成長期ビーグル犬のグレリンと成長ホルモン分泌様態

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Relationship Between Growth and Plasma Concentrations of Ghrelin and Growth Hormone in Juvenile Beagle Dogs

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ABSTRACT. Although the release of growth hormone (GH) is known to be regulated mainly by GH-releasing hormone (GHRH) and somatostatin (SRIF) secreted from the hypothalamus, ghrelin also may be involved in GH release during juvenile period. We have examined plasma concentrations of acylated ghrelin, desacyl ghrelin, and GH in juvenile beagle dogs. Plasma acylated and desacyl ghrelin levels changed through aging; however, there was no closely correlation between ghrelin, body weight and circulating GH levels during juvenile period. The increase in body weight was essentially linear until 8 months of age, whereas plasma GH concentrations exhibited bimodal peaks for the meanwhile. The results suggest that ghrelin may not play internal cueing in GH secretion in juvenile beagle dogs.
KEY WORDS: canine, ghrelin, growth hormone.

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As in many other species, prepubertal growth and development play an important role in determining the onset of puberty or sexual maturity in dogs. Equally striking differences are observed in head shape, body proportions, hair coat, and behavior. Several hormones are involved in promoting growth and skeletal muscle development, and of these, growth hormone (GH), insulin, and thyroid hormone appear to be of major importance. Many earlier studies indicate that the secretion of GH during growth is attributable to alterations in hypothalamic activity. GH secretion is regulated primarily as a result of the interplay between hypothalamic GH-releasing hormone (GHRH) and somatostatin (SRIF) as well as input from other factors including nutritional intake and neural transmitters [11]. In particular, GH secretagogues (GHS) are a group of synthetic compounds that induce GH secretion through the activation of the GHS receptor (GHS-R). Ghrelin, a recently discovered peptide hormone that is secreted mainly by the stomach, has been identified as the endogenous ligand of the GHS-R and has a potent GH-releasing effect [8]. The discovery of ghrelin introduces another regulatory input into the hypothalamic GHRH/SRIF-pituitary GH axis. Since ghrelin has only recently been discovered, the information available on its intrinsic role during prepubertal growth and development is limited. We and other group have recently found that exogenous ghrelin injection in beagle dogs stimulates prompt GH release [2, 16] and ghrelin-immunoreactive cells localize to the fundus and body of the stomach [12, 16], but a physiological role of ghrelin in energy homeostasis during the growth phase has not yet been established. Thus, the present study was conducted to determine the juvenile growth patterns in beagle dogs, to measure changes in the plasma concentrations of GH, acylated ghrelin, and desacyl ghrelin, and to establish whether there is any correlation between these hormone levels and body weight (BW) increments in the prepubertal stages of growth in these animals.

Healthy male and female beagle dogs were used for this study. All the dogs were kept in similar conditions throughout the study and were separately fed once a day a maintenance commercial canine laboratory diet (DS-A; Oriental Yeast, Chiba, Japan) that was formulated to contain 6.0% moisture, 24.7% crude protein, 8.2% crude fat, 7.0% crude ash, 3.9% crude fiber, 50.2% nitrogen-free extract, and 15.6 kJ/g metabolizable energy. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care. Whole blood samples were collected between 0900 and 1100 hr by jugular venipuncture after fasting overnight, transferred to ice-chilled tubes containing disodium ethylenediamine tetraacetic acid and 500 U aprotinin, and centrifuged at 4°C and 2,000 × g. Immediately after plasma collection, 100 μl of 1 N HCl was added per milliliter of plasma sample for use in an enzyme-linked immunosorbent assay (ELISA) for acylated and desacyl ghrelin. Plasma was then stored at –80°C until hormone analyses were performed. BW was recorded on the blood sampling days. Plasma acylated and desacyl ghrelin concentrations were determined using ELISA kits (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan) according to manufacturer's specifications. The minimum sensitivities of these kits were 2.5 fmol/m*l* and 12.5 fmol/m*l*, respectively, and the intra- and interassay coefficients of variation were both <10%. Since the kits were designed for use with rat, mouse, and human, we first verified that dog plasma contains suitable matrices. Plasma GH concentration was measured with the aid of commercial porcine/canine radioimmunoassay kits (Linco Research Inc, St Charles, MO, U.S.A.). The limit of sensitivity for the GH assay was 1 *n*g/m*l*, and the

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intra- and interassay coefficients of variation were both < 7%.

