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A Multi-faceted, Fat-defending Peptide Hormone: Ghrelin

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Abstract: Ghrelin is a novel gastric hormone recognized in 1999 as a mediator of growth hormone release. Ghrelin is a peptide hormone in which the third amino acid usually serine is modified by an acyl acid. This modification is essential for ghrelin's activity. Ghrelin may thus be an essential hormone for maintaining growth hormone release and energy homeostasis. Ghrelin has profound orexigenic, adipogenic and somatotrophic properties, increasing food intake and body weight. Secreted predominantly from the stomach, ghrelin is the natural ligand for the growth hormone secretagogue receptor in the pituitary gland, thus fulfilling the criteria of a brain-gut peptide. Ghrelin is an important component of an integrated regulatory system of growth and metabolism acting via the vagus nerve and is implicated in a variety of altered energy states as obesity, eating disorders, neoplasia and cachexia. It also enhances immune responses and potentially down-regulates anti-inflammatory molecules. The discovery of ghrelin has increased the current knowledge on feeding regulation, nutritional homeostasis and metabolic processes.

Key words: Energy homeostasis, food intake, ghrelin, growth hormone release

INTRODUCTION

Ghrelin was named from *ghre*, a word root in Proto-Indo-European languages for "grow" and the suffix-relin for "releasing substance". Its name also implies that this peptide stimulates Growth Hormone (GH) release. Ghrelin exists not only in mammalian species, but also in non-mammalian species such as frog, chicken and fish (Kojima *et al.*, 2004). The pituitary secretion GH is activated by the Growth Hormone Releasing Hormone (GHRH) and inhibited by somatostatin. In 1977, endorphin-derived peptides were shown to stimulate GH release and this led to the development of peptidic and non-peptidic synthetic molecules called Growth-Hormone Secretagogues (GHS). GHRH and GHS stimulate GH release through the cAMP pathway and the inositol triphosphate/calcium pathway, respectively, suggesting they are acting on distinct receptors. While the GHS receptor (GHSR 1a) was cloned from human pituitary in 1996, its natural ligand was only identified in 1999 and called ghrelin (de Vriese and Delporte, 2008). Ghrelin is a 28 amino acid peptide found in mainly two forms in blood plasma, the acylated on the serine in position 3 with medium chain fatty acid, usually n-octanoic acid namely Acyl-Ghrelin (AG) and the other, Des-Acyl Ghrelin (DAG). The stomach can only acylate ghrelin with medium-chain and not with short- or long-chain fatty acids. Because animals do not synthesize medium-chain fatty acids, octanoic acid from dietary sources is probably utilized for ghrelin adduction. This type of post-transnational modification is entirely unique to ghrelin within the animal kingdom and is required for the peptide to bind to and activate its classical receptor,

GHSR1a. Consequently, most biological actions of ghrelin, especially those involving endocrine and anabolic effects, require acylated ghrelin. Whether des-acyl ghrelin has physiologic roles is a controversial question (Cummings, 2006; Otto *et al.*, 2005).

The AG is the endogenous ligand for GHSR1a, whereas DAG is not. GHSR1a mediates AG stimulation of GH release and food intake. GHSR1a is predominantly expressed in the pituitary and at lower levels in the hypothalamic nuclei, including the Arcuate (ARC), Paraventricular (PVN), Ventromedial (VMH) and in some brain-stem nuclei (Shrestha *et al.*, 2009).

The discovery of ghrelin and its receptor was initially viewed as a milestone in understanding GH-related endocrine mechanisms. However, it soon became apparent that side from ghrelin's role as a GH secretagogue, it acts as an orexigen. In fact, the endogenous G protein-coupled-GHS receptor agonist administered peripherally and directly into the brain induces an acute and robust feeding response, only a bit lower in magnitude than the effect of Neuropeptide Y (NPY). Long-term injections of this peptide continue to stimulate consummatory behavior and promote weight gain by increasing energy intake and by reducing energy expenditure. While ghrelin comes from both peripheral and central sources, its hyperphagic properties to a large extent, arise from the activity at the brain level (Olszewski *et al.*, 2008).

Expression, regulation and turnover: Ghrelin is predominantly produced in the stomach and in the gastrointestinal tract (duodenum, jejunum, ileum, colon),

but small quantities have been found in the brain and may other tissues such as pituitary, hypothalamus, pancreas, placenta, salivary glands, lung, heart, liver, pancreas, kidney, thyroid, ovary and testis (Klok *et al.*, 2007; de Vriese and Delporte 2008; Shrestha *et al.*, 2009).

