Age-related Changes in Plasma Leptin from Early Growing to Late Finishing Stages of Castrated Holstein Steers: Utilizing Multi-species Leptin RIA

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ABSTRACT : This experiment was performed to understand the changes in plasma leptin in association with plasma IGF-1, body weight and ADG from early growing to late finishing stages of Holstein steers. Blood collection was performed by arterial vein puncture at selected monthly ages of 1 (54 kg), 2.6 (103 kg), 7.2 (205 kg), 13.5 (314 kg), 16.9 (414 kg), 22.2 (550 kg), 24.9 (626 kg) and 27.4 months (695 kg). The blood was analyzed for leptin using the multi-species leptin RIA with recombinant bovine leptin (rbleptin) as standard, plasma IGF-1 was also measured using RIA. Against the standard rbleptin, the multi-species Leptin RIA system's sensitivity, cross reactivity, slope and recovery of 41.0 ng/ml rbleptin in plasma were 4.9 ng/ml, 11.22%, -1.396 and 97.8%, respectively. Plasma leptin measured were more than 5.0 ng/ml, which enable multi-species RIA system to investigate plasma leptin in normal growing steers. Body weight resulted to a highly significant second-degree polynomial relationship with plasma leptin ($q=0.54$, $p<0.0001$) and plasma IGF-1 ($q=0.44$, $p<0.0001$) from 1 to 27.4 monthly ages. However, the second-degree polynomial curve of plasma leptin and IGF-1 differs showing a concave and convex curvilinear relationship, respectively. ADG was not significantly associated to plasma leptin ($r=0.06$, $p>0.05$) and plasma IGF=1 ($r=0.06$, $p>0.05$) from 1 to 27.4 monthly ages. Low coefficient, but significant associated increase of plasma leptin and IGF-1 ($r=0.12$, $p<0.008$) from 1 to 27.4 months was observed. The uncoordinated increases of plasma IGF-1 at growing and plasma leptin at fattening period, may indicate (1) indirect involvement of endogenous IGF-1 on leptin secretion, and (2) IGF-1 level may signify lean and bone accretion while plasma leptin may mirror body fatness across the monthly ages of Holstein steers. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 5 : 725-731)***

Key Words : Multi-species Leptin RIA, Holstein Steer, Leptin, IGF-1, Growth Stages

INTRODUCTION

Leptin is considered as the "lipostat" signal from adipose tissue that control body fatness through the regulation of feed intake and metabolism. In animal production the regulation of feed intake, utilization and partitioning of nutrients to produce economically important products such as meat and milk is a top priority.

Age-related pattern of circulating leptin concentration of domestic animals has been scarce. In male subjects, transitory increase in circulating leptin at the onset of puberty in boys (Mantzoros et al., 1997), similar leptin level observed at pre- and pubertal age (Arslanian et al., 1998), and declining level towards puberty (Blum et al., 1997) has been reported. These findings indicate that the involvement of leptin in relation to the regulation of physiological body weight before attaining sexual maturity remains unclear.

There is limited study on the association of plasma leptin and IGF-1. In Holstein bulls, pituitary and plasma GH decreases significantly from birth to puberty (Mc Andrews et al., 1993; Purchas et al., 1970), while plasma IGF-1 in humans increases from infancy to sexual maturity and gradually declines thereafter (Blum and Ranke, 1991). The profile of plasma leptin and IGF-1 across the monthly ages was considered to understand the relationship of plasma IGF-1 and leptin.

The correlation coefficient of plasma leptin and measure of body fat in sheep ranges from 0.30 to 0.83 (Blache et al., 2000; Delavaud et al., 2000; Ehrhardt et al., 2000). Higher coefficient depends upon sensitivity and stability of leptin assay and accuracy of body fat measurement. However, in weight matched group of obese human, those having low plasma leptin concentration gained body weight (Ravussin et al., 1997), suggesting low plasma leptin relation with ADG. Examining the changes in plasma leptin in relation to body weight and average daily gain (ADG) may be relevant to understand its physiological role. Since leptin research is very limited in ruminants, we realize the need to know the cross-reactivity and sensitivity of multi-species leptin RIA with recombinant bovine leptin (rbleptin) before establishing the relationship of plasma leptin to body weight and ADG across the monthly ages of castrated Holstein steers.

