Acta Biochim Biophys Sin (2009): 188–197 | © The Author 2009. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmp001.

Review

Ghrelin fluctuation, what determines its production?

Xuefeng Yin[1], Yin Li[1], Geyang Xu[1], Wenjiao An[1], and Weizhen Zhang[1,2*]

[1]Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing 100191, China
[2]Department of Surgery, University of Michigan Medical Center, Ann Arbor, MI 48109-0346, USA
*Correspondence address. Tel: +86-10-82802183; Fax: +86-10-82802183; E-mail: weizhenzhang@bjmu.edu.cn

Ghrelin, a 28 amino acid gut brain peptide, acts as an endogenous ligand for its receptor, the growth hormone secretagogue receptor, to exercise a variety of functions ranging from stimulation of growth hormone secretion, regulation of appetite and energy metabolism, and cell protection to modulation of inflammation. This review summarizes the advance in the regulation of ghrelin expression and secretion. We introduce the structure of ghrelin promoter, the processing and modification of ghrelin precursor, and the regulation mechanism in these processes. Then we discuss factors found to be important in the regulation of ghrelin production, including nutrients, hormones, and autonomic nervous system. Finally, we outline the alteration in the level of ghrelin in certain physiological and pathological status.

Keywords ghrelin; regulation; diet; secretion; biosynthesis

Received: September 2, 2008 Accepted: December 9, 2008

Introduction

Ghrelin is an acyl-peptide composed of 28 amino acids. It is synthesized mainly by X/A-like cells in the gastric mucosa, but also found in hypothalamus, pituitary gland, hippocampus, brain cortex, adrenal gland, intestine, pancreas, and many other human tissues [1–3]. Ghrelin has a unique structure, with an *N*-octanoyl group covalently linked to the hydroxyl group of its serine 3 residue. This octanoylation is necessary for ghrelin to bind with its receptor, the growth hormone secretagogue receptor (GHS-R) [4]. Through the mediation of this seven transmembrane G-protein-coupled receptor, ghrelin exerts neuroendocrine effects of eliciting growth hormone release, regulating appetite and energy metabolism, and carries many other functions in a variety of tissues and organs such as gastroenteropancreatic system, cardiovascular system [5], reproduction system [6], and immune system [7].

Although numerous results about the function of ghrelin in physiological and pathological conditions have been reported, emerging evidences suggest that the regulation of ghrelin expression and secretion is complicated yet precise. Regulation of ghrelin concentration may occur at different levels ranging from transcription, post-transcription, translation, post-translation modification to secretion, suggesting the remarkable complexity of its regulation. This review summarizes the recent progress in the regulation of ghrelin expression and secretion, explores the possible mechanism involved, and introduces factors which are important for its regulation such as nutrients, hormones, autonomic nervous system, and lastly discusses altered level of ghrelin in certain physiological and pathological status.

Transcriptional Regulation of Ghrelin

As shown in **Fig. 1**, ghrelin gene spans 5 kb of the genomic DNA on chromosome 3p25–26, consisting of four introns and five exons, including a non-coding exon 1. Ghrelin gene encodes a pre-proghrelin composed of 117 amino acid residues, which can be further processed into acyl ghrelin, des-acyl ghrelin, and obestatin. The 5′-upstream regulation region of the ghrelin gene consists of binding sites of several transcriptional factors such as upstream stimulatory factor−1/−2 [8], activator proten-1, CCAAT enhancer binding proteins, and cAMP response element binding protein [9], indicating that these transcription factors may regulate ghrelin expression. However, evidence for the direct regulation of these transcriptional factors on ghrelin gene expression is still lacking. Recently, a report revised the structure of ghrelin gene, demonstrating the presence of

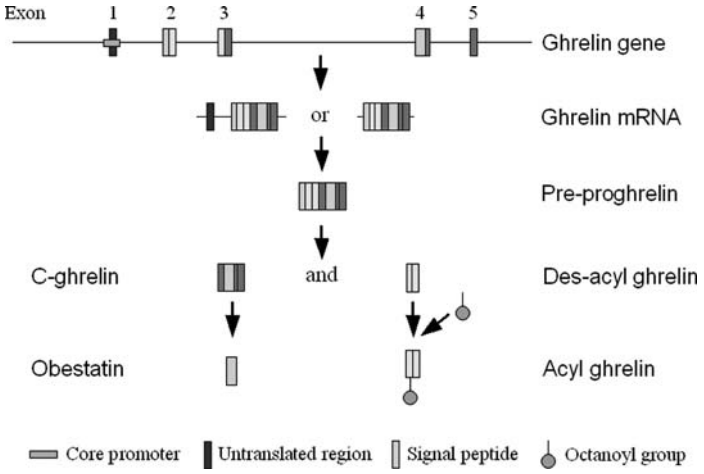

Fig. 1 Structure of human ghrelin gene and its processing.