Ghrelin cells can be classified into opened- and closed-type cells. Opened-type cells are in contact with the glandular lumen and closed-type cells do not have a luminal connection. The ghrelin in the stomach are closed-type cells, whereas in the duodenum, jejunum, ileum and colon opened- and closed-type of ghrelin cells are found and number of opened-type cells gradually increased in the direction from stomach to the lower gastrointestinal tract (Klok *et al.*, 2007).

Plasma ghrelin level is regulated by several factors. Age and gender do not seem to profoundly affect plasma ghrelin level. A key regulator of plasma ghrelin level is food intake. Plasma ghrelin level is elevated under starvation and decreases after food intake in response to an increase of glycemia. Plasma ghrelin level decreases more drastically and faster following a meal rich in carbohydrates rather than in fats, while a meal rich in proteins was described as having contradictory effects. Plasma ghrelin level is negatively associated with Body Mass Index (BMI). Indeed, plasma ghrelin level is increased in anorexia nervosa and cachexia and decreased in obesity (de Vriese and Delporte, 2008). Moreover, ghrelin levels fluctuate in a compensatory manner to body weight variations. Ghrelin levels decrease with weight gain resulting from overfeeding, pregnancy, or high-fat diet. Conversely, weight loss induces an increase of ghrelin levels. This effect is observed with weight loss due to food restriction and long-term chronic exercise, but not acute exercise, cachectic states induced by anorexia nervosa, severe congestive heart failure, lung cancer, breast and colon cancer (de Vriese and Delporte, 2007).

The normal plasma concentration of ghrelin in humans is 10-20 fmol/ml for mostly n-octanoyl ghrelin and 150-180 fmol/ml for total ghrelin, including both AG and DAG (Hosoda *et al.*, 2006).

Mechanisms of action

GH secretion: Ghrelin strongly and dose-dependently stimulates GH secretion, releasing more GH than GHRH. Combined administration of ghrelin and GHRH induces synergistic effects on GH release. Although ghrelin acts directly on the pituitary, ghrelin is not able to release GH in patients with organic lesions in the hypothalamic area and increases GHRH release from hypothalamic tissue *in vitro*. This suggests that ghrelin action is also mediated by the hypothalamus. Apart from GH release, ghrelin stimulates Adrenocorticotrophic Hormone (ACTH), cortisol and prolactin release (de Vriese and Delporte, 2008).

The secretion of ghrelin by the stomach depends largely on the nutritional state. Ghrelin levels show preprandial increases and postprandial decreases. In addition, ghrelin levels show a diurnal variation and seem to be influenced by BMI, GH, glucose and insulin (Klok *et al.*, 2007).

Appetite: Appetite is the internal driving force for the search, choice and ingestion of food. Appetite in humans can be measured in 2 ways. First, it can be measured with the help of subjective ratings. Humans have a capacity for introspection and can rate the strength of their conscious drive or motivation to eat. Second, appetite can be measured by actual food intake; that is, the amount of food eaten within a certain context can be considered as a measure of appetite. The degree to which actual food intake reflects appetite is debatable. There are many factors that may intervene between appetite and actual food intake: cognitive factors, such as dietary restraint, but also external factors, such as availability, hedonic properties of food and social circumstances. However, when measured under standardized conditions, actual food intake serves as a post hoc indicator of appetite (de Graaf *et al.*, 2004). Feeding is a basic behavior that is necessary for life. It is well accepted that appetite is controlled by the brain and that feeding behavior is regulated by complex mechanisms in the central nervous system, in particular, the hypothalamus (Hosoda *et al.*, 2006). In rats, Intracerebroventricular (ICV), intravenous or subcutaneous administration of ghrelin stimulates food intake and decreases energy expenditure, enhancing weight gain. In humans, intravenous ghrelin administration also increases appetite and stimulates food intake. Ghrelin is the only known circulating hormone to promote feeding following systemic administration. Moreover, ghrelin levels increase during fasting, before the onset of meals and decrease strongly 30 min after feeding, suggesting that ghrelin levels may serve as a signal for meal initiation. In fasting individuals, ghrelin levels display a circadian pattern similar to that described in people eating three times a day (de Vriese and Delporte, 2007).

