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Insulin Dynamics in Transition Dairy Cows as Revealed by Intravenous Glucose Tolerance Testing

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Abstract: Glucose tolerance and tissue sensitivity to glucose are considered to be important factors controlling metabolism around parturition. The objective of this study was to estimate the changes of physiological response to Glucose Tolerance Test (GTT) in transition dairy cows and clarify the metabolic characteristics of various physiological states from late gestation to early lactation. GTT was conducted at day prior to expected date of parturition (phase I) and 5 and 30 days after parturition (phases II and III), using five multiparous Holstein-Friesian cows. The levels of glucose at phases I and II sharply increased and then gradually decreased after infusion but the level at phase II was still higher than the basal value at 30 min post-infusion. The level of glucose at phase III 30 min post-infusion was kept at 1.3 times basal level at the end of GTT. Plasma insulin levels at phase I sharply increased at 15 min and immediately decreased after 30 min post-infusion. At phase II, the decline of insulin after 15 min post-infusion was gradual while levels at 120 min were still higher than the basal one. After 15 min, the rise of insulin at phase III was lower than those observed in others. The clearance rate of plasma insulin at phase I was twice as high as those at phases II and III. These findings suggested that these differences were caused by low insulin sensitivity due to onset of lactation and these changes were necessary for high producing dairy cows to maintain glucose homeostasis in early lactation.

Key words: Transition dairy cow, glucose tolerance, insulin, negative energy balance, plasma, metabolic characteristics

INTRODUCTION

The period from 3 weeks prior to 3 weeks after parturition so called transition period is the most critical time in the management of dairy cows (Grummer, 1995). Cows experience many drastic changes in physiology and metabolism during the period and some cows that cannot keep up with the changes often succumb to peripartum diseases (McNamara, 1991; Kelton *et al*., 1998; Ingvartsen *et al*., 2003; Ingvartsen, 2006). The energy requirement for milk production often exceeds the level of dietary sources because of a decrease in dry matter intake that often occurs during the final week prior to calving (Bremmer *et al*., 1999). High producing cows in early lactation also enter a period of Negative Energy Balance (NEB) in the early postpartum period (Drackley, 1999; Doepel *et al*., 2002). The increased rate of lipolysis in undernutrition such as NEB, releases Free Fatty Acids (FFA) at a greater rate than that needed for energy metabolism. In ruminants, the rate of FFA synthesis in the liver and the entry of FFA into the liver are very low (Vernon, 2005). Nevertheless, a rapid increase in energy demand just after calving could cause a large entry of FFA into the liver to produce ketone bodies. Thus, dairy cows show lower level of plasma glucose and higher concentrations of plasma FFA and ketone bodies in early lactation (Harrison *et al*., 1990). Because of the role of adipose tissue in buffering the mammary gland from the external environment and for providing the optimum mix of metabolites, more specific research is needed.

Continued selection for higher milk production has been questioned on a number of counts. Selection has been widely associated with deleterious effects on health, fertility and welfare of cows (Pryce and Veerkamp, 2001). Consistent with this, the genetic improvement should consider any physiological criteria (e.g., the homeorhetic process) with milk production. There have been many reports investigating the homeorhetic process in transition cows using various methods. An intravenous

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Glucose Tolerance Test (GTT) is a useful method for assessing the ability of animals to maintain endocrine homeostasis (Subiyatno *et al*., 1996; Gabai *et al*., 2002). However in Japan, quantitative estimations on glucose tolerance during transition periods have not been described in high producing dairy cows. Thus, the purpose of this study is to establish which characteristics of glucose tolerance were altered by different metabolic status around parturition.

MATERIALS AND METHODS

About 5 Holstein-Friesian multiparous cows which experienced parturition 2.2±0.49 (mean±SE) times before were used in this experiment. Each delivery was performed without any kind of nursing care. They were isolated from the dry period until 7 days after parturition, thereafter introduced into free-stall facilities as milking cows. The total mixed ration based on alfalfa hay, ort hay, beet pulp, concentrated feed and silage was fed to the cows twice a day after milking and water was available *ad libitu*m. Cows were milked twice a day at 08:30 and 16:00 in the milking parlor.

