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RESEARCH ARTICLE

Association of Prepartum Hypoleptinemia and Postpartum Subclinical Ketosis in Holstein Dairy Cows

Bao-xiang HE*[1], Xiang-hong DU[1], Yu-lan DU[1], Qi-qi HE[2] and Muhammad Ali Mohsin[1]

[1]Clinical Veterinary Laboratory, Guangxi University, Nanning 530005, China; [2]Department of Urology, Gansu Nephro-Urological Clinical Center, Second Hospital of Lanzhou University, Lanzhou,730030, Gansu, China
*Corresponding author: hebaox@gxu.edu.cn

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ABSTRACT

To assess the relationship between blood leptin concentration and the development of ketosis postpartum in Holstein dairy cows, blood plasma leptin, ketone body (KB), glucose and lipid concentrations of 15 cows were measured once per wk from zero wk before to 8 wk after calving. The BCS and backfat thickness of cows were measured one wk before calving. The cows were divided into subclinical ketosis (SK) and no subclinical ketosis (NSK) group accordance of KB, above parameters were compared between SK and NSK cows. The correlation between leptin and KB concentrations was analyzed in all experimental cows. Four of 15 cows developed SK with plasma KB concentrations reached above 1.722 mmol/L at least once in the eight weekly measurements following parturition, another 11 animals had NSK were regarded as normal. Cows which developed SK had significantly lower leptin concentration one wk before calving in comparison to the animals that did not. Plasma leptin concentrations measured at one wk before calving were negatively correlated with KB concentrations measured at calving as well as in the following eight wks. It may be concluded that the SK occurrence of Holstein dairy cows postpartum may be associated with the lower leptin level before calving. Low plasma leptin concentration before calving may serve as a prognostic factor for postpartum subclinical ketosis in dairy cow herd.

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INTRODUCTION

Leptin is an adipocyte-derived hormone not only with an important role in the central control of energy metabolism but also with many pleiotropic effects in different physiological system (Pérez-Pérez *et al.,* 2017). The hormone leptin is an important regulator of metabolic homeostasis, able to inhibit food intake and increase energy expenditure (D'Souza *et al.,* 2017). In dairy cattle, there are many studies on plasma leptin concentrations (Wylie *et al*., 2008; Dänicke *et al.,* 2018).

Subclinical ketosis (SK) is a common metabolic disorder of transition dairy cows during the early lactation period (Kaya *et al.,* 2016; Zhang *et al.,* 2016). The development of ketosis is associated with malfunction in the regulation of energy metabolism with excessive negative energy balance for long periods of time (Shin *et al.*, 2015; Jeong *et al*., 2018).

Previous studies have reported plasma leptin concentration and its dynamic characteristics around parturition in dairy cows (Holtenius *et al.,* 2003; Liefers *et al.,* 2003). However, information regarding dynamic changes of leptin and ketone body (KB) concentrations around parturition in cows with SK is very limited, and the relationship between leptin and KB concentrations in dairy cows with ketosis is particularly unclear. The current study was conducted to improve our understanding of the relationship between blood leptin concentration and the development of subclinical ketosis.

MATERIALS AND METHODS

Dairy cows: The experimental procedures and care of animals were in accordance with the guidelines of the Animal Care & Welfare Committee of Guangxi University. Fifteen Holstein dairy cows were randomly chosen from the Jinguang Dairy Cattle Experimental Farm of Guangxi University (in Nanning, China). All experimental animals were at second parity, housed in tie-stall barns when feeding. They were fed with the same diet keeping the same environmental and managerial conditions.

Sample collection: Blood samples were collected in the morning before milking and feeding. Blood was then centrifuged on site (750×g for 10min) to separate plasma. All blood plasma samples were kept on ice and were sent to our laboratory for measurements or were stored at -20ºC prior to leptin measurement.

Blood samples were collected at 1 wk prepartum, on the day of calving (Wk 0) and then once per wk for eight wks. The experimental cows were categorized into the sub-clinical ketosis (SK) and no sub-clinical ketosis (NSK) by the ketosis diagnostic criteria of ketone bodies above 1.722 mmol/L (10 mg/dL) (Li *et al.*, 2016).

