Twenty-four-hour Variation of Plasma Leptin Concentration and Pulsatile Leptin Secretion in Cattle

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ABSTRACT : We conducted this study to investigate 24 h leptin profiles and to ascertain whether leptin secretion occurs in a pulsatile manner in cattle. Plasma leptin concentrations were measured every 10 min for 24 h in five Holstein steers aged 10 months. Simultaneously, feeding behavior was recorded every 5 min during this experiment. In two of the five cattle, leptin showed diurnal rhythmicity, which could be described by a cosine, with peaks between 15:00 and 16:00 and nadirs at around midnight. Pulsatile leptin release was quantified by model-free Cluster analysis. Plasma leptin showed a pulsatile pattern in all cattle, with an average number of pulses at 15 peaks/24 h. The daily number of pulses was not related to total time spent eating, ruminating or chewing. However, when divided into six 4 h time intervals, time spent ruminating was positively related with pulse number ($p=0.05$) in cattle showing no diurnal plasma leptin variation. These results suggest that cattle may have unique diurnal variation and pulsatile patterns of plasma leptin, differing from those of monogastric animals. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 9 : 1209-1215)*

Key Words : Leptin, Circadian Rhythm, Cattle

INTRODUCTION

Leptin, the product of the *ob* gene, is a 16-kDa-protein hormone produced mainly by adipose tissue (Zhang et al., 1994; Halaas et al., 1995). After its release into the circulation, leptin undergoes rapid uptake from the blood into the brain (Banks et al., 1996) and acts to regulate food intake and energy expenditure (Campfield et al., 1995; Levin et al., 1996; Mistry et al., 1997). Expression of the *ob* gene and circulating leptin concentrations are highly correlated with percentage of body fat in rodents (Frederich et al., 1995) and with degree of obesity in humans (Considine et al., 1996). Recently, it has been reported that short-term fasting in cattle reduced leptin mRNA expression in adipose tissue (Tsuchiya et al., 1998; Amstalden et al., 2000) and circulating leptin concentrations (Amstalden et al., 2000) as compared with levels in animals in well-fed condition. Therefore, leptin is thought to play an important role in controlling energy balance in cattle as shown in humans and rodents. However, there has been produced no hard evidence concerning the role of leptin in cattle.

A diurnal variation in plasma leptin has been observed in humans with a maximum between midnight and early morning and a minimum around noon to afternoon (Sinha et al., 1996a; Schoeller et al., 1997), and it has been seen that plasma leptin also exhibits diurnal fluctuation in rodents (Ahima et al., 1998; Cha et al., 2000). The diurnal variation of plasma leptin is entrained to meal timing in men (Schoeller et al., 1997) and affected by sleep whatever the time when it occurs (Simon et al., 1998).

Plasma leptin levels have also shown a pulsatile secretary pattern in humans (Sinha et al., 1996b). Saad et al. (1998) have shown that obesity is associated with dampened pulsatility with sampling at 20 min intervals. On the other hand, Licinio et al. (1998) demonstrated a lack of significant correlation between mean 24 h leptin levels and pulsatility, with sampling at 7 min intervals, and emphasized the importance of sampling frequency.

In sheep, however, plasma leptin levels did not show diurnal variation (Tokuda et al., 2000; Blache et al., 2000). Ruminant animals have particular digestive functions, and sleeping time is short and transient in cattle (Arave and Albright, 1981). Therefore, the circadian or pulsatile pattern of plasma leptin levels in these animals may be different from that in monogastric animals.

The aim of this study was to ascertain whether circulating leptin appears to follow a circadian rhythm in cattle. Furthermore, we investigated whether leptin secretion occurs in a pulsatile manner in cattle as determined by sampling at 10 min intervals.