In the male dogs, the mean (± SEM) BW increased from 3.5 ± 0.3 kg at 2 months to 12.4 ± 0.5 kg at 12 months. In the female dogs, the mean BW increased from 3.2 ± 0.2 kg at 2 months to 11.4 ± 0.4 kg at 12 months (Fig. 1-A). All of the animals exhibited a healthy BW gain throughout the experimental period until reaching a mature BW by approximately 12 months of age, showing a sigmoid growth curve. Although these animals do not grow at the same rate between birth and puberty, the increase in BW with time was essentially linear between 2 and 8 months of age.

The mean plasma acylated and desacyl ghrelin concentrations of these growing dogs are shown in Fig. 1-B. In the male dogs, mean plasma acylated ghrelin concentrations decreased gradually from a start point of approximately 60.6 ± 8.3 fmol/m*l* at the beginning of the study until 8 months of age. A few episodic releases of acylated ghrelin were detected between 6 months and 12 months of age. In the female dogs, mean acylated ghrelin concentrations reached a high of 84.3 ± 18.0 fmol/m*l* at between 4 and 5 months of age. Thereafter, acylated ghrelin levels decreased and remained at low a level, exhibiting only a slight increase, until the age of 10 months. There was a subsequent increase to 74.9 ± 14.2 fmol/m*l* at 11 months of age. The peak and nadir of desacyl ghrelin concentration was related to the corresponding plasma acylated ghrelin concentrations during the experimental period for both genders.

Plasma GH concentrations were also determined from the blood sample collected at prepubertal stages of growth (Fig. 1-C). In the male dogs, GH levels were highest at 2 months of age. Thereafter, they steadily declined until 5 months of age and then increased from 6 to 7 months of age. Following this increase, the mean GH level decreased, remaining at a low level until 12 months of age. In the female dogs, the GH concentrations at 2 months of age was 9.9 ± 0.9 *n*g/m*l*; the mean level declined thereafter and, other than a transient peak at 8 months of age, the concentration remained at approximately 2.0 *n*g/m*l* until the end of the study. The increase in BW with time was essentially linear between 2 and 8 months of age; plasma GH concentrations, however, exhibited a biphasic pattern during this period.

The temporal changes in the increase in BW and the secretion profiles of the three hormones were similar between the male and female dogs. It seems, therefore, that in our study gender had no significant effect on either parameter.

A significant correlation between the plasma acylated ghrelin and desacyl ghrelin levels was observed (Fig. 2). A negative linear correlation was found between BW and plasma acylated ghrelin levels and plasma GH levels of the beagle dogs (Figs. 3-A and 3-B), but regression lines of the relationship these parameters showed the low coefficient of correlation. There was no correlation between BW and plasma desacyl ghrelin levels, and GH and plasma acylated ghrelin levels (Figs. 3-C and 3-D).