In Table 1, features which support the role of ghrelin in appetite and short-term food intake is shown (Gil-Campos *et al.*, 2006).

Leptin antagonist: The hypothalamic ARC nucleus is the main active site of ghrelin. The ARC nucleus is also the target site of leptin, an appetite-suppressing hormone from adipose tissues and NPY and Agouti-Related-Peptide (AgRP), which are appetite-stimulating peptides. NPY and AgRP are produced in the same neurons in the ARC nucleus and the appetite-stimulating effects of these peptides are directly inhibited by leptin. ICV injection of ghrelin induced the expression of protein in

Table 1: Features which support the role of ghrelin in appetite and short-term food intake

1. Most ghrelin is synthesized in the stomach, a well-positioned organ to detect recent intake of food.
2. The main effects of intraventricular and blood system ghrelin injection at times of minimal spontaneous intake is to trigger eating and to decrease the latency of feeding.
3. Human ghrelin secretion is suppressed immediately after a meal, the depth and duration of the suppression being proportional to the energy intake.
4. Ghrelin stimulates gastrointestinal motility and gastric and exocrine pancreatic secretions.
5. Ghrelin stimulates the secretion of neuropeptide Y and agouti-related protein, two well-known orexigens, in the arcuate nucleus of the hypothalamus.
6. Some ghrelin gene polymorphisms are associated with alterations in eating patterns.

NPY neural cells and increased the amount of NPY mRNA in the ARC nucleus. Moreover, ICV ghrelin injection increased the AgRP mRNA level in the hypothalamus. The intravenous injection of ghrelin also stimulates NPY/AgRP neurons in the hypothalamus. ICV injections of an antagonist of NPY receptor-I and AgRP inhibitor inhibited the appetite-stimulating effects of ghrelin. These results indicate that ghrelin exerts its feeding activity by stimulating NPY/AgRP neurons in the hypothalamus to promote the production and secretion of NPY and AgRP peptides. Ghrelin is thus a natural antagonist to leptin (Kojima *et al.*, 2004).

Food intake, body weight and energy homeostasis: The effects of ghrelin on energy homeostasis are at least in a large part mediated by the hypothalamus. Korbonits *et al.* (2004) proposed three different pathways for the appetite-stimulating effects of ghrelin. First, after release into the bloodstream by the stomach, ghrelin may cross the blood-brain barrier and bind to its receptors in the hypothalamus. Second, ghrelin may reach the brain through the vagal nerve and NTS. Third, ghrelin is produced locally in the hypothalamus, where it may directly affect the various hypothalamic nuclei. Although ghrelin has become widely known for its potential role as a short-term hunger signal, chronic administration increases body weight and endogenous ghrelin levels fluctuate with changes in body weight. One may wonder whether the elevation in ghrelin levels under these circumstances is merely a reflection of the reduced feeding; circulating ghrelin may be high because less energy is entering the gut and suppressing this hormone. It appears, however, that baseline (preprandial) ghrelin levels do correspond with body weight independent of food intake (Williams and Cummings, 2005). Leidy *et al.* (2004) conducted a study in which healthy women took part in a chronic exercise program while fed a diet designed to maintain body weight. Some remained weight stable whereas others lost weight. Over time, ghrelin levels increased only in the weight loss group. Foster-Schubert *et al.* (2005) also confirmed these results with sedentary postmenopausal women were randomized to either a chronic aerobic exercise group or a passive stretching control group for 1 year and were asked to maintain their typical diet. At the end of the intervention, the chronic exercise group

had lost a small but substantial amount of weight compared with the control group and ghrelin levels increased in relation to the amount of weight loss. Taken together, these results demonstrate that in the absence of reduced food intake, plasma ghrelin increases in response to a loss of body weight per se.