MATERIALS AND METHODS

Animals and procedures

Five 10-month-old Holstein steers were used. The mean body weight of these animals was 227±5.2 kg. Animals were housed individually in pens under natural light except for minimal supplemental lighting required during sampling at night. A catheter was placed in the jugular vein and was

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kept patent by means of heparinized solutions. Blood samplings were performed continuously throughout the 24 h experiment at 10 min intervals beginning at 09:00, at least 2 h after initial venipuncture. Blood samples were collected in heparinized tubes immediately centrifuged at 4°C and plasma was stored at -20°C until assay. Feed was offered in two equal portions at 09:00 and 16:00. Animals were fed 2.6 kg Italian ryegrass hay and 4 kg concentrate diet per day on a dry matter basis to promote 1.2 kg gain/day according to the Japanese Feeding Standard for Beef Cattle (Agriculture, Forestry and Fisheries Research Council Secretariat, 2000). The concentrate diet consisted mainly of flaked barley, flaked corn, wheat bran, and soybean meal. Animals had access to fresh water at all times. Time spent eating and ruminating were simultaneously recorded every 5 min over the 24 h experiment.

Plasma hormones and glucose assays

Plasma leptin levels were determined by radioimmunoassay (RIA) using a Multi-Species kit obtained from Linco Research (St. Charles, MO), with a lower sensitivity of 0.5 ng/ml human equivalent (HE). The intraassay and interassay of coefficients of variation were 10.7 and 11.4%, respectively. In order to demonstrate leptin linearity, two plasma samples from steers containing a high concentration of leptin were diluted and assayed, exhibiting a parallelism with the standard curve. Plasma insulin levels were determined using a RIA kit purchased from Eiken Chemical (Tokyo, Japan). Plasma glucose levels were measured by Glucose-test purchased from Wako Pure Chemicals (Osaka, Japan).

Data analysis

A cosinor method, which was developed to describe diurnal rhythm by a cosine curve, was used to test for a diurnal variation as previously reported using human subjects (Schoeller et al., 1997; Franceschini et al., 1999; Langendonk et al., 2000), assuming a period of 24 h according to Cornelissen et al. (1980). A correlation coefficient between the predicted and measured leptin levels above 0.273 was required for significance. In that case, we fitted a cosine curve and considered these animals having diurnal variation. Acrophase was defined as the time between reference time and time of peak value. Amplitude was defined as the half of the total predictable change in a rhythm. Mesor was defined as the average value of a cosinor curve fitted to the data.

Pulse analysis was performed using a largely model-free pulse analysis algorithm, Cluster (Veldhuis et al., 1986; Licinio et al., 1998). Samples obtained at 10 min intervals over the 24 h period were used to assess mean 24 h leptin level, pulse frequency (number of significant peak per 24 h), mean interpeak interval (time separating consecutive peak maxima), mean pulse duration in minutes, mean pulse height (maximal leptin concentration in a peak), pulse height as increase ratio over preceding baseline (1.0 corresponds to preceding baseline), interpulse valley mean (a valley has been defined as a region embracing nadirs without intervening peaks), and nadir concentrations. The variance model used in Cluster analysis was a power function variance model. Test Cluster sizes were 2×2 in the moving nadir and peak with t=2.0 as the significant level for both test upstrokes and downstrokes in the data.

Statistical analysis

Differences between the two groups, which were classified based on 24 h leptin variation, or among periods of 4 h intervals, were analyzed by GLM procedure of the Statistical Analysis Systems Institute (SAS, 1988). Correlations between parameters were evaluated using Pearson's correlation coefficient calculated by the CORR procedure of the SAS (1990).

RESULTS

Blood sampling at 10 min intervals over 24 h was completed in three of the five cattle. We did not obtain blood samples from cow no.1 from 22:50 to 23:30, 0:10 to 1:30, 2:00 to 2:40, and from cow no.4 from 2:50 to 4:00, 5:30 to 6:00 due to a technical problem.

Figure 1 illustrates individual 24 h leptin profiles from three animals from which were taken blood samples throughout the 24 h experiment. In the other two animals, there was no problem for analysis of diurnal variation although some of those blood samples were lacking. The profiles are presented as a ratio of changes from each individual's 24 h mean. In two of the five cattle, the 24 h variation of plasma leptin levels could adequately be described by a cosine (figure 1; cows 3 and 5). There, plasma leptin levels increased in the daytime, reached a maximum between 15:00 and 16:00, and then decreased progressively and attained a nadir at around midnight (table 1). In the other cattle, it did not appear to show diurnal variation (figure 1; cow 2).

