Elevated blood plasma concentrations of active ghrelin and obestatin in benign ovarian neoplasms and ovarian cancers

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Summary

Both ghrelin and obestatin are derived from preproghrelin by post-translational processing. The two peptides are secreted into the blood but circulating levels of these peptides have not been assessed in women with ovarian tumours. Therefore, the purpose of this study was to evaluate peripheral blood concentrations of active and total ghrelin and obestatin in patients with benign ovarian tumours and those with ovarian cancer. The studies were conducted on 22 patients operated due to benign ovarian tumours, and 31 patients operated due to ovarian cancer. A control group consisted of 32 women, 24 to 65 years of age. Both in women with benign ovarian tumours and those with ovarian cancer blood concentrations of active ghrelin and obestatin were higher than in the control group (active ghrelin: 90 ± 4, 84 ± 4 and 56 ± 9 pg/ml, respectively, obestatin: 660 ± 36; 630 ± 30 and 538 ± 31 ng/ml (x ± SE), respectively). In contrast, total ghrelin concentrations in blood were similar in the studied groups. The alterations resulted in increased values of active to total ghrelin concentration ratio in the peripheral blood of patients with benign ovarian tumours or with ovarian cancer (0.79 ± 0.02 and 0.93 ± 0.05, respectively vs 0.58 ± 0.02 in the control group). Due to the absence of any convincing proof for the presence of a functional GHS-R-1a receptor for ghrelin in human ovaries it did not seem probable that the observed elevated levels of active ghrelin and obestatin were directly linked to development of ovarian tumours.

Key words: Ovarian neoplasms; Blood ghrelin; Blood obestatin.

Introduction

Ghrelin represents an endogenous hormone, isolated from the rat stomach by Kojima *et al*. (1999) [1]. Ghrelin is a polypeptide of 28 amino acids, with serine in position 3, and molecular weight of 3,314. To evoke its biological effect, ghrelin must be acylated at the serine 3 position with an octanoyl group. The human ghrelin gene is located on chromosome 3 (3p25-26) and diverges from the rat gene by two amino acids [2-5]. Expression of the ghrelin gene and presence of its peptide has also been detected in the hypophysis, hypothalamus, hippocampus, cerebellum, liver, spleen, thymus, adrenal gland, prostate, myocardium, adipose tissue, testes, placenta, kidneys and in other organs [1, 2, 6-8]. A significant difference was noted in serum ghrelin concentrations between males and females [2].

Ghrelin represents an endogenous ligand of growth hormone (GHS) receptor, which was detected in the hypophysis and hypothalamus and cloned in 1996 [7]. Two subtypes of the receptor, GHS-R-1a and 1b were identified. GHS-R-1a is a polypeptide of 366 amino acids, containing seven transmembrane domains and manifesting biological activity. The 1b subtype of the polypeptide also contains 289 amino acids, includes five transmembrane domains but exhibits no biological activity [9, 10].

The almost universal expression of ghrelin receptor indicates that the peptide should exhibit a multiorgan and multidirectional effect on cells, tissues and organs. Such conclusion stems from the presence of GHS-R in the hypophysis, hypothalamus, adrenal glands, ovaries, testes, blood vessels, heart, lungs, liver, pancreas, skeletal muscles, kidneys, thyroid gland, adipose tissue, uterus, skin, and lymph nodes [11-13]. The main role of ghrelin involves GHS-R-1a-mediated stimulation of secretion of growth hormone and other hormones, including adrenocorticotropic hormone (ACTH) and prolactin [14, 15]. Ghrelin also provides an important control agent of energy homeostasis in the body [3, 4, 5, 8, 16, 17]. Moreover the peptide plays an important role in control of neoplastic cell proliferation, in particular in such hormone-dependent tumours as the prostate, mammary gland, ovarian, endometrial tumours but also in neoplastic lesions of the thyroid gland, intestines and pancreas [18-24].

The preproghrelin gene codes also for obestatin, the peptide of 23 amino acids [25, 26]. The biological activity of obestatin depends on amidation of its carboxyl terminus. Originally, obestatin was thought to represent a ligand of the GPR39 orphan receptor, belonging to the family of ghrelin receptors [25] but recent studies failed to confirm this [27-30]. At first, obestatin was suggested to evoke effects reciprocal to those exerted by ghrelin [25, 31]. However, recent studies did not corroborate the role of obestatin in control of food uptake and body weight, energy expenditure or secretion of growth hormones [32]. The role of obestatin in the control of metabolism remains to be a matter of dispute.

