters after treatment between patients with IHH and controls***

Tabela III. ***Porównanie danych antropometrycznych oraz parametrów hormonalnych i metabolicznych po leczeniu między grupą mężczyzn z IHH i grupą kontrolną***

Variables	IHH (n = 24)			Control (n=20)
	Baseline	After 6 months	P	
Weight [kg]	62.94 ± 8.81	69.38 ± 8.67	< 0.0001	61.70 ± 5.90
Body Mass Index [kg/m^2]	20.93 ± 2.12	22.58 ± 1.95	< 0.0001	20.81 ± 2.08
Body Fat Mass (%)	26.55 ± 6.51	11.13 ± 4.25	< 0.0001	12.75 ± 4.44
Lean Body Mass (%)	73.45 ± 6.51	88.88 ± 4.25	< 0.0001	87.25 ± 4.44
Total T [ng/mL]	1.66 ± 0.37	4.37 ± 0.93	< 0.0001	5.07 ± 1.16
Free T [pg/mL]	2.83 ± 1.24	21.57 ± 3.46	< 0.0001	22.42 ± 4.09
Oestradiol [pg/mL]	4.52 ± 2.45	26.70 ± 4.70	< 0.0001	30.26 ± 2.55
SHBG [nmol/mL]	30.22 ± 6.42	18.47 ± 4.26	< 0.0001	20.53 ± 4.60
Fasting Glucose [mg/dL]	85.17 ± 6.91	77.75 ± 7.09	< 0.0001	75.30 ± 4.99
Fasting Insulin [μU/mL]	25.61 ± 8.36	11.24 ± 3.98	< 0.0001	13.44 ± 4.27
HOMA-IR	5.41 ± 1.96	2.21 ± 0.86	< 0.0001	2.54 ± 0.95
QUICKI index	0.30 ± 0.01	0.34 ± 0.02	< 0.0001	0.34 ± 0.02
Ghrelin [fmol/mL]	2.80 ± 1.07	4.73 ± 1.45	< 0.0001	5.64 ± 1.12

IHH — idiopathic hypogonadotropic hypogonadism; T — testosterone; SHBG — sex hormone binding globulin; HOMA-IR — Homeostasis Model Assessment-Insulin Resistance; QUICKI-index — Quantitative Insulin Sensitivity Check Index. Data are shown as mean values ± SD

Discussion

The effect of T on body composition and muscle mass have been examined in several recently published studies. It has been proven by epidemiological studies that either free T or total T concentrations in healthy men are negatively correlated with intra-abdominal fat mass, coronary artery disease, and type 2 DM. Bhasin et al.

detected that replacement doses of T decrease the visceral fat accumulation in eugonadal men [24]. Studies in hypogonadal men revealed a decrease in body fat mass and an increase in lean body mass by replacement doses of T enanthate, T cypionate, and scrotal transdermal patch. In our study, it was confirmed that there is a negative correlation between T concentrations and body weight, BMI, and body fat mass in eugonadal men. Parallel to former studies, our data also revealed that body fat mass, tending to be higher at baseline in hypogonadal men, statistically significantly decreased after T replacement therapy (T propionate, T phenylpropionate, T isocaproate, and T decanoate), when compared with the control subjects, whereas body fat mass and lean body mass increased. In summary, our findings support the concept that even if different preparations are used, testosterone compounds have similar effects on body fat proportion and muscle mass.

Contradictory results have been reported about the effects of T replacement therapy on insulin sensitivity in male individuals with androgen deficiency. The frequency of hypoandrogenemia in patients with type 2 DM is high. In these patients, it was supposed that better glycaemic control could be achieved through the desirable effects of testosterone on the regulation of insulin sensitivity. Boyanov et al. assessed the effects of T replacement therapy in middle-aged men with type 2 DM and mild androgen deficiency [25]. Twenty-four subjects received testosterone and 24 subjects received no treatment. T replacement therapy gave a statistically significant reduction in body weight and body fat mass, and a decrease in blood glucose values and mean glycosylated haemoglobin (HbA_{1c}). However, Corralers et al. reported a high frequency of hypogonadism in patients with type 2 DM, and correction of the partial T deficiency was demonstrated not to have a meaningful effect on glycaemic control [26]. Androgen replacement in men with low serum levels of total T reduces insulin levels by decreasing insulin resistance [27]. Nevertheless, T replacement therapy does not appear to influence insulin resistance in male individuals who have T levels within the physiological range [28].

The effects of administration of T on insulin resistance have not been completely identified. Simon et al. treated 18 hypogonadal men with T, dihydrotestosterone in gel forms, and placebo [7]. After three months, a significant decrease in plasma fasting insulin and in the ratio of plasma insulin/plasma fasting glucose was reported in patients administered with an androgen replacement. In another study, no decrease in insulin sensitivity was detected in ten hypogonadal men following T replacement therapy in three months [29]. In our study, insulin resistance in hypogonadal men which was increased before the treatment was found to improve after six months of T replacement therapy. It supports the notion that insulin resistance can be broken down with long-term T replacement therapy. Using stepwise regression analysis in male subjects with IHH, IR was found to be affected independently by free T concentrations before T replacement therapy and by both body fat mass and free T concentrations after therapy.