MATERIALS AND METHODS

Care and management of livestock

Eight normally growing Holstein steers from 1 to 27.4

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months (54±1.8 to 695±22.3 kg) was utilized to understand the age-related changes in plasma leptin. The animals were castrated at 3 months (approximately 120 kg). The calves were bought from nearby dairy farm at about 7 to 10 days after birth. Calves were offered milk-replacer (150 grams) dissolved in one-liter lukewarm drinking water until seven weeks old. Then gradually the animals were shifted to hay and calf concentrate diet. The animals were housed in a pen with continuous provision of drinking water, while commercial hay and concentrate feed were offered twice daily at 9:00 and 17:00 h. The three months old calves were given hay and calf concentrate diet of 2.6% DM (17.2% CP and 75% TDN) per day of kilogram body weight. The 13.5 months old fattening steers were offered hay and concentrate diet of 2.0% DM (11.85% CP and 71.15% TDN) per kg body weight everyday, while the 23.6 months old finishing steers were offered 1.6% DM of hay and concentrate diet having 12.5% CP and 75% TDN. The kinds of feed offered according to monthly ages or body weight including the nutrient contents and TDN consumed (%/kg metabolic body weight) are shown in table 1. The diets were formulated based on the recommendation of the Japanese Feeding Standard for Beef Cattle (AFFRC, 1995). The body weight of the animals was measured at least once every month. Blood was collected through the arterial vein at selected monthly ages of 1 (54 kg), 2.6 (103 kg), 7.2 (205 kg), 13.5 (314 kg), 16.9 (414 kg), 22.2 (550 kg), 24.9 (626 kg) and 27.4 months (695 kg) with their corresponding bodyweight. Since daytime plasma leptin does not vary in ruminants (Blache et al., 2000), blood samples were collected only once in the afternoon at about 2:00 PM.

Plasma IGF-1 measurement

Plasma IGF-1 was measured by double antibody RIA utilizing human anti-IGF-1 (Biogenesis, UK lot# 003), standard IGF-1 (Amersham, lot # 30) and labeled ^{125}I-IGF-1 (Amersham, code IM172). Before the RIA, plasma samples were extracted according to the method of Daughaday et al (1980). The sensitivity of the IGF-1 assay was 0.82 ng/ml, and the inter- and intra- assays CV were 11.3 and 6.2, respectively.

Recombinant bovine leptin production and plasma leptin assay

The recombinant bovine leptin was obtained from those produced at Animal Metabolism and Physiology Lab. (Obihiro Univ. of Agric and Vet Med, Japan) and more detailed procedure will be published separately. Total mRNA from subcutaneous adipose tissue obtained by biopsy in adult Japanese Black steer was reversed and transcribed by Hokkaido System Science Co., Ltd. (Japan). The resulting cDNAs were used in PCR to amplify the whole native bovine leptin cDNA (Genebank accession #U50365). PCR was performed with forward primer 5'CCA

Table 1. Kind of feed with its nutrient content offered according to monthly ages or mean body weight as well as TDN intake/kg metabolic body weight (MBW)

Age (months)/Mean body weight (kg)	1 54	2.6 103	7.2 205	13.5 314	16.9 414	22.2 550	24.9 626	27.4 695
	Period of feed offered							
Kinds of feed:								
Milk replacer[1]								
Concentrate for;								
Calf starter								
Fattening								
Finishing								
Hay 1								
Hay 2								
Hay 3								
TDN consumed (%)[2]		6.26		5.99		5.90		
	Concentrate feed (DM basis)			Timothy hay (DM basis)				
	Calf	Fattening	Finishing	1	2	3		
Dry matter, (%)	92.0	88.21	88.16	85.2	90.8	89.6		
Crude protein, (%)	21.3	13.3	12.98	6.9	6.7	5.0		
Ether extract, (%)	7.1	3.4	3.1	1.9	1.8	1.2		
Crude fiber, (%)	7.0	3.5	3.2	33.4	36.7	66.4[3]		
Ash, (%)	8.1	3.6	3.4	5.0	4.7	4.8		
TDN, (%)	79.3	73.4	71.9	54.9	59.2	39.5		

[1] The milk replacer was composed primarily of skim milk, soybean, fish meal, vitamins and minerals.
[2] Percent TDN consumed per kg metabolic body weight was determined at 3.07, 13.5 and 23.6 monthly ages.
[3] Measured as Nutrient Detergent Fiber (NDF).