At 20 days prior to expected date of parturition (phase I), 5th and 30th day after parturition (phases II and III), a GTT was performed. Because the gestation length of most cows was slightly shorter or longer than expected, the interval between the first GTT and actual date of parturition ranged from 13-28 days (mean: 20.0±2.5 days). All tests were conducted at approximately 13:00 h to minimize the effect of feeding Glucose [sterile 50% solution (wt/vol)] was injected via the udder vein at 300 mg $kg^{-1}BW^{0.75}$ over 3 min. Stress was avoided as much as possible; cows generally continued to ruminate and appeared relaxed during the test. Blood samples were 88 obtained from the udder vein on the opposite side at 0, 15, 30, 60 and 120 min after infusion. Zero time was set immediately before the start of glucose infusion.

Approximately, 5 mL of blood which was collected into a tube containing 10 mg sodium heparin was centrifuged (3,500 rpm, 15 min) immediately after collection and plasma was stored at -20°C until the analyses of glucose, FFA and insulin. The plasma concentrations of glucose and FFA were measured using a commercial kit (Glucose CII-Test Wako and NEFA C-Test Wako, Wako Pure Chemical Industries Ltd., Osaka, Japan). Plasma insulin levels were measured with time-resolved fluoro-immuno assay. Insulin assay was conducted as described previously (Takahashi *et al*., 2006). The insulin concentration was measured by competitive solid-phase immunoassay using Eu-labeled synthetic bovine insulin and polystyrene microtiter strips (Nalge Nunc Int., Tokyo, Japan) coated with anti-guinea pig γ-globulin. Intra- and inter-assay coefficients of variation were 4.7 and 5.5%, respectively. Least detectable dose in this assay system was 0.133 ng mL^{-1}. Insulin clearance rates (k) are the fractional turnover rate of plasma insulin as determined using the following equation (Kaneko, 1997):

$$k\ (\%\ min^{-1}) = \frac{\{(\ln[t_1]-\ln[t_2])}{(t_2-t_1)\}} \times 100$$

Where:

$[t_1]$ = The concentration of plasma insulin at time 1 (t_1)
$[t_2]$ = The concentration of plasma insulin at time 2 (t_2)

Statistical analyses of the differences among the measurements were done using the Tukey-Kramer test. All data were analyzed using the commercial package Statview (Version 5, SAS Institute, Cary, USA, 1998). Differences were considered to be significant when the value $p<0.05$.

RESULTS AND DISCUSSION

Multiparous Holstein cows in this study produced an average 10,322 kg of milk for the year. Body weight measurements indicated a linear decrease from a high of 747.5 kg on phase I to 663.7 kg on phase II and 642.5 kg on phase III. The concentrations of plasma glucose, FFA and insulin and the ratio of insulin to glucose before glucose infusion (zero time) in transition cows are shown in Table 1. Dairy cows showed NEB just after parturition (Bertics *et al*., 1992) and sympathicotonia response on cows in NEB remained until the 10th day after parturition (Terao *et al*., 2008). In response to this condition, early lactation cows mobilized body fat, increasing plasma FFA concentrations. As expected, plasma glucose concentration at phase II was significantly lower than that at phase I ($p<0.05$) and the level of plasma FFA at phase II was twice as high as those at phases I and III ($p<0.05$).

These results showed that cows at phase II appeared to be in NEB. The ratio of insulin to glucose at phase III was higher than that at phase I ($p<0.05$) but the ratio did not have significant difference at each time after glucose

Table 1: Plasma glucose, FFA and insulin concentration and the ratio of insulin to glucose before glucose infusion in an intravenous glucose tolerance test around parturition (Mean±SE)

	Phase		
Parameters	I	II	III
Glucose (mg dL^{-1})	58.4±3.5^{a}	40.3±4.5^{b}	34.2±5.2^{b}
FFA (μmol L^{-1})	112.5±23.8^{b}	263.9±47.9^{a}	130.7±24.6^{b}
Insulin (μIU mL^{-1})	50.4±6.3	39.0±11.3	51.7±8.8
Insulin: glucose (IU mol^{-1})	15.6±1.8^{b}	16.9±3.9^{a,b}	27.6±2.5^{a}