an additional novel exon, detecting several ghrelin gene-derived mRNA splice variants, including transcripts which probably encode C-ghrelin, obestatin or novel protein isoforms, and antisense non-coding regulatory transcripts [10]. This study reveals that the ghrelin gene locus may be far more complex than previously recognized and the transcription regulation mechanism remains to be unraveled.

Post-translational Modification of Ghrelin Precursor Protein

Ghrelin is the only protein currently known to be octanoylated. This unique modification is necessary for ghrelin to bind with its receptor, GHS-R1a. The des-acyl ghrelin, which is the dominant form of ghrelin in plasma, cannot bind with the GHS-R1a receptor and was once considered inactivated. However, emerging evidence has challenged this notion. Several studies have suggested that des-acyl ghrelin may stimulate [11], or inhibit [12,13], food intake in a GHS-R1a independent pathway, probably through a novel receptor distinct from the classical ghrelin receptor GHSR-1a. In addition, there are some naturally occurring variants of ghrelin such as decanoyl and decanoyl ghrelin based on the different acylation of the serine-3 residue, and des-Gln14 ghrelin resulting from alternative splicing of ghrelin gene [14]. These variant molecules exhibit physiological function similar to acyl ghrelin [14].

The enzyme that catalyzes the octanoylation of ghrelin is identified by two individual studies and designated as ghrelin *O*-acyltransferase (GOAT) [15,16]. GOAT is a member of the family membrane-bound *O*-acyltransferases, with conserved structure among different species. Analysis of GOAT reveals its highly specific expression in the gastric mucosa. This discovery manifests its significance in the regulation of ghrelin secretion, because the amount and/or activity of this specific enzyme likely affect the level of acyl ghrelin. An *in vitro* study has demonstrated that GOAT activity could be inhibited potently by an octanoylated ghrelin pentapeptide and other end-products [17], suggesting the existence of a negative feedback regulation on the production of acyl ghrelin. This insight may promote the design of useful GOAT inhibitors as anti-obesity and anti-diabetic drugs.

In addition, a recent report demonstrates that ghrelin can be phosphorylated by protein kinase C α, β, and δ at serine 18 residue and this phosphorylation affects the secondary structure and membrane binding property of ghrelin [18]. The cellular role of phosphorylated ghrelin remains to be determined, but the phosphorylation probably relates with subcellular localization of ghrelin, especially des-acyl ghrelin.

In vitro and *in vivo* studies have shown that the prohormone convertase 1/3 (PC1/3) is the only identified enzyme responsible for processing of proghrelin into ghrelin [19]. This endoprotease co-localizes with ghrelin in gastric ghrelin-positive cells and processes the 94 amino acids human ghrelin precursor into the 28 amino acids mature ghrelin through limited proteolytic cleavage at a site determined to be LQPR↓ALAG [20]. However, as PC1/3 is also capable of processing other

hormones including glucagon-like peptide-1 (GLP-1) [21] and cholecystokinin [22], the regulation mechanism, if exists, is probably not specific for ghrelin.

The circulating level of ghrelin is determined by the balance among its secretion rate, degradation rate, and clearance rate. Plasma esterases have been reported to des-acylate acyl ghrelin, whereas plasma proteases account for the degradation of circulating ghrelin [23]. Clearance of circulating ghrelin includes being captured by its receptor and excreted in urine [23]. Besides, acyl ghrelin can transport across blood–brain barrier bidirectionally through specific transport system in humans [24]. As ghrelin carries out the orexigenic [25] and other important functions [26] by interacting with hypothalamic neurons, the efficacy of this yet to be identified transport system may involve in the regulation of ghrelin as well.

