【Original Article】

A study on the clinical application of insulin sensitivity tests on late pregnancy dairy cows and adaptability probability to predictively diagnose the development of peripartum diseases.

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Summary

This study investigated insulin sensitivity in clinically healthy dairy cows (n=32) in late pregnancy through insulin-stimulated glucose disposal rate (GDR value, %), and examined the associations between GDR value and peripartum diseases. GDR value obtained 10 days before expected calving from dairy cows which developed peripartum diseases(n=6) was 35.8 ± 7.4%, and the value from dairy cows which did not develop those diseases(n=26) was 46.1 ± 8.1%, showing a significantly lower value in the dairy cows that developed diseases (P<0.05). GDR value of cows in late pregnancy showed that the occurrence of disease increases with a decrease in GDR value, but the plasma biochemical components, which have been thought to be highly associated with the development of peripartum diseases in dairy cows, did not indicate obviously abnormal values in late pregnancy, even in the group where peripartum diseases dominantly occurred. In addition, in the analysis for predicting the development of disease, the odds ratio of GDR value was 8 and remarkably higher than those of plasma biochemical components. From these results, this study suggests that the insulin sensitivity test is very useful in predicting the development of peripartum disease after calving, and has significance in evaluating risks of peripartum and productive diseases.
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Key Words: Insulin sensitivity test, Dairy cow, Predictive diagnosis of peripartum diseases, Insulin-stimulated glucose disposal rate

Introduction

The incidence of cows suffering from peripartum diseases such as a hepatic lipidosis, displaced abomasum, ketosis, mastitis, placental retention and breeding disorders is extremely high in the periparturient period, and about 65% of illness in cows are reported to have developed during the periparturient period in Japan[1)]. The main causes of the diseases are considered to be the remarkable hyperlipidemia, hepatic fatty changes and energy shortage in the tissue cells induced by fat mobilization from the tissues based on the significant negative energy balances during from late pregnancy to early lactation[2)]. Recently, the reduction of insulin sensitivity suggests its involvement in the occurrence factor of peripartum diseases[3,4,5)], but there are really very few reports that specifically prove this.

On the other hand, in today's clinical human medicine, it has been revealed that insulin sensitivity level declines in diseases such as diabetes, hypertension, hyperlipidemia and obesity. Furthermore, many of the studies have reported recently that patients with insulin sensitivity

who have these diseases share a common genetic background. It has been revealed that patients with this shared background are predisposed to complications of these diseases[6,7].

However, many of the studies on insulin sensitivity profiles in dairy cows have focused on hepatic fatty changes[8,9] and liver function disorder[8] after calving, and no studies on the participation of insulin sensitivity to evaluate the development of peripartum diseases in dairy cows have been reported.

In this study, we compared the relationships among the results of insulin sensitivity, changes of plasma biochemical component value and development of peripartum diseases in dairy cows in late pregnancy, and we examined whether the results of insulin sensitivity are able to be applied to a predictive diagnosis of peripartum diseases.

Materials and Methods

Dairy Cows and Feeding

Thirty-two Holstein-Friesian cows in late pregnancy without clinical abnormality were used for this study. They were raised on four different farms in Maebashi City, Gunma Prefecture (age range: 3 to 8 years old), and calved between December 2004 and April 2005 (Table 1). The cows on each farm were given feed designed to satisfy the NRC 1998 version standard feed requirements. Cows in late pregnancy were given two- to three- kilograms of formula feed, consisting mainly of high-quality hay. Cows in their early lactation period were given the same formula feed, but the feed and several kinds of feed additives were adjusted depending on the yield of milk they produced.

Blood Samples and Biochemical Analysis

Blood samples were collected 10 and 30 days before expected calving, between 6 and 8 hours after feeding. Blood was taken from the jugular vein into a heparin-coated vacuum tube. Plasma was separated and stored at –40℃ until use.