Ovary expression of both ghrelin and GHS-R-1a has been described in normal human ovaries as well as in dif-

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ferent human metaplastic and neoplastic lesions [33, 34]. Manifestation of the receptor may suggest involvement of ghrelin in the development of various ovarian tumours. The available references contain no mention of ghrelin and obestatin levels in women with ovarian tumours. Therefore, peripheral blood levels of active ghrelin, total ghrelin and obestatin were estimated in patients with benign ovarian tumours or with ovarian cancer.

Materials and Methods

The Ethics Committee of Poznan University of Medical Sciences approved the study protocol. Before the study, every participant signed an informed consent form.

The study included 32 women in the control group and 53 patients who underwent surgery at the Department of Oncology, Medical University of Poznan due to a benign ovarian tumour or ovarian cancer. The general characteristics of the women are listed in Table 1. The control group was comprised of 32 women, 24 to 65 years of age (mean: 42.2 years). The study group was divided into two subgroups: 22 patients subjected to surgery due to benign ovarian tumours, aged 22 to 66 years (mean 44.4 years) who were subjected to extirpation of the tumour or to uni- or bilateral salpingo-oophorectomy or to hysterectomy plus salpingo-oophorectomy, depending on size, location and type of the tumour and on patient age. In these patients, postoperative histopathology disclosed an endometrial ovarian cyst in seven cases, adult cystic teratoma in four cases, serous ovarian cystadenoma in six cases, inflammatory cysts (salpingitis and purulent oophoritis) in two cases, ovarian adenofibroma, ovarian fibrothecoma, and ovarian serous cystitis in a single case each. The other subgroup included 31 patients subjected to surgery due to ovarian cancer, aged 31 to 78 years (mean 55.4 years). Depending on stage of the neoplastic process the surgery involved hysterectomy plus salpingo-oophorectomy, omentectomy with/without lymphadenectomy and with/without subsequent chemotherapy. In these patients, postoperative histopathology disclosed mucinous ovarian carcinoma in seven cases, serous ovarian adenocarcinoma in nine cases, planoepithelial, partially keratinizing carcinoma in two cases, borderline papillary serous cystadenoma in five cases, endometrioid cystadenocarcinoma in five cases, a poorly differentiated ovarian carcinoma and a partially serous, partially clear-cell adenocarcinoma in a single case each.

Fasting blood was taken in all women from the cubital vein, mixed with EDTA, centrifuged and the plasma was frozen at -80°C. Active ghrelin and total ghrelin levels were estimated using commercial immunoenzymatic tests (Linco Research, Inc., St. Charles, MO, USA, EZGRA-88K and EZGRT-89K, respectively). Parameters of the test for total ghrelin were as follows: specificity for active ghrelin: 80%, for des-octanoyl human ghrelin: 100%, sensitivity: 5 pg/ml, intra-assay reproducibility of the test: CV 0.9- 1.9%, inter-assay reproducibility of the test: CV 5.2-7.8%. Parameters of the test for active ghrelin were: specificity for active ghrelin: 100%, for des-octanoyl human ghrelin: 0%, sensitivity: 8 pg/ ml, intra-assay reproducibility: CV 0.9-3.6%, inter-assay reproducibility: CV3.6-13.0%.

Determination of obestatin levels in the above samples took advantage of the tests made by Peninsula Laboratories (Peninsula Laboratories, San Carlos, CA; S-1284.0001) manifesting the following parameters: sensitivity: 1.3 ng/ml, specificity for human obestatin: 100%. Individual stages of the estimation were conducted as recommended by the manufacturer.

Table 1. — *Basic clinical data of the studied women with benign ovarian neoplasms, ovarian cancer, and the control group*.

Group	Age (years)	Body wieght (kg)	Height (cm)	BMI	BF%
Control (n = 32)	42 ± 2	62 ± 2	163 ± 1	23.37 ± 0.66	32.16 ± 1.19
Benign ovarian neoplasms (n = 22)	45 ± 3	63 ± 2	162 ± 1	24.18 ± 0.94	33.91 ± 1.55
Ovarian cancer (n = 31)	57 ± 2**	70 ± 2*	161 ± 1	27.11 ± 0.77*	40.36 ± 1.18**

BMI - body mass index [kg/m^2], BF% - body fat percentage. The results are means ± SE. Number of studied persons is shown in brackets. Statistical significance of differences in relation to the control group was evaluated using the unpaired Student's t-test: * $p < 0.05$; ** $p < 0.001$.

