

## The Influence of Feeding Pattern on Changes in Plasma Ghrelin in the Holstein Cow

Hiroshi MIURA<sup>1)\*</sup>, Nachi HOJO<sup>1)</sup>, Ryoji TAKAHASHI<sup>1)</sup>, Motohiro KIKUCHI<sup>1)</sup>, Masayasu KOJIMA<sup>2)</sup>, Kenji KANGAWA<sup>3)</sup>, Yoshihisa HASEGAWA<sup>1)</sup> and Minoru SAKAGUCHI<sup>1)</sup>

<sup>1)</sup>School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada-shi, Aomori 034-8628, Japan

<sup>2)</sup>Kurume University, Fukuoka 830-0011, Japan

<sup>3)</sup>National Cardiovascular Center Research Institute, Osaka 565-8565, Japan

(Received 13 February 2014/Accepted 4 April 2014/Published online in J-STAGE 12 May 2014)

**ABSTRACT.** We measured the plasma ghrelin and cortisol concentrations in non-lactating cows under fixed-time feeding conditions followed by an acute or gradual fasting treatment. During the 4 days before fasting, animals in Group 1 were fed a fixed amount of rations at 0800 and 1600 hr, and those in Group 2 were fed a gradually reduced amount. Thereafter, the plasma ghrelin concentrations of each animal were measured for 40 hr. The plasma ghrelin concentrations, which were low at the onset of fasting, increased before and after 0800 during fasting in Group 1, but not in Group 2. There were no significant differences in the plasma cortisol concentration within or between the groups. It was demonstrated that acute fasting induces elevation of the plasma ghrelin concentration, but that gradual fasting does not. This result suggests that fixed-time and fixed-quantity feeding caused a daily ghrelin rhythm in the cow and that this rhythm influenced changes in plasma ghrelin.

**KEY WORDS:** acute fasting, cortisol, ghrelin, gradual fasting, Holstein cow

doi: 10.1292/jvms.14-0079; *J. Vet. Med. Sci.* 76(8): 1137–1139, 2014

Ghrelin is secreted from the stomach into the systemic circulation and influences growth hormone secretion and feeding behavior, thereby playing an essential role in the maintenance of energy balance in humans and laboratory animals [1, 9]. Generally, this hormone increases before feeding and decreases with feeding [3]. In cattle, the plasma ghrelin concentration has been shown to increase during a negative energy balance and decrease during a positive energy balance [2].

We previously investigated the changes in plasma ghrelin concentrations in cows fed at scheduled times [6]. In this study, cows were fed at 0800 and 1600 hr, and the plasma ghrelin concentrations gradually increased for 8–10 hr before feeding, decreased at feeding time and then formed a daily ghrelin rhythm. This result agreed with previous studies of sheep fed at scheduled times [7, 8].

In the present study, we investigated the effect of acute or gradual fasting on plasma ghrelin. We also measured the plasma cortisol concentration to evaluate the stress reaction to restricted feeding. Eight non-lactating Holstein cows (age, 5–12 years) housed in a free stall barn of Kitasato University were given free access to food (dried Italian ryegrass hay) and water for 3 months before the experiment. All animal use conformed to the guidelines governing animal experimentation published by Kitasato University.

The first group of animals (Group 1, n=4, body weight 731.3 (102.8) kg) was fed 6 kg (dry matter) of long-stemmed Italian ryegrass hay and 3 kg (DM) of long-stemmed alfalfa hay (dairy hay) at 0800 hr and 1600 hr on days –3 to day 0. The amount of feed was based on the Japanese Feeding Standard for Dairy Cattle (1999, National Agriculture and Food Research Organization). After the feeding at 1600 hr on day 0, the animals were fasted until 0800 hr on day 2 (for 40 hr). The second group of animals (Group 2, n=4, body weight 725 (61.2) kg) had the same feeding schedule as Group 1 on day –3. Thereafter, the quantity of food was reduced by 25% on day –2, 50% on day –1 and 75% on day 0. Then, the animals were fasted until 0800 hr on day 2 (for 40 hr).

Blood sampling started just after feeding at 1600 hr on day 0. Blood was collected from the jugular vein at 2-hr intervals until 0800 on day 2 (for 40 hr). Samples were immediately placed into heparinized tubes containing aprotinin (10 IU/ml of blood; Trasylol; Bayer Yakuin Ltd., Osaka, Japan) and centrifuged at 1,600 g for 10 min at 4° C. Plasma was stored at –80°C.

