Endocr (2008) 34:81–86
DOI 10.1007/s12020-008-9102-x

ORIGINAL PAPER

Plasma ghrelin levels in males with idiopathic hypogonadotropic hypogonadism

Cevdet Duran · Arif Yonem · Ihsan Ustun · Omer Ozcan · Osman Metin Ipcioglu · Cihat Cinar Basekim

Received: 14 July 2008 / Accepted: 8 September 2008 / Published online: 23 October 2008

Abstract It has recently been shown that ghrelin is a pleitropic modulator with effects on diverse biological functions, such as energy homeostasis and reproduction. In this study, ghrelin levels and its relationship between metabolic and biochemical parameters were investigated in male subjects with idiopathic hypogonadotropic hypogonadism (IHH). Patients in the study were composed of 33 men with IHH, and controls were composed of 36 healthy age-matched men. The patients' group had significantly higher waist/hip ratio (WHR), and lower testis volume, luteinizing hormone (LH), follicle stimuling hormone (FSH) and total testosterone (TT) levels when compared with controls. Plasma total ghrelin levels were significantly lower in patients than in controls (96.4 ± 29.1 ng/ml vs. 146.1 ± 28.9 ng/ml, $P < 0.001$, respectively). No correlation of ghrelin was found with body mass index, waist/hip ratio, homeostasis model assessment insulin resistance index, testis volume, LH, FSH and TT levels in both patients and controls. The present study showed that ghrelin levels were significantly lower in men with IHH than in controls. However, further studies are needed to better understand the relationships between ghrelin, and metabolic and reproductive systems.

Keywords Hypogonadotrophic hypogonadism · Ghrelin · Insulin resistance · Obesity

C. Duran (✉)
Division of Endocrinology, Metabolism and Internal Medicine, Konya Education and Research Hospital, Meram, Konya, Turkey
e-mail: drcduran@gmail.com

A. Yonem
Department of Endocrinology, Metabolism and Internal Medicine Gulhane School of Medicine, Haydarpasa Training Hospital, Tibbiye Cad, Uskudar, Istanbul, Turkey

I. Ustun
Division of Endocrinology, Metabolism and Internal Medicine, Mersin State Hospital, Mersin, Turkey

O. Ozcan · O. M. Ipcioglu
Department of Biochemistry, Gulhane School of Medicine, Haydarpasa Training Hospital, Tibbiye Cad, Uskudar, Istanbul, Turkey

C. C. Basekim
Department of Radiology, Gulhane School of Medicine, Haydarpasa Training Hospital, Tibbiye Cad, Uskudar, Istanbul, Turkey

Introduction

Ghrelin, a hormone produced mainly by stomach, was identified originally as the endogenous ligand of the growth hormone secretagogue receptor. Ghrelin might also be synthesized in other organs, where it might have autocrine or paracrine effects, indicating that ghrelin has other effects, as well as stimulating the release of growth hormone. In recent years, there has been increasing evidence suggesting that ghrelin may play a role in the central regulation of reproduction [1]. The immunoreactivity and gene expression for ghrelin and its functional receptor have been found in the hypothalamus, known as the most important area in the control of reproduction, hypophysis, testicular Leydig cells, ovary in rats, sheep and humans, indicating that ghrelin has a role in the regulation of reproductive function [2]. It is suggested that ghrelin has predominantly an inhibitory effect on hypothalamic gonadotropin-releasing hormone (GnRH) secretion or GnRH induced gonadotropin secretion, but direct stimulatory effect on basal luteinizing (LH) and follicle stimulating hormone (FSH) secretions in vitro. In most studies, exogenous

ghrelin has been reported to inhibit LH secretion in vivo and to decrease LH responsivenes to GnRH in vitro [3–8]. On the other hand, it has interestingly been shown that ghrelin stimulates LH release from pituitary cells of rats and of goldfish [9]. In the testis, ghrelin was capable of inhibiting testosterone secretion in vivo and in vitro, and testosterone replacement therapy restores low-ghrelin levels in hypogonadal men [10–12].

To date, data indicating the plasma ghrelin levels and related to the effects of ghrelin on the control of the reproductive system have generally been obtained from animal studies, but investigations about plasma levels in hypogonads and the effects on human reproductive system are insufficient. In this study, our aim was to evaluate plasma ghrelin levels in male patients with idiopathic hypogonadotropic hypogonadism (IHH) and its relation to different metabolic and biochemical parameters.