Nutrients Regulating Ghrelin Expression and Secretion

Glucose markedly inhibits ghrelin secretion. Oral infusion of glucose can decrease the plasma concentration of total ghrelin 30 min after ingestion in humans [27] and in rats [28]. Ingestion of crude fiber has the similar effect with glucose [28]. Insulin-induced hypoglycemia up-regulates ghrelin mRNA expression [29] and serum acyl ghrelin level [30] in the stomach. It is, however, not clear whether glucose inhibits directly the expression and secretion of ghrelin, or indirectly by the mediation of a yet to be identified mechanism.

Ingestion of either medium-chain fatty acids (*n*-octanoic acid) or medium-chain triglycerides (glyceryl trioctanoate) increases the stomach contents of acyl ghrelin without changing the total ghrelin in mouse [31]. This study suggests that medium-chain fatty acids can be utilized directly for the acyl modification of ghrelin as proposed by the author, but may also indicate a decrease in ghrelin secretion and thus explain the decrement in serum ghrelin level. Infusion of intralipid, a mixture of long-chain triglycerides, decreases plasma total ghrelin in humans [32]. Intraduodenal administration of C12 dodecanoic acid decreases plasma ghrelin significantly, whereas C10 decanoic acid has no effect [33]. The above discoveries suggest that the effects of fatty acids and triglycerides on ghrelin secretion are dependent on the length of their chain. Generally, lipid ingestion leads to a smaller decline in ghrelin relative to the administration of glucose or amino acids [34]. This observation may explain the weight gain effect of high-fat dietary.

Oral ingestion of a physiological dose of essential amino acids leads to a continuous rise in serum ghrelin level in humans [35,36], which unexpectedly contradicts with the inhibitory effect of protein on ghrelin as discussed later in chapter 7. Insoluble dietary fiber ingestion may influence ghrelin level as well [37].

Hormones Regulating Ghrelin Expression and Secretion

Insulin

In rats, gastric artery perfusion of insulin inhibits ghrelin release from isolated stomach tissue significantly [38]. Administration of insulin in central nervous system reduces serum total ghrelin concentration [39]. Several observations in humans also indicate that insulin may inhibit ghrelin secretion. Infusion of insulin significantly decreases plasma ghrelin level while maintaining euglycemia [40,41]. Fasting plasma acyl ghrelin level is negatively related to insulin concentration [30]. Total ghrelin level is also negatively related to homeostasis model assessment insulin resistance index (HOMA-R) [42], and positively related to insulin sensitivity [43]. GLP-1, a potent stimulator for insulin secretion, has been reported to alleviate the pre-prandial rise of ghrelin in humans [44]. This inhibitory effect of insulin may underlie the suppression of glucose on ghrelin and the inverse relationship between body weight and ghrelin level. It may also explain the low ghrelin level in patients of type 2 diabetes mellitus as discussed in chapter 8. However, Toshinai *et al.* [29] observed increment in ghrelin after insulin administration. This can be explained as a result of severe hypoglycemia induced by rapid injection of high dose of insulin. As suggested by Flanagan *et al.* [41], the influence of insulin and glucose on ghrelin secretion is probably contradictory and independent. Also, it is worth to note that insulin sensitivity, rather than insulin itself, may play a more important role in the regulation of ghrelin [45].

It has been reported that the insulin-induced hypoglycemia is independent of growth hormone level [46]. But there is also a study showing that the negative correlation between insulin and ghrelin disappears in patients with growth hormone disorder [30]. Therefore, it cannot be excluded that insulin interacts with growth hormone axis to regulate ghrelin level.

Glucagon

Glucagon may contribute to the pre-prandial surge of ghrelin as evidenced by the following observations.

First, glucagon receptor is present in endocrine cells in gastric mucosa [47]. Second, glucagon concentration increases during fasting. Third, plasma acyl ghrelin concentration rises transiently while des-acyl ghrelin increases persistently after administration of glucagon in rats [47]. In addition, ghrelin released from the rat stomach is augmented by glucagon perfusion [38].

Glucagon may directly stimulate the gene transcription of ghrelin. The molecular mechanism on how glucagon affects the expression of ghrelin remains to be explored. One study reports that glucagon significantly elevates the activity of ghrelin gene promoter *in vitro* [8] by the mediation of the second messenger cAMP.

However, it has also been reported that glucagon suppresses ghrelin secretion [48] by the mediation of hypothalamus pituitary axis [49], because glucagon may increase growth hormone and glucocorticoids which then inhibit ghrelin secretion.