Ghrelin is an endogenous orexigenic peptide. In humans, ghrelin administration stimulates appetite and increases food intake. Plasma ghrelin levels increase before meals and decrease strongly postprandially, indicating that ghrelin plays a role in meal initiation. Ghrelin stimulates appetite by acting on the hypothalamic ARC nucleus, a region known to control food intake. Circulating ghrelin and ghrelin synthesized locally in the hypothalamus activate neurons expressing NPY and AgRP in the ARC nucleus, but also neurons expressing orexin in the lateral hypothalamic area. Moreover, through the activation of GHS on vagal afferents in the stomach, the signal induced by ghrelin reaches the Nucleus of Tractus Solitarius (NTS), which communicates with the hypothalamus (de Vriese and Delpote, 2008). *In vivo*, chronic administration of ghrelin increases body weight by stimulating food intake, decreasing energy expenditure, decreasing utilization of fat and increasing utilization of carbohydrates. Ghrelin may thus influence adipocyte metabolism. *In vitro*, ghrelin stimulates differentiation of adipocytes, inhibits adipocyte apoptosis and antagonize lipolysis. Chronic central infusion of ghrelin inhibits lipid oxidation and increases lipogenesis and triglyceride uptake in white adipocytes. Ghrelin also improves lean body mass retention (de Vriese and Delpote, 2007, 2008).

Fasting ghrelin levels are elevated in subjects with anorexia nervosa and cachexia and return to normal levels after weight recovery. Conversely, fasting ghrelin levels are reduced in obese subjects and increase after diet-induced weight loss. Thus, ghrelin seems to play an important role in the regulation of energy homeostasis. Since compensatory mechanisms to ghrelin loss exist, the physiological importance of ghrelin as a regulator of energy homeostasis is still unclear (de Vriese and Delpote, 2008).

In Table 2, features which support the role of ghrelin in the regulation of energy homeostasis is summarized (Gil-Campos *et al.*, 2006).

Table 2: Features which support the role of ghrelin in the regulation of energy homeostasis

1. Serum ghrelin levels are inversely correlated with BMI, age and insulin concentrations.
2. Ghrelin levels are influenced by nutritional status, increasing in response to weight loss resulting from low-energy diets, life-style modifications and diseases leading to malnutrition
3. Obese children and adults have lower ghrelin plasma levels than lean subjects and they exhibit a lower postprandial decline and a more rapid returning towards baseline levels
4. Bariatric surgery is associated with reduced ghrelin plasma levels
5. Peripheral or central chronic administration of ghrelin increases body weight and reverses the effects of leptin
6. Ghrelin decreases energy expenditure limiting fat catabolism, lipolysis and adipocyte apoptosis
7. Acute ghrelin blockade in animals using anti-ghrelin antibodies, ghrelin receptor antagonists or antisense oligonucleotides decreases food intake and weight loss

Gastrointestinal effects: Ghrelin increases gastric acid secretion and gastric motility in rats. Circulating ghrelin levels accelerated gastric emptying rate in humans. Stimulatory or inhibitory effects of ghrelin may depend on experimental conditions and models. Ghrelin stimulates gastric motility by inducing the migrating motor complex and accelerating gastric emptying. Moreover, ghrelin exerts a gastroprotective effect against stress- and ethanol-induced ulcers. This effect depends on sensory nerve fiber integrity and is mediated by the nitric oxide system (Wu and Kral, 2004; de Vriese and Delporte, 2008).

Cardiovascular effects: Increasing evidence supports a functional role of ghrelin in myocardial growth associated with improved cardiac function. Both ghrelin and GHS have been detected in the aorta and myocardium, indicating that ghrelin may modulate cardiovascular parameters (Wu and Kral, 2004). Ghrelin has diverse cardiovascular effects. *In vitro*, ghrelin inhibits apoptosis of cardiomyocytes and endothelial cells. Moreover, by inhibiting natural killer B activation in human endothelial cells and mononuclear cell adhesion, ghrelin might oppose inflammation of the cardiovascular system. Ghrelin exerts vasodilatory effects by an endothelium-independent mechanism. Administration of ghrelin decreases mean arterial pressure without changing the heart rate. Ghrelin improves cardiac contractility and left ventricular function in chronic heart failure and reduces infarct size (de Vriese and Delporte, 2008).

Effects on glucose homeostasis: Ghrelin seems to influence glucose and lipid metabolism. Acute ghrelin administration increases plasma glucose levels and amplifies the hyperglycemic effect of arginine. This effect could be due to glycogenolysis activation, indirectly by stimulation of catecholamine release or directly by acting on hepatocytes where ghrelin might modulate gluconeogenesis. *In vivo*, chronic ghrelin administration induces adiposity in rodents and *in vitro*, ghrelin stimulates differentiation of preadipocytes and antagonizes lipolysis (Broglia *et al.*, 2005).