Absorbance was measured using a photometer with microplate reading attachment (Multiscan, Labsystem) at the wavelengths of 450 nm and 620 nm.

Data are expressed as the mean ± SEM and the statistical comparison was done by the unpaired Student's t-test.

Results

As indicated in Table 1, body weight of patients with benign ovarian tumours did not differ from body weight of women in the control group. Patients with ovarian cancer clearly manifested much higher body mass index (BMI) values and a higher body fat percentage. Both patients with benign ovarian tumours and those with ovarian cancer had higher concentrations of active ghrelin and obestatin in the peripheral blood than those in the control group (Figure 1). In the respective groups of patients concentration of active ghrelin amounted to 90 ± 4, 84 ± 4 and 56 ± 9 pg/ml and concentration of obestatin was 660 ± 36, 630 ± 30 and 538 ± 31 ng/ml (x ± SE). In contrast, the total ghrelin concentration in blood was similar in the three groups of patients. Due to such alterations, the ratio of active ghrelin to total ghrelin in peripheral blood was increased in patients with benign ovarian tumours or with ovarian cancer (0.79 ± 0.02 and 0.93 ± 0.05, respectively, vs 0.58 ± 0.02 in the control group) while concentration ratios of active ghrelin to obestatin and total ghrelin to obestatin did not differ from those in the control group (Figure 2).

Discussion

The human ghrelin gene can generate multiple active molecules by alternative splicing and/or post-translational modifications [4, 35, 36]. These molecules are classified into three groups: ghrelin and analogs, C-ghrelin and obestatin. The major active product of the ghrelin gene is a 28 amino acid peptide acylated at the serine 3 position with an octanoyl group while the non-modified des-n-octanoyl form of ghrelin, designated as des-acyl ghrelin, is probably an inactive form [4, 37, 38]. Numerous molecular forms of ghrelin are present in plasma and the concentration of the unacylated form of ghrelin is higher than that of active ghrelin. Of interest is that in humans, plasma RIA-immunoreactive obestatin accounted for 5.21% of the ghrelin concentration, which indicates that in peripheral blood the ghrelin concentration is around 20-fold higher than that of obestatin [39].