Growth hormone/insulin-like growth factor-1 (IGF-1) axis

Growth hormone therapy in growth hormone deficient patients significantly decreases the serum acyl ghrelin concentration [50]. Administration of growth hormone in cultured rat gastric tissue time dependently inhibits total ghrelin secretion [51]. Administration of growth hormone in rats significantly decreases the gastric mRNA content and plasma ghrelin level, with no changes in gastric ghrelin level which may due to the reduction in ghrelin releasing [52]. The above information supports the notion that growth hormone exerts a negative feedback action on ghrelin production and secretion.

The concept that IGF-1 may promote ghrelin secretion is supported by the following studies. Administration of recombinant human IGF-1 in severely under-nutritioned patients elevates plasma total ghrelin concentration [53]. The IGF-1/IGFBP-3 complex significantly increases ghrelin level in children with low birth weight [54]. Since IGF-1 functions to inhibit growth hormone secretion, IGF-1 may induce ghrelin secretion either directly or indirectly through the reduction of growth hormone.

Somatostatin

Somatostatin probably inhibits ghrelin synthesis directly, as shown by the observation that plasma acyl and total ghrelin levels fall after the infusion of somatostatin or somatostatin analog octreotide [55] and the presence of somatostatin receptor in rat stomach [56]. Somatostatin knockout mice display an increase in stomach ghrelin mRNA and serum total ghrelin, but appear no alteration in hypothalamic and pituitary ghrelin mRNA and serum acyl ghrelin concentration [57]. Since ghrelin increases the level of somatostatin in plasma [58], the inhibitory effect of somatostatin on ghrelin may be considered as a negative feedback modulation.

Leptin

Although some studies demonstrate that leptin positively correlates with serum acyl ghrelin in normal weight woman [59] and up-regulates ghrelin mRNA in mice stomach [29], it is generally agreed that leptin inhibits ghrelin synthesis. Leptin is mainly synthesized and secreted by adipose tissue [60]. Leptin concentration in obese is significantly higher than normal, whereas ghrelin is lower [61]. Leptin correlates with ghrelin in a complex pattern, which depends on the body weight (normal or obesity) and insulin sensitivity or insulin concentration [59,62]. As shown by recent studies, ghrelin mRNA increases in stomach during fasting whereas leptin and leptin mRNA decrease [63]. Leptin dose-dependently inhibits ghrelin transcription *in vitro* [63] and decreases ghrelin release from isolated rat stomach [38]. Central leptin gene therapy decreases plasma leptin level and increases ghrelin level significantly in the mouse fed with high-fat diet [64], indicating that leptin exerts its inhibition on ghrelin secretion only in peripheral tissues. Thus, peripheral, especially gastric leptin, probably represses ghrelin expression through its receptor in gastric mucosa cells.

Estrogen

Many studies report that estrogen up-regulates ghrelin level. Administration of estrogen elevates plasma total ghrelin concentration in female patients with anorexia nervosa [53]. Ghrelin mRNA level rises significantly after estrogen administration in cultured stomach cells [65]. Estrogen has been well documented to regulate food intake by modulating meal size and to stimulate growth hormone secretion. These effects may be partially mediated through ghrelin.

However, there also exist discrepant results on the effect of estrogen on ghrelin. Estrogen replacement therapy in post-menopausal women induces serum total [66] and acyl [67] ghrelin secretion only to an insignificant extent, or even decreases [68] serum total ghrelin level. Plasma acyl ghrelin concentration, ghrelin expressing cells and ghrelin mRNA level in stomach, increases transiently after ovariectomy in the female rats [69]. These contradictions may be attributed to the variation in methods used for estrogen administration such as per oral or transdermal

administration [67,68], duration of estrogen administration, age [69] and physiological status (such as obesity [68] vs. normal weight, post- or pre-menopausal of experimental subjects), the outcome index measured (total ghrelin or acyl ghrelin), and other experimental methods.