The beagle dogs used in our study exhibited an essen-

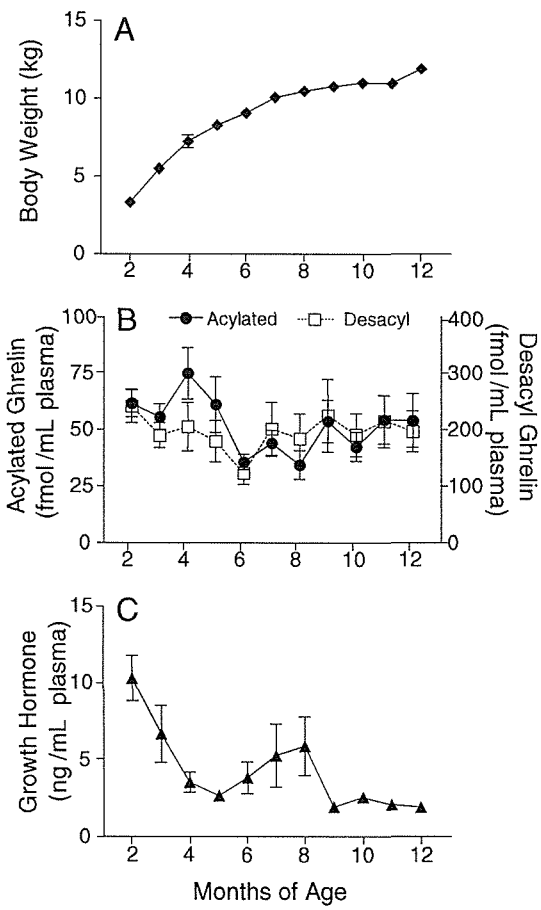

Fig. 1. Changes in body weight (A), plasma acylated ghrelin and desacyl ghrelin levels (B), and plasma growth hormone (GH) levels (C) with age in beagle dogs. The symbols and vertical lines represent the mean and ± SEM of five males and five females, respectively (n=10).

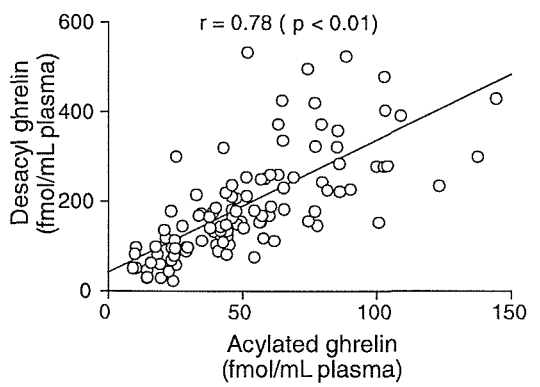

Fig. 2. Correlation between plasma acylated ghrelin and desacyl ghrelin levels in beagle dogs (r=0.78, p<0.01; Spearman).

