Acta Veterinaria (Beograd), Vol. 46. No. 5-6, 245–254, 1996.

UDK 619:612.018.2:636.2

GLUCOSE TOLERANCE AND PROPIONATE LOADING TESTS IN THE ASSESSMENT OF ENDOCRINE PANCREATIC FUNCTION IN HEALTHY AND KETOTIC COWS

H. ŠAMANC*, J. ANNA NIKOLIĆ**, V. STOJIĆ*, R. ĐOKOVIIĆ*, Z. DAMNJANOVIĆ* and I. IVANOV*

*Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Yugoslavia ** INEP - Institute for the Application of Nuclear Energy, Zemun-Belgrade, Yugoslavia

(Received, 4 October 1996)

An attempt to assess the secretory capacity of endocrine pancreas beta-cells was made in healthy and ketotic cows by determination of glucose and insulin concentrations in the peripheral circulation during glucose tolerance and propionate loading tests.

Each test was carried out in the second week of lactation with twenty cows, of which ten were healthy and ten were exhibiting the first clinical signs of ketosis. In both tests mean initial blood glucose concentrations were higher in the healthy group of cows than in the ketotic group, while basal serum insulin concentrations did not show statistically significant differences between the respective groups.

Following intravenous loading with glucose the increment and rate of disappearance of glucose from the peripheral circulation was similar in the healthy and ketotic cows indicating similar degrees of glucose tolerance. However, the insulin response, while following the same dynamics, was significantly smaller in the group of ketotic cows as demonstrated by markedly lower serum insulin concentrations at 30 minutes ($P < 0.01$) and 60 minutes ($P < 0.05$) after glucose injection. Namely, assuming that liver insulin uptake was not higher in the ketotic group, it may be concluded that the glucose tolerance test can be used to detect reduced pancreatic secretory capacity in cows in the earliest phase of lactation.

After intravenous loading with sodium propionate there was a similar difference in serum insulin response between the control and ketotic groups of cows. Namely, the insulin increment 8 minutes after injection, taken to reflect the immediate pancreatic response, was statistically significant only in the group of healthy cows. Thereafter, insulin concentrations returned to the initial values to be followed by a second later increase which reflected the increase in glycemia. Since the glycemic response to intravenous propionate is a measure of liver gluconeogenic capacity, the late differences in insulin concentration between the healthy and ketotic cows reflect the differences in glucose concentration and are therefore primarily the result of differences in liver function, although a contribution of differences in glucagon secretion (pancreatic alpha-cell function) cannot be excluded.

Thus, the propionate loading test may provide information about both pancreatic beta- cell secretory capacity as well as liver function in cows.

Key words: propionate, ketosis, cows, insulinemia, glycemia

INTRODUCTION

Ketosis is a complicated disorder in the metabolism of carbohydrates and fats characterised by hypoglycemia, ketonemia and ketonuria. Many authors agree that the first metabolic alteration to appear in primary ketosis is hypoglycemia (Kronfeld, 1971; Bergman, 1973; Stamatović et al., 1983; Šamanc and Damjanović, 1987). This leads to a series of metabolic changes in the organism which are directed towards the securing of new sources of glucose and energy. Increased gluconeogenesis can supply more glucose but Krebs (1966) showed that large quantities of ketone bodies are also formed at the same time. However, the causes of the original hypoglycemia have not been clearly defined, although temporary hyperinsulinemia has been suggested as one of the primary etiological factors potentiating hypoglycemia (Kronfeld, 1971). Thus, Mitin and coworkers (1977) found higher concentrations of insulin in the blood serum of ketotic cows than in healthy cows. In contrast to this, other authors (Hove, 1978; Hove and Halse, 1978) observed low insulin levels in ketotic cows. Moreover, there was a combined state of hypoglycemia and hypoinsulinemia in almost all cows exhibiting ketosis at the beginning of lactation (Schwalm and Schultz, 1976), even though no differences in prepartal insulin, glucose, non-esterified fatty acid (NEFA), triacylglycerol and cholesterol concentrations were detected between cows which remained healthy and those which became ketotic after calving.