Autonomic Nervous System Regulating Ghrelin Expression and Secretion

Autonomic nervous system, especially the parasympathetic nerve, plays an important role in the regulation of ghrelin. Excitation of the vagus nerve can stimulate ghrelin secretion. In rats and humans, ghrelin level rises after administration of muscarinic agonists and falls after administration of muscarinic antagonists [70,71]. Because ghrelin-producing cells are governed by enteric nervous system in stomach mucosa, this stimulation probably is a direct effect. And this nervous regulation likely contributes to the pre-prandial reflexive surge of ghrelin, as shown by the report that vagotomy or blockade of vagus nerve by atropine attenuates the increment of ghrelin induced by fasting [72]. On the other hand, vagotomy blocks the stimulatory effect of ghrelin on food intake. Thus, the efferent fiber of vagus nerve regulates the synthesis of ghrelin, whereas its afferent fiber is critical for ghrelin to carry out its function.

In addition, plasma acyl ghrelin concentration is induced by α-adrenergic antagonist and β-adrenergic agonist, indicating that sympathetic nervous system is also involved in the regulation of ghrelin [73]. It is reported that vagotomy inhibits the secretion of gastric ghrelin acutely, but activates its secretion in long term, suggesting that ghrelin secretion is modulated by the balance between cholinergic and adrenergic tones that control the enteric nervous system [73].

Physiological Status Influencing the Level of Ghrelin

Fasting and feeding

Food intake is the most important factor that influences ghrelin level. Circulating ghrelin concentration rises before meal and falls after meal. Total ghrelin level increases in night and decreases after breakfast in humans [74]. Serum ghrelin increases steadily during long term of fasting in humans [75] and rats [28,29] and returns to normal after re-feeding [76]. But, a new report indicates that only acyl ghrelin but not total ghrelin changes after fasting [77], suggesting that fasting stimulates acylation of ghrelin. In addition, the content of total ghrelin in gastric fundus is decreased when fasting and returns to normal when re-feeding [29], showing that fasting has more profound stimulation on the secretion of ghrelin than on the biosynthesis.

The post-prandial decrease of ghrelin can be attributed mainly to the increase of the serum glucose concentration. Total ghrelin, acyl ghrelin, and des-acyl ghrelin all decrease significantly after a high-carbohydrate meal [78], in accordance with the response of ghrelin after glucose ingestion. Other nutrients probably contribute as well. High-fat meal induces minor [79] but more persistent [80,81] post-prandial suppression in circulating total ghrelin than high-carbohydrate isoenergetic meal in humans. Long-term high-fat diet reduces the plasma total ghrelin level and stomach ghrelin content in mouse [82]. In contrast, it is also reported that serum ghrelin remains the same [83] or increases [84] after a high-fat meal. Protein is generally believed to be more satiety than glucose, which is consistent with a more sustainable suppression on ghrelin by protein [85]. As reported, high-protein meal decreases serum acyl [81] and total ghrelin [74,80,86] in humans in both lean and obese subjects [87]. But, contradicting results have also been reported. A protein-rich meal increases [84] or has no effect on ghrelin level [35,42,88]. The variance in meal composition may account for the discrepancies to large extent.

The pre-prandial surge of ghrelin may be induced largely by the expectation of food [89]. The signal is discharged from the central nervous system and transmitted to stomach through the efferent fiber of vagus nerve. But this cephalic control probably is not involved in the post-prandial regulation [80].

Body weight

Many reports show that ghrelin level is negatively correlated with body mass index in humans in physiological and many pathological statuses [42,43,67]. Plasma ghrelin concentration is low and post-prandial decrease in ghrelin is attenuated in the obese population [62,74]. Patients with anorexia nervosa have significant elevated serum total and acyl ghrelin level [90], which returns to normal when the disease is cured and the body weight restored [91]. Furthermore, total ghrelin level is inversely associated with fat cell volume [43] and specifically in women with total fat mass and fat mass/lean mass ratio, whereas in men it is associated with abdominal fat mass and fat distribution index [92].

Age

In mouse, the level of acyl ghrelin steadily increases in suckling stage (the first 3 weeks after birth). After initiation of weaning, however, acyl ghrelin falls sharply, though the total ghrelin level remains generally unchanged [23]. This observation suggests that certain lipid composition in breast milk may notably stimulate ghrelin synthesis and acylation. Similar change of ghrelin expression during development has been reported by another study [93]. This study demonstrates that the ghrelin mRNA level declines, but the protein concentration remains unchanged as adult mice are aging. In humans, total ghrelin is inversely related to age in children [94]. Fasting acyl ghrelin [95] and total ghrelin [43,96] are significantly lower in the aged population than in the youth. Besides, ghrelin mRNA level also decreases as aging in the human adrenal cortex [97]. However, this age-dependent decline of ghrelin is not observed in the obese population [98]. Despite the elevated basal ghrelin level, the malnutrition-induced increase of plasma ghrelin levels may be lacking in elderly human [99]. Additionally, the orexigenic effect of peripheral ghrelin may also be influenced by age, as shown by experiments in rats [100,101].