Fig. 1

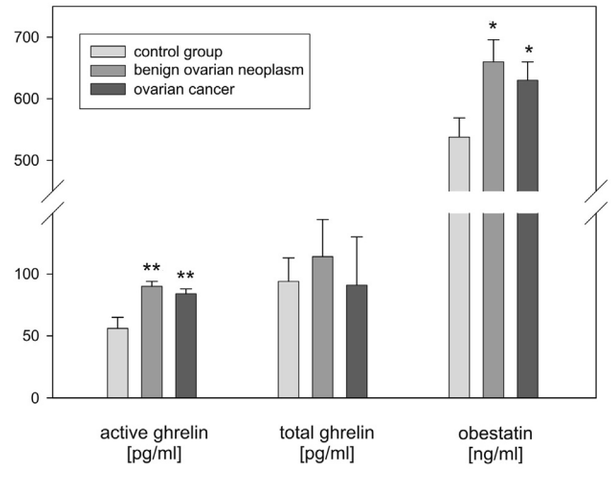

Fig. 2

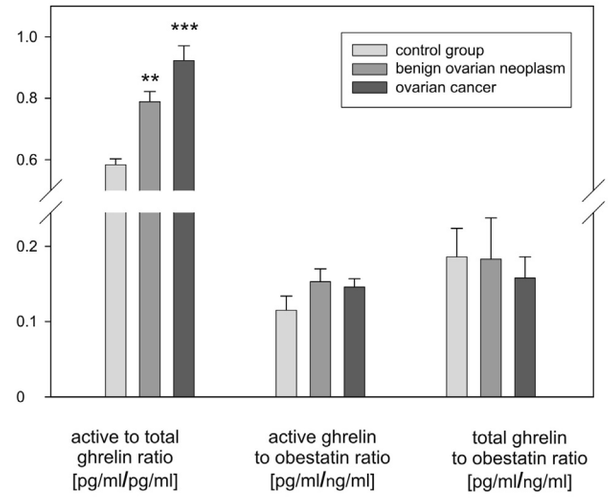

Figure 1. — Blood plasma concentrations of active [pg/ml] and total [pg/ml] ghrelin and obestatin [pg/ml] in women with benign ovarian neoplasms (n = 22), ovarian cancer (n = 31) and in controls (n = 32). Bars represent means ± SE. Statistical significance of differences in relation to the control group was evaluated using the unpaired Student's t-test: * $p < 0.05$; ** $p < 0.01$.

Figure 2. — Ratios of circulating active ghrelin [pg/ml]/total ghrelin [pg/ml], active ghrelin [pg/ml]/obestatin [μg/ml], and total ghrelin [pg/ml]/obestatin [μg/ml] in women with benign ovarian neoplasms (n = 22), ovarian cancer (n = 31) and in controls (n = 32). Bars are means ± SE. Statistical significance of differences in relation to the control group was evaluated using the unpaired Student's t-test: ** $p < 0.01$; *** $p < 0.001$.

The applied kits of Linco in the study permitted the determination of blood concentrations of total human ghrelin (both intact and des-octanoyl forms) and of active ghrelin (specific for the biologically active form of ghrelin with the octanoyl group). Both in patients with benign ovarian neoplasms and those with ovarian cancer blood concentrations of active ghrelin and obestatin were higher than those in the control group while concentrations of total ghrelin remained unchanged. Thus, both benign ovarian neoplasms and ovarian cancers were accompanied by elevated levels of active ghrelin and obestatin. It is difficult to speculate which factors are responsible for such alterations. It has been recognised that two-thirds of circulating ghrelin is secreted by oxyntic mucosa of the stomach while the remaining part is secreted by the small intestine [7] and in this situation the potential for secretion of active ghrelin and obestatin by neoplastically altered ovaries can hardly be taken into account. In view of the unchanged concentrations of total ghrelin it seems more probable that a mechanism other than increased secretion must be responsible for the increase in active ghrelin and obestatin levels in patients with ovarian neoplasms [40]. In women with benign ovarian tumours and those with ovarian cancer such changes led to elevated ratios of active to total ghrelin in the peripheral blood while ratios of active ghrelin to obestatin and total ghrelin to obestatin remain unchanged. Elevated ghrelin levels in serum have also been reported in patients with cachexia of various origins, i.e., in cachexia associated with chronic heart failure, in cancer cachexia, in chronic liver disease and in uterine myomata [40-46]. This means that elevated active ghrelin levels in the serum are specific neither for cachexia nor for ovarian neoplasms.

Only a few reports have dealt with obestatin levels in human blood [39, 47-49]. Obestatin levels are significantly lower in obese than in normal weight and anorectic women and in obese and anorectic patients increased total ghrelin to obestatin ratio has been observed [47, 50]. In contrast to obesity our study demonstrated augmented blood obestatin levels in cases of benign ovarian neoplasms and ovarian cancers. Similarly to the case of ghrelin, the effect seems non-specific for the studied type of the ovarian disease.

Using the QPCR technique, expression of the ghrelin gene and its receptor gene, GHS-R-1b, but not GHS-R-1a, can be noted in a normal human ovary [51, 52]. Nevertheless, recent studies have shown that both 1a and 1b isoforms of the GHS-R are expressed in human granulosa-lutein cells and in vitro, acting through GHS-R-1a, ghrelin inhibits their steroidogenesis [53]. In contrast to the data originating from PCR studies, immunocytochemical studies of normal human ovaries and different metaplastic and neoplastic lesions of the human ovary have documented the expression of both ghrelin and GHS-R-1a in various structural elements of the ovary [24, 33, 34]. However, the role of ghrelin in the control of ovarian function remains to be fully clarified. GHS-R-1a expression detected in the ovary by immunohistochemistry has not been confirmed by PCR techniques. Employing immunohistochemistry, expression of GHS-R-1a has been detected, i.e., in the surface of ovarian epithelium but malignant tumours which derive from this source have shown weak or no GHS-R-1a immunoreactivity. These observations fail to support a potential role of ghrelin in promotion or development of benign tumours or cancers of the ovary.