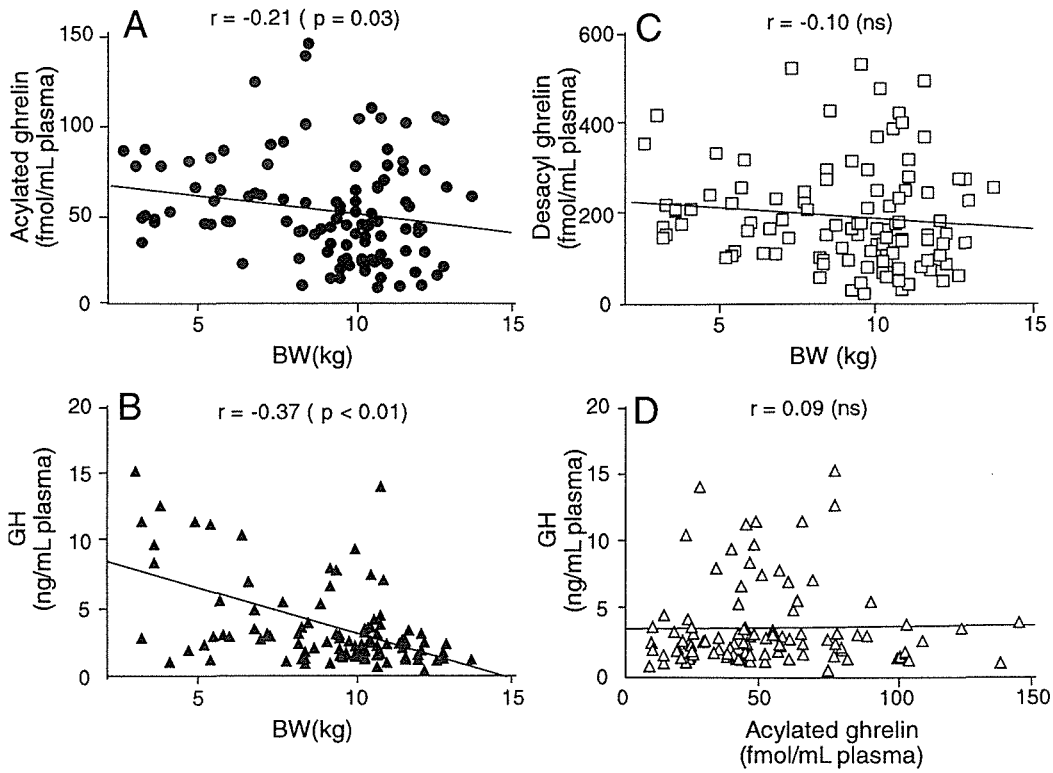

Fig. 3. A, B and C: Relationships of BW with plasma acylated ghrelin, desacyl ghrelin, and GH levels in beagle dogs. D: Relationship between plasma acylated ghrelin and plasma GH levels in beagle dogs.

tially similar prepubertal growth pattern to that reported for adult medium-sized breeds of dog, when comparing absolute BW with beagles of the same age [6]. This is the first study to examine whether there is a relationship between acylated and desacyl ghrelin levels and BW increments during the prepubertal stages of growth in beagle dogs. There was significant relationship between the mean increase in BW of the animals used in the present study and the corresponding mean plasma acylated ghrelin and GH concentrations, throughout the experimental period. However there was only a low correction between BW and plasma acylated ghrelin levels and plasma GH levels.

It is known that exogenous administration of ghrelin stimulates GH release and appetite in beagle dogs [16]. Previous investigations have suggested that ghrelin plays an important role in the regulation of metabolic balance. In the present study, the concentrations of acylated and desacyl ghrelin fluctuated between intermediate and high levels without any clear age-associated trend. The physiological significance of ghrelin in GH secretion and/or the prepubertal growth of juvenile dogs therefore remain unclear. Although further *in vivo* studies are required to establish the details of any correlation with various aspects of growth, it may be that ghrelin levels are also regulated by physiology of anabolism, feeding behavior, and nutritional homeostasis for GH secretion. In particular, the effects of ghrelin on the somatotroph remain to be classified.

One recent study [10] in which a GHS-R antagonist was used, revealed that circulating ghrelin in the peripheral blood may not play a role in generating pulsatile GH secretion. Moreover, deletion of ghrelin impairs neither growth nor appetite, indicating that ghrelin is not essential for GH secretion [14]. Another study, however, demonstrated that the *in vivo* attenuation of GHS-R expression results in a reduction in food intake and growth, suggesting a physiological role of the ghrelin-GHS-R system in the secretory regulation of GH [13]. Our observations in juvenile dogs may not support the concept that plasma ghrelin plays a crucial role as an endocrine mediator of GH secretion.

Desacyl ghrelin, whose plasma concentrations in rats is at least 2.5-fold higher than that of acylated ghrelin [7], neither activates GHS-R nor exhibits endocrine activity [7–9]. In contrast, transgenic mice over expressing desacyl ghrelin are phenotypically smaller than the norm [1]. This observation may indicate a role of desacyl ghrelin in the regulation of GH secretion. It was observed in the present study that plasma desacyl ghrelin levels were greater by about four-fold than that of acylated ghrelin, based on evaluations of individual animals. The significance of any physiological role of desacyl ghrelin is not clear at this time. It is possible, however, that circulating desacyl ghrelin in dogs is regulated in the same manner as in humans and rodents.

A limited number of studies have reported the basal level of GH in dogs. Eigenmann and Eigenmann [5] reported a

mean ± SEM GH level of 1.92 ±0.14 $\mu g/l$ for a group of 63 healthy adult dogs. A more recent report indicates that differences in final body size between medium-sized (beagles) and giant (Great Danes) dog breeds are associated with differences in GH release at a young age [6]. During the entire observation period of that study, the basal plasma GH levels of the beagles remained at a stable level [6]. In contrast, in the juvenile beagle dogs employed in the present study, the high GH levels observed exhibited a bimodal distribution, the peak being observed at 2 and 8 months of age. The position of the first peak is at a level similar to that observed until 7 weeks of age by Favier *et al.* [6]. Because the experimental period of Favier *et al.* lasted from 6 until 24 weeks of age (c.a. 6 months), the second peak after the 24 weeks might have observed only in the current study. The neonatal period in humans is also characterized by relatively high GH concentrations [4]. Neonatal hypersomatotropism in human beings is characterized by pulsatile GH secretion with a high pulse amplitude and a high pulse frequency [3, 15]. It is speculated that in our results the first excessive secretion of GH is also associated same manner with the immediate postnatal rise of GH secretion in the human newborn. The second peak observed at 8 months in the present study may be associated with the timing of changes in nutritional status or sexual maturity. In our observation, the plasma acylated and desacyl ghrelin levels were unaffected by the first, but not the second distribution of GH, therefore the reasons behind this rise remain enigmatic.

