Changes in Blood Values of Glucose, Insulin and Inorganic Phosphorus in Healthy and Ketotic Dairy Cows after Intravenous Infusion of Propionate Solution

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Abstract

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The aim of the present study was to determine the degree of blood glucose utilization by peripheral tissue on the basis of changes in blood concentrations of glucose, insulin and inorganic phosphorus in healthy (n = 10) and ketotic cows (n = 10) after intravenous infusion of propionate solution.

Blood samples were taken in both groups of examined cows at the following time intervals: just before (time 0) and 8, 30, 60, 120, 180, 240 and 480 min after the intravenous infusion of 1.84 mol·l^{-1}solution of propionate in the amount of 1ml·kg^{-1} of body weight. Glucose and insulin blood serum values in both groups of cows increased significantly within 120 min of the experiment ($p < 0.05$). Significantly lower values ($p < 0.05$) of glucose in blood of ketotic cows, compared to the blood value of glucose in healthy cows were established within 30, 60, 120 and 240 min of the experiment, as a consequence of the decreased gluconeogenic ability of the liver in the ketotic cows. Significantly lower values ($p < 0.05$) of insulin in blood of ketotic cows in comparison with healthy ones were established within 240 and 480 min of the test. That indicates that the ability of beta cells of the endocrine pancreas to release insulin is reduced in cows suffering from ketosis. After intravenous administration of propionate, it was established that values of inorganic phosphorus were reduced in blood in both groups of cows after 8, 30, 60, 120, 240 and 480 min. Within 480 min of the test there was a significant decrease ($p < 0.05$) in blood value of inorganic phosphorus in ketotic cows in comparison with healthy ones. This is linked with the active entry of glucose into glucolytic pathway of peripheral tissues. It can thus be concluded that there is a higher degree of blood glucose utilization by peripheral tissues in ketotic cows.

Gluconeogenesis, ketosis, glucose utilization, peripheral tissue

The optimal supply of liver and extrahepatic tissues with glucose has an important role in preserving the health of dairy cows in the early stage of lactation. Glucose concentration in blood of dairy cows in early lactation completely depends on the process of gluconeogenesis in the liver. If the degree of gluconeogenesis does not satisfy increased needs of glucose in dairy cows, the state of hypoglycaemia, ketonemia and ketonuria occurs (Young 1977).

The first metabolic change in primary ketosis in dairy cows in early lactation is hypoglycaemia. It causes serious metabolic changes in the cow's body, which are manifested through lipid mobilisation from body reserves and ketogenesis and lipogenesis in the liver (Bruss et al. 1986; Veenhuizen et al. 1991).

Propionate loading test in ruminants is used for estimating the intensity of gluconeogenesis in the liver, such as the ability of endocrine pancreas for synthesis and secretion of insulin (Gröhn 1985; Sano et al. 1993; Šamanc et al. 1996; Constable 2000). The ability of endocrine pancreas for insulin release after intravenous infusion of glucose is well known. The insulin concentration in blood is reduced in ketotic cows, compared to healthy animals

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before and after infusion of a glucose solution (Hove 1978; De Cupere et al. 1991; Šamanc et al. 1996). Similar results have been reported by other authors after intravenous infusion of propionate solution, because propionic acid directly stimulates pancreatic secretion of insulin in ruminants (Peters et al. 1983; Peters and Elliot 1984; Sano et al. 1993; Šamanc et al. 1996; Lee and Hossner 2002).

During the propionate loading test, it is very difficult to estimate on the basis of glycaemia whether the metabolic disorder was induced by liver disease or hypofunction of the endocrine pancreas. At the same time, an estimation of the concentration of inorganic phosphorus in blood can be helpful. Namely, the decrease of its concentration in blood after intravenous infusion of glucose is linked with the active utilization of glucose into the glycolytic pathway of peripheral tissue, especially muscles, while a considerably smaller amount of glucose is used for glycogenesis in the liver (Stojić 1993; Bringhurst et al. 2003).

The aim of the present study was to determine the degree of blood glucose utilization by peripheral tissues on the basis of changes in blood concentrations of glucose, insulin and inorganic phosphorus in healthy and ketotic cows after intravenous infusion of propionate solution.