Summing up, the present experiments have documented elevated concentrations of active ghrelin and obestatin in the blood of patients with benign ovarian neoplasms or ovarian cancer while levels of total ghrelin resembled that of the control group. Such changes in the peripheral blood of patients with benign ovarian tumours or ovarian cancer have resulted in an elevated ratio of active ghrelin to total ghrelin concentrations. Due to the lack of convincing proof for the presence of a functional GHS-R-1a ghrelin receptor in the human ovary, the observed elevated levels of active ghrelin and obestatin do not seem to be directly linked to the development of ovarian tumours.

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References

[1] Kojima M., Hosoda H., Date Y., Nakazato M., Matsuo H., Kangawa K.: "Ghrelin is a growth-hormone-releasing acylated peptide from stomach". *Nature*, 1999, *402,* 656.

[2] Korbonits M., Bustin S.A., Kojima M., Jordan S., Adams E.F., Lowe D.G. *et al*.: "The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors". *J. Clin. Endocrinol. Metab*., 2001, *86*, 881.

[3] Ueno H., Yamaguchi H., Kangawa K., Nakazato M.: "Ghrelin: a gastric peptide that regulates food intake and energy homeostasis". *Regul. Pept*., 2005, *126*, 11.

[4] Kojima M., Kangawa K.: "Ghrelin: structure and function". *Physiol. Rev.,* 2005, *85,* 495.

[5] Pazos Y., Casanueva F.F., Camiña J.P.: "Basic aspects of ghrelin action". *Vitam Horm*., 2008, *77*, 89.

[6] Ariyasu H., Takaya K., Tagami T., Ogawa Y., Hosoda K., Akamizu 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.

[7] Date Y., Kojima M., Hosoda H., Sawaguchi A., Mondal M.S., Suganuma T. *et al*.: "Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans". *Endocrinology,* 2000, *141,* 4255.

[8] Hosoda H., Kojima M., Kangawa K.: "Ghrelin and the regulation of food intake and energy balance". *Mol. Interv.,* 2002, *2,* 494.

[9] Howard A.D., Feighner S.D., Cully D.F., Arena J.P., Liberator P.A., Rosenblum C.I. *et al*.: "A receptor in pituitary and hypothalamus that functions in growth hormone release". *Science,* 1996, 273, 974. Comment in: *Science,* 1996, *273*, 923.

[10] Young Cruz C.R., Smith R.G.: "The growth hormone secretagogue receptor". *Vitam Horm,* 2008, *77*, 47.

[11] Palyha O.C., Feighner S.D., Tan C.P., McKee K.K., Hreniuk D.L., Gao Y.D. *et al*.: "Ligand activation domain of human orphan growth hormone (GH) secretagogue receptor(GHS-R) conserved from Pufferfish to humans". *Mol. Endocrinol.,* 2000, *14*, 160.

[12] Smith R.G., Feighner S., Prendergast K., Guan X., Howard A.: "A New Orphan Receptor Involved in Pulsatile Growth Hormone Release". *Trends Endocrinol. Metab*., 1999, *10,* 128.

[13] Smith R.G., Leonard R., Bailey A.R., Palyha O., Feighner S., Tan C. *et al*.: "Growth hormone secretagogue receptor family members and ligands". *Endocrine*, 2001, *14,* 9.

[14] Amato G., Carella C., Fazio S., La Montagna G., Cittadini A., Sabatini D. *et al*.: "Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses". *J. Clin. Endocrinol. Metab*., 1993, *77,* 1671.

[15] Takaya K., Ariyasu H., Kanamoto N., Iwakura H., Yoshimoto A., Harada M. *et al*.: "Ghrelin strongly stimulates growth hormone release in humans". *J. Clin. Endocrinol. Metab*., 2000, *85,* 4908.

[16] Broglio F., Arvat E., Benso A., Papotti M., Muccioli G., Deghenghi R. *et al*.: "Ghrelin: endocrine and non-endocrine actions". *J. Pediatr. Endocrinol. Metab.,* 2002, *15,* 1219.

[17] Cummings D.E.: "Ghrelin and the short- and long-term regulation of appetite and body weight". *Physiol. Behav.,* 2006, *89*, 71.