TAT GGT GCC CAT CCG CAA GGT C 3', and the reverse primer 5'GGG ATC CTC AGC ACC CGG GAC TGA G 3'. The recombinant bovine leptin still undergo purification and it had 85% purity when utilized as standard in plasma bovine leptin measurement using multi-species leptin RIA kit (Linco Research, Inc., St Louis, MO). The intraassay means of SD and CV from two duplicate quality control standards were 0.66 and 7.36%, respectively. Also, recombinant human leptin (NIDDK, California) was utilized as another reference standard, and it showed parallelism with the multi-species leptin RIA standard (data not shown).

Statistical analysis

Cross-reactivity of multi-species leptin antibody to rbleptin was computed based on procedures and information provided by Dr. A.F. Parlow on ovine growth hormone RIA manual (NIDDK, California). Linear regression analysis between hormones and monthly ages was performed before utilizing ANOVA and Duncan Multiple Range Test (DMRT). As linear regression was found significant between plasma leptin and monthly ages ($r=0.49$, $p<0.0001$) and plasma IGF-1 and monthly ages ($r=0.32$, $p<0.0001$), one-way ANOVA was performed followed by DMRT between monthly ages comparison of hormone levels. Scatter plot shows the second-degree polynomial relationship between plasma leptin and body weight and plasma IGF-1 and body weight. Linear association of plasma leptin and IGF-1 across the monthly ages was demonstrated using scatter plot. General Linear Model (GLM) was used for ANOVA and Regression Model for linear regression as well as second-degree polynomial or quadratic relationship ($Y=\alpha+\beta_1X+\beta_2X^2$, where α is the intercept, $\beta_{1,2}$ is the partial coefficient and Y as plasma leptin or IGF-1) utilizing SAS system statistical software (SAS, 1988). The q represents the R^2 of quadratic relationship.

RESULTS

The steers obtained normal growth from acquisition until it reaches desired slaughter weights as shown in figure 1. Comparably lower ADG was obtained when the calves experienced the first winter season, their low average daily gains were 0.33, 0.48 and 0.46 kg/d, during 8.6, 10.0 and 10.9 months old, respectively. The TDN consumption (%/kg of metabolic body weight) recorded was high at 3 months old and lower at 13.5 and 23.6 months old (table 1). The energy consumed across the monthly ages was high above the maintenance requirement of the animal as manifested by positive average daily gain. In steers the low TDN consumption may have no effect on plasma IGF-1, as low feed intake (1.22% DM) does not cause significant