Gender

Many studies report an elevated serum ghrelin level in female subjects relative to male ones [42,92,96]. In humans, total ghrelin level is about 3-fold higher [102] in women during the late follicular stage of the cycle than in men. Similarly, ghrelin level is higher in female mice than in male, especially in aged ones [93].

Pathological Status that Influences the Level of Ghrelin

Ghrelin level alters in several disease states. In Prader–Willi syndrome, ghrelin level is elevated, despite the increased body weight [103]. Therefore, the excessive ghrelin secretion may be the cause of hyperphagia and obesity in these patients.

In the case of illness-induced cachexia [104] and anorexia nervosa, ghrelin is increased. This increase in ghrelin level may occur either as an adaptive response to correct the abnormal energy status or as a result of relative resistance to ghrelin. Ghrelin level is decreased in patients with metabolic syndrome [105] and patients with polycystic ovarian syndrome [106], in accordance with the negative correlation between ghrelin and body weight. In the cases of diabetes mellitus type 1 and type 2, the level of ghrelin is generally decreased and the response of ghrelin after meal consumption is attenuated or remains similar with normal people. For details, please refer to the review by Pusztai *et al.* [107].

Inflammatory diseases such as ulcerative colitis and Crohn's disease potently increase ghrelin level [108]. This increase in ghrelin is probably a protective response because ghrelin has a potent anti-inflammatory effect. In addition, ghrelin is reduced in *Helicobacter pylori* infection [109] and other diseases associated with gastric atrophy or removal [110].

Summary

As summarized in **Table 1**, ghrelin level is controlled by neuroendocrine system, increased at the time of negative energy balance and decreased at the time of positive energy balance. Therefore, ghrelin is probably an important member of the survival kit of nature [5] and may function as a signal communicating the nutrition states of the body to the central nervous system and help the body adjusting to its energy status, likely through stimulation of food intake.

It has been discovered that ghrelin has a vast range of physiological functions, thus the abnormality in its

Table 1 Alterations of serum ghrelin level under different conditions

Group	Elevated ghrelin level	Depressed ghrelin level
Nutrients	Fatty acids[a], amino acids[a]	Glucose, fatty acids[a]
Hormones	Glucagon[a], IGF-1, estrogen[a]	Insulin, growth hormone, somatostatin, leptin[a], estrogen[a]
Autonomic nervous system	Vagus nerve activation	Sympathetic nerve activation
Physiological status	Fasting, lean, youth	Feeding[a], obesity, aging[a]
Pathological status	Prader–Willi syndrome, anorexia nervosa, cachexia	Metabolic syndrome, diabetes mellitus

[a]Information incomplete or controversial.

secretion possibly leads to hyper or hypophagia, obesity and other metabolic syndrome, growth retardation, cardiovascular and/or reproduction system disorder, and many other pathological changes. However, the current understanding about the regulation of ghrelin level, especially its mechanism, is far from satisfaction, with much discrepancy among studies. Unanimous conclusion on some critical topics is still lacking, reflecting the remarkable complexity in the regulation system, again indicating the important biological role of ghrelin. In addition, many previous reports identify only correlation between ghrelin and a certain agent, but cannot distinguish whether the change in ghrelin level is the cause or effect, or they are actually independent. Thus, future works need to discover agents that have more direct and significant effect on ghrelin secretion, confirm the causality, and elucidate the underlying mechanism of its regulation.

It is also worth noting that some of the current reports do not distinguish between total ghrelin and acyl ghrelin, partially because the limitation of the detecting methods they used and partially because the ratio between the two has been reported as constant. Since des-acyl ghrelin, the major form of ghrelin in circulation, has now been recognized as being able to exercise physiological roles distinct from acyl ghrelin, further study to examine how the ghrelin and des-acyl ghrelin are differentially influenced and to explore the change in GOAT activity will unravel the mystic of ghrelin regulation.

Funding

This work was supported by grants from the National Natural Science Foundation of China (30890043, 30740096, 30871194 and 30700376), and the '985' Program at Peking University (985-2-097-121).

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