Materials and method

This study was conducted at Endocrinology Department of Haydarpasa Training Hospital, Gulhane School of Medicine, Istanbul, between February 2007 and September 2007. The subjects in the study were composed of two groups: patients and controls. Under the current Turkish Military Act, the patients with hypogonadism must be evaluated in detail by hospitalizing to assess whether they are appropriate for completing their military service or not. Patients' group was composed of 33 hypogonadal males, and controls were composed of 36 healthy age-matched soldiers, who serve as security personnel, from our military hospital. Andropometric and biochemical characteristics of both groups are shown in Table 1. Based on the clinical history, physical examination and routine laboratory measurements, patients with diabetes, primary hypogonadism, hypopituitarism except for secondary hypogonadism, or other endocrine, renal, liver and cardiovascular diseases, were not included in the study. None of the patients and controls had any caloric restrictions, and the rates of their calorie intake were at almost similar levels. The fact that testosterone levels were under 3.5 ng/ml was defined as IHH when gonadotropin levels were close to lower limit or lower than normal range. Magnetic resonance imaging test was performed in all patients to exclude any pituitary lesion. The study protocol was approved by the local research ethics committe, in accordance with the declaration of Helsinki, and written informed consent forms were obtained from all participants.

Table 1 Baseline characteristics of both groups

	Patients	Controls	*P* value
N	33	36	ns
Age	22.9 ± 4.1	22.2 ± 2.5	ns
BMI (kg/m^2)	22.9 ± 4.4	22.9 ± 2.7	ns
WHR	0.93 ± 0.07	0.86 ± 0.05	0.001
Testis volume (ml)	7.4 ± 4.8	20.7 ± 1.7	<0.001
LH (mIU/ml)	0.38 ± 0.6	3.8 ± 1.7	<0.001
FSH (mIU/ml)	0.7 ± 0.8	2.9 ± 1.5	<0.001
Total testosterone (ng/ml)	1.4 ± 1.6	7.0 ± 1.4	<0.001
HOMA-IR	1.93 ± 1.25	1.01 ± 0.62	<0.001
Plasma ghrelin level (ng/ml)	96.4 ± 29.1	146.1 ± 28.9	<0.001

BMI, body mass index; WHR, waist/hip ratio; LH, luteinizing hormone; FSH, follicle stimulating hormone; HOMA-IR, homeostasis model assessment insulin resistance

Anthropometric measurements

In all study participants, body weight (kg) and height (m) were measured in undressed condition. Waist circumference was measured as the minimum size between iliac crest and lateral costal margin, while hip circumference was determined as maximum size over buttocks, using a measuring tape in centimetres (cm).

Biochemical and hormonal assays

After an overnight fasting, serum levels of insulin, LH, FSH and total testosterone (TT) were measured by chemiluminometric immunoassay methods (Abbott Laboratories, Abbott Park, Chicago, IL, USA). Fasting blood glucose levels were measured by using an autoanalyser (Beckman Coulter LX-20, USA). The insulin resistance score in the study was computed with the formula homeostasis model assessment insulin resistance (HOMA-IR): fasting plasma glucose (mmol/l) times fasting serum insulin (mIU/l) divided by 22.5.

For ghrelin measurement, blood samples of the patients and healthy individuals after an overnight fasting were taken into test tubes containing aprotinin (0.6 IU/ml of blood), and these samples were immediately chilled via ice boxes, and then, serum samples were separated by centrifugation and stored in deepfreeze at −70°C until being analyzed. Plasma total ghrelin levels were measured with a commercially available ELISA kit (the range of detection: 0–100 ng/ml, sensitivity: 0.08 ng/ml, Phoenix International, USA).

Testis ultrasonography

The anatomy and structure of the testis were evaluated with an ultrasonography device (Esaote AU 5, Italy) by one of the radiologists of our hospital (all measurements were

made by the same radiologist). Testis volume was calculated using this formula [length (cm) × width (cm) × height (cm) × 0.52].

Statistical analysis

Clinical and laboratory data were expressed as mean ± SD. Distributions of variations were assessed using Shapiro–Wilk test. Comparisons between groups were performed using Mann–Whitney *U* test. Correlations between variables were investigated by Pearson correlation test. All statistical analyses were performed through a PC compatible statistics programme (SPSS v.13, Chicago, IL, USA) and *P*-values less than 0.05 were considered statistically significant.

