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Expression of GHS-R1 in the Stomach of Male and Female Rats after High-Fat, High-Carbohydrate Diet

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The aim of our work was to investigate the expression of ghrelin receptor (GHS-R1) in the stomach of dietary-manipulated rats by high-fat-high-carbohydrate-diet (HFHCD). Wistar rats (5 male, 5 female) were fed HFHCD for 16 weeks. Control rats (5 male, 5 female) were fed with standard rat chew for the same period of time. Metabolic control was determined by measuring body weight gain and BMI. Immunohistochemical study was performed on the stomach of both groups with primary ghrelin receptor GHS-R1 antibody. Results: We found positive expression of GHS-R1 in the stomach fundus and antral glandular cells of the experimental rats. The reaction had moderate and high intensity in single and clusters of cells. HFHCD activates the expression of GHS-R1 in the gastric mucosa in both sexes.GHS-R1 presence indicates the ability of ghrelin to affect the secretory activity of the ghrelin-producing cells in paracrine/autocrine way and allow for autonomous regulation of gastric secretion, different from other hormonal and nerve pathways.

Key words: ghrelin, GHS-R1, stomach, high-fat-high-carbohydrate diet, rats

Introduction

The involvement of ghrelin in the pathogenetic mechanisms of obesity is intensively studied but still not evaluated in details [19]. The ghrelin secretion depends on humoral factors such as blood glucose levels or insulin secretion, respectively; as well as external factors – the composition of the ingested nutrients. The hormonal regulation of the ghrelin secretion is affected by a number of circulating and gastrointestinal hormones [4,23,25]. Intravenous administration of glucagon caused transient increases of ghrelin levels and this effect was shown to be mediated by glucagon receptors on ghrelin cells [14]. De la Cour et al. found that epinephrine, norepinephrine, endothelin, and secretin stimulated ghrelin release [8]. GHS-R1A is a mature polypeptide expressed in brain and some peripheral tissues, GHS-R1B is an immature polypeptide [1,5,11,16]. Kitazawa et al. [15] reported of presence of GHS-R1 in gastrointestinal tract of rat and guinea pig.There is an evidence for an endocrine/paracrine role for ghrelin in the reproductive tissues [17]. Ghrelin and GHS-R1 have been found to regulate the proliferation of cancer cells in astrocytoma and glioblastoma by an autocrine/paracrine mechanism [9,18]. Some authors explore the potential functional role of ghrelin and its receptor in hormone-dependent cancers, such as

prostate and breast cancer [12]. There are fine autoregulatory mechanisms, performed by the autocrine/paracrine effect of ghrelin on the ghrelin-producing cells themselves in the stomach [10,22]. It is concluded that ghrelin may affect gastrointestinal motility via specific ghrelin receptors [20]. Obesity and insulin resistance may play an important role in the release of ghrelin [24]. Obesity selectively impairs the stimulatory effect of a caseinhydrolyaste on ghrelin release in the fundus [26].

The aim of our work is to investigate the immunohistochemical (IHC) expression of ghrelin receptor GHS-R1 in the stomach of dietetic manipulated male and female rats by high-fat-high-carbohydrate diet.

Materials and Methods

Male and female Wistar rats (5 male, 5 female)were fed with high-fat-high-carbohydrate diet (HFHCD) for 16 weeks. Control male and female rats (5 male, 5 female) were fed a normal diet for the same period of time. Metabolic control was determined by measuring body weight and BMI. Two-way ANOVA statistic analysis was used.Immunohistochemical study was performed on the stomach of the both groups with primary ghrelin receptor antibody (goat polyclonal antibody GHS-R1: sc-10351 – Santa Cruz Biotechnology USA). GHS-R1, was diluted in PBS in 1:100 ratio. We used a semi-quantitative evaluation method for the obtained results. We accept the presence of expression of the primary antibody as a positive result, and as a negative – the lack of its expression. Positive reaction for GHS-R1 was reported in the presence of fine brown granulation in the cell cytoplasm. The specificity of immunohistochemical reactions was confirmed by negative controls in which the specific antibodies are substituted with a buffer (PBS). In them there was a complete lack of a product of the respective reaction. Observation and photo documentation of microscopic preparations were performed with digital photo microscopic camera of a light microscope "Olympus BX51".

