The analysis of exogenous ghrelin plasma activity and tissue distribution

Marek Ruchala [1], Ludmila Rafinska [2], Jerzy Kosowicz [1], Edyta Gurgul [1], Piotr Sawinski [2], Maciej Biczysko [3], Andrzej Lukaszyk [2], Jerzy Sowinski [1]

1 Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Poznan, Poland
2 Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
3 Department of General, Gastroenterological and Endocrinological Surgery, Poznan University of Medical Sciences, Poznan, Poland

Correspondence to: Prof. Marek Ruchala
Department of Endocrinology, Metabolism and Internal Medicine,
Poznan University of Medical Sciences
Przybyszewskiego Str. 49, 60-355 Poznan, Poland.
TEL: +48618691330; FAX: +48618691682; E-MAIL: mruchala@ump.edu.pl

Submitted: 2011-10-19 Accepted: 2012-01-15 Published online: 2012-04-25

Key words: **ghrelin; half-life time; biodistribution**

Neuroendocrinol Lett 2012; **33**(2):191–195 **PMID:** 22592200 NEL330212A09

Abstract

OBJECTIVES: Ghrelin presents a multiplicity of biological functions, what is consistent with widespread expression of this peptide and its receptors. Ghrelin may act locally, but it may also influence distant cells. The aim of the study was to assess plasma activity of exogenous ghrelin and its distribution in rats.

DESIGN: Plasma radioactivity of ^{125}I-ghrelin (cpm) was analyzed in blood specimens collected after ^{125}I-ghrelin administration. Tissue uptake of ^{125}I-ghrelin (cpm/mg) was evaluated in 27 tissues obtained during an autopsy performed 1, 2 and four hours after ^{125}I-ghrelin administration. The radioactivity of the tissue specimen (cpm) was divided by the weight of the specimen (mg).

RESULTS: Plasma ^{125}I-ghrelin radioactivity decreased rapidly after peptide administration. The half-life time of ^{125}I-ghrelin was 15–18 minutes. The analysis of ^{125}I-ghrelin distribution revealed three profiles of its tissue uptake. The first profile was characterized by decreasing radioactivity (e.g. brain, kidney, liver). Increasing tissue radioactivity followed by a gradual decrease (second profile) was observed for example in stomach, intestine and thyroid. The third profile was described as a relatively stable radioactivity (e.g. lung, myocardium). Despite of Lugol's solution administration, thyroid uptake of ^{125}I-ghrelin was notably higher than in other tissues (second and third profile).

CONCLUSIONS: Exogenous ghrelin uptake in tissues that produce this peptide suggests, that ghrelin influences the biology and function of these cells also in endocrine way. Similarly, the accumulation of peptide observed in the third profile (e.g. thyroid) may reflect a potential role of ghrelin in these organs.

ORIGINAL ARTICLE

INTRODUCTION

Ghrelin is a 28-amino-acid-long peptide isolated in 1999 from the mucous membrane of rat stomach (Kojima *et al.* 1999). It was discovered as the first endogenous ligand for the orphan growth hormone secretagogue receptor (GHS-R). Although stomach is the main source of ghrelin, other tissues considerably support the synthesis of this peptide (Ariyasu 2001). Ghrelin's expression has been demonstrated in hypothalamus, pituitary, small and large intestine, liver, pancreas, spleen, kidney, lung, myocardium, thyroid, adrenal gland, ovary, breast and many other tissues (Gnanapavan *et al.* 2002; Raghay *et al.* 2006; Ghelardoni *et al.* 2006; Grönberg *et al.* 2008; Dagli *et al.* 2009; Ueberberg *et al.* 2009). Growth hormone secretagogue receptors – active GHS-R1a and assumed inactive GHS-R1b – are highly expressed in hypothalamus and pituitary, as well as in other central and peripheral organs (Howard *et al.* 1996; Guan *et al.* 1997; Hattori *et al.* 2001; Gnanapavan *et al.* 2002; Dixit *et al.* 2004; Leite-Moreira & Soares 2007).

Ghrelin presents a multiplicy of physiological functions. It proved to possess strong, dose-related GH–releasing properties, acting even more potently than GHRH (Arvat *et al.* 2000; Takaya *et al.* 2000). Ghrelin is also an orexigenic factor and an essential regulator of metabolic processes in humans and animals (Tschop *et al.* 2000; Wren *et al.* 2000; Wren *et al.* 2001; Lawrence *et al.* 2002). Furthermore, the biological role of ghrelin includes cardiovascular functions, the regulation of cell proliferation and inflammatory response, as well as the influence on cognition and behavioral processes (Baldanzi *et al.* 2002; Jeffery *et al.* 2002; Nagaya *et al.* 2003; Dixit *et al.* 2004; Diano *et al.* 2006).