Hove (1978) monitored peripheral insulin concentrations after intravenous glucose administration to fed or starving healthy cows and to cows with ketosis. There was a marked increase in peripheral insulin concentrations only in the healthy fed cows which indicated that pancreatic beta cell secretory activity was decreased in ketotic cows.The lower responses to glucose loading found in ketotic cows by De Cupere and coworkers (1991) and Takeo and coworkers (1993) support this conclusion.

In ruminants it has been established that short-chain fatty acids like butyrate and propionate can directly stimulate pancreatic secretion of insulin (Horino et al., 1968; Bartoš et al., 1970; De Jong, 1982; Istasse and Ørskov, 1984; Mineo et al., 1990). This has led to the suggestion that propionate may contribute in the regulation of insulinemia under physiological conditions (Peters et al., 1983; Peters and Elliot, 1984).

The aim of the present investigation was to assess the secretory capacity of the beta-cells of the endocrine pancreas to release insulin after glucose or propionate loading and, on the basis of the results obtained in each test, to evaluate their functional state in healthy and ketotic cows in the same phase of lactation.

MATERIALS AND METHODS

Animals. A total of twenty healthy cows and twenty cows suffering from ketosis were chosen from a Holstein dairy herd. The diagnosis of ketosis was made on the basis of clinical symptoms (reduced appetite, rumen atony, behavioural changes) and urinary ketone concentrations above 17.2 mmol/1. Ketotic animals were included in the experiment 1-2 days after exhibiting clinical symptoms and before commencing medical treatment. The cows were of similar body weight (650kg), 4-6 years old, had had an average of 2.8 lactations with a mean milk yield of 7625 l (calculated over 305 days) in the previous lactation and were all in the earliest stage of lactation (7-14 days post partum).

Experimental procedure. Tests were carried out in the morning at 09 h about 3 h after feeding.

1. Glucose tolerance test. A total of 500 ml of 50% glucose solution (Zdravlje, Leskovac) was injected within 5 min. into one jugular vein of each animal. Blood samples (10 ml) were taken from the contralateral vein by puncture immediately before glucose administration and 30, 60, 120, 180 and 240 minutes thereafter.

2. Propionate loading test. Sodium propionate solution (1.84 mol/1; Zdravlje, Leskovac) was injected intravenously during 5 min. at the dose of 1 ml/kg body weight. Blood samples (10 ml) were taken from the opposite jugular vein before and 8, 30, 60, 120, 180, 240 and 480 minutes after the injection.

Portions of the blood samples were allowed to coagulate spontaneously at room temperature. The serum was then decanted, centrifuged at 3000 rpm and preserved at - 18^{0}C until analysed.

Assays. Glucose concentration was determined in fresh whole blood using Dextrostix tracks and the values read on an Eyeton Refractans colorimeter.

Serum concentrations of insulin were determined using a heterologous radioimmunoassay which included standard solutions of bovine insulin (Nikolić et al., 1989).

Statistical analysis. The mean values and standard deviations (SD) for each group of cows were calculated at each time interval. Differences between the group means within each treatment were evaluated after two-factor analysis of variance and estimation of the statistical significance by the least significant difference (LSD) test at probability levels of $P < 0,05$and $P < 0.01$.

RESULTS

Changes in glucose and insulin concentrations in the peripheral circulation of the cows given glucose intravenously are shown in Figures 1 and 2.

Initial blood glucose concentrations in the group of cows exhibiting ketosis (1.69 ± 0.35 mmol/l) were significantly lower than in the healthy cows (3.03 ± 0.51 mmol/l; $P < 0.01$; Figure 1). Although this difference appeared to be maintained at 30 minutes, there were wide variations within the group of healthy animals leading to non-homogeneity of variance between the two groups. Overall,

the increment in glucose concentrations and the rate of decline appeared to be similar in both groups of cows indicating that net rates of glucose utilisation did not differ between them. Mean values at the end of the experiment were still slightly higher than the initial values.