Ghrelin was measured by time-resolved fluoroimmunoassay at the High-tech Research Center of Kitasato University. The antibody for ghrelin and ghrelin standard were gifted by M. Kojima and K. Kangawa [4, 5]. The methods for measuring the concentration of this hormone are well established, and they were used in our previous research [6]. Each assay was performed in triplicate. The intra- and inter-assay CVs were 12% and 14%, respectively.

Cortisol concentrations were measured by RIA in a clinical laboratory test at SRL Inc. (Tokyo, Japan). The reliability of this system is high, and it is regularly used for diagnostic purposes at the Kitasato University Veterinary Teaching Hospital. The intra-assay CV was under 10%.

Significant differences in hormone concentrations

\*CORRESPONDENCE TO: MIURA, H., School of Veterinary Medicine and Animal Sciences, Kitasato University, 23–35–1 Higashi, Towada-shi, Aomori 034–8628, Japan.  
e-mail: miurah@vmas.kitasato-u.ac.jp

©2014 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.

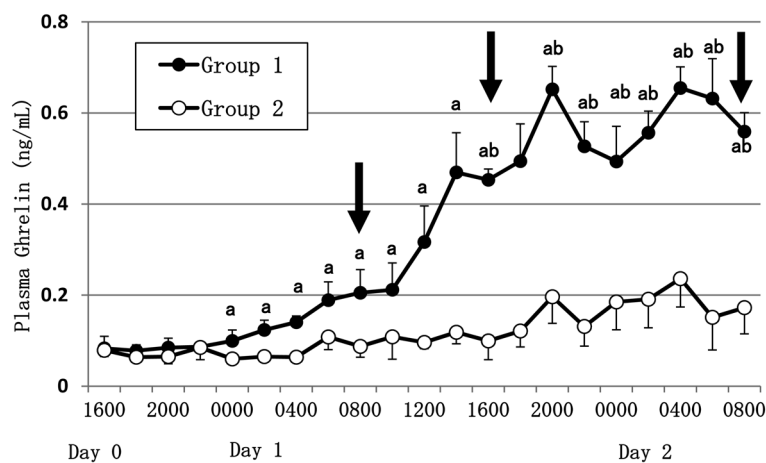


Fig. 1. Changes in plasma ghrelin concentration (mean  $\pm$  SD). A significant effect between groups and significant change in Group 2 were detected ( $P < 0.001$ ). The letter "a" indicates a significant difference between Group 1 and Group 2 ( $P < 0.01$ ). The letter "b" indicates a significant increase in ghrelin in Group 1 starting from 1600 on day 0 ( $P < 0.01$ ).

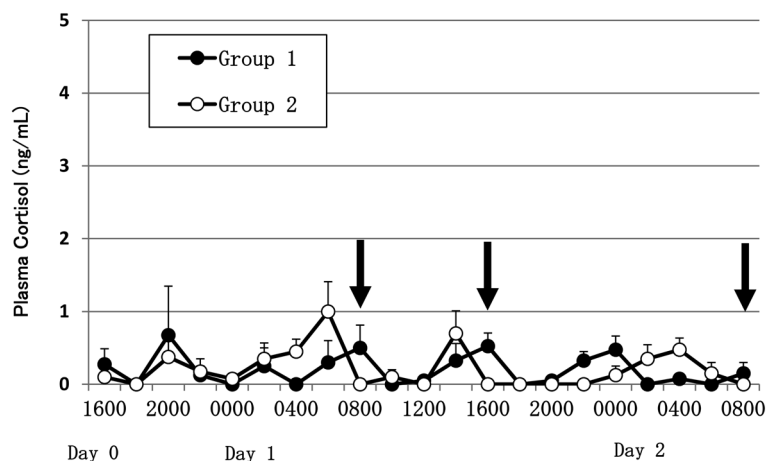


Fig. 2. Changes in plasma cortisol concentration (mean  $\pm$  SD). There was no significant difference between the two groups or within the groups.

between the groups were analyzed by repeated two-way ANOVA and Tukey's test ( $P < 0.01$ ), and significant changes in hormone concentrations were analyzed by Dunnett's test ( $P < 0.01$ ) using Excel Toukei (SSRI, Osaka, Japan).

In Group 1, the plasma ghrelin concentration was about 0.1 ng/ml at the start, increased to 0.2 ng/ml after 6–8 hr and remained at that level until 0800 on day 1. After 0800 on day 1, the plasma ghrelin concentration increased and reached 0.5–0.6 ng/ml. In Group 2, the plasma ghrelin concentration was about 0.1 ng/ml at the start and gradually increased up to 0.2 ng/ml. After 2400 on day 0, the plasma ghrelin concentrations of Group 1 were significantly higher than those of Group 2. No significant increase was observed before or after 0800 on day 1 (Fig. 1).