Pancreatic effects: Ghrelin has been reported to influence the endocrine pancreatic function. Depending

on the experimental conditions, ghrelin either stimulates or inhibits insulin secretion. The effects of ghrelin on exocrine pancreas are also controversial. In rat pancreas, ghrelin was demonstrated to inhibit pancreatic protein secretion or to increase protein output. These effects are indirect and may involve cholecystokinin and reflex vagal pathways (de Vriese and Delporte, 2008). Immunohistochemical analyses have shown co-localization of ghrelin proteins with glucagon in the α -islets cells of the pancreas. In addition, ghrelin was found to increase cytosolic-free calcium concentration in β -cells and stimulate insulin secretion in isolated rat pancreatic islets. Thus, ghrelin functions as a gastro-entero-pancreatic hormone, participating in the regulation of glucose metabolism, insulin release and pancreatic exocrine secretion to integrate the hormonal and metabolic responses to fasting (Wu and Kral, 2004).

Reproductive effects: Ghrelin participates in the regulation of the reproductive function. In the testis, ghrelin inhibits testosterone secretion. Besides having gonadal effects, ghrelin may participate in the regulation of gonadotropin secretion and may influence the timing of puberty. In the pituitary, ghrelin inhibits luteinizing hormone (de Vriese and Delporte, 2008).

Anti-inflammatory effects: Ghrelin has anti-inflammatory effects. Indeed, ghrelin inhibits production of proinflammatory cytokines in human endothelial cells, T cells, monocytes and in a rat model of endotoxic shock. Also, ghrelin attenuates the development of acute pancreatitis in rats. Ghrelin dose-dependently inhibits proliferation of anti-CD3 activated splenic T lymphocytes in mice (de Vriese and Delporte, 2008).

Effects on bone formation: Ghrelin stimulates bone formation by stimulating *in vitro* osteoblastic cell proliferation and differentiation, inhibiting apoptosis and increasing *in vivo* bone mineral density in both normal and GH-deficient rats (Fukushima *et al.*, 2005).

Effects on immune function: Ghrelin and GHSR signaling system were detected in human T cells, B cells and neutrophils, regardless of the maturity of the cell types. Ghrelin induced significant increases in

peripheral blood lymphocytes and thymic cellularity and differentiation in young and old mice, respectively. In addition, ghrelin-treated mice exhibited significant increases in cytotoxic lymphocytes, cycling of lymphoid cells in the spleen and reduction in tumor initiation and subsequent metastases. These observations indicated an immune enhancing property of ghrelin, with clinical implications for the treatment of immunocompromised states, eg, in aging, transplantation and Acquired Immune Deficiency Syndrome (AIDS) (Wu and Kral, 2004).

Effects on neoplasia: Ghrelin mRNA has been found in normal pituitary tissue and pituitary tumors. Non-functioning adenomas expressed the highest level of ghrelin mRNA, followed by GH- and gonadotropin-producing adenomas, while prolactinomas appeared to express the lowest level. There was an inverse correlation between the level of mRNA expression and size in GH-producing adenomas. In addition, has also been linked to several cancer cell lines and neuroendocrine tumors (Wu and Kral, 2004).

Ghrelin in breast-milk: Ghrelin in breast-milk is likely synthesized and secreted from the breast. Different serum ghrelin concentration in infants were detected for exclusively breast-fed or formula-fed infants, with higher values in the latter. A negative correlation was observed between ghrelin concentration and infant weight gain, suggesting that also in healthy infants with a physiologic energy balance, ghrelin may play a role in the regulation of body weight. A positive correlation has been observed between serum ghrelin concentration, infants' age, weight, head circumference and height, suggesting that this hormone could exert an influence on growth in the first months of life (Savino and Ligouri, 2008).

Conclusion: Since the discovery of ghrelin in 1999, the volume of literature on its biology is impressive. In addition to affecting energy homeostasis, ghrelin may have a number of effects on several systems. The effect of ghrelin on appetite is mediated in hypothalamus through stimulation of NPY and AgRP release. The effect of ghrelin on body weight is mediated by stimulating food intake, but also by reducing energy expenditure and promoting adiposity. The isolation of ghrelin can be considered a landmark in the GH field, allowing new insights into understanding the regulation of the GH system, food intake, body fat composition and gastrointestinal functions. Ghrelin may prove to be an effective probe for elucidating the normal and pathological regulation of GH release and appetite in humans, it should be explored in the clinical setting as a potential diagnostic and therapeutic tool. Ghrelin agonists seem useful for the treatment of several

diseases, including cachexia due to cancer, cardiovascular diseases, etc. All these clinical applications of ghrelin need further investigation. Our view of ghrelin as an orexigenic agent may soon need to be broadened from a relatively narrow perspective of a molecule that merely affects energy intake itself to a neural agent that prepares the organism for an effective and safe consummatory activity. This complexity of ghrelin's influence on consumption control through a variety of mechanisms require further studies.