Ghrelin may act locally in auto- or paracrine way, but there is also a clear evidence, that peptide circulating in blood may influence distant cells. The aim of the study was to assess plasma activity of exogenous ghrelin and describe its distribution in various tissues in rats.

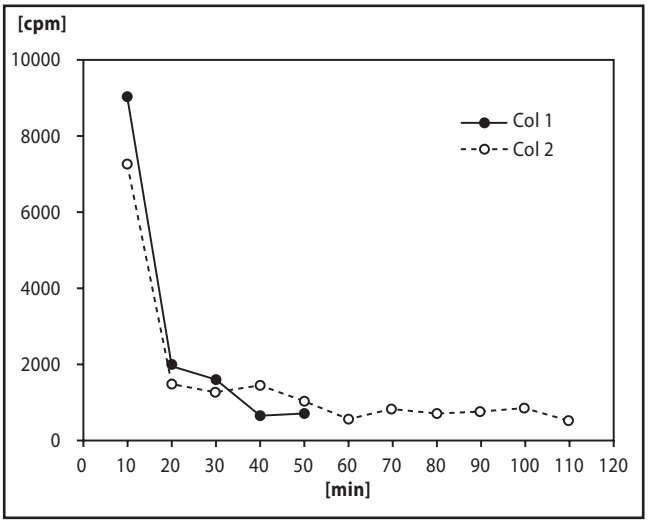

Fig. 1. The radioactivity of ^{125}I-ghrelin in rat plasma (col 1 – in the first hour, col 2 – in the first two hours of the experiment). T ½ of ^{125}I-ghrelin was 15–18 minutes.

MATERIAL AND METHODS

The study was conducted in accordance with the acceptance of the Local Animal Ethics Committee. 90-days old male Wistar rats were purchased from Poznan University of Medical Sciences Laboratories. The animals did not present any signs of disease or injury and were housed in air-conditioned animal quarters, given food and water *ad libitum*. The thyroid ^{125}I uptake was inhibited by the administration of Lugol's solution. Anesthesia was induced by the intramuscular injection of ketamine (1 mg/100 g, i.m.) or xylamine (0.2 mg/100 g, i.m.). The ^{125}I-labeled ghrelin (Peninsula Laboratories, INC) was administered intravenously by the cannula inserted into the jugular vein (1.25 μCi per rat).

The plasma radioactivity of ^{125}I-ghrelin was analyzed in blood specimens (0.1 ml) collected every 10 minutes in the first 2 hours after ^{125}I-ghrelin administration. The activity of ^{125}I was counted in scintillation gamma-counter (LKB/Wallac). The ^{125}I radioactivity in plasma was expressed by counts per minute (cpm).

The tissue distribution of ^{125}I-ghrelin was evaluated after autopsy. The autopsy was performed 1, 2 and four hours after ^{125}I-ghrelin administration and peptide distribution was analyzed in 27 tissues, assuming that the ^{125}I-labeled ghrelin complex was stable. The activity of ^{125}I in tissues was counted in scintillation gamma-counter (cpm). The tissue uptake of ^{125}I-ghrelin (cpm/mg) was estimated by the radioactivity of the tissue specimen (cpm) divided by the weight of the specimen (mg).

RESULTS

Plasma ^{125}I-ghrelin radioactivity decreased rapidly after peptide administration and after 40–60 minutes reached a level, that remained relatively stable for at least 2 hours (Figure 1). The half-life time (T ½) of ^{125}I-ghrelin was 15-18 minutes.

The uptake of ^{125}I-ghrelin was different in various tissues and organs (Figure 2). Despite of Lugol's solution administration, thyroid uptake of ^{125}I-ghrelin was notably higher than in other tissues. Thus, to maintain the clarity of the graphs the radioactivity of ^{125}I-ghrelin was not inserted. The radioactivity of ^{125}I-ghrelin in thyroid was 43 000 cpm one hour after administration, 178 000 cpm after two hours and 125 000 cpm after four hours.

The analysis of ^{125}I-ghrelin distribution revealed three profiles of its tissue uptake. The first profile was characterized by decreasing radioactivity, very similar to the plasma ghrelin activity. This profile was observed in brain, kidney, liver, urinary bladder, prostate gland, seminal capsule and skeletal muscle (Figure 3).