Table 1. Cosinor analysis parameters of the 24 h plasma leptin rhythmicity in two cattle

Cattle no.	Acrophase (hour after 09:00)	Mesor (ng/ml HE)	Amplitude (ratio to mesor)	Cosinor p[1)]
3	6.84	2.82	0.22	0.0001
5	6.20	2.61	0.17	0.0001
Mean	6.56	2.72	0.20	0.0001
SE	0.32	0.10	0.02	

[1)] Cosinor analysis was used to appraise the presence of 24 h leptin variations.

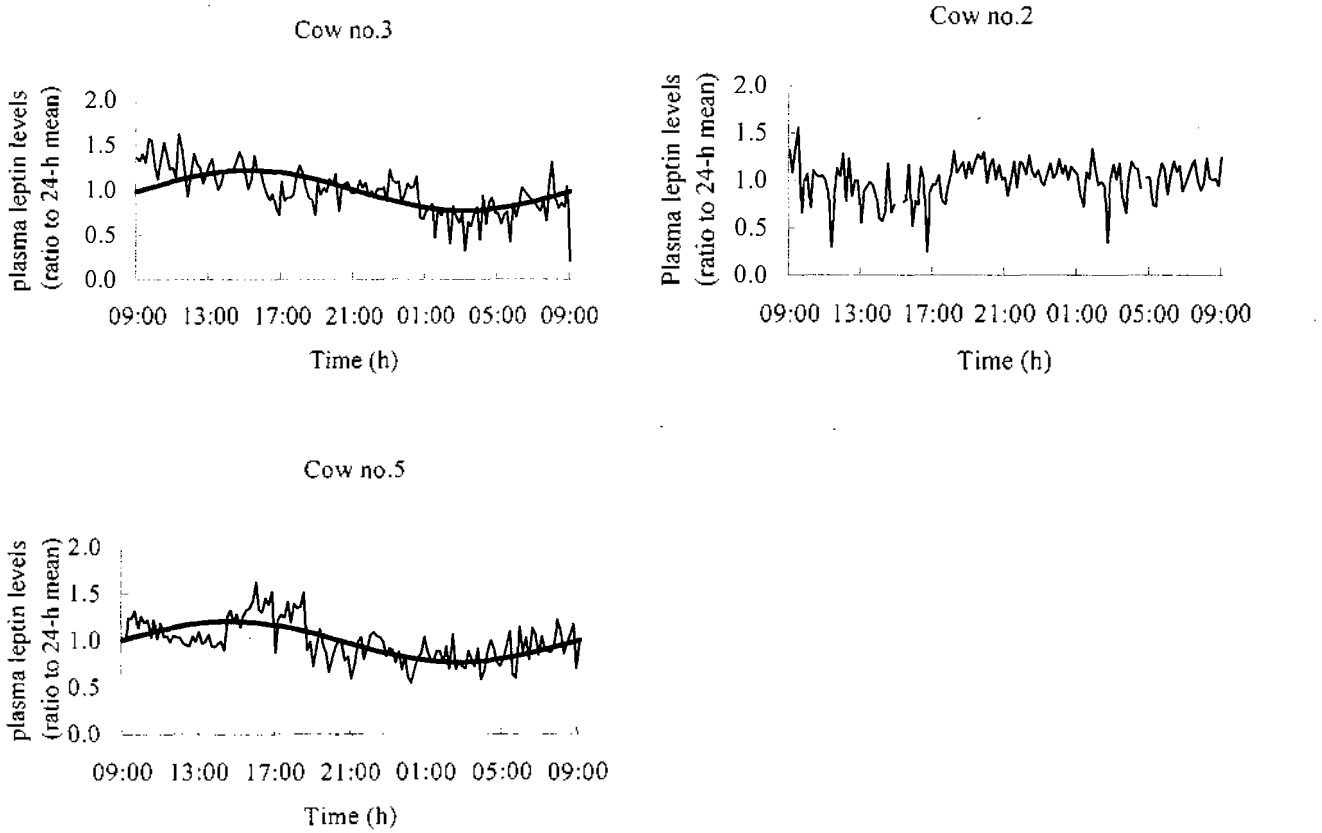

Figure 1. Individual profiles of 24 h plasma leptin levels in three cattle, from which were taken blood samples throughout the 24 h experiment. Cows 3 and 5 show diurnal variation and cow 2 shows no diurnal variation. Plasma leptin levels are expressed as a ratio of each individual's 24 h leptin mean. The cosine curve corresponds to the best-fitted model obtained by cosinor analysis, which was developed to apply diurnal rhythm to a cosine curve. A correlation coefficient between the predicted and measured levels above 0.273 was required for significance. Feed was offered at 09:00 and 16:00 in two equal portions.

All series had significant pulsatile attributes of leptin release as assessed by Cluster analysis (table 2). In Holstein aged 10 months, the mean 24 h leptin level was 2.84±0.24 ng/ml HE (mean±SE), the number of significant leptin pulses was 15±1.1/24 h, interpeak interval was 97.4±10.2 min, pulse duration was 77.3±8.5 min, and pulse height (increase ratio) was 1.43±0.05. There was no difference between animals showing diurnal plasma leptin variation and those showing no diurnal variation in either mean 24 h leptin levels or leptin pulsatility. Mean 24 h leptin levels were significantly correlated with the concentration-dependent pulse parameters, which are pulse height and nadir of valley concentrations. However, there was no significant correlation between mean 24 h leptin levels and pulse number, interpeak interval, pulse duration, or pulse height expressed as increase ratio.

