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# Purification and characterization of feline ghrelin and its possible role

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**Abstract**

Ghrelin, a novel 28-amino acid peptide with an *n*-octanoyl modification at $Ser^3$, has been isolated from rat and human stomach as an endogenous ligand for the growth hormone secretagogue receptor. Here, we purified feline ghrelin and examined its possible physiological role in cats. The major active form of feline ghrelin is a 28-amino acid peptide octanoylated (C8:0) at $Ser^3$; except for one amino acid residue replacement, this structure is identical to those of rat and human ghrelins. However, much structural divergence in peptide length and fatty acid modification was observed in feline ghrelin: peptides consisting of 27 or 26 amino acids lacking $Gln^{14}$ and/or $Arg^{28}$ were found, and the third serine residue was modified by octanoic acid (C8:0), decanoic acid (10:0), or unsaturated fatty acids (C8:1, C10:1 and C10:2). In agreement with the structural divergence, two kinds of cDNA with different lengths were isolated. Administration of synthetic rat ghrelin increased plasma growth hormone levels in cats, with a potency similar to that in rat or human. Plasma levels of ghrelin in cats increased approximately 2.5-fold after fasting. The present study indicates the existence of structural

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divergence in feline ghrelin and suggests that, as in other animals, ghrelin may play important roles in GH release and feeding in cats.


*Keywords:* Ghrelin; Cat; Growth hormone; Feeding behavior

## 1. Introduction

Ghrelin, a novel 28-amino acid peptide, was originally isolated from rat stomach as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) [1]. A unique feature of the structure of ghrelin is modification of the $Ser^3$ residue by *n*-octanoic acid. This octanoyl modification is essential for receptor binding and subsequent expression of biological activity, such as growth hormone (GH) release. Structural divergence has been observed in rat and human ghrelins. For example, ghrelins lacking $Gln^{14}$ or $Arg^{28}$ have been isolated from rat and human, respectively [2,3]. In addition to octanoylated (C8:0) ghrelin, decanoylated (C10:0) and decanoylated (C10:1) ghrelins were also found in human stomach [3]. These structural divergences of peptide length and fatty acid modification have also been reported in non-mammalian (rainbow trout, chicken and bullfrog) ghrelins [4–6]. Although GH release induced by the cognate ghrelin has been confirmed in various animals, potency differs according to peptide length and fatty acid modification [2–6].

In addition to stimulation of GH secretion in vivo and in vitro, ghrelin has been reported to stimulate food intake, body weight gain and adiposity when administered peripherally or centrally to rodents, and these activities are independent of GH secretion [7,8]. The effect of peripheral ghrelin on appetite is mediated via the gastric afferent vagal nerve [9]. On the other hand, its central effect is thought to occur via neuropeptide Y and agouti-related peptide secretion from the arcuate nucleus in the hypothalamus [8]. These results suggest that ghrelin plays important roles in the regulation of food intake and energy expenditure.

Obesity and anorexia have become serious problems in humans. Administration of ghrelin increases food intake in cancer patients with anorexia [10]. Human gastrectomy reduces plasma ghrelin levels by one-half, after which levels gradually increase as a result of compensation by other tissues [3]. Increasing ghrelin levels might restore food intake in patients, and research on the clinical application of ghrelin for anorexia is now in progress. In the veterinary field, obesity and anorexia have also become serious problems in companion animals, especially dogs and cats [11–13]. Cats have long been used as an important model to study the regulation of feeding, since lesions in the ventromedial hypothalamus produce rapid hyperphagia and abnormal body weight gain that persist for a long time [14]. In addition, diabetes in cats closely resembles type 2 diabetes in humans [15]. Recently, it has been reported that plasma levels of ghrelin change in rats with hyperphagia induced by streptozotocin-induced diabetes [16], and that a ghrelin $Arg^{51}Gln$ mutation is a risk factor for type 2 diabetes and hypertension in middle-aged humans [17]. Therefore, it appears important to determine the structure of feline ghrelin and the physiological role of ghrelin in cats. In the present study, we purified feline ghrelin from the stomach. During the course of purification, we found several minor peptides with ghrelin-like activity but with charac-