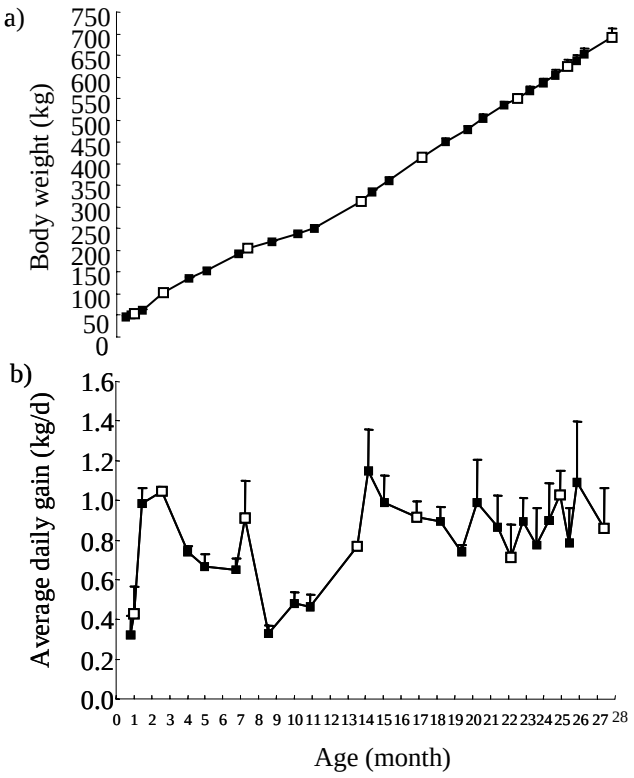

Figure 1. Means of (a) body weight (kg) and, (b) average daily gain (kg/d) of Holstein steers across the monthly ages. Open squares represent the monthly ages of blood collection.

influence on plasma IGF-1, only during high feed intake (2.43% DM) (Lee et al., 2000a) and during fasting (Lee et al., 2000b). In sheep high and low lupin grain supplement did not influence plasma leptin (Blache et al., 2000). Hence, relatively low yet above the maintenance requirement of TDN consumption may have no effect on plasma leptin and IGF-1 of castrated Holstein steers.

In cattle, limited plasma leptin research could be caused by unavailability of commercial leptin assay kit; hence evidence of multi-species leptin RIA system's cross reactivity and sensitivity was performed against rbleptin. The parallelism of multi-species' standard assay is shown in figure 2. The cross reactivity and slope of standard rbleptin utilizing multi-species RIA were 11.22% and -1.396, respectively. The percent of non-specific binding and binding of ^{125}I-labelled rhleptin to anti-human leptin was 1.13 and 41.31%, respectively. The sensitivity of multi-species leptin RIA with rbleptin was 4.9 ng/ml and the range of plasma leptin obtained from 54 to 695 kg in castrated steer was 5.0 to 58.4 ng/ml. The recovery of 41.0 ng/ml rbleptin when added to bovine plasma was 97.8%.

Figure 3 shows the plasma leptin and IGF-1 concentration from calf hood to finishing period of castrated Holstein steers. Plasma leptin non-significantly decline

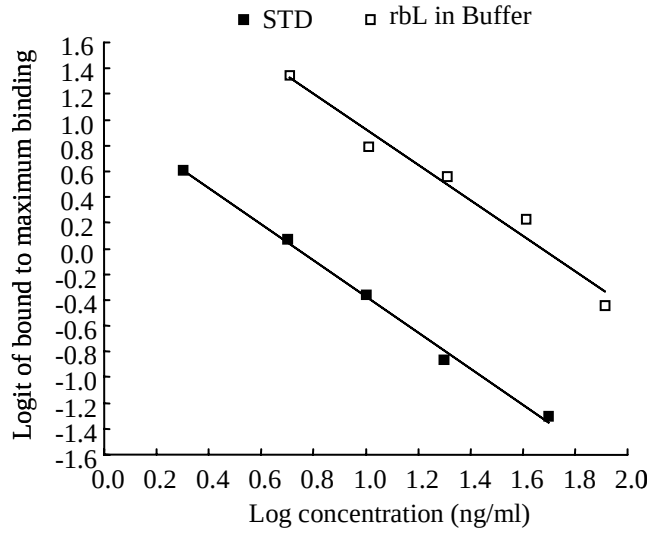

Figure 2. Cross reactivity of 11.22% and parallelism of native recombinant bovine leptin to the standard used in the multi-species leptin RIA system.