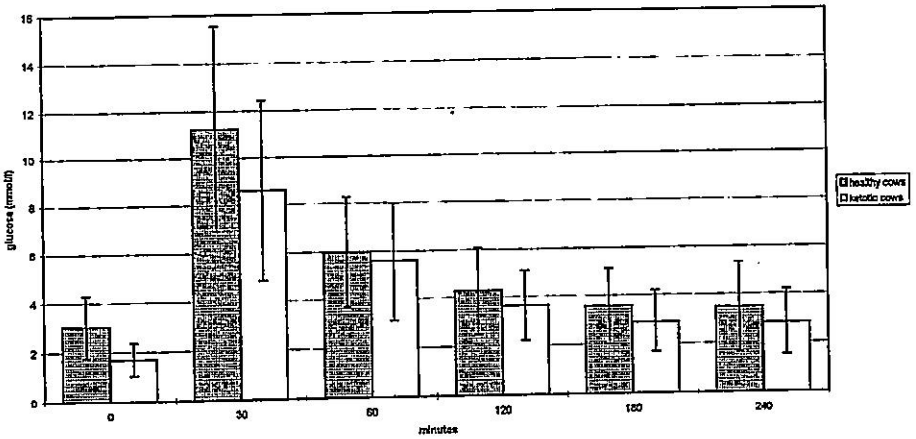

Figure 1. Blood glucose concentrations (mmol/l) in healthy and ketotic cows (n = 20) before and after intravenous administration of glucose solution.

Mean initial concentrations of serum insulin were slightly but not significantly lower in the ketotic group (10.7 ± 2.7 mIU/1) than in the control group of cows (14.7 ± 5.0 mIU/l) but there was a large difference in the mean response to glucose administration between the two groups (Figure 2). Namely, values

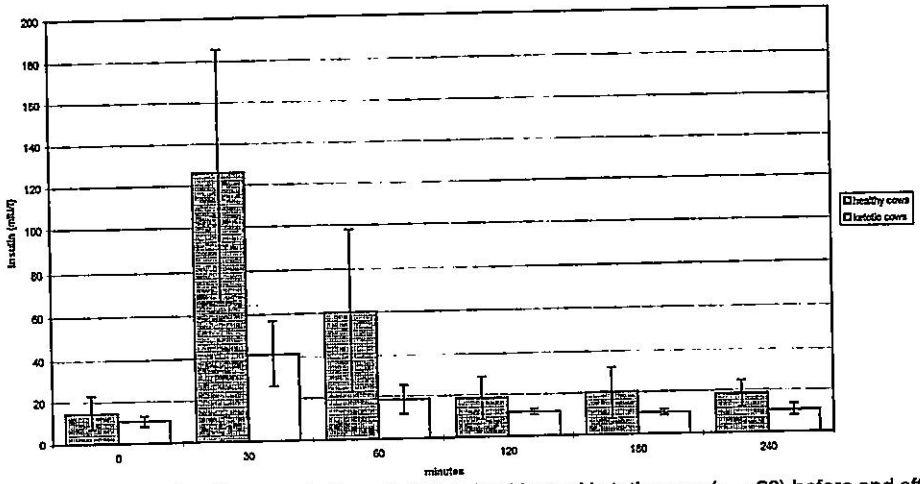

Figure 2. Serum insulin concentrations (mIU/l) in healthy and ketotic cows (n = 20) before and after intravenous administration of glucose solution.

increased to 126.0 ± 60.0 mIU/l at 30 minutes in the healthy cows compared with 41.5 ± 15.5 mIU/l in the ketotic cows ($P < 0.01$). This marked difference was maintained at 60 minutes after glucose administration ($P < 0.05$) but the insulin response was over at 120 minutes (Figure 2) in both groups of cows, which is similar to the dynamics observed in lactating ewes (Bassett, 1989).

The glycemic and insulinemic responses to intravenous propionate administration in healthy and ketotic cows are shown in Figures 3 and 4. As in the first experiment, initial blood glucose levels were lower in the ketotic (1.94 ± 0.30 mmol/l) than in the healthy group of cows (2.67 ± 0.29 mmol/l). Propionate injection led to a sustained increase in glycemia in the healthy cows (Figure 3) which peaked at 60 minutes and then slowly declined similarly to the pattern observed by Peters and Elliot (1984). In the group of cows with ketosis, mean blood glucose concentration was significantly greater than the respective initial value only at 8 minutes after propionate administration ($P < 0.05$; Figure 3). Blood glucose levels in these cows then decreased to levels significantly below those in the healthy cows, returning at the end of the experiment to concentrations below the normal physiological limit similar to the initial values (2.08 ± 0.67 mmol/l).