The Group 1 animals were fed at fixed times until day 0

as in our previous study [6]. Under these feeding conditions, the plasma ghrelin concentration decreased to about 0.1 ng/ml just after feeding time and increased to 0.2 ng/ml at 6–8 hr after feeding. Therefore, if the Group 1 animals had been fed at 0800 on day 1, the plasma ghrelin concentration might have fallen and the daily rhythm might have been maintained. The results suggested that fasting at the feeding time caused the further increase in ghrelin. The ghrelin increase before and after 0800 on day 1 was not observed in Group 2, probably because a daily rhythm was not maintained when the amount of food was gradually reduced.

It is interesting that the starting concentrations of ghrelin were the same in Groups and lower in Group 2 at the end of study, because the group 2 animals had been fed reduced rations and might have been in a more negative energy balance

condition than Group 1. Because the cortisol concentration did not change in either group of animals, it was suggested that these acute and gradual fasting animals were not under severe stresses (Fig. 2). Van der Walt *et al.* [10] reported that the plasma cortisol concentration remained within the normal range for 72 hr of food deprivation in oxen.

Some researchers reported that the plasma ghrelin concentration changed diurnally in sheep fed at fixed times, but not in animals fed *ad libitum* [8]. They asserted that this was probably stimulated by psychological factors. Animals fed at fixed times memorize the feeding time and expect food, and when food is not given at that time, the anxiety over food might increase, and ghrelin secretion might be stimulated. Our results are in agreement with this supposition, with this discussion, though further research is required to consider the psychological factors.

In conclusion, the present study suggested that the plasma ghrelin concentration is influenced by a diurnal rhythm of feeding and that if the rhythm is disturbed, ghrelin secretion may be stimulated.

**ACKNOWLEDGMENT.** This work was supported in part by the “High-Tech Research Center” Project for Private Universities of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

#### REFERENCES

1. Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K. and Nakao, K. 2001. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity concentrations in humans. *J. Clin. Endocrinol. Metab.* **86**: 4753–4758. [[Medline](#)] [[CrossRef](#)]
2. Bradford, B. J. and Allen, M. S. 2008. Negative energy balance increases periprandial ghrelin and growth hormone concentrations in lactating dairy cows. *Domest. Anim. Endocrinol.* **34**: 196–203. [[Medline](#)] [[CrossRef](#)]
3. Cummings, D. E. 2006. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol. Behav.* **89**: 71–84. [[Medline](#)] [[CrossRef](#)]
4. Hayashida, T., Murakami, K., Mogi, K., Nishihara, M., Nakazato, M., Mondal, M. S., Horii, Y., Kojima, M., Kangawa, K. and Murakami, N. 2001. Ghrelin in domestic animals: Distribution in stomach and its possible role. *Domest. Anim. Endocrinol.* **21**: 17–24. [[Medline](#)] [[CrossRef](#)]
5. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402**: 656–660. [[Medline](#)] [[CrossRef](#)]
6. Miura, H., Tsuchiya, N., Sasaki, I., Kikuchi, M., Kojima, M., Kangawa, K., Hasegawa, Y. and Ohnami, Y. 2004. Changes in plasma ghrelin and growth hormone concentrations in mature holstein cows and three-month-old calves. *J. Anim. Sci.* **82**: 1329–1333. [[Medline](#)]
7. Sugino, T., Hasegawa, Y., Kikkawa, Y., Yamaura, J., Yamagishi, M., Kurose, Y., Kojima, M., Kangawa, K. and Terashima, Y. 2002. A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. *Biochem. Biophys. Res. Commun.* **295**: 255–260. [[Medline](#)] [[CrossRef](#)]
8. Sugino, T., Yamaura, J., Yamagishi, M., Ogura, A., Hayashi, R., Kurose, Y., Kojima, M., Kangawa, K., Hasegawa, Y. and Terashima, Y. 2002. A transient surge of ghrelin secretion before feeding is modified by different feeding regimens in sheep. *Biochem. Biophys. Res. Commun.* **298**: 785–788. [[Medline](#)] [[CrossRef](#)]
9. Toshinai, K., Mondal, M. S., Nakazato, M., Date, Y., Murakami, N., Kojima, M., Kangawa, K. and Matsukura, S. 2001. Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem. Biophys. Res. Commun.* **281**: 1220–1225. [[Medline](#)] [[CrossRef](#)]
10. Van der Walt, J. G., Hattlingh, J., Petty, D. B., Grobler, M. J. and Ganhao, M. F. 1993. Effect of a 72 hour fast on physiological stress indicators in feedlot cattle. *J. S. Afr. Vet. Assoc.* **64**: 39–42. [[Medline](#)]