Figure 2 illustrates feeding behavior and number of leptin pulses for six 4 h time periods (09:00 - 13:00, 13:00 - 17:00, 17:00 - 21:00, 21:00 - 01:00, 01:00 - 05:00, 05:00 - 09:00). There was no significant difference between animals showing diurnal variation and those showing no diurnal variation relating to feeding behavior. Pulse frequency was not significantly affected by two feeding times (09:00 and 16:00), but the number of pulses from 01:00 to 05:00 tended to be more frequent than that from 21:00 to 01:00 ($p<0.07$). Total time spent eating, ruminating, or chewing per 24 h did not correlate with mean 24 h leptin levels or leptin pulsatile attributes. However, time spent ruminating during 4 h time periods was positively related to their respective pulse frequency in cattle showing no diurnal plasma leptin variation ($r=0.539$, $p=0.05$, figure 3), but not in cattle showing diurnal variation.

Leptin levels, averaged for six 4 h time periods starting

Table 2. Twenty-four hour pulsatility parameters in five cattle

Cattle No.	Average 24-h leptin (ng/ml HE)		Pulse frequency (peaks/24 h)	Interpeak interval (min)		Pulse duration (min)		Pulse height (ng /ml HE)		Pulse height (increase ratio)		Nadir of valley (ng/ml HE)	
	Mean	SE		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1[1)]	3.60	0.07	11	137	33.5	104.5	34.8	4.36	0.33	1.27	0.07	3.26	0.17
2	2.12	0.04	15	94.3	17.4	68.0	15.3	2.60	0.09	1.53	0.07	1.43	0.11
3	2.86	0.07	17	79.4	12.3	57.1	9.9	3.49	0.20	1.41	0.08	2.17	0.20
4[2)]	2.98	0.06	15	90.0	21.9	68.7	16.9	3.66	0.17	1.51	0.12	2.30	0.22
5	2.65	0.05	12	86.4	19.6	88.3	35.0	3.06	0.16	1.42	0.03	1.90	0.08
Mean	2.84		15[3)]	97.4		77.3		3.43		1.43		2.21	
SE	0.24		1.13[3)]	10.2		8.47		0.30		0.05		0.30	

[1)] Samples of 22:50 to 23:30, 0:10 to 1:30 and 2:00 to 2:40 are lacking.
[2)] Samples of 2:50 to 4:00 and 5:30 to 6:00 are lacking.
[3)] These data were calculated without the results of cattle nos. 1 and 4 because of a lack of samples.