Biochemical components of the plasma were measured using an automatic analyzer (HITACHI 7170, HITACHI, Tokyo, Japan) in order to determine aspartate aminotransferase (AST), gamma-glutamyl transferase (γ-GTP), total cholesterol (T-Ch), free fatty acid (FFA), beta-hydroxybutyric acid (BHB), blood glucose (GL), total protein (TP), blood urea nitrogen (BUN), and calcium (Ca).

Insulin Sensitivity (Tolerance) Test

The insulin sensitivity test was conducted according to previous reports 9）. Rapid acting insulin (Novolin R, Nobo Nordisk, Tokyo, Japan) was injected intravenously into the cows at 0.05 IU/kg 10 days before calving. Their venous blood was collected 30 minutes before and after the injection into vacuum tubes containing EDTA・NaF for blood glucose analysis.

The insulin-stimulated glucose disposal rate (GDR) was calculated according to the following equation: GDR (%) = (G0 – G1) / G0 x 100, where G0 and G1 are the blood glucose levels 30 minutes before and after the insulin injection, respectively.

Table1　Farms and Dairy Cows Used in Study

Farm	No. of Cows Raised	No. of Cows Delivered	No. of Cows Used in Study
OK Farm	64	9	8
SK Farm	55	12	12
OT Farm	65	5	5
AT Farm	72	10	7
Total		36	32

Statistical Analysis

Results are expressed as means ± SD. The statistical analysis of each biochemical component was conducted using analysis of variance (ANOVA) after assessing the normality of data in order to determine the differences among groups.

Statistical significance in differences between the two groups was determined by Student's t-test.

For analysis to predict the development of peripartum diseases, odds ratios[10,11] calculated from GDR value and the measurements of several biochemical components were compared. Also, the odds ratio was determined using the following equation: odds ratio = the incidence of the diseases against the number of cows predicted to develop peripartum diseases / the incidence of the diseases against the number of cows not predicted to develop peripartum diseases.

Results

The results of insulin sensitivity test obtained from all dairy cows 10 days before expected calving（before calving：0.3 ± 5.3 days） show that GDR ranged from 28.4 % to 60.2 % with the mean ± standard deviation of 44.1 ± 8.8%. Many of the tested cows did not have any clinically abnormal symptoms 30 days after calving, but 6 cows developed retained placenta, milk fever, and left displaced abomasums after calving (18.8%). Those 6 cows had a significantly lower GDR value than the 26 dairy cows which did not develop these diseases after calving, at 35.8 ± 7.4% and 46.1 ± 8.1%, respectively ($P < 0.05$).

All the studied cows were classified into four groups, with each group corresponding to a 10% range GDR value, and the occurrence of postpartum diseases between the groups after calving was compared (Table 2). The cows were divided into 4 groups based on GDR value; Group I (GDR value not less than 50 %, n = 10), Group II (40 – 49 %, n = 12), Group III (30 – 39%, n = 7), and Group IV (20 – 29%, n = 3), (Table 2).

GDR values and the incidence of postpartum diseases for each group are presented in Table 2. Group I had no incidence of the diseases. Group Ⅱ had an incidence in 3 out of 12 cows (8.3%), and Group Ⅲ had a disease incidence in 3 out of 7 cows (42.9%). Group Ⅳ had 2 cows out of 3 which developed diseases (66.7%).

Body weight and body condition score (BCS) were measured 10 days before expected calving. Body weight did not show any significant differences among groups, although average weight was the lowest in Group I, and it increased in order in Group III, II, and IV. BCS also did not show significant differences, but the average score was the lowest in Group I, and it increased in order in Group II, III, and IV (Table 2).

The plasma biochemical parameters at 30 and 10 days before expected calving are shown in Table 3.