Results

Metabolic control. The obtained results showed that from the beginning of the study until the 11th week there were no significant differences in the body weight of the animals. From the 12th week until the end of the experiment the HFHCD had a significant main effect on the body weight ($P<0.05$). At the end of the experiment the dietary-manipulated (male and female) rats had higher body weight in comparison with the controls (357.33 ± 12.24 g vs 320.42 ± 12.24 g, $P<0.05$) (**Fig. 1.**). At the end of the experiment dietary-manipulated rats had a higher BMI as compared to the control rats (0.69 ± 0.02 g·cm-² vs 0.63 ± 0.02 g·cm-², $P < 0.01$).

Immunohistochemical expression of GHS-R1 in the stomach.The immunohistochemical response for GHS-R1in the stomach of the rat control group was negative (**Fig.2.A.**). The glands in the stomach mucosa showed typical characteristics. There was no expression of GHS-R1 in the different types of cells - parietal, chief, foveolar, as well as in the enteroendocrine cells (**Fig. 2.B.**). In the experimental groups of animals, we detected a positive immunohistochemical reaction for ghrelin receptor GHS-R1. We observed fine brown granules in the cytoplasm of some cells from gastric glands (**Fig. 3A**). Some of the GHS-R1-positive cells were located in groups along the tubular glands of the stomach body (**Fig. 3.B**). In the transverse sections of the gastric glands the expression engaged the basal parts of the cells (**Fig. 4.A**). The larger number of GHS-R1-positive cells had a moderate intensity of the immunohistochemical reaction. Some cells with high intensity of the immunohistochemical reaction were also observed (**Fig. 4.B**).

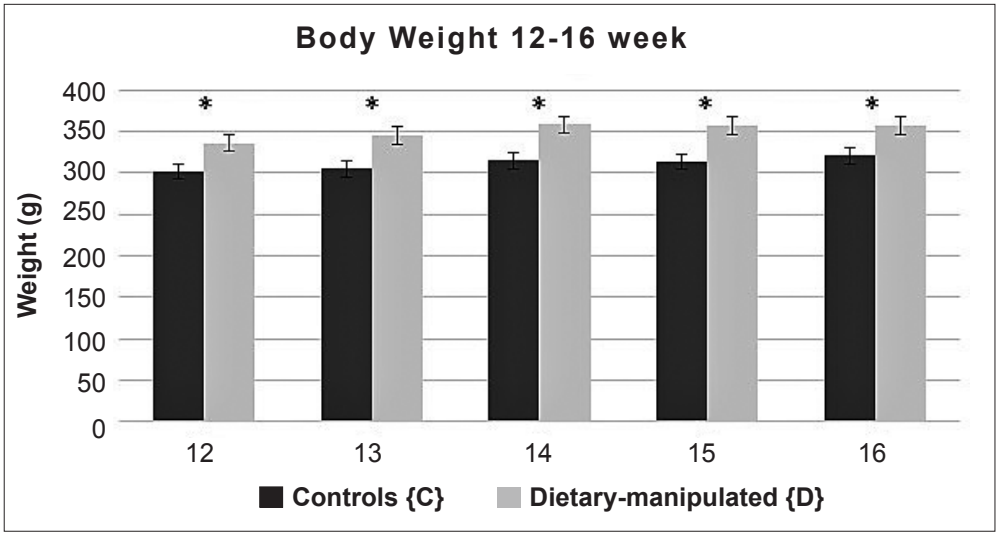

Fig. 1. Weight (g) of dietary-manipulated and control groups from the 12th week until the end of the experiment. *P<0.05, dietary-manipulated vs controls.