Measurements of blood plasma leptin, ketone body, glucose and lipid concentrations: Plasma leptin concentration was measured with a double-antibody radioimmunoassay according to the method described earlier (Cheng *et al.*, 2007). The inter- and intra-assay coefficients of variation (CV) were 9 and 6% and the sensitivity was 0.2 ng/mL for leptin. Plasma KB, including acetone, acetoacetate and β-hydroxybutyrate, was measured by salicylaldehyde spectrophotometry (Li *et al.*, 2016). Plasma glucose concentrations were measured by enzymatic colorimetric assays. While plasma lipid concentration was measured using a phosphovanillin spectrophotometry method (Frings and Dunn, 1970). The inter-assay CV was ≤9.7% and the intra-assay CV was ≤5.1% for all measurements except leptin.

Measurements of body condition score and backfat thickness: Body condition score (BCS) was measured as a method of Edmonson *et al.* (1989). Backfat thickness (BFT) is the thickness of subcutaneous fat layer. Measurements of BCS and BFT were implemented at 7±3 days before calving.

Statistical analyses: SPSS statistics software was used in this study, one-way repeated measure analysis of variance was used to determine significant differences. The dynamic linear correlations were analyzed as Pearson Correlation coefficients. The Mann-Whitney test was used for BCS and the Student's *t*-test was used for BFT in SK and NSK Cows. Differences were considered significant when P<0.05.

RESULTS

Among 15 cows used in this study, none developed obvious symptoms related to ketosis, but 4 cows had KB above 1.722mmol/L at least once and were then classified into the SK group according to the diagnostic criteria as stated earlier. The other 11 cows had KB below 1.722mmol/L at all measurement time points and were classified into the NSK group. The incidence of SK was 26.7% among all experimental animals. During the experimental period, the daily milk yields per cow in SK and NSK cows were 20.1±2.4 and 21.1±1.6 kg, respectively, while SK cows consumed 1.1 kg less concentrate per cow per day than NSK cows. Notably, milk yield increased 45.2% from wk one to wk four postpartum in SK cows, which was associated with the onset of SK, while milk production increased only 13.9% in NSK cows in the same time period. In the first 10 days after the onset of SK, the SK cows produced 0.66 kg less milk per cow per day than NSK cows.

Leptin concentration in SK and NSK animals: Plasma leptin concentrations differed between SK and NSK cows in the entire experimental period as shown in Fig.1A. The average plasma leptin concentrations in SK and NSK cows were 0.73±0.6 and 2.15±0.46 ug/L, respectively, and there was a significant difference between two groups (P<0.001). At one-wk prepartum, leptin concentrations for the four cows which eventually developed SK were either undetectable or very low, resulting in a mean leptin concentration of 0.06 ug/L, which is 27.8 fold lower (P<0.001) than the mean leptin level in the 11 cows that did not develop SK (Fig. 1A). In the dynamic changes of plasma leptin concentrations, SK cows had a long-time wave from wk 0 to wk 7 postpartum, then jumped up to the peak, while NSK cows had several smaller waves during the same time (Fig. 1A).

Ketone body, glucose and lipid concentrations in SK and NSK cows: Along with changes in leptin concentrations, plasma KB also showed significant differences between NSK and SK cows. The average plasma KB concentration was very significantly higher in SK cows than that in NSK cows (0.98±0.44 *vs* 0.38±0.07 mmol/L, P<0.001). Notably, the KB concentrations were not significantly different between NSK and SK animals at one wk prepartum (Fig. 1B). However, KB levels increased steadily in the first four wks after parturition in SK animals. In contrast, the KB levels of the NSK animals increased only slightly at calving, and then maintained a lower level during the early lactation period. As a result, the SK animals had KB levels significantly higher than those of the NSK animals at 0, 2, 3, 4, 5, 6 and 8 wks postpartum. The peak of average plasma concentration occurred at four wks postpartum in the current study (Fig. 1B).