Results

The group of patients was composed of 33 men with IHH, controls were 36 healthy men. On comparing the two groups, patients had significantly higher levels of WHR and HOMA index, and lower levels of testis volume, LH, FSH and TT. Plasma ghrelin levels were significantly lower in patients when compared with controls (96.4 ± 29.1 ng/ml vs. 146.1 ± 28.9 ng/ml, $P < 0.001$, respectively) (Table 1).

The group composed of patients with six anosmic subjects. Plasma ghrelin levels showed no significant difference between anosmic and normal smelling patients (94.8 ± 27.2 vs. 103.3 ± 39.8 ng/ml, $P > 0.05$). No correlation of ghrelin was found with body mass index, waist/hip ratio, homeostasis model assessment, insulin resistance index, testis volume, LH, FSH and TT levels in both groups.

Discussion

The present study is the first human study with the highest number of cases investigating plasma ghrelin levels and its relation with some metabolic and hormonal parameters. In this study, plasma total ghrelin levels were found to be lower in men with IHH than in healthy controls, and no correlation related to ghrelin was found between age, BMI, WHR, HOMA, testis volume, LH, FSH and TT levels.

Patients with low serum testosterone and gonadotropin levels are diagnosed with hypogonadotropic hypogonadism. While some cases are genetic in origin (KAL1, FGFR1), others are considered to be idiopathic. During the foetal development, GnRH secreting neurons and olfactory neurons migrate together from the olfactory area to hypothalamus, and GnRH neurons eventually reside in this area [13]. When this transport does not take place, hypogonadotrophic hypogonadism and anosmia (also called Kallman's syndrome) ensues.

Ghrelin is mainly secreted from the stomach, hypothalamus and, to a lesser extent, from testis and other tissues. The best known effect of ghrelin is on growth hormone secretogogue reseptor type 1a (GHS-R1a) and thus causing growth hormone to be secreted. GHS-R1a is highly expressed in the areas of hypothalamus and pituitary glands, mainly involved in the control of appetite, food and energy intake and reproduction [14]. In light of the literature, some studies have been consistently suggested the potential effects of ghrelin on the control of the reproductive system in humans and rodents. While ghrelin has an effect on gonadotropin secretion, gonadotropin has also an effect on ghrelin secretion. Some studies (in vivo) have shown that ghrelin has inhibitory effects on the control of pulse frequency of LH secretion in overectomized rodents [3], overectomized rhesus monkeys [5], and in cyclic and overectomized female rats [7]. On the other hand, it has been shown that ghrelin stimulates LH release from the rat pituitary cells (in vitro) depending on the phase of estrous cycle [4]. This interaction between ghrelin and gonadotropins has been proposed as a protective mechanism to decrease energy requirement of the body in the presence of energy insufficiency [15]. This situation indicates that ghrelin might be responsible for inhibiting reproductive function in the state of malnutrition to avoid the excess metabolic demands, such as pregnancy and sexual intercourse [16].

Gonadotropins appear to be involved in the modulation of ghrelin expression or secretion. In a study, it was reported that ovarian ghrelin mRNA expression was disrupted by blockade of preovulatory gonadotropin surge as shown by administiration of a potent GnRH antagonist [16]. In a different study by the same authors, it was reported that Leydig cell-specific expression of ghrelin in rat testis is under the control of LH, but not of FSH [17]. On the other hand, Pagotto et al. [12] reported no correlation between ghrelin levels and gonadotropins.

On the other hand, it was reported in some previous studies that ghrelin has a direct effect on testicular functions. In the testicular tissue, ghrelin is mainly located in Leydig cells, Sertoli cells and seminifer tubuli [17], and ghrelin receptor has been demonstrated to exist in testis [18]. In the rat testis, ghrelin secretion is mainly under the control of pituitary LH secretion [11, 17, 19]. In the study performed by Barrelio et al. [17], it was reported that mature foetal and adult Leydig cells contain ghrelin, and that testicular ghrelin mRNA and protein expression are decreased after long-term hypophysectomy, and human chorionic gonadotropin replacement therapy increases ghrelin mRNA and peptide levels. In another study,

reported by the same group, it was suggested that intratesticular injection of ghrelin inhibits the proliferative activity of immature Leydig cells in vivo and regulates stem cell factor. It was proposed in this study that acquisiton of ghrelin expression by Leydig cell precursors during differentiation may operate as a self-autoregulatory signal for the inhibiton of proliferative activity in these cells [20]. In the testis, it was shown that ghrelin could inhibit testosterone secretion in vivo and in vitro [10, 11]. On the other hand, testosterone replacement theraphy increases ghrelin levels [12]. The major source of androgen secretion in the testis is Leydig cells, and ghrelin and its receptors are also mainly present in Leydig cells. As was understood from this interaction between ghrelin and testosterone, ghrelin could be proposed to play a role on testosterone secretion by causing an autocrine or paracrine effect, as well as gonadotropins.