Materials and Methods

Healthy cows (C; control group; n = 10) and ketotic cows (E; experimental group; n = 10) were chosen from a Holstein dairy herd. Ketosis was diagnosed on the basis of clinical symptoms (reduced appetite, rumen atonia, behavioural changes) and determined urinary ketone in high concentrations. The presence of ketone bodies in urine was examined by using Lestradet test (Rosenberger 1979; Kégl and Gaál 1992). Ketotic animals were included in the experiment 1 - 2 days after the occurrence of clinical symptoms and before commencing the medical treatment. Healthy cows did not show clinical symptoms of ketosis and urinary ketone bodies were not determined in those cows. The trial cows were kept in tie-up stalls in barn housing. Cows were of similar body mass (650 kg), 4 - 6 years old, an average of 2.8 lactations with a mean milk yield of 7,750 l (calculated over 305 days) in the previous lactation and were all in the earliest stage of lactation (7 - 14 days post partum). The meal was prepared to meet the energy needs of animals in early lactation. Both groups were fed the same diet consisting of 4 kg lucerne hay, 15 kg maize silage (30% DM), 8 kg lucerne haylage, 4 kg maize ear silage (68% DM), 2 kg dry sugar beet pulp, 2 kg extruded soybean grains, 4.5 kg concentrate (30% CP). The dietary nutrient content for dairy cows with milk production of 35 l is given in Table 1.

Table 1. Nutrient contents in daily ration for dairy cows with milk yield 35 litres

Dry matter (DM), kg	21.5
Net energy of lactation (NEL), MJ	153.2
Crude protein (CP), % DM	18.3
Rumen non-degradable protein (RUP), % CP	39.69
Fat, % DM	4.92
Fibre, % DM	17.2
Acid detergent fibre (ADF), % DM	22.6
Neutral detergent fibre (NDF), % DM	37.16

The test was carried out in the morning at 09:00 h, ca 3 h after feeding. A solution of sodium propionate (1.84 mol·l^{-1}; Zdravlje, Leskovac) was injected intravenously during 5 min into a jugular vein of each animal at the dose 1 ml·kg^{-1} body mass. Blood samples (10 ml) were taken from the opposite jugular vein before and 8, 30, 60, 120, 240 and 480 min after injection.

Portions of the blood samples were allowed to coagulate spontaneously at room temperature. The serum was then decanted, centrifuged and preserved at -18 °C until analysis.

Glucose concentrations were determined in fresh whole blood using Dextrostix tracks and the values were read on an Eyeton Refractans colorimeter. Blood serum concentrations of insulin were determined using heterologous radioimmunoassay (RIA method) which included standard solutions of bovine insulin (Nikolić et al. 1989). Blood serum concentrations of inorganic phosphorus were determined using the UV method (023200) reagent Serbolab (Serbia). Biochemical indicators in the blood serum were assayed at the laboratory of Institute for the Application of Nuclear Energy in Agriculture (INEP) Zemun.

The mean values and standard deviations (SD) for each group of cows were calculated at each time interval.

The differences between the group means within each treatment were evaluated by analysis of variance according to ANOVA procedure, and the statistical significance was estimated by the least significance difference (LSD) test at a probability levels of $p < 0.05$ and $p < 0.01$ (Microsoft STATISTICA ver.5.0, Stat. Soft. Inc. 1995).

Results

Changes in glucose, insulin and inorganic phosphorus values in the peripheral circulation of the cows given propionate intravenously are shown in Figs 1, 2 and 3.

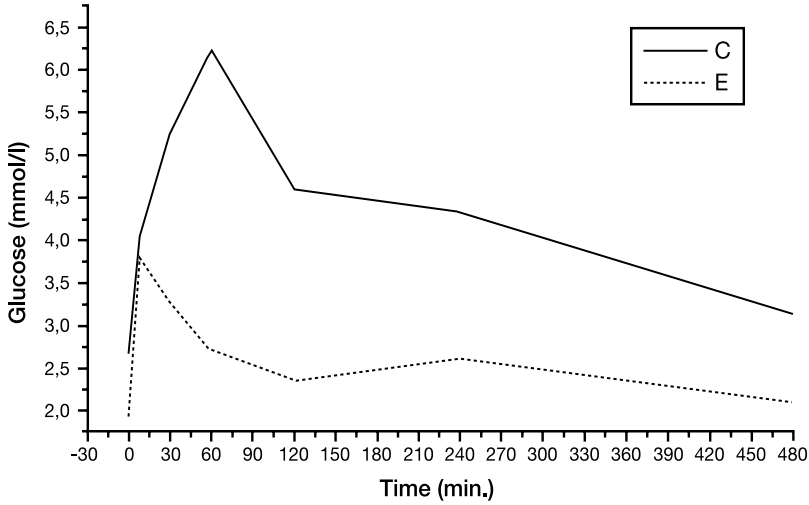

Fig. 1. Concentrations of glucose in blood serum (mmol·l^{-l}) of healthy (C - control group) and ketotic cows (E - experimental group) after intravenous infusion of sodium propionate solution.