^{a,b}Means within a row with different superscripts differ ($p<0.05$), (I) 20 days prior to expected date of parturition. (II) 5th day after parturition. (III), 30th day after parturition

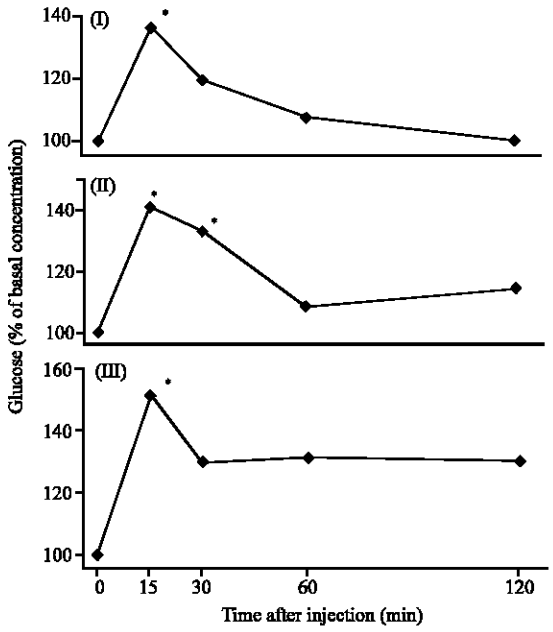

Fig. 1: Percentage of basal plasma glucose concentration in an intravenous glucose tolerance test around parturition. I) 20 days prior to expected date of parturition. II) 5th day after parturition. III) 30th day after parturition. All data were showed as means±SE. Asterisks indicate means which are significantly different ($p<0.05$) as compared to preinfusion level

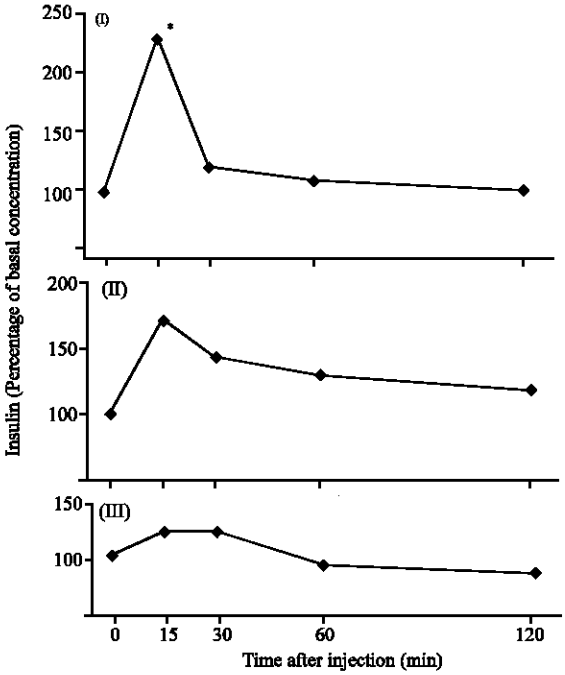

Fig. 2: Percentage of basal plasma insulin concentration in an intravenous glucose tolerance test around parturition. I) 20 days prior to expected date of parturition. II) 5th day after parturition. III) 30th day after parturition. All data were showed as means±SE. Asterisks indicate means which are significantly different ($p<0.05$) as compared to preinfusion level

infusion (data not shown). The ratio of insulin to glucose can be considered a crude index of tissue sensitivity to insulin (Subiyato *et al*., 1996). At phase III, plasma glucose concentration before glucose infusion was also lower than phase I ($p<0.05$) due to the large amount of milk production. These results indicated that low insulin sensitivity in peripheral tissues during early lactation was due to shunting glucose toward lactose synthesis (Bell and Bauman, 1997).