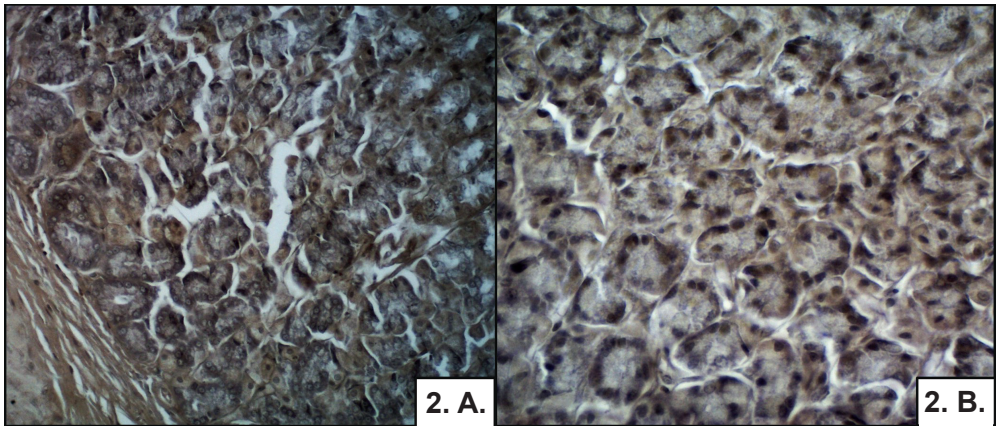

Fig. 2. Control group. IHC reaction for GHS-R1. A. Female rats. Stomach – body. Negative IHC reaction for GHS-R1 in fundic glands. Magn. × 200. B. Male rats. Stomach – body. Cross-section of fundic glands without expression of GHS-R1 in the different cell types. Magn. × 400.

Discussion

We report for the first time of influence of HFHCD on the expression of ghrelin receptor in the stomach of rats of both sexes. In our study the rats from the experimental groups subjected to a long-term HFHC diet demonstrated obesity with significant differences in body weight and BMI compared to the animals of the control groups.We detected presence of expression of GHS-R1 in the stomach body and antrum of the experimental rats, while the reaction for the receptor in the control groups was negative. Some authors report for GHS-R1 expression in the gastrointestinal tract of rat but this expression is located in

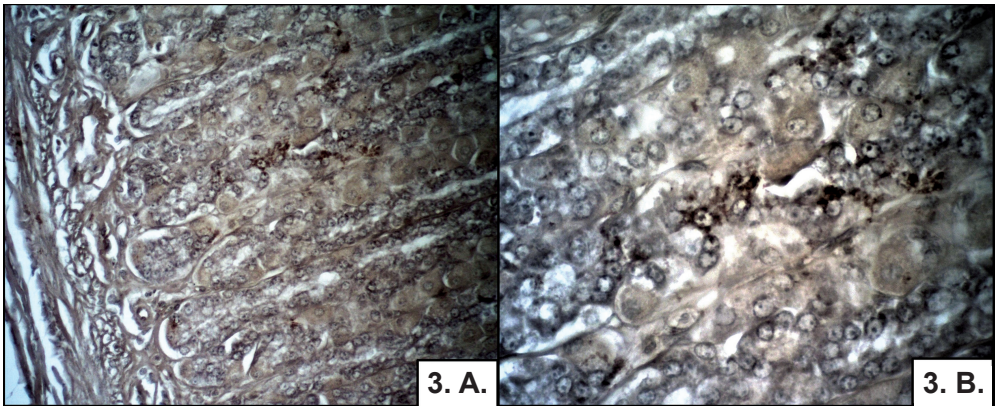

Fig. 3. Experimental group. IHC reaction for GHS-R1. A. Female rats. Stomach – body. GHS-R1-positive cells cells in the fundic glands with moderate intensity of the reaction. Magn. × 200. B. Female rats. Stomach – body. A group of GHS-R1-positive cells in the fundic glands. Magn. × 400.