The average plasma glucose concentrations during the experiment in SK and NSK cows were 3.96±0.3 and 4,21±0.2 mmol/L, respectively, and there was a significant difference between them (P<0.01). The average plasma lipid concentrations in SK and NSK cows were 2.9±0.6 and 3.1±0.6 mmol/L, respectively, with no difference between the two groups. In the dynamic changes of plasma concentrations, unlike leptin and KB, glucose (Fig. 1C) and lipid (Fig. 1D) concentrations showed no consistent changes during the entire experimental period. Plasma glucose concentration seemed to be slightly high at one wk before calving in both the SK and NSK animals and then slightly reduced at the day of calving, but the decrease was minor and not significant (Fig. 1C). However, the plasma glucose concentration in the SK animals appeared lower than in the NSK animals at 0-4 wk postpartum, and there was a significant difference between two groups at 4 wks, although no difference was observed at any other time points. Plasma lipid levels seemed to be similar from one wk prepartum till three wks postpartum, and then increased slightly afterwards. These changes were not significant either (Fig. 1D). No difference in lipid levels was observed between SK and NSK animals at any stage of observation in the current study.

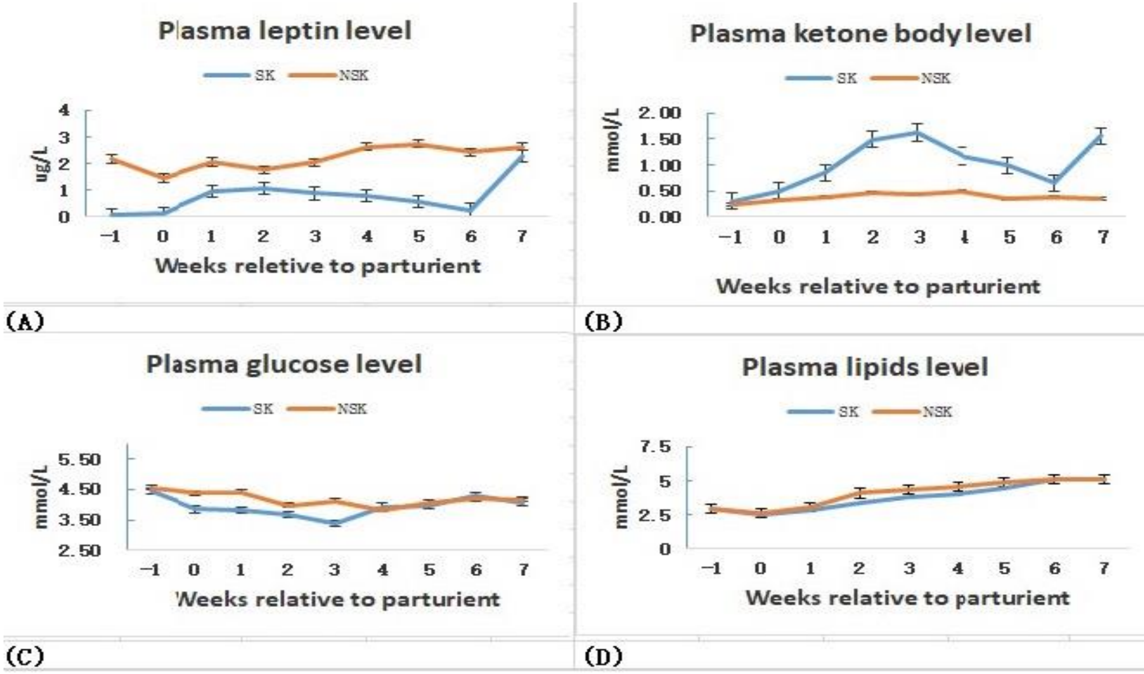

Fig. 1: Plasma dynamic levels of leptin, ketone body, glucose, and lipids. The effect of wk relation to parturition on plasma level of leptin (A), KB (B), glucose(C), and lipids(D) in dairy cows classified as suffering from sub-clinical ketosis (SK, n=4) on no sub-clinical ketosis (NSK, n=11). Data were presented as means with standard error.