REFERENCES

- Broglio, F., F. Prodham, E. Me, F. Riganti, B. Lucatello and R. Granata, 2005. Ghrelin: Endocrine, metabolic and cardiovascular actions. *J. Endocrinol. Invest.*, 28: 23-25.
- Cummings, D.E., 2006. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol. Behavior.*, 89: 71-84.
- de Graaf, C., A.M.W. Blom, A.M.P. Smeets, A. Stafleu and H.F.J. Hendriks, 2004. Biomarkers of satiation and satiety. *Am. J. Clin. Nutr.*, 79: 946-991.
- de Vriese, C. and C. Delporte, 2007. Influence of ghrelin on food intake and energy homeostasis. *Curr. Opin. Clin. Nutr. Metab. Care.*, 10: 615-619.
- de Vriese, C. and C. Delporte, 2008. Ghrelin: A new peptide regulating growth hormone release and food intake. *Int. J. Biochem. Cell. Biol.*, 40: 1420-1424.
- Foster-Schubert, K.E., A. McTieman, R.S. Frayo, R.S. Schwartz, K.B. Rojan and Y. Yasui, 2005. Human plasma ghrelin levels increase during a one year exercise program. *J. Clin. Endocrinol. Metab.*, 90: 820-825.
- Fukushima, N., R. Hanada, H. Teranishi, Y. Fukue, T. Tachibana and H. Ischikawa, 2005. Ghrelin directly regulates bone formation. *J. Bone. Miner. Res.*, 20: 790-798.
- Gil-Campos, M., M.C. Aguilera, R. Canete and A. Gil, 2006. Ghrelin: A hormone regulating food intake and energy homeostasis. *Br. J. Nutr.*, 96: 201-226.
- Hosoda, H., M. Kojima and K. Kangawa, 2006. Biological, physiological and pharmacological aspects of ghrelin. *J. Pharmacol. Sci.*, 100: 398-410.
- Klok, M.D., S. Jakobsdottir and M.L. Drent, 2007. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review. *Obes. Rev.*, 8: 21-34.
- Kojima, M., H. Hosoda and K. Kangawa, 2004. Ghrelin, a novel growth-hormone-releasing and appetite-stimulating peptide from stomach. *Best. Practice. Res. Clin. Endocrinol. Metab.*, 18: 517-530.
- Korbonits, M., A.P. Goldstone, M. Gueorguiev and A.B. Grossman, 2004. Ghrelin-a hormone with multiple functions. *Front. Neuroendocrinol.*, 25: 27-68.

- Leidy, H.J., J.K. Gardner, B.R. Frye, M.L. Snook, M.K. Schuchert and E.L. Richard, 2004. Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women. *J. Clin. Endocrinol. Metab.* 89: 2659-2664.
- Olszewski, P.K., H.B. Schiöth and A.S. Levine, 2008. Ghrelin in the CNS: From hunger to a rewarding and memorable meal? *Brain. Res. Rev.*, 58: 160-170.
- Otto, B., J. Spranger, C.S. Benoit, J.D. Clegg and M.H. Tschöp, 2005. The many faces of ghrelin: New perspectives for nutrition research? *Br. J. Nutr.*, 93: 765-771.
- Savino, F. and S.A. Ligouri, 2008. Update on breast milk hormones: Leptin, ghrelin and adiponectin. *Clin. Nutr.*, 27: 42-47.
- Shrestha, Y.B., K. Wickwire and S. Giraud, 2009. Effect of reducing hypothalamic ghrelin receptor gene expression on energy balance. *Peptides*, 30: 1336-1341.
- Williams, D.L. and D.E. Cummings, 2005. Regulation of ghrelin in physiologic and pathophysiologic states. *J. Nutr.*, 135: 1320-1325.
- Wu, T.J. and G.J. Kral, 2004. Ghrelin, integrative neuroendocrine peptide in health and disease. *Ann. Surg.*, 239: 464-474.