[18] Chopin L.K., Herington A.C.: "A potential autocrine pathway for growth hormone releasing hormone (GHRH) and its receptor in human prostate cancer cell lines". *Prostate,* 2001, *49*, 116.

[19] Asa S.L., Kovacs K., Thorner M.O., Leong D.A., Rivier J., Vale W.: "Immunohistological localization of growth hormone-releasing hormone in human tumors". *J. Clin. Endocrinol. Metab*., 1985, *60,* 423.

[20] Kanamoto N., Akamizu T., Hosoda H., Hataya Y., Ariyasu H., Takaya K. *et al*.: "Substantial production of ghrelin by a human medullary thyroid carcinoma cell line". *J. Clin. Endocrinol. Metab*., 2001, *86,* 4984.

[21] Khorram O., Garthwaite M., Grosen E., Golos T.: "Human uterine and ovarian expression of growth hormone-releasing hormone messenger RNA in benign and malignant gynecologic conditions". *Fertil. Steril*., 2001, *75*, 174.

[22] Papotti M., Cassoni P., Volante M., Deghenghi R., Muccioli G., Ghigo E.: "Ghrelin-producing endocrine tumors of the stomach and intestine". *J. Clin. Endocrinol. Metab*., 2001, *86,* 5052.

[23] Skinner M.M., Nass R., Lopes B., Laws E.R., Thorner M.O.: "Growth hormone secretagogue receptor expression in human pituitary tumors". *J. Clin. Endocrinol. Metab*., 1998, *83,* 4314.

[24] Leontiou C.A., Franchi G., Korbonits M.: "Ghrelin in neuroendocrine organs and tumours". *Pituitary,* 2007, *10,* 213.

[25] Zhang J.V., Ren P.G., Avsian-Kretchmer O., Luo C.W., Rauch R., Klein C. *et al*.: "Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake". *Science,* 2005, *310*, 996.

[26] Pemberton C.J., Richards A.M.: "Biochemistry of ghrelin precursor peptides". *Vitam. Horm*., 2008, *77,* 13.

[27] Chartrel N., Alvear-Perez R., Leprince J., Iturrioz X., Reaux-Le Goazigo A., Audinot V. *et al*.: "Comment on Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake". *Science*, 2007, *315,* 766; author reply 766. Comment on: *Science*, 2005, *310,* 996.

[28] Holst B., Egerod K.L., Schild E., Vickers S.P., Cheetham S., Gerlach L.O. *et al*.: "GPR39 signaling is stimulated by zinc ions but not by obestatin". *Endocrinology,* 2007, *148*, 13.

[29] Lauwers E., Landuyt B., Arckens L., Schoofs L., Luyten W.: "Obestatin does not activate orphan G protein-coupled receptor GPR39". *Biochem. Biophys. Res. Commun*., 2006, *351*, 21.

[30] Tremblay F., Perreault M., Klaman L.D., Tobin J.F., Smith E., Gimeno R.E.: "Normal food intake and body weight in mice lacking the G protein-coupled receptor GPR39". *Endocrinology*, 2007, *148*, 501.

[31] Lagaud G.J., Young A., Acena A., Morton M.F., Barrett T.D., Shankley N.P.: "Obestatin reduces food intake and suppresses body weight gain in rodents". *Biochem. Biophys. Res. Commun.,* 2007, *357,* 264.

[32] Nogueiras R., Pfluger P., Tovar S., Arnold M., Mitchell S., Morris A. *et al*.: "Effects of obestatin on energy balance and growth hormone secretion in rodents". *Endocrinology,* 2007, *148*, 21.

[33] Gaytan F., Barreiro M.L., Chopin L.K., Herington A.C., Morales C., Pinilla L. *et al*.: "Immunolocalization of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in the cyclic human ovary". *J. Clin. Endocrinol. Metab*., 2003, *88,* 879.

[34] Gaytan F., Morales C., Barreiro M.L., Jeffery P., Chopin L.K., Herington A.C. *et al*.: "Expression of growth hormone secretagogue receptor type 1a, the functional ghrelin receptor, in human ovarian surface epithelium, mullerian duct derivatives, and ovarian tumors". *J. Clin. Endocrinol. Metab.,* 2005, *90,* 1798.

[35] Hosoda H., Kojima M., Mizushima T., Shimizu S., Kangawa K.: "Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing". *J. Biol. Chem*., 2003, *278,* 64.