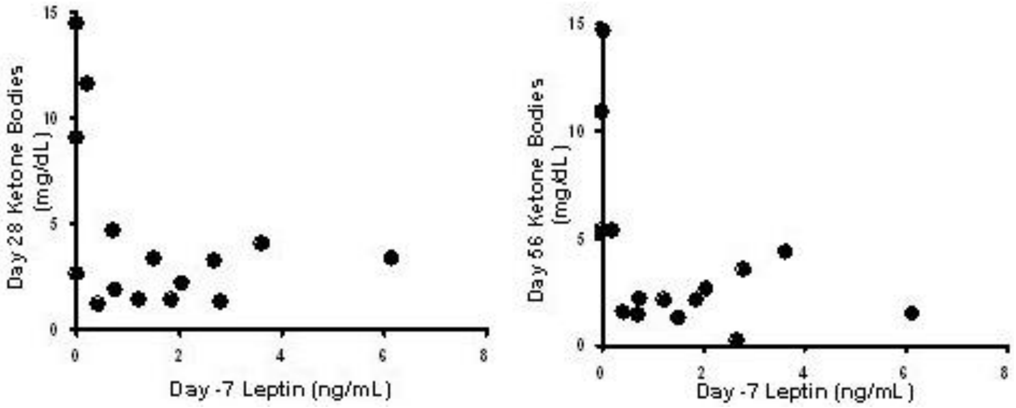

Fig. 2: Correlation between preparturition leptin and postpartum ketone body concentrations. Plasma leptin concentration measured at one wk preparturition (Day-7) showed consistent negative correlations with plasma KB concentrations measured at 4 (Day 28, r=-0.5190, P<0.0474) and 8 (Day 56, r=-0.4352, P<0.1050) wks postpartum. Negative correlations were also obvious between preparturition leptin and postparturition KB measured at other time points.

Correlation between leptin and ketone body concentrations: In Table 1, it has been shown that positive correlations existed between postpartum leptin and preparturition leptin concentrations, the positive correlations also existed between postpartum KB concentrations and preparturition KB in Table 2. It was revealed negative correlations existed between postpartum leptin concentrations and preparturition KB concentrations except wk 2 in Table 1. The negative correlations also existed between postpartum KB concentrations and preparturition leptin concentrations in Table 2, the higher negative correlations among them appeared wk 4 and wk 8 showed as Fig. 2.

Body condition score and backfat thickness in SK and NSK cows: All 15 cows in the present study had an average BCS of 2.7±0.3 at one wk prepartum, and there was no significant difference between SK and NSK cows. The average BFT at one wk prepartum was 4.2±0.4 mm, and there was a significant difference between SK and NSK cows (3.7±0.1 *vs* 4.4±0.4 mm, P<0.01).

DISCUSSION

A unique feature of our current study is that we investigated a natural herd of dairy cows without experimental manipulation before or after the study. The herd has an average BCS of 2.66±0.29 at 1 wk prepartum which is very low in comparison with other studies such as 3.1-3.3 (Abuajamieh *et al.*, 2016), 3.5-3.7 (Shin *et al.*, 2015) and 3.4-3.5 (Gillund *et al.*, 2001). The average BFT of 4.2±0.2 mm prepartum is also lower than 4.9-6.1 mm from (Vandehaar *et al.*, 1999), >6 mm from (Reist *et al.*, 2003), and 5.4 mm from (Carriquiry *et al.*, 2009). It is clear that the cows in the present study were much leaner than those reported by others as referred above.