Increasing tissue radioactivity with the maximum 2 hours after ^{125}I-ghrelin administration and with a grad-

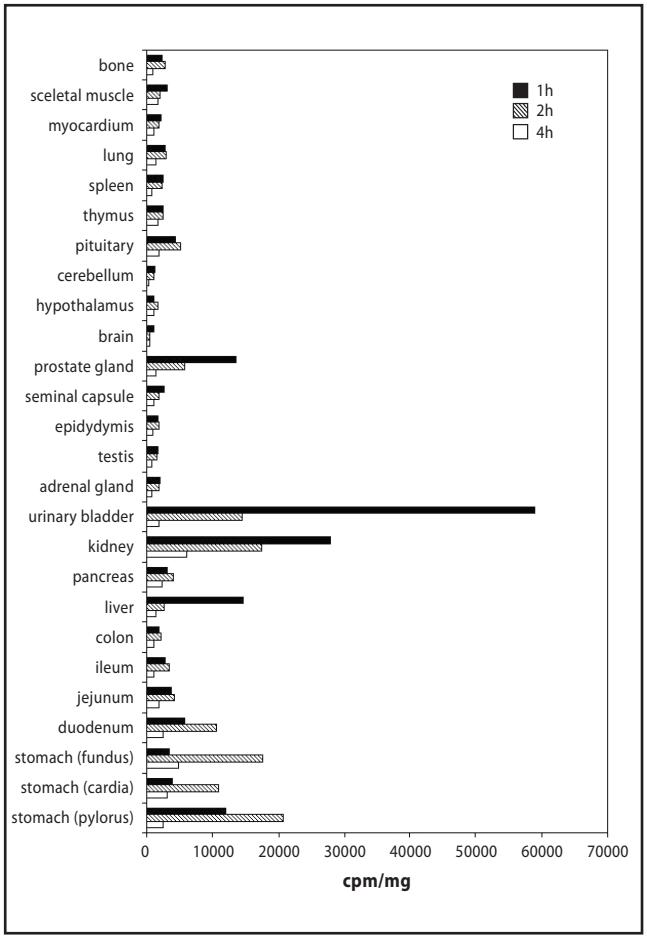

Fig. 2. The biodistribution of ^{125}I-ghrelin.

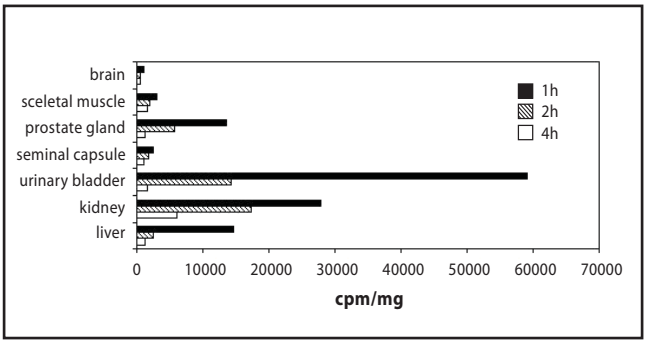

Fig. 3. Tissue and organ distribution of ^{125}I-ghrelin (the first profile)

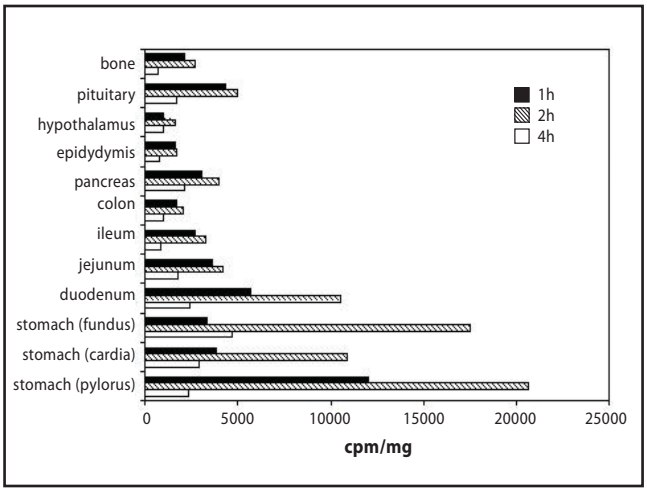

Fig. 4. Tissue and organ distribution of ^{125}I-ghrelin (the second profile). Thyroid uptake of ^{125}I-ghrelin was not inserted into the graph.

ual decrease after 4 hours of the experiment (second profile) was typical for hypothalamus, pituitary, thyroid, pancreas, stomach, duodenum, ileum, jejunum, colon, bone, epididymis (Figure 4). The half-life time of ^{125}I-ghrelin in stomach and duodenum exceeded 2 hours.