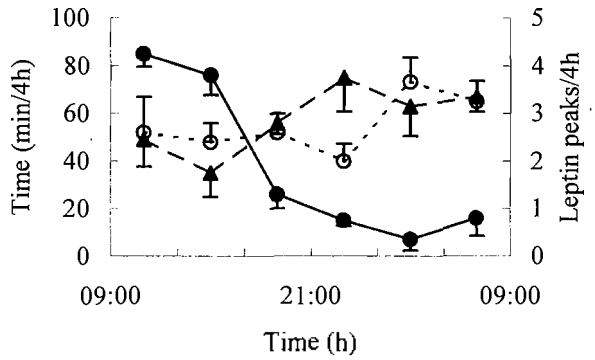

Figure 2. Time spent eating (●), ruminating (▲), and number of leptin pulses (○) during 4 h intervals over 24 h in cattle (n=5). Times spent eating and ruminating are represented on the left y-axis, and number of leptin pulse on the right. Leptin pulses from 21:00 to 01:00, 01:00 to 05:00, and 05:00 to 09:00 were averaged from 3, 4, and 4 cattle because we did not obtain blood samples from cow no.1 from 22:50 to 23:30, 0:10 to 1:30, 2:00 to 2:40, and from cow no.4 from 2:50 to 4:00, 5:30 to 6:00 due to a technical problem. Data are the mean ± SE.

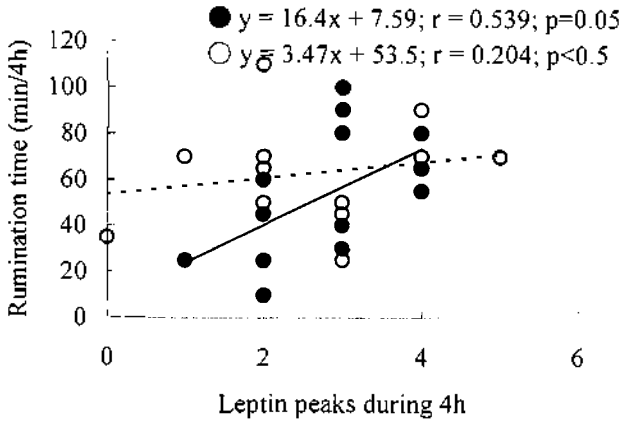

Figure 3. Relation between time spent ruminating and number of pulses during 4 h intervals from cattle showing no diurnal variation (n=3, ●, continuous line) and those showing diurnal variation (n=2, ○, dashed line).

at 09:00, and expressed as a ratio of the 24 h mean, were positively correlated with their respective eating times during the same periods in cattle showing diurnal variation (r=0.81, p<0.01), but negatively in those showing no diurnal variation (r=-0.45, p=0.06, figure 4).

Plasma glucose and insulin levels, expressed as a ratio of changes from each individual's 24 h mean are shown in figure 5. Plasma glucose concentrations were almost constant in all animals. Insulin levels exhibited a tendency to increase after feeding in all animals. The increase over the 4 h period after the morning feed in animals showing no diurnal variation seemed to be greater than those showing diurnal variation (p=0.14).

DISCUSSION

In this study, we show the presence (n=2) or absence (n=3) of diurnal variation of plasma leptin in cattle by a cosinor method. In cattle showing diurnal variation, leptin levels peaked at around evening. Plasma leptin exhibits diurnal fluctuation in humans (Sinha et al., 1996a) and rodents (Ahima et al., 1998; Cha et al., 2000). Leptin levels are maximal between midnight and early morning and minimal at around noon to mid-afternoon in normal subjects (Sinha et al., 1996a; Licinio et al., 1998). The times of peak and nadir of diurnal leptin observed in cattle were those of nadir and peak in humans, respectively. Sinha et al. (1996a) suggested that the nocturnal rise in leptin secretion in humans may be related to appetite suppression during sleep. Schoeller et al. (1997) have reported that diurnal variation in plasma leptin levels is entrained to meal timing in young healthy men. Furthermore, under continuous enteral nutrition, the diurnal rhythm of leptin in men is