[36] Soares J.-B., Leite-Moreira A.F.: "Ghrelin, des-acyl ghrelin and obestatin: Three pieces of the same puzzle". *Peptides,* 2008, *29,* 1255.

[37] Hosoda H., Kojima M., Matsuo H., Kangawa K.: "Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor". *J. Biol. Chem.,* 2000a, *275*, 21995.
[38] Hosoda H., Kojima M., Matsuo H., Kangawa K.: "Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue". *Biochem. Biophys. Res. Commun.,* 2000b, *279,* 909.
[39] Mondal M., Toshinai K., Ueno H., Koshinaka K., Nakazato M.: "Characterization of obestatin in rat and human stomach and plasma, and its lack of acute effect on feeding behavior in rodents". *J. Endocrinol.,* 2008, *198*, 339.
[40] Garcia J.M., Garcia-Touza M., Hijazi R.A., Taffet G., Epner D., Mann D. *et al*.: "Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia". *J. Clin. Endocrinol. Metab*., 2005, *90,* 2920.
[41] Nagaya N., Uematsu M., Kojima M., Date Y., Nakazato M., Okumura H. *et al*.: "Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors". *Circulation,* 2001, *104,* 2034.
[42] Shimizu Y., Nagaya N., Isobe T., Imazu M., Okumura H., Hosoda H. *et al*.: "Increased plasma ghrelin level in lung cancer cachexia". *Clin. Cancer Res*., 2003, *9*, 774.
[43] Tacke F., Brabant G., Kruck E., Horn R., Schöffski P., Hecker H. *et al*.: " Ghrelin in chronic liver disease". *J. Hepatol.,* 2003, *38,* 447.
[44] Wolf I., Sadetzki S., Kanety H., Kundel Y., Pariente C., Epstein N. *et al*.: "Adiponectin, ghrelin, and leptin in cancer cachexia in breast and colon cancer patients". *Cancer,* 2006, *106*, 966.
[45] Wang H.S., Oh D.S., Ohning G.V., Pisegna J.R.: "Elevated serum ghrelin exerts an orexigenic effect that may maintain body mass index in patients with metastatic neuroendocrine tumors". *J. Mol. Neurosci,* 2007, *33,* 225.
[46] Markowska A., Ziolkowska A., Nowinka K., Malendowicz L.K.: "Elevated blood active ghrelin and normal total ghrelin and obestatin concentrations in uterine myomatosis". *Eur. J. Gynaecol. Oncol.,* 2009, *3*, 281.
[47] Guo Z.F., Zheng X., Qin Y.W., Hu J.Q., Chen S.P., Zhang Z.: "Circulating preprandial ghrelin to obestatin ratio is increased in human obesity". *J. Clin. Endocrinol. Metab.,* 2007, *92,* 1875.
[48] Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Woltz M *et al*.: "Serum retinol-binding protein-4 is reduced after weight loss in morbidly obese subjects". *J. Clin. Endocrinol. Metab.,* 2007, *92,* 1168.
[49] Qi X., Li L., Yang G., Li K., Tang Y., Liou H. *et al*.: " Circulating obestatin levels in normal subjects and in patients with impaired glucose regulation and type 2 diabetes mellitus". *Clin. Endocrinol*., 2007, *66,* 593.
[50] Zamrazilová H., Hainer V., Sedláčková D., Papežová H., Kunešová M., Bellisle F. *et al*.: "Plasma obestatin levels in normal weight, obese and anorectic women". *Physiol. Res.,* 2008 [Epub ahead of print].
[51] Gnanapavan S., Kola B., Bustin S.A., Morris D.G., McGee P., Fairclough P. *et al*.: "The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans". *J. Clin. Endocrinol. Metab*., 2002, *87,* 2988.
[52] Skommer J., Katulski K., Poreba E., Meczekalski B., Slopień R., Plewa R. *et al*.: "Gherlin expression in women with polycystic ovary syndrome-a preliminary study". *Eur. J. Gynaecol. Oncol.,* 2005, *26,* 553.
[53] Viani I., Vottero A., Tassi F., Cremonini G., Sartori C., Bernasconi S. *et al*.: "Ghrelin inhibits steroid biosynthesis by cultured granulosa-lutein cells". *J. Clin. Endocrinol. Metab.,* 2008, *93,* 1476.

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