In a different study reported by Pagotta et al. [12], circulating ghrelin levels were significantly decreased in obese hypogonadal men, compared to their overweight matched controls, and a positive correlation was reported to exist between ghrelin, and total and free testosterone. In the same report, testosterone replacement therapy of 6 weeks was reported to increase circulating ghrelin levels to those found in normal control subjects and to decrease HOMA index [12], but the limitation of this study was the small number and the heterogenity of the subjects investigated in the study (of total 7 subjects with hypogonadism, 4 were with Klinifelter's syndrome; 1 with primary hipogonadism due to varicosel, and 2 with Kallman's syndrome, and controls, including 9 obese and 10 normal weight subjects, were composed of two control groups). In the same study, ghrelin levels were reported to be lower insignificantly in patients with primary hypogonadism than in those with secondary hypogonadism [12]. In our study, ghrelin levels were demonstrated to be lower in men with IHH than in healthy controls. Due to the above mentioned causes, the fact that levels of gonadotropins and testosterone are decreased in IHH cases could explain the low levels of plasma total ghrelin in these cases. However, our study showed that there is no correlation between ghrelin and gonadotropins in both patients and controls although previous studies reported that ghrelin has negative effects on gonadotropins. The fact that correlation emphasized in previous studies was not indicated in our study was linked to the existence of secretion defects of gonadotropins due to pituitary and/or hypothalamic causes in our patients. Although ghrelin increases the secretion of gonadotropins from pituitary gland in the absence of GnRH [4], and GnRH is absent in some of our patients, a positive correlation may be expected between ghrelin and gonadotropins levels in patients' group. However, this effect was also linked to secretion defects of gonadotropins from pituitary. Considering the results of our study, low levels of gonadotropins and androgen were thought to cause low levels of ghrelin levels. In addition, the restriction of our study is the absence of a group with primary hypogonadism in the study. The fact that patients with IHH have lower gonadotropin levels may have an addional effect on patients with lower ghrelin levels when compared to patients with primary hypogonadism.

Another limitation in our study was that the effect of the treatment on ghrelin levels could not be investigated although the testosterone and/or gonadotropin replacement therapies were applied to patients. Follow-up visits by the patients were unlikely because our patients had been referred to the center from distant troops, and then returned their towns and provinces after completing detailed investigations.

Although a negative correlation between BMI and ghrelin levels was reported [21–24], no correlation as to BMI and ghrelin levels could be indicated in our study due to close BMI levels between nearly 22–24 kg/m^2, except for only three and one obese subjects in patients and controls, respectively (BMI levels of three obese subjects among patients were 30.5, 32.2 and 37.9 kg/m^2, and that of one obese subject in controls was 33.4 kg/m^2), and only one subject indicated lower BMI level among patients (16.7 kg/m^2). In our study, the fact that high WHR levels exist in patients indicated higher abdominal obesity in patients than in controls in spite of similar BMI values, and suggested that higher WHR levels in IHH could contribute to the detection of low ghrelin levels in this group. Although a negative correlation was reported between age rates and ghrelin levels [22], no significant correlation was found in our study.

Obesity and insulin resistance are frequently encountered in hypogonadal patients when compared with healthy individuals. Lower ghrelin levels could be the reason of obesity and insulin resistance in hypogonads. In previous studies, it was reported that ghrelin inhibits the secretion of insulin [22, 24] and increases glucose output in primary hepatocytes, while des-acyl ghrelin, most abundant form of ghrelin in the body, decreases glucose output in primary hepatocytes [25], and that ghrelin inhibits the secretion of adiponectin, a hormone decreasing insulin resistance [26] and causes an increase in the levels of counter-insulinary hormones, such as growth hormone and cortisol [27]. In addition, low plasma ghrelin levels are associated with several components of metabolic syndrome, such as obesity, insulin resistance and high blood pressure, indicating that ghrelin might be a useful biomarker for the metabolic syndrome [28–30]. Interestingly, insulin also inhibits ghrelin secretion [31], which could be seen as a vicious circle. In our study, although there was no correlation between ghrelin and HOMA indexes in both groups,

HOMA indexes were found to be higher in IHH when compared with controls, and so the association between HOMA and ghrelin expressed above suggested that HOMA indexes higher in IHH group could cause low levels of ghrelin via direct or indirect effects.