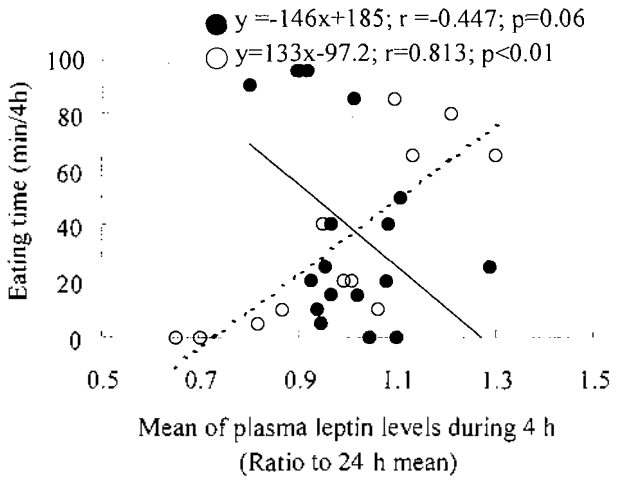

Figure 4. Relation between time spent eating and averaged leptin levels, expressed as a ratio of each cow's 24 h mean, during 4 h intervals from cattle showing no diurnal variation (n=3, ●, continuous line) and those showing diurnal variation (n=2, ○, dashed line).

modulated by both rectal temperature and sleep (Simon et al., 1998). Cattle sleep for only short and transient periods without loss of vigilance or consciousness (Arave and Albright, 1981). The difference of time course of the diurnal rhythm of leptin between these species may be related to the differences in their digestive systems and (or) sleep behaviors.

The diurnal secretion of leptin observed in elderly men shows a similar mesor mean value but decreased amplitude compared with that in middle-aged subjects (Franceschini et al., 1999). Saad et al. (1998) showed that the magnitude of the relative diurnal change in leptin was higher in men than in women, and higher in lean than in obese subjects. In addition, Langendonk et al. (1998) have shown that the relative amplitude, expressed as a ratio of the 24 h mean of circadian rhythm of leptin, was lower in obese women than in women of normal weight (0.22 vs 0.34). In our study, the amplitude of leptin in cattle showing diurnal variation was lower compared with that in the normal subjects observed in those studies. Blunted relative diurnal excursions may be due to the fact that these cattle in this study were in the progress of fattening, that is, in a condition that could be regarded as obese.

A significant positive correlation was observed in cattle showing diurnal variation between time spent eating during 4 h time periods and their respective leptin levels, averaged for 4 h intervals and expressed as a ratio of the 24 h mean. The positive correlation is thought to be in consequence of the circadian leptin levels, which peaked at around evening, a time to eat, and decreased around the night, a time not to eat. On the other hand, that correlation was negative in those showing no diurnal variation. In cattle, fasting reduces leptin mRNA in adipose tissue (Tsuchiya et al., 1998; Amstalden et al., 2000) and circulating leptin