A relatively stable radioactivity of ^{125}I-ghrelin during 4 hours of the experiment was observed in cerebellum, lung, myocardium, thymus, thyroid, adrenal gland, spleen, testis and epididymis (third profile) (Figure 5).

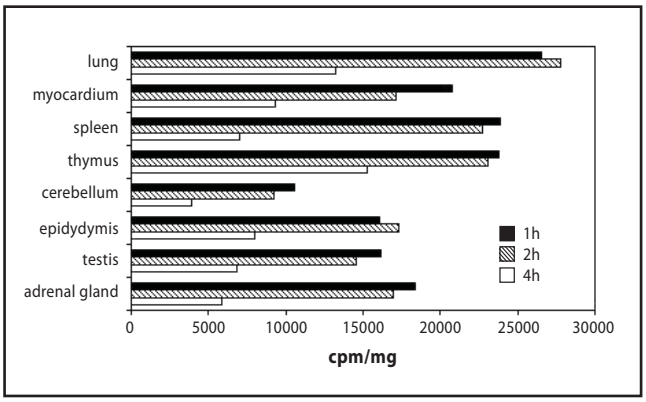

Fig. 5. Tissue and organ distribution of ^{125}I-ghrelin (the third profile). Thyroid uptake of ^{125}I-ghrelin was not inserted into the graph.

DISCUSSION

The analysis of ^{125}I-ghrelin plasma radioactivity revealed a rapid clearance of the peptide with a half-life time of 15–18 minutes. This observation is consistent with the previous studies. Akamizu *et al.* showed, that exogenous ghrelin administered in bolus to healthy volunteers is quickly removed from plasma with a half-life time of 27–31 minutes (Akamizu *et al.* 2005). Ghrelin administered in intravenous infusion presented a similar model of clearance (T ½ was 24.2±2.5 min) (Vestergaard *et al.* 2007).

Short half-life time of ghrelin has been proven also in studies, that evaluated ghrelin's levels throughout the day. Since ghrelin is a major stimulator of appetite, peptide secretion depends on nutritional status (Cummings *et al.* 2001; 2004; Callahan *et al.* 2004; Barazzoni *et al.* 2007). The highest levels of ghrelin are observed at fast, decrease rapidly after food intake and rise again at

hunger (Cummings *et al.* 2001; Shiiya *et al.* 2002; Yildiz *et al.* 2004).

The analysis of ^{125}I-ghrelin biodistribution showed, that ghrelin circulating in blood reaches various tissues and therefore is able to influence the biology and function of distant cells in endocrine way. The bounding sites are probably GHS-R1a receptors (Papotti *et al.* 2000; Gnanapavan *et al.* 2002). However, GHS-R1b or other unknown receptor cannot be excluded (Baldanzi *et al.* 2002).

To our knowledge, this is the first research, that evaluated the biodistribution of exogenous ghrelin in such wide range. The analysis of ^{125}I-ghrelin tissue uptake revealed, that it can be classified into three profiles. The first profile was very similar to the activity of ^{125}I-ghrelin in plasma with a rapid decrease in the early phase (observed in brain, skeletal muscle, prostate gland, seminal capsule, urinary bladder, kidney and liver).

The second profile was characterized by an increasing tissue radioactivity with the maximum noted two hours after ^{125}I-ghrelin administration and with a following gradual decrease (hypothalamus, pituitary, thyroid, pancreas, stomach, duodenum, ileum, jejunum, colon, bone, epididymis). Noteworthy, considerable ^{125}I-ghrelin's uptake was observed in stomach and duodenum, which are the main ghrelin-secreting organs. The half-life time of exogenous ghrelin in these organs exceeded 2 hours. Long-lasting reactivity of ^{125}I-ghrelin proves, that this peptide may influence the biology and function of these cells in endocrine way. Possible differences between local (auto- or paracrine) and endocrine effects of ghrelin remain to be explored.