Conclusion

In conclusion, the present study showed that male patients with IHH had lower ghrelin levels when compared with healthy males. Low ghrelin levels in the patients with IHH could result from low levels of gonadotropins, the tendency of the patients with IHH to obesity and insulin resistance. However, further studies should be performed to enlighten the relationships between ghrelin and reproductive system in the humans in both genders.

Acknowledgements We thank Ilker Ercan (Assoc. Prof. of Uludag University, Bursa, Department of Biostatistics) for his valuable assistance for statistical analysis and Numan Duran for language editing.

References

1. A.F. Leite-Moreira, J.B. Soares, Physiological, pathological and potential therapeutic roles of ghrelin. Drug Discov. Today **12**, 276–288 (2007)
2. D.W. Miller, J.L. Harrison, Y.A. Brown, U. Doyle, A. Lindsay, C.L. Adam et al., Immunohistochemical evidence for an endocrine/paracrine role for ghrelin in the reproductive tissues of sheep. Reprod. Biol. Endocrinol. **3**, 60 (2005)
3. M. Furuta, T. Funabashi, F. Kimura, Intracerebroventricular administration of ghrelin rapidly supresses pulsatile luteinizing hormon secretion in ovariectomized rats. Biochem. Biophys. Res. Commun. **288**, 780–785 (2001)
4. R. Fernandez-Fernandez, M. Tena-Sempere, E. Aguilar, L. Pinilla, Ghrelin effects on gonadotropin secretion in male and female rats. Neurosci. Lett. **362**, 1037 (2004)
5. N.R. Vulliémoz, E. Xiao, L. Xia-Zhang, M. Germond, J. Rivier, M. Ferin, Decrease in luteinizing hormone pulse frequency during a five-hour peripheral ghrelin infusion in the overectomized rhesus monkey. J. Clin. Endocrinol. Metab. **89**, 5718–5723 (2004)
6. J. Iqbal, Y. Kurose, B. Canny, I.J. Clarke, Effects on central infusion of ghrelin on food intake, and plasma levels of growth hormone, luteinizing hormone, prolactin and cortisol secretion in sheep. Endocrinology **147**, 510–519 (2006)
7. R. Fernandez-Fernandez, M. Tena-Sempere, V.M. Navarro, M.L. Barreiro, J.M. Castellano, E. Aguilar et al., Effects of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretion in adult female rats: In vivo and in vitro studies. Neuroendocrinology **82**, 245–255 (2005)
8. A.C. Martini, R. Fernandez-Fernandez, S. Tovar, V.M. Navarro, E. Vigo, M.J. Vazquez et al., Comparative analysis of the effects of ghrelin and unacylated ghrelin on luteinizing hormone secretion in male rats. Endocrinology **147**, 2374–2382 (2006)
9. S. Unniappan, Peter RE: In vitro and in vivo effects of ghrelin on luteinizing hormone and growth hormone release in goldfish. Am. J. Physiol. Regul. Integr. Comp. Physiol. **286**, R1093–R1101 (2004)
10. R. Fernandez-Fernandez, V.M. Navarro, M.L. Barreiro, E.M. Vigo, S. Tovar, A.V. Sirotkin et al., Effects of chronic hyperghrelinemia on puberty onset and pregnancy outcome in rat. Endocrinology **146**, 3018–3025 (2005)
11. M. Tena-Sempere, M.L. Barreiro, L.C. Gonzalez, F. Gaytan, F.P. Zhang, J.E. Caminos et al., Novel expression and functional role of ghrelin in rat testis. Endocrinology **143**, 717–725 (2002)
12. U. Pagotta, A. Gambineri, C. Pelusi, S. Genghini, M. Cacciari, B. Otto et al., Testosterone replacement therapy restores normal ghrelin in hypogonadal men. J. Clin. Endocrinol. Metab. **88**, 4139–4143 (2003)
13. L.C. Layman, Hypogonadotropic hypogonadism. Endocrinol. Metab. Clin. North Am. **36**, 283–296 (2007)
14. A.D. Howard, S.D. Feighner, D.F. Cully, J.P. Arena, P.A. Liberator, C.I. Rosenblum et al., A receptor in pituitary and hypothalamus that functions in growth hormone release. Science **273**, 9747 (1996)