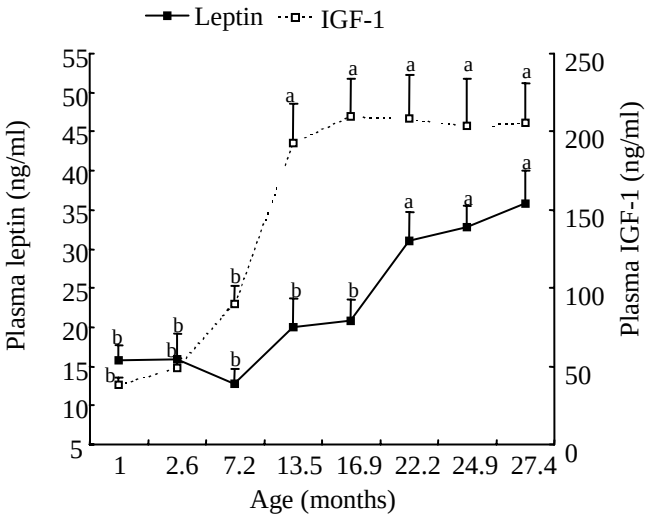

Figure 3. Means of plasma leptin (ng/ml) and IGF-1 (ng/ml) across the monthly ages of castrated Holstein steers. The vertical lines in every point represent the upper limit of SEM, and those having different alphabet across the monthly ages shows significant differences at $p<0.0001$.

from 1 to 7.2 months and starts to rise from 7.2 to 27.4 months. On the other hand, plasma IGF-1 significantly increases from 1 to 13.5 months and then plateaus until attaining the slaughter weight. Both plasma leptin and IGF-1 obtained significant second-degree polynomial relationship with body weight from 1 to 27.4 months, as shown in figure 4. However, plasma leptin shows a concave curvilinear while plasma IGF-1 shows a convex curvilinear relationship with body weight. Plasma IGF-1 significantly increases from 1 to 16.9 months old, implying the IGF-1 bioactivity with age. Plasma leptin and IGF-1 linear association obtained low coefficient but it reached significant association from 1 to 27.4 months, indicating that both hormones increases with monthly ages. ADG was high from 1 to 2.6 months of age (1.037 kg/day) while the average plasma leptin was coincidentally low (12.9 ng/ml). ADG computed one month before and after blood collection at selected monthly ages (from 1 to 27.4 months) did not show significant relationship with plasma leptin ($r=0.006$, $p=0.55$) and plasma IGF-1 ($r=0.06$, $p=0.06$), indicating that plasma leptin and IGF-1 are poor predictors of body weight gain.

DISCUSSION

This is the first across the monthly ages experiment in castrated Holstein steers, which describe the changes in plasma leptin concentration from early growing to late finishing stage. This was performed to determine the developmental pattern of plasma leptin in association with IGF-1 across the monthly ages or body weight.

Absence of linear relationship of plasma leptin measured by multi-species leptin RIA and specific bovine RIA was reported in bull calves undergoing high and low feed intake (Ehrhardt et al., 2000). This absence of linear relationship can be explained by low plasma leptin in the low feed intake (within 4 to 5 ng/ml) compared to high feed intake (about 5.5 to 8 ng/ml) bull calves. The range of plasma leptin we observed from 54 to 103 kg BW was 5.0 to 22 ng/ml, which is wider than those observed by Ehrhardt et al. (2000). The narrower range of their result could be attributed to higher sensitivity and stability of specific RIA system. Also in ovine, the relationship of plasma leptin measured utilizing specific leptin RIA and multi-species RIA in 56 animals showed curvilinear response and non-linear response can be noticed below 5.0 ng/ml (Delavaud, 2000), which consistently supports our finding. Since parallelism exists and our measured bovine plasma leptin were above the assay sensitivity, the use of multi-species leptin RIA system is justified in this study.

In male subjects and monkey experiments, leptin involvement in relation to the regulation of body weight at sexual maturity remains unclear (Urbanski et al., 1998; Arslanian et al., 1998; Blum et al., 1997). Compared to research on male rhesus monkey of Urbanski et al. (1998), our data does not include newly born calves and utilizes the same animals across the monthly ages. The non-significant depression of plasma leptin we observed from 1 to 7.2 months old may have been affected by castration at 3 months old. Somehow the report in sheep that plasma leptin was not significantly different between rams and castrated sheep (Kauter, 2000) upholds our finding that