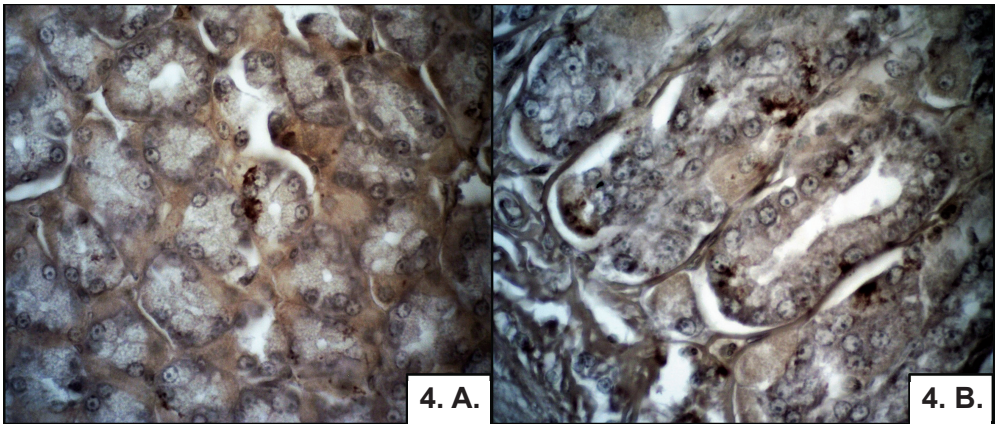

Fig. 4. Experimental group. IHC reaction for GHS-R1. A. Male rats. Stomach - body. Single GHS-R1-positive cells in the fundic glands. Expression of GHS-R1 in the basal part of the cells. Magn. × 400. B. Male rats. Stomach – anthrum. GHS-R1-positive cells in the pyloric glands with moderate to high intensity of the reaction. Magn. × 400.

the smooth muscle cells of the wall [15]. The GHS-R1 positive expression in the gastric mucosa showed that ghrelin could have an endocrine and paracrine effect on the cellsin the gastric glands. Ghrelin could affect in an autocrine/paracrine way the ghrelin-producing cells themselves. Changes in expression level and/or cell density are supposed to be accompanied with a consumption of high HFHCD. Feeding a HFHCD might affect the expression of fatty acid receptors or the number of lipid sensing cells as well as ghrelin-producing cell populations.

The long-term intake of dietary fat is supposed to be associated with adaptive reactions of the organism and it is assumptive that this is particularly true for fat responsive epithelial cells in the mucosa of the gastrointestinal tract [27]. May be HFHCD is also associated with similar adaptive reactions through the ghrelin-producing cells in the gas-

tric glands via the activation of the ghrelin receptors. Whether these changes are a consequence of the direct exposure to high fats and carbohydrates in the luminal content or a physiological response to them in the body remains elusive. Ghrelin shows orexigenic effect through its action on the hypothalamic appetite-regulating pathways, while in the periphery it increases adipose tissue accumulation [2]. In contrast to other forms of obesity, patients with Prader-Willi syndrome display reduced visceral adiposity and high levels of ghrelin [21]. Peripheral ghrelin induces a depot-specific increase in white adipose tissue mass by GHS-R1A-mediated lipolysis [7]. Although the precise mechanisms governing the export of free fatty acids from adipocytes remain to be elucidated [3,13]. Some authors concluded that exposure to ghrelin appears to induce adipocyte hypertrophy by enhancing lipid retention in responsive adipocytes [7]. Ghrelin can exert its effects in the regulation of feeding behavior and energy homeostasis and through systemic or autocrine/paracrine actions [6,22].

Conclusion

The HFHCD applied to male and female rats activates the expression of ghrelin receptor GHS-R1 in the gastric mucosa. These results imply that the diet leads to significant changes in the cellular repertoire of the stomach mucosa. GHS-R1 presence in the gastric mucosa cells indicates the ability of ghrelin to affect the secretory activity of the ghrelin-producing cells in both paracrine and autocrine way. Auto/paracrine action of ghrelin allows autonomous regulation of gastric secretion, different from other hormonal and nerve pathways. This possibility makes the stomach adaptive to the excessive conditions such as HFHCD.