The third profile of tissue uptake showed relatively stable radioactivity of ^{125}I-ghrelin (observed in cerebellum, lung, myocardium, thymus, thyroid, adrenal gland, spleen, testis and epididymis). Such accumulation of ghrelin may reflect its potential role in this organs. To be more specific, recently several studies brought up a potential relationship between ghrelin and thyroid function (Riis *et al.* 2003; Ruchala 2007; Braclik *et al.* 2009). The expression of ghrelin and its receptors in thyroid cells has been widely proved (Cassoni *et al.* 2000; Papotti *et al.* 2000; Gnanapavan *et al.* 2002, Ruchała 2007; Ueberberg *et al.* 2009). Furthermore, the immunohistochemical reactivity of ghrelin in tyreocytes was observed mainly in the apical part of these cells, which is directly connected with thyroid hormones secretion (Ruchala 2007). Several clinical studies revealed, that ghrelin plasma concentrations are significantly increased in hypothyroidism and decreased in hyperthyroidism in comparison to healthy subjects (Riis *et al.* 2003; Rojdmark *et al.* 2005; Giménez-Palop *et al.* 2005; Altinova *et al.* 2006; Ruchala 2007; Gjedde *et al.* 2008; Braclik *et al.* 2009). The expression of ghrelin and its receptors in thyroid tissue may reflect the functional activity of ghrelin in thyroid gland. Since ghrelin acts as a major regulator of metabolism and thyroid hormones are essential to maintain energy balance, the connection between the thyroid gland and ghrelin production appears fairly probable.

CONCLUSIONS

Ghrelin is a short-lived peptide, that when administered intravenously is rapidly removed from plasma. Biodistribution of ghrelin is characterized by widespread tissue uptake, that may be classified into three profiles. Long-lasting activity of exogenous peptide in certain tissues may reflect its biological activity in this localization.

ACKNOWLEDGEMENTS

The study was supported by the grant from the Polish Ministry of Science no 3951/P01/2006/31 and Poznan University of Medical Sciences no 501-01-02221355-06171.

REFERENCES

1 Akamizu T, Shinomiya T, Irako T *et al.* (2005). Separate measurement of plasma levels of acylated and desacyl ghrelin in healthy subjects using a new direct ELISA assay. J Clin Endocrinol Metab. **90**: 6–9.
2 Altinova AE, Törüner FB, Aktürk M *et al.* (2006). Reduced serum acylated ghrelin levels in patients with hyperthyroidism. Horm Res. **65(6)**: 295–299.
3 Ariyasu H, Takaya K, Tagami T *et al.* (2001). Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab. **86**: 4753–4758.
4 Arvat E, Di Vito L, Broglio F *et al.* (2000). Preliminary evidence that ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. J Endocrinol Invest. **23**: 493–495.
5 Baldanzi G, Filigheddu N, Cutrupi S *et al.* (2002). Ghrelin and desacyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. J Cell Biol. **159**(6): 1029–1037.
6 Braclik M, Marcisz C, Giebel S *et al.* (2008). Serum leptin and ghrelin levels in premenopausal women with stable body mass index during treatment of thyroid dysfunction. Thyroid. **18**(5): 545–550.
7 Cummings DE, Purnell JQ, Frayo RS *et al.* (2001). A preprandial rise in plasma ghrelin suggests a role in meal initiation in human. Diabetes. **50**: 1714–1719.
8 Cummings DE, Frayo RS, Marmonier C *et al.* (2004). Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. Am J Physiol Endocrinol Metab. **287**(2): E297–304.
9 Dagli AF, Aydin S, Karaoglu A *et al.* (2009). Ghrelin expression in normal kidney tissue and renal carcinomas. Pathol Res Pract. **205**(3): 165–173.
10 Diano S, Farr SA, Benoit SC *et al.* (2006). Ghrelin controls hippocampal spine synapse density and memory performance. Nat Neurosci. **9**: 381–388.
11 Dixit VD, Schaffer EM, Pyle RS *et al.* (2004). Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. J Clin Invest. **114**(1): 57–66.
12 Ghelardoni S, Carnicelli V, Frascarelli S *et al.* (2006). Ghrelin tissue distribution: comparison between gene and protein expression. J Endocrinol Invest. **29**: 115–121.