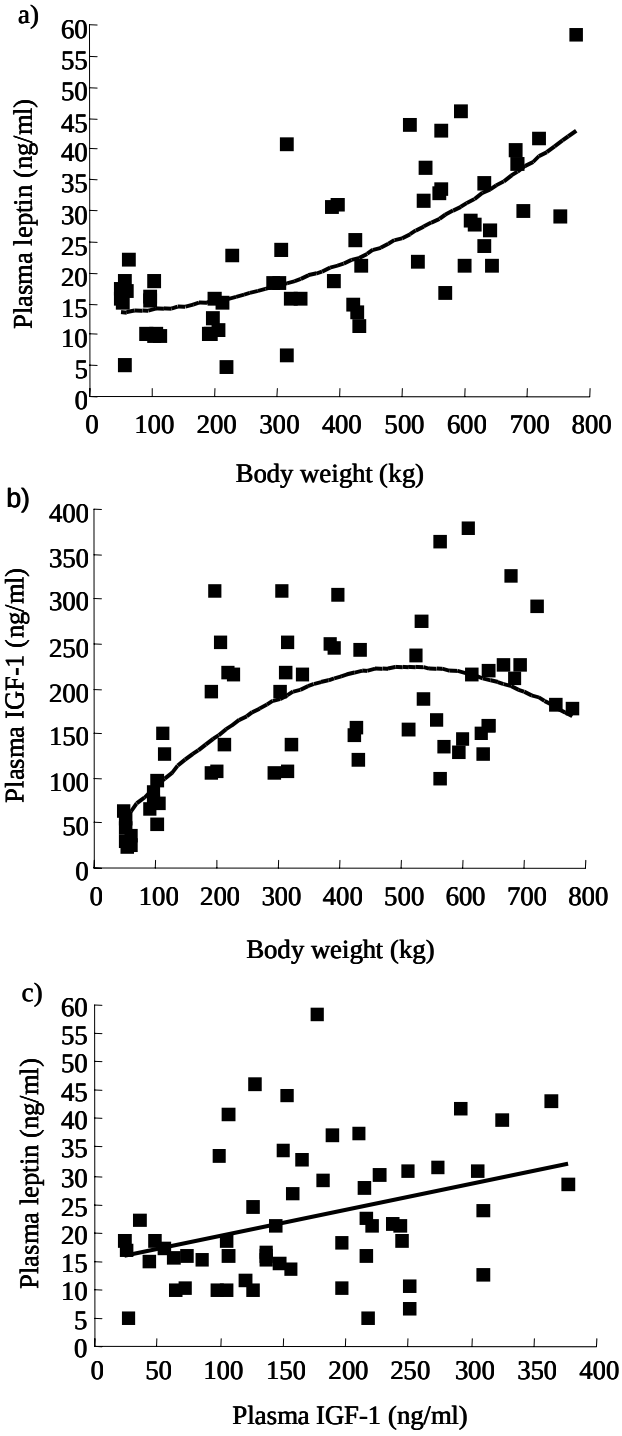

Figure 4. Scatter plot showing second-degree polynomial relationship between (a) plasma leptin and body weight ($y=5E-05x^2-0.0004x+13.575$; $q=0.54$; $p<0.0001$), (b) plasma IGF-1 and body weight ($y=-0.0008x^2+0.8x+17.82$; $q=0.44$; $p<0.0001$) and (c) linear association of plasma leptin and IGF-1 ($y=-0.0452x+15.02$; $r=0.12$; $p<0.008$) from 1 to 27.4 months old steers. The q represents the coefficient of second-degree polynomial relationship.

plasma leptin was not significantly different at this period.

Leptin has been positively correlated to body weight and body fat measurements across species such as in humans (Takahashi et al., 1996; Arslanian et al., 1998) in pigs (Robert et al., 1998) and in sheep (Blache et al., 2000; Delavaud et al., 2000). We observed linear relationship of plasma leptin to body weight ($r=0.49$, $p<0.0001$) and plasma IGF-1 to body weight ($r=0.31$, $p<0.0001$) from 1 to 27.4 months in steers. In search for proper fitness of curve, we found the second-degree polynomial relationship of plasma leptin and IGF-1 to body weight better than the linear relationship, because both hormones obtained highly significant relationship and higher coefficient with the second-degree polynomial model. The concave curvilinear relationship of plasma leptin to body weight may likely reflect the degree of body fatness of steers from early growing until slaughter weight, although valid evidence is still necessary. Moreover, the beginning age of significant body fat development cannot be generalized because this is dependent on environment, nutrition and breed. On the other hand, plasma IGF-1 demonstrates a convex curvilinear pattern of relationship with body weight from 1 to 27.4 monthly ages. The convex curvilinear pattern exhibited by IGF-1 mirrors its biological activity at early growing period for the formation and deposition of bones, muscles and other tissues.