The initial blood glucose values in healthy cows were 2.67 ± 0.29 mmol·l^{-1}, whereas in ketotic cows hypoglycaemia was determined (1.94 ± 0.30 mmol·l^{-1}; $p > 0.05$, Fig. 1). Propionate injection led to a significant increase ($p < 0.05$) in glycaemia in healthy cows within 8, 30, 60, 120 min of the experiment, which peaked within 60 minutes and then slowly declined (Fig. 1). In the group of cows with ketosis, mean blood glucose value was significantly higher than the respective initial value only within 8 minutes after propionate administration ($p < 0.05$, Fig. 1). Blood glucose value in these cows then decreased to values significantly ($p < 0.05$) below those in healthy cows within 30, 60, 120 and 240 min of the experiment, returning to values below the physiological limit at the end of the experiment (2.08 ± 0.67 mmol·l^{-1}).

The insulin response to propionate was biphasic in both groups of cows (Fig. 2) with the first maximum, probably due to the direct action of propionate on the endocrine pancreas, within 8 min after administration. The increase from 13.40 ± 2.68 to 21.44 ± 6.70 mIU·l^{-1} was statistically significant ($p < 0.05$) in healthy cows but not in ketotic cows (12.06 ± 4.02 to 16.08 ± 4.82 mIU·l^{-1}, $p > 0.05$). After a return to initial values within 30 min in both groups of cows, mean serum insulin values then showed a mild but continuous significant increase within 120, 240 and 480 min in healthy cows up to 25.46 ± 5.36 mIU·l^{-1} at the end of the experiment ($p < 0.01$; Fig. 2). In ketotic cows the later response cumulated with maximum insulin values (18.76 ± 4.52 mIU·l^{-1}) within 120 min after propionate injection which was significantly higher than the initial value ($p < 0.05$). However, insulin concentrations in ketotic cows then declined, leading to statistically significant differences ($p < 0.05$) between them and healthy cows within 240 and 480 min.

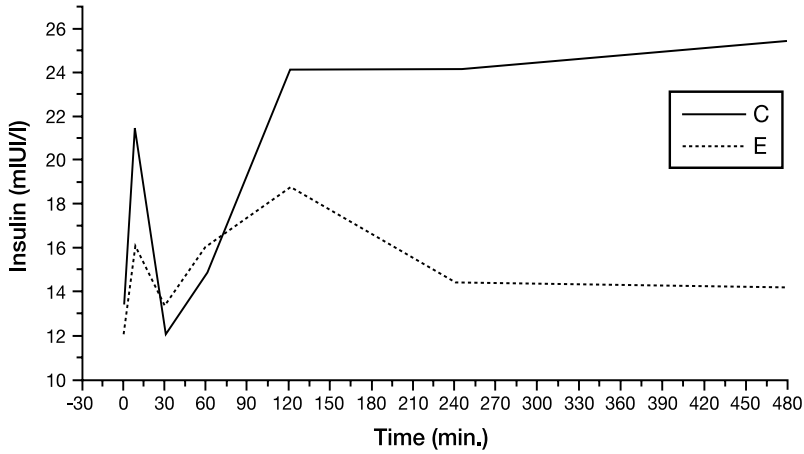

Fig. 2. Concentrations of insulin in blood serum (mIU·l^{-1}) of healthy (C - control group) and ketotic cows (E - experimental group) after intravenous infusion of propionate solution.

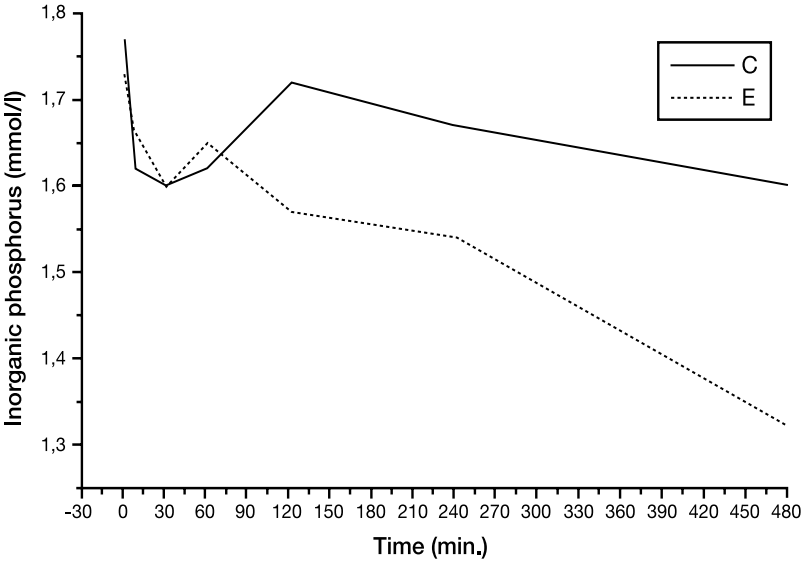

Fig. 3. Concentrations of inorganic phosphrus in blood serum (mmol·l^{-1}) of healthy (C - control group) and ketotic cows (E - experimental group) after intravenous infusion of propionate solution.