Table 1: Correlation coefficients of postpartum leptin concentrations with leptin and ketone body concentrations measured at prepartum

Post-partum period (week)	Preparturition leptin	P value	Preparturition KB	P value
0	0.7081	0.0031	-0.3201	0.2447
1	0.5542	0.0321	-0.0218	0.9384
2	0.6469	0.0091	0.0834	0.7677
3	0.0961	0.7334	-0.0667	0.8132
4	0.2715	0.3277	-0.3528	0.1922
5	0.4094	0.1296	-0.3903	0.1503
6	0.3728	0.1711	-0.4580	0.0860
7	0.5010	0.0571	-0.5146	0.0497
8	0.0718	0.7993	-0.3723	0.1717

Preparturition measurements were made at 7±3 day before calving. Week 0 represents measurements made at the day of calving. Data shown are Pearson correlation coefficient from measurements of 15 cows. KB: Ketone body.

Table 2: Correlation coefficients of postpartum ketone body concentrations with leptin and ketone body concentrations measured at prepartum

Post-partum period (week)	Preparturition Leptin	P value	Preparturition KB	P value
0	-0.2513	0.3664	0.6315	0.0150
1	-0.1777	0.5264	0.4508	0.0917
2	-0.2283	0.4132	0.4846	0.0671
3	-0.3400	0.2150	0.3620	0.1849
4	-0.5190	0.0474	0.4006	0.1389
5	-0.1488	0.5967	0.1599	0.5692
6	-0.2380	0.3930	0.2682	0.3338
7	-0.1087	0.6998	0.2375	0.3490
8	-0.4352	0.1050	0.0111	0.9686

Preparturition measurements were made at 7±3 day before calving. Wk 0 represents measurements made at the day of calving. Data shown are Pearson correlation coefficient from measurement of 15 cows. KB: Ketone body.

In the present study, the dynamic changes of plasma leptin concentrations in SK and NSK cows were reported (Fig.1A), from which two points may be concluded. First, the leptin concentration of SK cows had a larger increase postpartum relative to its lowest level prepartum. It increased 17.6 times in the first wave from trough of 0.06ng/mL at one wk prepartum to the crest at 3 wks postpartum. This number increased 8.4 times in the 2nd wave from the trough of 0.27ng/mL at 7 wks to the crest at 8 wks postpartum, while the leptin level of NSK cows only showed a small increase postpartum relative to its lowest level (1.46 ng/mL) at calving day, no more than 2 times from trough to crest among all waves. After pondering this situation we analyzed the correlation between leptin and KB concentration in SK and NSK cows and found the correlative coefficient ($r=0.497$, $n=40$, $P<0.01$) was higher in SK cows than that ($r=0.251$, $n=110$, $P<0.05$) in NSK cows. From these results it may be suggested that the more the plasma leptin concentration increases from its lowest level before calving to the peak in early lactation stage in cows, the higher the KB concentration is, the higher the risk of ketosis is. Second, the fewer waves in dynamical change of leptin level in SK cows during test suggested another abnormal characteristic of plasma leptin hormone secretion. The reason for this is probably related to the over-mobilization of body fat for long periods of time. Because of the sustaining higher energy demand for lactation in the early lactation stage, the body of SK cows needs to produce more leptin relative to itself for a long time, so as to accelerate energy supply by adipolysis, thus body fat becomes over-mobilized, so ketosis occurred. In NSK cows, leptin concentration showed more fluctuations in the same period.

In the present study, mean leptin concentration decreased from one wk before calving and reached the lowest level (1.46 ng/mL) at calving day, stayed lower at wk one (1.55 ng/mL) and then increased and maintained relatively normal levels for the following wks in NSK cows. This dynamic change of leptin concentration in NSK cows is consistent with changes (Block *et al*., 2001; Holtenius *et al*., 2003). However, the dynamic changes of leptin level were different in SK animals, in which the leptin level increased very largely from its lowest level around calving, even so it was still lower than that of the NSK cows at the same time. Our results are in accordance with those reported by Kerestes *et al.* (2009), in which the normoketonemic cows had higher plasma leptin levels than hyperketonemic cows during the test. However, the leptin levels of hyperketonemic cows did not increase after calving, which is different from our results, and this difference may have been caused by the unequal distribution of sampling time points. The leptin dynamic change of SK cows in our study is very similar to that in primiparous cows with BCS<3 (Meikle *et al.*, 2004). This is probably related to the fact that both studies have cows with similar parities (2 *vs* 1) and BCS of less than 3, especially with higher NEFA and KB levels. From our results, it is obvious that cows which eventually developed SK had a lowest leptin level at one wk prepartum and retained it until to calving, then it increased very largely relative to its the lowest value. In comparison, cows that did not develop SK had normal levels of leptin in blood plasma at late gestation and these normal leptin levels continued into early lactation.