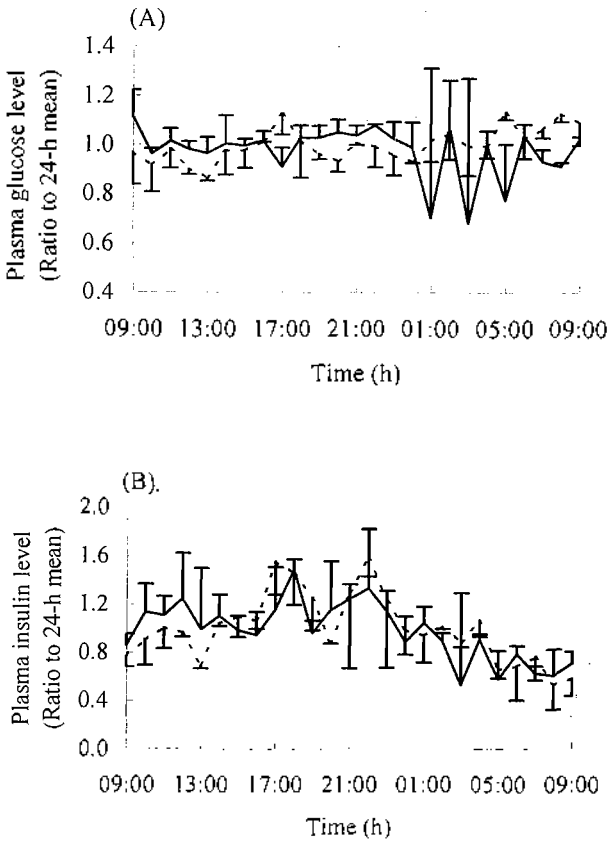

Figure 5. Twenty-four-hour plasma glucose (A) and insulin (B) levels of cattle showing no diurnal variation (n=3, continuous line) and those showing diurnal variation (n=2, dashed line). Plasma levels (mean±SE) are expressed as the ratios of each cow's 24 h mean. Feed was offered at 09:00 and 16:00 in two equal portions.

concentrations (Amstalden et al., 2000) compared with levels in well-fed condition. These results indicate that leptin is partly involved in feed intake in cattle. Our results showing a negative correlation between mean leptin levels and time spent eating during 4 h intervals in cattle showing no diurnal rhythm might reflect leptin function, suppression of appetite, as in humans and rodents.

Furthermore, plasma leptin levels showed a pulsatile secretion pattern in cattle. We found an average of 15 pulses during the 24 h experiments as determined by sampling at 10 min intervals. Licinio et al. (1998) have shown 30 pulses/24 h in humans using 7 min interval sampling, whereas Bergendahl et al. (2000) have shown 14 pulses /24 h by sampling at 10 min intervals. Leptin pulse frequency in cattle might be similar to that in humans using the same sampling intervals. However, variability in interpeak interval or duration time within each animal was higher in cattle than in humans, which may indicate the irregular periodicity of leptin pulses in cattle. The number of the pulses was not affected by time spent eating or ruminating

when calculated per 24 h; however, time spent ruminating correlated positively with the number of pulses when divided into six 4 h intervals in cattle showing no diurnal variation, suggesting that ruminating behavior could partly induce pulsatile leptin secretion or, conversely, that pulsatile leptin secretion could induce rumination in these animals.

In three cattle, which showed no diurnal plasma leptin variation, insulin levels over the 4 h period after the morning feed were higher than in those showing diurnal variation. Utriainen et al. (1996) showed that infusion of insulin could increase plasma leptin concentrations in 4 to 6 h. It is plausible, therefore, that repeated daytime postprandial insulin release could induce an increase in plasma leptin levels in the afternoon and during the night, and conversely, that the nocturnal plasma insulin diminution could cause a decline in plasma leptin in the early morning in humans. The increase in insulin release after morning feed in three of the five cattle might cause a rise in plasma leptin in the night, and not apparently induce diurnal leptin variation. With twice daily feedings, insulin concentrations after feeding were higher in a high concentrate diet group than in a low concentrate diet group (Sutton et al., 1988). In our study, cattle showing no diurnal leptin variation might have eaten by preference only a concentrate diet just after feeding in the morning, and consequently, that might have contributed to the rise in insulin release compared with the animals showing diurnal leptin variation. The difference of insulin secretion between animals showing diurnal leptin variation and those showing no diurnal leptin variation might account for the difference of the relation between leptin pulse frequency and time spent ruminating.

Tokuda et al. (2000) and Blache et al. (2000) have shown that plasma leptin levels showed no clear diurnal rhythm in sheep. Furthermore, contemporary reports have shown that the specific RIA system for cattle or sheep can detect circulating leptin more sensitively than can a Multi-Species kit obtained from Linco Research (Delavaud et al., 2000; Blache et al., 2000; Ehrhardt et al., 2000). If we use the specific RIA system, it could possibly clarify the 24 h plasma leptin variation in cattle.

In conclusion, the present study demonstrated that diurnal variation of leptin secretion in cattle dose not show the same pattern in all animals. In cattle showing diurnal rhythm by a cosinor method, plasma leptin levels reached a maximum in the daytime and dropped to a minimum around the dead of the night, and their excursions were blunted as compared with the normal humans previously reported. In addition, leptin secretion showed a pulsatile pattern, but leptin pulses showed irregular periodicity in cattle.

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