The effects of T on insulin sensitivity are dose dependent. In a study, the effects of T replacement on insulin sensitivity were determined in castrated rats by the euglycaemic hyperinsulinemic clamp technique [9]. In another study, administration of T has been shown to increase insulin sensitivity as much as in healthy rats. On the other hand, supra-physiological doses of T have resulted in an increase in the IR. The relationship between hyperandrogenism and IR in PCOS, which is primarily characterized by chronic anovulation, still appears to be an interesting issue. It is predicted that the development of hyperinsulinaemia is the result of the overproduction of gonadal and adrenal androgens [30]. Hyperandrogenism may play an important role in gender associated IR.

In several studies, it has been demonstrated that normal weight subjects tend to present with higher circulating ghrelin levels in comparison to overweight/ /obese eugonadal males. In agreement with the study designed by Pagotto et al., we reported that circulating ghrelin levels in hypogonadal men are lower when compared with the eugonadal control group [20]. Ikezaki et al. have reported that percent overweight and down regulation of ghrelin secretion may be a consequence of a higher IR [31]. Furthermore, in a recent population-based epidemiological study, it appears that the positive correlation between low ghrelin concentrations and high insulin levels may be a risk factor for IR and type 2 DM (15). Additionally, Shiiya et al. reported normal ghrelin concentrations among lean patients with type 2 DM [32]. Studying the relationship between plasma ghrelin levels and IR, we confirmed a negative correlation between these parameters. These findings further support the hypothesis that low ghrelin levels may be a pharmacodynamic indicator of IR.

In this study, we reported a positive correlation between ghrelin levels and T concentrations in both eugonadal male individuals and in control subjects. After administration of T, the positive correlation in IHH males remained, and circulating ghrelin levels tended to correlate closely with the levels in control subjects. We predict that the same circulating ghrelin levels can be achieved following long-term T replacement therapy. Considering these results, we estimate that the interaction between the two parameters correlates independently with body fat mass and IR. Pagotto et al. reported that plasma ghrelin levels were inversely cor-

related with parameters of IR in obese women with PCOS, compared with weight-matched control subjects, and no significant correlation between changes of ghrelin and IR after treatment was found [12]. These findings confirm that androgens play an important role in ghrelin metabolism. In summary, we propose that T replacement therapy in androgen deficiency status may lead to weight gain and may induce a positive energy balance by restoring ghrelin concentrations to normal range.

Mechanisms by which abnormal androgen conditions may alter ghrelin concentrations are at present unknown. Theoretically, androgens may act directly on both peptide expression and synthesis as well as on its metabolic pathways. Additionally, it is believed that androgens may modulate ghrelin levels through other regulatory factors such as free fatty acids. It is well known that adipose visceral depots are important sites of production of free fatty acids and that androgens influence visceral fat mass in a gender specific manner. In men, T stimulates lipolysis, inducing an increase of free fatty acids release from the visceral fat depots, whereas in females, it accelerates lipogenesis in visceral depots [33]. Therefore, low T concentrations in men and hyperandrogenism in women are usually associated with an increased abdominal adipose proportion [34]. A link between free fatty acids and ghrelin has been shown by Broglio et al., demonstrating that infusion of free fatty acids reduces the ability of ghrelin to induce GH secretion from the pituitary [35]. On the other hand, there are also studies in humans showing no changes in ghrelin levels caused by lipid infusion, a stimulus known to increase circulating free fatty acid levels [36]. Therefore, the putative role of free fatty acids on ghrelin regulation is yet to be defined.

Although it does not completely clarify the mechanisms of the effects of androgens on ghrelin concentrations, our data supports the notion that ghrelin may constitute an important link between the regulation of reproduction and the control of metabolic homeostasis. Additionally, our study demonstrates the relationship between androgens and adipose tissue. Further highlights on the treatment of IR will be achieved by discovering the interactions of the pathways causing these results. Male individuals with IHH included in our study were a specific aetiological group of hypoandrogenism, which gave us an opportunity to investigate only the effects of androgen deficiency.