Table 2 Body Weight, BCS and Incidence of Diseases in Each Group Classified According to GDR Value

Group	Range of GDR Value	No. of Cows	Body Weight (Kg)[1)]	BCS[1)]	Incidence of Postpartum Diseases	
					No. of Cows	Incidence (%)
Group Ⅰ	≧ 50%	10	728.4 ± 70.5	3.1 ± 0.4	0	0
Group Ⅱ	40-49%	12	792.7 ± 70.5	3.4 ± 0.5	1[2)]	8.3
Group Ⅲ	30-39%	7	765.4 ± 75.7	3.5 ± 0.5	3[3)]	42.9
Group Ⅳ	20-29%	3	802.7 ± 62.8	3.8 ± 0.3	2[4)]	66.7

1) Analysis of variance ($P > 0.05$)
2) Retained placenta
3) Milk fever (n=1) and left displaced abomasum (n=2)
4) Subclinical ketosis and left displaced abomasum（n=1), retained placenta (n=1)
BCS: body condition score

A significant difference between groups was not observed on either sampling date, although AST activities tended to be higher in Group Ⅳ, and FFA levels tended to be higher in Groups Ⅲ and Ⅳ.

The value of predictive decision or "predictive value"[11] to diagnose the occurrence of peripartum diseases was calculated as an odds ratio. The predictive value was obtained using a calculation expression computed by deducting one standard deviation value from the average value of GDR of the cows without the peripartum diseases, and the value was used as a normal borderline.

The predictive value calculated was 38.0%. The predictive values from plasma concentration of FFA, T-Ch and glucose were calculated with the equation, and their odds ratio was also determined (Table 4). The results showed that the odds ratio of GDR was 8, and remarkably higher than those of FFA, T-Ch and glucose.

Discussion

The significance of insulin in energy metabolism has been well recognized. Many of the metabolic diseases in dairy cows predominantly occur during the periparturient period, and most of them can be attributed to a disturbed energy metabolism. Thus it is important to understand the mechanisms of insulin secretion, insulin - receptor interactions, and their association with pathophysiology in dairy cows. However, there has been a paucity of studies in this field. In particular, few studies have been conducted to assess the relation between GDR value in late pregnancy and peripartum diseases. We examined the relationship among the incidence of peripartum diseases, GDR value, and plasma biochemical parameters of dairy cows in late pregnancy.

Clearly, an incidence of peripartum diseases increased in the opposite direction of reduction of GDR value, and especially, the incidence showed a higher value at 30% or less.

The plasma biochemical parameters did not show abnormal values even in the groups with a higher disease incidence. BCS, body weight, and plasma FFA tended to be higher in Groups III and IV compared to other groups. A negative association between insulin sensitivity and FFA

Table 3　Plasma Biochemical Parameters at 30 Days (d 30) and 10 Days (d 10) Before Calving (mean± s.d.)

		Group Ⅰ		Group Ⅱ		Group Ⅲ		Group Ⅳ		ANOVA (P Value)	
		d 30	d 10	d 30	d 10	d 30	d 10	d 30	d 10	d 30	d 10
AST	(IU/L)	64.7 ± 17.2	62.0 ± 17.3	55.8 ± 9.9	55.2 ± 8.1	52.0 ± 11.6	54.0 ± 10.2	80.3 ± 59.5	98.0 ± 92.3	0.19	0.13
γ-GTP	(IU/L)	25.6 ± 5.0	22.5 ± 3.0	24.9 ± 4.4	23.8 ± 5.2	21.8 ± 6.5	20.3 ± 7.0	26.0 ± 11.0	22.9 ± 9.0	0.58	0.66
T-Ch	(mg/dL)	103.9 ± 17.3	84.0 ± 15.8	121 ± 47.7	76.8 ± 12.1	90.7 ± 11.8	82.3 ± 19.2	109.7 ± 30.9	77.7 ± 16.0	0.29	0.70
FFA	(μEq/L)	132.8 ± 74.2	245.7 ± 195.7	155.3 ± 72.7	295.3 ± 236.1	136 ± 45.2	363.1 ± 268.8	161 ± 86.0	365.0 ± 271.9	0.84	0.75
BHB	(μmol/L)	481.5 ± 164.4	456.2 ± 124.5	403.5 ± 114.0	496.0 ± 123.2	474.6 ± 121.2	486 ± 94.5	358 ± 190.5	528.0 ± 53.3	0.42	0.76
GL	(mg/dL)	66.4 ± 5.2	65.5 ± 4.0	64.1 ± 4.9	62.7 ± 4.6	67.6 ± 6.7	64.7 ± 4.9	66.3 ± 6.7	65.3 ± 2.9	0.58	0.46
TP	(g/dL)	7.3 ± 0.4	7.1 ± 0.5	7.4 ± 0.4	6.9 ± 0.6	7.8 ± 0.9	7.3 ± 0.6	7.7 ± 0.5	7.3 ± 0.1	0.23	0.23
ALB	(g/dL)	3.6 ± 0.3	3.6 ± 0.2	3.7 ± 0.2	3.5 ± 0.3	3.9 ± 0.3	3.7 ± 0.2	3.4 ± 0.3	3.4 ± 0.4	0.06	0.25
BUN	(mg/dL)	9.1 ± 2.2	8.8 ± 3.5	8.3 ± 3.2	7.7 ± 3.2	9.9 ± 3.6	9.1 ± 2.7	8.0 ± 5.3	8.6 ± 0.6	0.74	0.75
Ca	(mg/dL)	9.1 ± 0.3	9.0 ± 0.3	9.0 ± 0.4	9.0 ± 0.4	9.2 ± 0.4	8.9 ± 0.3	9.0 ± 0.2	8.7 ± 0.1	0.81	0.66