Figure 1 shows the relative levels of plasma glucose in transition cows measured by GTT. Plasma glucose levels at each phase changed with time ($p<0.001$) following intravenous glucose infusion during GTT. Although, the levels of glucose at phases and II sharply increased ($p<0.05$) and then gradually decreased after infusion, the patterns of the change were different between phases: glucose level at phase II was still higher than the basal one at 30 min post-infusion ($p<0.05$). It is likely that this response to GTT at phase II might be due to glucose intolerance caused by metabolic changes caused by parturition and onset of lactation. Glucose intolerance is a symptom of insulin resistance that is related to impaired insulin action at the receptor or postreceptor levels (Kahn, 1978) and caused by gradual decline in insulin concentration around parturition (Doepel *et al*., 2002). In ruminants, the regulation of insulin secretion differs from that in nonruminants wherein short-chain fatty acids at supraphysiological concentrations are more potent stimulators of insulin secretion than is glucose (Brockman, 1995). It was suggested that elevated FFA concentration during lactation might interfere with glucose-induced insulin secretion (Bossaert *et al*., 2008). Therefore, it has been reported that elevated circulating FFA levels is one of the factors that may account for the impaired hepatic insulin extraction in nonruminants (Lewis *et al*., 2002). Thus, it is likely that NEB of particularly high FFA value observed during the periparturient period may be a key factor triggering low glucose tolerance in dairy cows.

The relative levels of plasma insulin in transition cows during GTT are shown in Fig. 2. Plasma insulin levels at phase I sharply increased at 15 min ($p<0.05$) and immediately decreased at 30 min post-infusion. Also, the

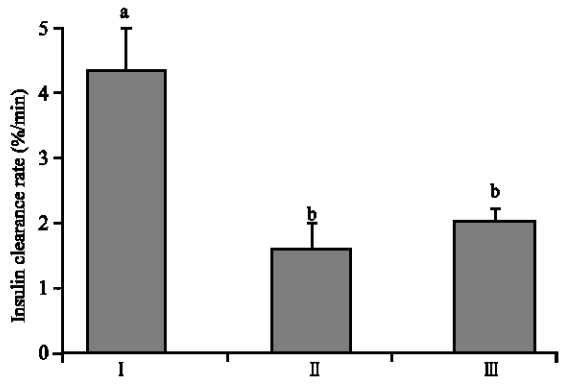

Fig. 3: Insulin clearance rate for the first 30 min after glucose infusion in an intravenous glucose tolerance test around parturition. I) 20 days prior to expected date of parturition. II) 5th day after parturition. III) 30th day after parturition. All data were showed as means±SE. Different letters are significantly different at $p<0.05$

level at phase II increased after 15 min but not significantly different compared with the basal one. However, the decline pattern at 15 min post-infusion was gradual and the levels at 120 min were still higher than the basal ones. As for the pattern of change at phase III, the rise at 15 min was lower than others and registering little change. Holtenius *et al.* (2003) reported reduced insulin response to glucose infusion during the post partum period compared to the prepartum period. These results are in good agreement with the observations (Fig. 2). Taken together, the findings indicated that the responsiveness of insulin secretion to glucose and likewise insulin sensitivity in peripheral tissues, might be decreased during early lactation. Figure 3 shows insulin clearance rate for the first 30 min after glucose infusion in transition cows. The clearance rate of plasma insulin (%/min) at phase I was 4.3±0.7 and is twice as high as those at phases II and III ($p<0.05$). It was reported that insulin clearance rates had a tendency to decrease during the postpartum period (Bossaert *et al.*, 2008). At phase III, the ratio of insulin to glucose before glucose infusion was higher than at phase I (Table 1) and glucose level 1.3 times the basal level at the end of GTT. It is hypothesized that the reduced clearance of glucose observed at postpartum was due to a greater degree of insulin resistance which gave rise to more pronounced net lipolysis from the adipocytes (Holtenius *et al.*, 2003; Pires *et al.*, 2007). These qualitative changes in metabolism occurring during lactation are necessary to support a homeostatic state (Bauman and Currie, 1980; Lomax *et al.*, 1979; Hayirli, 2006).

CONCLUSION

In the present study, the characteristics of glucose tolerance in transition dairy cows were clarified. The differences caused by high FFA concentration and low insulin sensitivity at the onset of lactation were necessary for high producing dairy cows to maintain glucose homeostasis in early lactation. In order to use this information in the management of transition dairy cows in avoiding metabolic diseases such as ketosis, further research is necessary to shed more light on the relationship between glucose and lipid metabolism.

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