The results of this study demonstrate that the observed increase in BW is significantly correlated to the corresponding plasma acylated ghrelin and GH concentrations, but not desacyl ghrelin during the period of prepubertal growth in beagle dogs. However BW is not closely correlated with plasma acylated ghrelin and GH levels. Although further *in vitro* and *in vivo* studies are required to establish the regulation of GHRH and SRIF secreted from the hypothalamus, the alterations of the GH response pattern and of acylated and desacyl ghrelin reported in the present study are valuable in the comparison of the relative contributions of the two hormones in growing beagle dogs.

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REFERENCES

1. Ariyasu, H., Takaya, K., Iwakura, H., Hosoda, H., Akamizu, T., Arai, Y., Kangawa, K. and Nakao, K. 2005. *Endocrinology* **146**: 355–364.
2. Bhatti, S. F. M., De Vliegher, S. P., Van Ham, L. and Kooistra, H. S. 2002. *Mol. Cell Endocrinol.* **197**: 97–103.
3. Deiber, M., Chatelain, P., Naville, D., Putet, G. and Salle, B. 1989. *J. Clin. Endocrinol. Metab.* **68**: 232–234.
4. De Zegher, F., DeVlieger, H. and Veldhuis, J. D. 1993. *J. Clin. Endocrinol. Metab.* **76**: 1177–1181.
5. Eigenmann, J. E. and Eigenmann, R. Y. 1981. *Acta Endocrinol.* **98**: 514–520.
6. Favier, R. P., Mol, J. A., Kooistra, H. S. and Rijnberk, A. 2001. *J. Endocrinol.* **170**: 479–484.
7. Hosoda, H., Kojima, M., Matsuo, H. and Kangawa, K. 2000. *Biochem. Biophys. Res. Commun.* **279**: 909–913.
8. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K. 1999. *Nature (Lond.)* **402**: 656–660.
9. Matsumoto, M., Hosoda, H., Kitajima, Y., Morozumi, N., Minamitake, Y. and Tanaka, S. 2001. *Biochem. Biophys. Res. Commun.* **287**: 142–146.
10. Okimura, Y., Ukai, K., Hosoda, H., Murata, M., Iguchi, G., Iida, K., Kaji, H., Kojima, M., Kangawa, K. and Chihara, K. 2003. *Life Sci.* **72**: 2517–2524.
11. Plotsky, P. M. and Vale, W. 1985. *Science* **230**: 461–463.
12. Rindi, G., Necchi, V., Savio, A., Torsello, A., Zoli, M., Locatelli, V., Raimondo, F., Cocchi, D. and Solcia, E. 2002. *Histochem. Cell Biol.* **117**: 511–519.
13. Shuto, Y., Shibasaki, T., Otagiri, A., Kuriyama, H., Ohata, H., Tamura, H., Kamegai, J., Sugihara, H., Oikawa, S. and Wakabayashi, I. 2002. *J. Clin. Invest.* **109**: 1429–1436.
14. Sun, Y., Ahmed, S. and Smith, R. G. 2003. *Mol. Cell Biol.* **23**: 7973–7981.
15. Wright, N. M., Northington, F. J., Miller, J. D., Veldhuis, J. D. and Rogal, A. D. 1992. *Pediatr. Res.* **32**: 286–290.
16. Yokoyama, M., Nakahara, K., Kojima, M., Hosoda, H., Kangawa, K. and Murakami, N. 2005. *Eur. J. Endocrinol.* **152**: 155–160.