15. M.C. Garcia, M. Lopez, C.V. Alvarez, F. Casanueva, M. Tena-Sempere, C. Dieguez, Role of ghrelin in reproduction. Reproduction **133**, 531–540 (2007)
16. J.E. Caminos, M. Tena-Sempere, F. Gaytan, J.E. Sanchez-Criado, M.L. Barreiro, R. Nogueiras et al., Expression of ghrelin in the cyclic and pregnant rat ovary. Endocrinology **144**, 1594–1602 (2003)
17. M.L. Barreiro, F. Gaytan, J.E. Caminos, L. Pinilla, F.F. Casanueva, E. Aguilar et al., Cellular location and hormonal regulation of ghrelin expression in rat testis. Biol. Reprod. **67**, 1768–1776 (2002)
18. M.L. Barreiro, J.S. Suominen, F. Gaytan, L. Pinilla, L.K. Chopin, F.F. Casanueva et al., Developmental, stage-specific, and hormonally regulated expression of growth hormone secretogogue receptor Messenger RNA in rat testis. Biol. Reprod. **68**, 1631–1640 (2003)
19. F. Gaytan, M.L. Barreiro, J.E. Caminos, L.K. Chopin, A.C. Herington, C. Morales et al., Expression of ghrelin and its functional recepor, type 1a growth hormone secretogogue receptor, in normal human testis and testicular tumors. J. Clin. Endocrinol. Metab. **89**, 400–409 (2004)
20. M.L. Barreiro, F. Gaytan, J.M. Castellano, J.S. Suominen, J. Roa, M. Gaytan et al., Ghrelin inhibits the proliferative activity of immature Leydig cells in vivo and regulates stem cell factor messenger ribonucleic acid expression in rat testis. Endocrinology **11**, 4825–4834 (2004)
21. M. Tschöp, C. Weyer, P.A. Tataranni, V. Devenerayan, E. Ravussin, M.L. Heiman, Circulating ghrelin levels are decreased in human obesity. Diabetes **50**, 7079 (2001)
22. A.M. Haqq, I.S. Farooqi, S. O'Rahilly, D.D. Stadler, R.G. Rosenfeld, K.L. Pratt et al., Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J. Clin. Endocrinol. Metab. **88**, 174–178 (2003)
23. D.E. Cummings, Ghrelin and short- and long-term regulation of appetite and body weight. Physiol. Behav. **89**, 71–84 (2006)
24. A. Salehi, C. Dornonville de la Cour, R. Hakanson, I. Lundquist, Effect of ghrelin on insulin and glucagon secretion: A study of isolated pancreatic islet and intact mice. Regul. Pept.**8**, 143–150 (2004)
25. C. Gauna, P.J. Delhanty, L.J. Hofland, J.A. Janssen, F. Broglio, R.J. Ross et al., Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. J. Clin. Endocrinol. Metab. **90**, 1055–1060 (2005)
26. V. Ott, M. Fasshauer, A. Dalski, B. Meier, N. Perwitz, H.H. Klein et al., Direct peripheral effects of ghrelin include suppression of adiponectin expression. Horm. Metab. Res. **34**, 640–645 (2002)
27. M.M. Malagon, R.M. Luque, E. Ruiz-Guerrero, F. Rodriguez-Pacheco, S. Garcia-Navarro, F.F. Casanueva et al., Intracellular

signaling mechanisms mediating ghrelin-stimulated growth hormone release in somatotropes. Endocrinology **144**, 5372–5380 (2003)
28. S.M. Pöykkö, E. Kellokoski, S. Hörkkö, H. Kauma, Y.A. Kesaniemi, O. Ukkola, Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes **52**, 2546–2553 (2003)
29. U. Mager, V. Lindi, J. Lindström, J.G. Eriksson, T.T. Valle, H. Hamalainen et al., Association of the Leu72Met polymorphism of the ghrelin gene with the risk o Type 2 diabetes in subjects with impaired glucose tolerance in the Finnish diabetes prevention study. Diabet. Med. **23**, 685–689 (2006)
30. O. Ukkola, S.M. Pöykkö, Y. Antero Kesaniemi, Low plasma ghrelin consentration is an indicator of the metabolic syndrome. Ann. Med. **38**, 274–279 (2006)
31. D.E. Flanagan, M.L. Eyans, T.P. Mondsod, F. Rife, R.A. Heptulla, W.V. Tamborlane et al., The influence of insulin on circulating ghrelin. Am. J. Physiol. Endocrinol. Metab. **284**, 313–316 (2003)