Table 4　Odds Ratio of Postpartum Disease Incidence

	GDR	FFA	T-Ch	GL
Odds Ratio	8.0	1.1	1.1	0.52
95% CI [1)]	1.6 to 43.3	0.1 to 12.1	0.1 to 12.1	0.02 to 11.3
Predictive Value [2)]	38.0 (%)	475.2 (μ Eq/L)	64.3 (mg/dL)	59 (mg/dL)

1) confidence interval around the odds ratio　(Woolf Method)

2) GDR, T-Ch and GL：The average GDR of cows in late pregnancy - 1SD
FFA：The average GDR of cows in late pregnancy ＋ 1SD

value has been reported in dairy cows[12]. It has been reported, moreover, that swollen adipose cells stimulate production of TNF[13] and decrease the concentration of insulin receptors[8,15], which induces the decrease in insulin sensitivity. This study also suggests that higher FFA values and swollen adipose cells in Group III and Group IV may be related to the decrease in insulin sensitivity. The decline in insulin sensitivity of dairy cows has been reported in postpartum period fatty livers[8,9], disturbed liver function[8], fat cow syndrome[14], displaced abomasum[5], the raising of obese cows[15], and others. In addition, studies on pathogenesis of peripartum diseases during periparturient periods have reported the effects of insulin levels in tissue on impaired glucose metabolism[3] and the increase in serum TNF activity from dry periods to immediately postpartum[13].

Koiwa et al.[8], reported that all postpartum cows with liver disease which showed a decline in insulin sensitivity tended to be overweight during dry periods, and 91% of them had complications of displaced abomasum after calving. Our study also found that the development of peripartum diseases, such as retained placenta, milk fever, and left displaced abomasum, was higher in cows in Group Ⅳ which showed remarkably lower GDR values. Though it has not yet been generally accepted that a glucose or energy metabolic disorder is the main cause of the diseases, our study strongly suggests the importance of a decline in insulin sensitivity as one of the risk factors during periparturient periods.

Statistically significant difference was found between dairy cows with peripartum diseases after calving and those without the diseases after calving.

Recently, epidemiologic studies have reported the possibility of and risks for occurrence of diseases. One way to compare the occurrence of diseases in a relative manner is the use of odds ratio. The odds ratio is used to estimate the contribution ratio for the occurrence of diseases having a pathogeny of which the occurrence rate cannot be directly calculated[11]. Studies on predictive diagnosis of peripartum diseases have been conducted by applying odds ratios of serum lecithin cholesterol acyltransferase (LCAT) activity[10], but there are few studies that used odds ratios of insulin sensitivity (GDR value).

In this study, GDR value had an extremely higher odds ratio rate than that of the plasma biochemical components of this study. Therefore, it can be seen that the development of peripartum diseases increases with a decline in insulin sensitivity or GDR value.