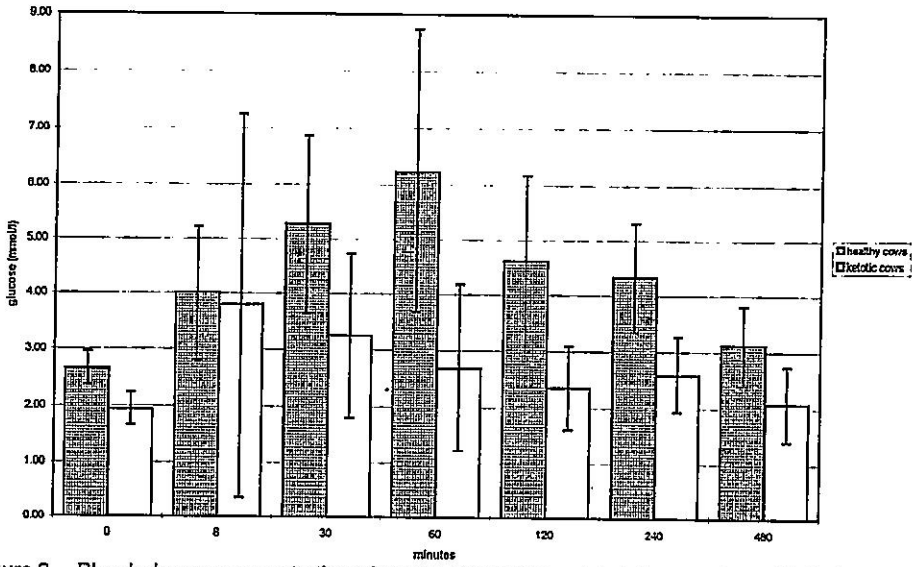

Figure 3. Blood glucose concentrations (mmol/l) in healthy and ketotic cows (n = 20) before and after intravenous administration of sodium propionate solution.

The insulin response to propionate was biphasic in both groups of cows (Figure 4) with the first maximum, probably due to the direct action of propionate on the endocrine pancreas, at 8 minutes after administration. The increase from 13.4 ± 2.6 to 21.4 ± 6.1 mIU/l was statistically significant in the healthy cows but not in the ketotic cows (12.1 ± 4.0 to 16.2 ± 5.4 mIU/l). After a return to initial levels at 30 minutes in both groups of cows, mean serum insulin concentrations

then showed a mild but continuous increase in the healthy cows up to 25.5 + 5.4 mIU/l at the end of the experiment (Figure 4). In the ketotic cows the later response cumulated with maximum insulin levels (18.8 + 4.2 mIU/l) at 120 minutes after propionate injection which was significantly higher than the initial value ($P < 0.05$). However, insulin concentrations in the ketotic cows then declined leading to statistically significant differences between them and the healthy cows at 240 and 480 minutes.

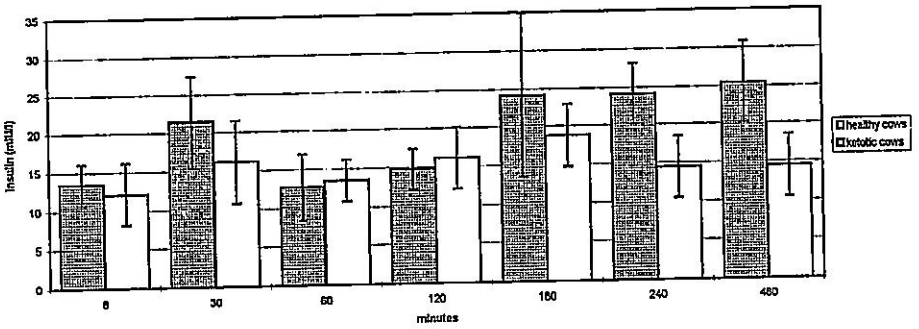

Figure 4. Serum insulin concentrations (mIU/l) in healthy and ketotic cows (n = 20) before and after intravenous administration of sodium propionate solution.