The study of Ravussin et al. (1997) suggesting that low plasma leptin preceding body weight gain is not applicable in meat production because their study is limited to abnormally obese group and they are beyond the linear growth of development. We utilized castrated steer to represent the condition in beef cattle industry experiencing four seasons. We found that the 2.6-months old steers, possessing highest ADG and low plasma leptin was just a coincidence, because the relationship of blood leptin concentration and ADG from 1 to 27.4 months failed to show statistical significance. Hence, plasma leptin is a poor indicator of weight gain in Holstein steers.

Generally IGF-1 is an anabolic hormone responsible for the bone, organs muscles and other tissues depositions. The elevation of plasma IGF-1 concentration towards sexual maturity which is supported by similar pattern in humans (Blum and Ranke, 1991) may be manifested by the peak of pituitary GH (the primary regulator of IGF-1) at 4 months (Purchas et al., 1970), and the decrease in baseline, mean and amplitude of GH from 1 to 42 weeks of age (Mc Andrews et al., 1993). The inverse relationship of plasma IGF-1 and GH level was suggested from birth to puberty (Mc Andrews et al., 1993), which was supported by the ability of IGF-1 to block GH response to GRF observed in prepubertal lamb (Blanchard et al., 1988). In the present study, the age-related increase in plasma IGF-1 from 1 to 13.5 months may suppose to decrease GH secretion by feedback mechanism and seems supportive of the

suggestion by Mc Andrews (1993). Furthermore it was reported that high plane of nutrition modulate plasma IGF-1 (Lee et al., 2000a), which may have contributed for its elevation at later period. Leptin has been implicated in the regulation of pituitary GH because of the presence of leptin receptors in the arcuate nuclei of hypothalamus (Dyer et al., 1997). In sheep, changes in diets indicate that plasma leptin readily passes to cerebrospinal fluid (Blache et al., 2000). Conflicting results of central leptin infusion on GH in well-fed sheep (Morrison et al., 2001), and pigs (Barb et al., 1998) was observed. Hence, the involvement of leptin in the GH-IGF-1 axis of normally fed animals remains unclear. In bovine, GH indirectly regulates leptin expression by attenuating the stimulatory effects of insulin (Houseknecht et al., 2000), also exogenous GH rapidly reduced leptin mRNA expression in porcine (Spurlock et al., 1998). In this study we did not measure plasma GH with monthly ages, and the assumption that plasma GH attenuate plasma leptin indirectly during early period may not be valid because of delayed rise in plasma leptin (16.9 months). Further study is needed to clarify the GH relationship with plasma leptin. The leveling-off of plasma IGF-1 and the significant increase of plasma leptin from 13.5 to 27.4 months old may imply that endogenous IGF-1 is not directly involve in the leptin secretion from adipose tissue. However, more detailed research is needed to justify this concept.

This 27 months of cross sectional study on circulating leptin and IGF-1 of castrated Holstein steers showed curvilinear and linear-curve pattern of relationship with body weight from 1 to 27.4 months, respectively. The non-significant changes of plasma leptin concentration at growing period suggest limited body fat accumulation and possible attenuation of leptin mRNA expression by GH (Houseknecht et al., 2000). The leveling-off of plasma IGF-1 and linear increase of plasma leptin from 13.5 to 27.4 months suggest indirect involvement of IGF-1 in the secretion of leptin from adipose tissue. Briefly, the results suggest that (1) plasma IGF-1 concentration denotes its significant role in bone, organ, muscles and other tissues deposition, while (2) plasma leptin reflects body fat accumulation across the monthly ages of castrated Holstein steers.

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