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References

1. **Albarrán-Zeckler, R. G., R. G. Smith.** The ghrelin receptors (GHS-R1a and GHS-R1b). – *Endocr. Dev.,* **25**, 2013, 5-15.
2. **Álvarez-Castro, P., L. Pena, F. Cordido.** Ghrelin in obesity, physiological and pharmacological considerations. – *Mini Rev. Med. Chem.,* **13**, 2013, 541-552.
3. **Bonen, A., A. Chabowski, J. J. Luiken, J. F. Glatz.** Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence. – *Physiology (Bethesda),* **22**, 2007, 15-29.
4. **Broglio, F., Pv. Koetsveld, A. Benso, C. Gottero, F. Prodam, M. Papotti, G. Muccioli, C. Gauna, L. Hofland, R. Deghenghi, E. Arvat, A. J. Van Der Lely, E. Ghigo.** Ghrelin secretion is inhibited by either somatostatin or cortistatin in humans. – *J. Clin. Endocrinol. Metab.,* **87**, 2002, 4829-4832.
5. **Callaghan, B., J. B. Furness.** Novel and conventional receptors for ghrelin, desacyl-ghrelin, and pharmacologically related compounds. – *Pharmacol. Rev.,* **66**, 2014, 984-1001.
6. **Castañeda, T. R., J. Tong, R. Datta, M. Culler, M. H. Tschöp.** Ghrelin in the regulation of body weight and metabolism. – *Front. Neuroendocrinol.,* **31**, 2010, 44-60.
7. **Davies, J. S., P, Kotokorpi, S. R. Eccles, S.K. Barnes, P. F. Tokarczuk, S. K. Allen, H. S. Whitworth, I. A. Guschina, B. A. Evans, A. Mode, J. M. Zigman, T. Wells.** Ghrelin induces abdominal obesity via GHS-R-dependent lipid retention. – *Mol. Endocrinol.,* **23**, 2009, 914-924.
8. **De la Cour, C. D., P. Norlén, R. Håkanson.** Secretion of ghrelin from rat stomach ghrelin cells in response to local microinfusion of candidate messenger compounds: a microdialysis study. – *Regul. Pept.,* **143**, 2007, 118-126.