From these results, this study suggests that the insulin sensitivity test is very useful in predicting the insulin sensitivity of peripartum disease after calving, and has significance in evaluating the risks of peripartum and productive diseases.

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References

1) Kohiruimaki M: Primary study on the introduction of immuno-profile test for the control of perinatal diseases in the dairy cow, Jpn. J. Large Anim. Clin. **29:** 58-71 2008 (in Japanese)
2) Hoedemaker M, Prange D, et al.: Peripartal propylene glycol supplementation and metabolism, animal health, fertility, and production in dairy cows, J. Dairy Sci. **87:** 2136-2145 2004
3) Bell AW, Burhans W, et al.: Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows, Proc. Nutr. Soc. **59:** 119-126 2000
4) Holtenius P: Hormonal regulation related to the development of fatty liver and ketosis, Acta Vet . Scand. **89:** 55-60 1993

5) Pravettoni D, Doll K, et al.: Insulin resistance and abomasal motility disorders in cows detected by use of abomasoldenal electromyography after surgical correction of left displaced abomasum.Am, J. Vet. Res. **65:** 1319-1324 2004
6) DeFronzo RA, Ferrannini E: Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease, Diabetes Care. **14:** 173-194 1991
7) Reaven G M: Role of insulin resistance in human disease, Diabetes. **37:** 1595-1607 1988
8) Koiwa M, Hatsugaya A, et al.: Relationship between the histological findings and insulin resistance in dairy cows, J. Vet. Clin. **45:** 787-793 1998 (in Japanese with English abstract)
9) Ohtsuka H, M. Koiwa M, et al.: Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver, J. vet. Med. Sci. **63:** 1021-1025 2001
10) Nakagawa-Ueta H, Katoh N: Monitoring of serum lecithin: cholesterol acyltransferase activity during the peripartum period for prediction of postparturient production diseases in dairy cows, Vet. Biochem. **37:** 17-22 2000 (in Japanese with English abstract)
11) Sibano K, Inoue M, et al.: Prediction of the Development of Fatty Liver using Lecithin: Cholesterol Acyltransferase Activities in Dairy Cows, J. Jpn. Vet. Med. Assoc. **58:** 815-819 2005 (in Japanese with English abstract)
12) Oikawa S, Oetzelt R: Decreased insulin response in dairy cows following a four-day fast to induce hepatic lipidosis, J. Dairy Sci. **89:** 2999-3005 2006
13) Ohtsuka H: Changes in serum TNF activity in dairy cows during peripartum periods, J . V. Clin. **48:** 735-740 2001 (in Japanese with English abstract)
14) Holtenius P: Hormonal regulation related to the development of fatty liver and ketosis, Acta Vet . Scand. **89:** 55-60 1993
15) Mccann JP, Reimers TJ：glucose response to exogenous insulin and kinetics of insulin metabolism in obese and lean heifers, J. Anim. Sci. **61:** 612-618 1985

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乳牛の妊娠末期におけるインスリン感受性試験の診断的意義と周産期病の発生予知診断への応用

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要　旨

臨床的に健康な妊娠末期乳牛 (n=32) のインスリン感受性をインスリン刺激糖低下率（GDR 値、%）から調査し、GDR 値と分娩後の周産期病との関連について検討した。分娩予定日 10 日前の周産期病発症牛群（n=6）の GDR 値は 35.8 ± 7.4%で、非周産期病発症牛群（n=26）の GDR 値 46.1 ± 8.1%より有意（P<0.05）に低値であった。GDR 値は、低下するに従って分娩後周産期病発症率が高値を示したが、乳牛の周産期病の発病との関連が高いとされている血漿生化学成分値では、周産期病が多発した牛群においても、明確な異常値を示さなかった。また、周産期病の発生予知を目的とした GDR のオッズ比は 8 で、血中生化学成分の各オッズ比と比較し高い値を示した。以上の成績から、乳牛の妊娠末期におけるインスリン感受性試験は、分娩後の周産期病発生の予知や、周産期病と生産病のリスク評価に有用であることが示唆された。

キーワード：インスリン感受性試験、乳牛、周産期病の予知診断、インスリン刺激糖低下率

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