13 Giménez-Palop O, Giménez-Pérez G, Mauricio D *et al.* (2005). Circulating ghrelin in thyroid dysfunction is related to insulin resistance and not to hunger, food intake or anthropometric changes. Eur J Endocrinol. **153**(1): 73–79.
14 Gjedde S, Vestergaard E, Gormsen LC *et al.* (2008). Serum ghrelin levels are increased in hypothyroid patients and become normalized by L-thyroxine treatment. J Clin Endocrinol Metab. **93**(6): 2277–2280.
15 Gnanapavan S, Kola B, Bustin SA *et al.* (2002). The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab. **87**: 2988–2991.
16 Grönberg M, Tsolakis AV, Magnusson L *et al.* (2008). Distribution of obestatin and ghrelin in human tissues: immunoreactive cells in the gastrointestinal tract, pancreas and mammary glands. J Histochem Cytochem. **56**: 793–801.
17 Gualillo O, Caminos J, Blanco M *et al.* (2001). Ghrelin, a novel placental-derived hormone. Endocrinology. **142**(2): 788–794.
18 Guan XM, Yu H, Palyha OC *et al.* (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res. **48**: 23–29.
19 Hattori N, Saito T, Yagyu T *et al.* (2001). GH, GH receptor, GH secretagogue receptor, and ghrelin expression in human T cells, B cells, and neutrophils. J Clin Endocrinol Metab. **86**(9): 4284–4291.
20 Howard AD, Feighner SD, Cully DF *et al.* (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release. Science. **273**: 974–977.
21 Jeffery PL, Herington AC, Chopin LK (2002). Expression and action of the growth hormone-releasing peptide ghrelin and its receptor in prostate cancer cell lines. J Endocrinol. **172**: R7–11.
22 Kojima M, Hosoda AH, Date Y *et al.* (1999). Ghrelin is a growth hormone releasing acylated peptide from stomach. Nature. **402**: 656–660.
23 Lawrence CB, Snape AC, Baudoin FM *et al.* (2002) Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology. **143**: 155–162.
24 Leite-Moreira AF, Soares JB (2007). Physiological, pathological and potential therapeutic roles of ghrelin. Drug Discov Today. **12**: 276–288.
25 Nagaya N, Uematsu M, Kojima M *et al.* (2001). Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation. **104**: 1430–1435.
26 Papotti M, Ghe C, Cassoni P *et al.* (2000). Growth hormone secretagog binding sites in peripheral human tissues. J Clin Endocrinol Metab. **85**(10): 3803–3807.
27 Raghay K, Garcia-Caballero T, Nogueiras R *et al.* (2006). Ghrelin localization in rat and human thyroid and parathyroid glands and tumors. Histochem Cell Biol. **89**: 400–409.
28 Riis AL, Hansen TK, Moller N *et al.* (|2003). Hyperthyroidism is associated with suppressed circulating ghrelin level. J Clin Endocrinol Metab. **88**: 853–857.
29 Rojdmark S, Calissendorff J, Danielsson O *et al.* (2005). Hunger-satiety signals in patients with Graves' thyrotoxicosis before, during, and after long-term pharmacological treatment. Endocrine. **27**: 55–61.
30 Ruchala M (2007). Ghrelina i somatostatyna jako modulatory sekrecji hormonów tarczycy: badania doświadczalne in vivo i in vitro oraz wynikające z nich implikacje kliniczne (Ghrelin and somatostatin as modulators of thyroid hormones secretion: experimental studies in vivo and in vitro and their clinical implications - ethichabilitation thesis). Wydawnictwo Naukowe Uniwersytetu im. K. Marcinkowskiego. Poznań
31 Shiiya T, Nakazato M, Mizuta M *et al.* (2002). Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab. **87**: 240–244.
32 Takaya K, Ariyasu H, Kanamoto N *et al.* (2000). Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab. **85**: 4908–4911.
33 Tschop M, Smiley DL, Heiman ML (2000). Ghrelin induces adiposity in rodents. Nature. **407**: 908–913.
34 Ueberberg B, Unger N, Saeger W *et al.* (2009). Expression of ghrelin and its receptor in human tissues. Horm Metab Res. **41**(11): 814–821.
35 Vestergaard ET, Hansen TK, Gormsen LC *et al.* (2007). Constant intravenous ghrelin infusion in healthy young men: clinical pharmacokinetics and metabolic effects. Am J Physiol Endocrinol Metab. **292**(6): E1829–36.
36 Wren AM, Small CJ, Ward HL *et al.* (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology. **141**: 4325–4328.
37 Wren AM, Seal LJ, Cohen MA *et al.* (2001). Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab. **86**: 5992–5995.
38 Yildiz BO, Suchard MA, Wong ML *et al.* (2004). Alternations in the dynamics of circulating ghrelin, adiponectin and leptin in human obesity. Proc Natl Acad Sci USA. **101**: 10434–10439.