9. **Dixit, V. D., A. T. Weeraratna, H. Yang, D. Bertak, A. Cooper-Jenkins, G. J. Riggins, C. G. Eberhart, D. D. Taub.** Ghrelin and the growth hormone secretagogue receptor constitute a novel autocrine pathway in astrocytoma motility. – *J. Biol. Chem.,* **281**, 2006, 16681-16690.
10. **Higgins, S. C., M. Gueorguiev, M. Korbonits.** Ghrelin, the peripheral hunger hormone. – *Ann. Med.,* **39**, 2007, 116-136.
11. **Howard, A. D., S. D. Feighner, D. f. Cully, J. P. Arena, P. A. Liberator, C. I. Rosenblum M. Hamelin, D. L. Hreniuk, O. C. Palyha, J. Anderson, P. S. Paress, C. Diaz, M. Chou, K. K. Liu, K. K. McKee, S. S. Pong, L. Y. Chaung, A. Elbrecht, M. Dashkevicz, R. Heavens, M. Rigby, D. J. Sirinathsinghji, D. C. Dean, D. G. Melillo, A. A. Patchett, R. Nargund, P. R. Griffin, J. A. DeMartino, S. K. Gupta, J. M. Schaeffer, R. G. Smith, L. H. Van der Ploeg.** A receptor in pituitary and hypothalamus that functions in growth hormone release. – *Science,* **273**, 1996, 974-977.
12. **Jeffery, P. L., A. C. Herington, L. K. Chopin.** The potential autocrine/paracrine roles of ghrelin and its receptor in hormone-dependent cancer. – *Cytokine Growth Factor Rev.,* **14**, 2003, 113-122.
13. **Kampf, J. P., A. M. Kleinfeld.** Is membrane transport of FFA mediated by lipid, protein, or both? An unknown protein mediates free fatty acid transport across the adipocyte plasma membrane. – *Physiology (Bethesda),* **22**, 2007, 7-14.
14. **Katayama, T., S. Shimamoto, H. Oda, K. Nakahara, K. Kangawa, N. Murakami.** Glucagon receptor expression and glucagon stimulation of ghrelin secretion in rat stomach. – *Biochem. Biophys. Res. Commun.,* **357**, 2007, 865-870.
15. **Kitazawa T, T. Nakamura, A. Saeki, H. Teraoka, T. Hiraga, H. Kaiya.** Molecular identification of ghrelin receptor (GHS-R1a) and its functional role in the gastrointestinal tract of the guinea-pig. – *Peptides,* **32**, 2011, 1876-1886.
16. **Laviano, A., A. Molfino, S. Rianda, F. Rossi Fanelli.** T he growth hormone secretagogue receptor (Ghs-R). – *Curr. Pharm. Des.,* **18**, 2012, 4749-4754.
17. **Miller, D. W., J. L. Harrison, Y. A. Brown, U. Doyle, A. Lindsay, C. L. Adam, R. G. Lea.** Immunohistochemical evidence for an endocrine/paracrine role for ghrelin in the reproductive tissues of sheep. – *Reprod. Biol. Endocrinol.,* **3**, 2005, 60.
18. **Okada, Y., Y. Sugita, K.Ohshima, M. Morioka, S. Komaki, J. Miyoshi, H. Abe.** Signaling of ghrelin and its functional receptor, the growth hormone secretagogue receptor, promote tumor growth in glioblastomas. – *Neuropathology,* **36**, 2016, 535-543.
19. **Papandreou, D., C. Karavolias, F. Arvaniti, E. Kafeza, F. Sidawi.** Fasting. Ghrelin Levels Are Decreased in Obese Subjects and Are Significantly Related With Insulin Resistance and Body Mass Index. – *Open Access Maced. J. Med. Sci.,* **5**, 2017, 699-702.
20. **Peeters, T. L.** Central and peripheral mechanisms by which ghrelin regulates gut motility. – *J. Physiol. Pharmacol.,* **54**, 2003, 95-103.
21. **Scerif, M., A. P. Goldstone, M. Korbonits.** Ghrelin in obesity and endocrine diseases. – *Mol. Cell. Endocrinol.,* **340**, 2011, 15-25.
22. **Shiiya, T., M. Nakazato, M. Mizuta, Y. Date, M. S. Mondal, M. Tanaka, S. Nozoe, H, Hosoda, K. Kangawa, S. Matsukura.** Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. – *J. Clin. Endocrinol.Metab.,* **87**, 2002, 240-244.
23. **Shimada, M., Y. Date, M. S. Mondal, K. Toshinai, T. Shimbara, K. Fukunaga, N. Murakami, M. Miyazato, K. Kangawa, H. Yoshimatsu, H. Matsuo, M. Nakazato.** Somatostatin suppresses ghrelin secretion from the rat stomach. – *Biochem. Biophys. Res. Commun.,* **302**, 2003, 520-525.
24. **Stylianou, C., A. Galli-Tsinopoulou, D. Farmakiotis, I. Rousso, M. Karamouzis, G. Koliakos, S. Nousia-Arvanitakis.** Ghrelin and leptin levels in obese adolescents. Relationship with body fat and insulin resistance. – *Hormones (Athens),* **6**, 2007, 295-303.
25. **Tan, T. M., M. Vanderpump, B. Khoo, M. Patterson, M. A. Ghatei, A. P. Goldstone.** Somatostatin infusion lowers plasma ghrelin without reducing appetite in adults with Prader-Willi syndrome. – *J. Clin. Endocrinol. Metab.,* **89**, 2004, 4162-4165.
26. **Vancleef, L., T. Thijs, F. Baert, L. J. Ceulemans, E. Canovai, Q. Wang, S. Steensels, A. Segers, R. Farré , J. Pirenne, M. Lannoo, J. Tack, I. Depoortere.** Obesity Impairs Oligopeptide/Amino Acid-Induced Ghrelin Release and Smooth Muscle Contractions in the Human Proximal Stomach. – *Mol. Nutr. Food Res.,* **62**, 2018. doi: 10.1002/mnfr.201700804.
27. **Widmayer, P., H. Goldschmid, H. Henkel, M. Küper, A. Königsrainer, H. Breer.** High fat feeding affects the number of GPR120 cells and enteroendocrine cells in the mouse stomach. – *Front. Physiol.,* **6**, 2015, 53.