References

1. Hunter SJ, Garvey WT. Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. Am J Med 1998; 105: 331–335.
2. Gnacińska M, Małgorzewicz S, Lysiak-Szydłowska W et al. The serum profile of adipokines in overweight patients with metabolic syndrome. Endokrynol Pol 2010; 61: 36–41.
3. Lichtenstein MJ, Yarnell JWG, Elwood PC et al. Sex hormones, insulin, lipids, and prevalent ischemic heart. Am J Epidemol 1987; 126: 647–657.
4. Pasquali R, Casimirri F, Cantobelli S et al. Effects of obesity and body fat distribution on sex hormones and insulin in men. Metabolism 1991; 40: 101–104.
5. Phillips GB. Relationship between sex hormones and glucose, insulin, and lipid abnormalities in men with myocardial infarction. Proc Nat Acad Sci 1977; 74: 1729–1733.
6. Seidell JC, Bjorntop P, Sjostrom LS et al. Visceral fat accumulation in men is positively associated with insulin, glucose, and c-peptide levels, but negatively with testosterone levels. Metabolism 1990; 39: 897–901.
7. Simon D, Preziosi P, Barrett-Connor E et al. Interrelationship between plasma testosterone and plasma insulin in healthy adult men: the telecom study. Diabetologia. 1992; 35: 173–177.
8. Cohen JC, Hickman R. Insulin resistance and diminished glucose tolerance in power lifters ingesting anabolic steroids. J Clin Endocrinol Metab 1987; 64: 960–963.
9. Holmang A, Bjorntorp P. The effects of testosterone on insulin sensitivity in male rats. Acta Physiol Scand 1992; 146: 505–10.
10. Shoupe D, Lobo RA. The influence of androgens on insulin resistance. Fertil Steril. 1984; 41: 385–388.
11. Marsden PJ, Murdock AP, Taylor R. Adipocyte insulin action in hypogonadotropic hypogonadism. Hum Reprod. 2000; 15: 1672–1678.
12. Pagotto U, Gambineri A, Vicennati V et al. Plasma ghrelin, obesity, and the polycystic ovary syndrome. Correlation with insulin resistance and androgen levels. J Clin Endocrinol Metab. 2002; 87: 5625–5629.
13. Kojima M, Hosoda H, Date Y. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999; 402: 656–660.
14. Ariyasu H, Takaya K, Tagami T et al. Stomach is a major source of circulating ghrelin and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab. 2001; 86: 4753–4758.
15. Pöykkö SM, Kellokoski E, Hörkkö S et al. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes 2003; 52: 2546–2553.
16. Barkan AL, Dimaraki EV, Jessup SK et al. Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by ambient growth hormone levels. J Clin Endocrinol Metab. 2003; 88: 2180–2184.
17. Skoczylas A, Adamczak M, Chudek J et al. Cilazapril increases plasma ghrelin concentration in obese patients with arterial hypertension. Endokrynol Pol 2010; 61: 21–27.
18. Nagaya N, Kojima M, Uematsu M et al. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. Am J Physiol Regul Integr Comp Physiol 2001; 280: 1483–1487.
19. Tschöp M, Weyer C, Tataranni PA et al. Circulating ghrelin levels are decreased in human obesity. Diabetes. 2001; 50: 707–709.
20. Pagotto U, Gambineri A, Pelusi C et al. Testosterone replacement therapy restores normal ghrelin in hypogonadal men. J Clin Endocrinol Metab. 2003; 88: 4139–43.
21. Siemińska L, Wojciechowska C, Kos-Kudła B et al. Serum concentrations of leptin, adiponectin and interleukin-6 in postmenopausal women with Hashimoto's thyroiditis. Endokrynol Pol 2010; 61: 112–116.
22. Reilly JJ, Wilson J, Mccoll JH et al. Ability of bioelectric impedance to predict fat-free mass in prepubertal children. Pediatr Res 1996; 39: 176–179.
23. Naharci MI, Pinar M, Bolu E et al. Effect of testosterone on insulin sensitivity in men with idiopathic hypogonadotropic hypogonadism. Endocr Pract 2007; 13: 629–35.
24. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. J Endocrinol 2001; 170: 27–38.
25. Boyanov MA, Boneva Z, Christov VG. Testosterone supplementation in men with type 2 diabetes, visceral obesity and partial androgen deficiency. Aging Male 2003; 6: 1–7.
26. Corrales JJ, Burgo RM, Garca-Berrocal B et al. Partial androgen deficiency in aging type 2 diabetic men and its relationship to glycemic control. Metabolism. 2004; 53: 666–672.
27. Marin P, Holmang S, Jonsson L. The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. Int J Obes Relat Metab Disord 1992; 16: 991–997.
28. Singh AB, Hsia S, Alaupovic P. The effects of varying doses of testosterone on insulin sensitivity, plasma lipids, apolipoproteins, and c-reactive protein in healthy young men. J Clin Endocrinol Metab 2002; 87: 136–143.
29. Tripathy D, Shah P, Lakshmy R et al. Effect of testosterone replacement on whole body glucose utilization and other cardiovascular risk factors in males with idiopathic hypogonadotrophic hypogonadism. Horm Metab Res. 1998; 30: 642–645.
30. Dunaif A. Insulin resistance and the polycytic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997; 18: 774–800.
31. Ikezaki A, Hosoda H, Ito K et al. Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes 2002; 51: 3408–3411.
32. Shiiya T, Nakazato M, Mizuta M et al. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab. 2002; 87: 240–244.
33. Bjorntop P. The regulation of adipose tissue distribution in humans. Int J Obes 1996; 20: 291–302.
34. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the Metabolic Syndrome. Endocr Rev 2000; 21: 697–738.
35. Broglio F, Benso A, Gottero C et al. Effects of glucose, fatty acids or arginine load on the Gh-releasing activity of ghrelin in humans. Clin Endocrinol 2002; 57: 265–271.
36. Mohlig M, Spranger J, Otto B et al. Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans. J Endocrinol Invest 2002; 25: 36–38.