In this study, we analyzed the relationship between plasma leptin and KB concentrations during the experimental time period around calving. It is of interest to note that leptin concentration measured at one wk prepartum was positively correlated with leptin concentrations measured at calving as well as during the first eight wks of lactation, although the correlation started strong and significant at wks 0, 1, and 2, and then weakened over time. As opposed to this, leptin concentrations from wk 0 to wk 8 were negatively correlated with prepartum KB concentration, with the correlation reaching a significant level at wk 7 (Table 1). These correlations indicate that leptin concentration in lactating cows could be positively associated with leptin levels during late gestation and could be negatively related to KB levels during late gestation.

An important finding from our current study is the negative correlation between prepartum leptin concentration and postpartum KB concentration in our experimental animals. The negative relation was consistent from wk 0 to wk 8 of which the strongest correlations occurred at wks 4 ($r=-0.51$, $P=0.047$) and wk 8 ($r=-0.44$, $P=0.105$, Fig. 2). At 4 wks postpartum, plasma KB concentration can be predicted by prepartum leptin concentration as follows: Ketone-Wk 4 (mg/dL) = 5.7900 – 0.8916 × Prepartum Leptin (ng/mL). Findings of positive correlations between pre- and post-partum KB concentrations were expected and would be easily understandable: animals with higher KB levels during late gestation may have a higher probability to have high KB levels during early lactation with a higher risk for the development of clinical or sub-clinical ketosis. Still, the negative correlations between prepartum leptin and postpartum KB levels we found in our current study may also provide us with a potential prognostic measure for ketosis in lactating animals.

The average leptin concentration at one wk prepartum was 1.6 ±1.7 ug/L in all cows in our study. This is lower than those reported by others (Kokkonen *et al.,* 2005; Kerestes *et al.,* 2009; Loiselle *et al.,* 2009). We further analyzed the relationships between prepartum leptin concentration and BCS and BFT, and found that there was a significant correlation between leptin and BFT (r=0.56, P=0.044), but not between leptin and BCS (r=0.15, P=0.06). Our results support the fact that plasma leptin concentration is related to body fatness (Ingvartsen and Boisclair, 2001), or that plasma leptin reflects primarily differences in body fatness (Chilliard *et al.,* 2005). Our findings, however, differ from the generally accepted theory. i.e. ketotic cows had higher BCS at calving and during the first wks postpartum than healthy cows (Gillund *et al.,* 2001; Kokkonen *et al.,* 2005).

Our current study showed a different case in which the SK cows had lower BFT and lower leptin levels prepartum than NSK cows. It is suggested that the extremely lean cows may also be more susceptible to lipid mobilization in the early lactation than normal cows, which may be related to the development of ketosis in underfeeding cows (lean *et al*., 1992).

So, we presume that a cow too fatty or too lean will be more susceptible to ketosis comparing with normal one. Being fatty or lean is only a predisposing factor whereas, the actual factor is excessive mobility of lipids that sets the stage for SK development.

Conclusions: Leptin dynamic abnormal is associated with development of subclinical ketosis. The hyperketonemia is also easy to appear in lean cows, but the most important etiology is the body lipid over-mobilization that may be regulated by leptin, not the lipid deposition. The hypoleptinemia prepartum may provide us with a potential prognostic measure for ketosis in early lactation period.

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Authors contribution: BH performed design, data analysis, manuscript writing and revising. XD carried out samples collection, trials implementation, data analysis and manuscript writing. YD performed statistical analysis. QH and MAM wrote manuscript. All authors approved final version of the manuscript.

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