DISCUSSION

It is now established that glucose directly stimulates insulin secretion primarily by increasing the concentration of cytoplasmic calcium ions, the process depending on the ability of beta cells to transport into the cell and metabolise the sugar (Flatt et al., 1991). Other than mannose, some amino acids and short-chain fatty acids (in ruminants) which act directly, beta cell function may be enhanced by cholinergic stimulation, the neuropeptide cholecystokinin, glucagon and gut hormones such as gastric inhibitory peptide. Long term exposure to fatty acids, such as palmitate, inhibits the insulin secretory response, probably by decreasing the rate of intracellular glucose oxidation (Randle, 1995). These mechanisms may explain the lower insulin response to inravenous glucose in ketotic cows observed by Hove (1978), De Cupere and coworkers (1991), Takeo and coworkers (1993) and confirmed in this investigation. Namely, inappetance would lead to lower gut hormone and glucagon secretion, while mobilisation of fatty acids raises blood non-esterified fatty acid concentrations.

The fact that glucose levels after intravenous administration showed similar increments and similar rates of decline in healthy and ketotic cows indicates that the mean rate of glucose disposal was similar in both groups of animals. Thus, the pancreatic islets were subjected to almost identical glucose stimuli. Hence, assuming that insulin uptake in the liver of ketotic cows is not markedly greater than in healthy cows, it may be concluded that the glucose tolerance test

uncovers a refractory state in pancreatic beta cells to the action of glucose which is not apparent from the basal insulinemic status.

Concerning the propionate loading test, the results for insulinemia at 8 minutes after injection also reflect, most probably, the ability of the endocrine pancreas to respond to changes in peripheral blood propionate concentrations which was different in healthy and ketotic cows. Namely, significantly increased insulinemia was observed only in the healthy group of cows.

Originally the propionate loading test was developed to evaluate hepatic function because propionate is the major precursor of glucose in ruminants and the liver is the main site of gluconeogenesis and propionate metabolism (Elliot, 1980). The lower gluconeogenic effect of propionate observed here in the ketotic group of cows, as expressed by the reduced glycemic response, confirms earlier observations (Bruss et al., 1986; Šamanc et al., 1994).

The finding that propionate administration may also increase serum cortisol and glucagon concentrations (de Jong, 1982; Peters and Elliot, 1984; Šamanc et al., 1993) provides a further mechanism possibly involved in maintaining relatively high glycemia for a considerable time in the group of healthy cows. This probably acted as a continued stimulus for the well expressed late insulin response in these animals.

While the overall rate of propionate utilization in ketotic cows may be lower due to impaired liver function or Vitamin B_{12} deficiency (Corse and Elliot, 1970), the metabolic pathway to glucose may be additionally compromised by decreased glucagon secretion. Thus, it has been shown that hepatocytes from ruminating calves respond to glucagon by increasing gluconeogenesis from propionate while insulin has no effect (Donkin and Armentano 1995), but the increment of both counterregulatory hormones, glucagon and cortisol, is lower after intravenous propionate in ketotic ruminants than in healthy animals (Bruss et al., 1986; Šamanc et al., 1994). The lack of a prolonged glycemic response to propionate in the ketotic cows is most probably responsible for the later significant differences in insulinemia observed between the two groups after propionate injection. It may be concluded that the differences reflect differences primarily in liver function but also partly in pancreatic alpha cell and adrenocortical function. Hence, the propionate loading test carried out carefully may provide more information than the glucose tolerance test.

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PRIMENA TESTA OPTEREĆENJA GLUKOZOM I PROPIONATOM U OCENI ENDOKRINE FUNKCIJE PANKREASA KOD ZDRAVIH I OD KETOZE OBOLELIH KRAVA

H. ŠAMANC., J. ANA NIKOLIĆ., V. STOJIĆ., R. ĐOKOVIĆ., Z. DAMNJANOVIĆ I I. IVANOV

SADRŽAJ

Za procenu funkcionalnog stanja endokrinog pankreasa u lučenju insulina kod zdravih i od ketoze obolelih krava primenjen je test opterećenja glukozom i propionatom. Utvrđeno je da se test opterećenja sa glikozom može sa uspehom koristiti u otkrivanju smanjene sekretorne aktivnosti endokrinog pankreasa kod krava u ranoj laktaciji.

Test opterećenja sa propionatom, pored podataka o funkcionalnom stanju endokrinog pankreasa, pruža uvid i u funkcionalno stanje jetre u pogledu aktivnosti procesa glukoneogeneze kod zdravih i ketoznih krava.