The inorganic phosphorus blood values in the healthy group of cows before infusion of propionate solution were 1.77 ± 0.15 mmol·l^{-1}, whereas they were 1.73 ± 0.29 mmol·l^{-1} in ketotic cows, although not significantly lower ($p > 0.05$). After intravenous infusion of propionate solution, a decrease of inorganic phosphorus value in the blood of healthy and ketotic cows was determined during the test period (Fig. 3). The blood value of inorganic phosphorus was determined as significantly lower ($p < 0.05$) within 480 min of the test period in ketotic cows.

Among the tested groups of cows, statistically significant differences of the values of inorganic phosphorus in the blood were determined within 480 min of the test period ($p < 0.05$; Fig. 3).

Discussion

The 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 sugar. Apart from mannose, some amino acids and short-chain fatty acids (in ruminants) that act directly, beta cell function may be enhanced by cholinergic stimulation, the neuropeptide cholecystokinin, glucagon and gut hormones such as gastric inhibitory peptide (Flatt et al. 1991).

Basically, the propionate loading test is used to estimate functional ability of the liver cells because propionate is a major precursor for glucose in ruminants and liver is the major site for gluconeogenesis and propionate metabolism (Bruss et al. 1986; Constable 2000).

After propionate infusion the significant increase of the concentration of glucose in blood occurs in both groups of cows, which clearly shows that propionate in the liver converts into glucose, which is in accordance with the results of other authors (Peters et al. 1983; Peters and Elliot 1984; Sano et al. 1993; Šamanc et al. 1996).

The initial blood glucose values in healthy cows were within the physiological range (2.2 - 4.0 $mmol \cdot l^{-1}$; Rosenberger 1979; Stamatović et al. 1983; Radostits et al. 2000; Stojić 2003). Hypoglycaemia determined in ketotic cows is explained as a decreased degree of gluconeogenesis in liver (fatty liver). Namely, the lipid infiltration of liver cells in cows in early lactation results in a significant decrease of its functional ability of glucose and glycogen synthesis (Gröhn 1985; Veenhuizen et al. 1991; Vázquez-Añón et al. 1994; Codorniga-Valino et al. 1997; Constable 2000). The statistically significant increase ($p < 0.05$) in blood insulin values in healthy and ketotic cows after the infusion of the propionate solution within 120 min of the test period compared to the starting values shows the ability of propionate to influence the synthesis and release of insulin from beta cells of endocrine pancreas. Furthermore, a significantly lower ($p < 0.05$) value of insulin was estimated in the blood of ketotic cows, compared to the healthy ones, within 240 and 480 min of the test. The low secretory responses of insulin in ketotic cows are therefore probably the result of a low insulin secretory capacity of the pancreas, developed during the days or weeks of hypoglycaemia that regularly accompanies high ketosis. The obtained results show the preserved function (relative insufficiency) of the beta cells of the endocrine pancreas of ketotic cows. Other authors have reported similar results in testing cows and sheep (Hove 1978; Peters et al. 1983; Peters and Elliot 1984; De Cupere et al. 1991; Sano et al. 1993; Šamanc et al. 1996; Lee and Hossner 2002).

The answer of insulin to the propionate infusion was biphasic in both groups of cows; the first maximum was probably the consequence of direct acting of propionate upon endocrine pancreas within 8 min of the test, after returning to the starting values, the concentrations of insulin in blood were increasing constantly, probably as a consequence of increased glycaemia.

In healthy cows, the greatest decrease of the value of inorganic phosphorus was determined within 480 min of the testing. It was 14.2% lower than the starting value, although not significantly lower ($p > 0.05$). On the other hand, the blood value of inorganic phosphorus within 480 min of the testing was determined as significantly lower ($p < 0.05$) in ketotic cows, which was 23.7% lower than the starting value.

The significant decrease ($p < 0.05$) of the value of inorganic phosphorus in the

blood of ketotic cows, compared to healthy ones, after 480 min of infusing propionate could be the sign of the increased usage of blood glucose by the peripheral tissue in ketotic cows as well as a significantly negative correlation ($r = -52$; $p < 0.05$) between concentrations of insulin and inorganic phosphorus in blood in the group of ketotic cows. The obtained results are in accordance with the statements of Stojić (1993) and Bringhurst et al. (2003).

In this experiment, it was determined that after 120 min of infusing the propionate solution, insulinaemia increased significantly in both groups of cows ($p < 0.05$). Thus the assumption that blood glucose synthesised by gluconeogenesis from propionate is used for energy purposes by peripheral tissue is confirmed. It can be concluded on the basis of this study that there is a higher degree of blood glucose utilization by peripheral tissues in ketotic cows.

Změny koncentrací glukózy, insulinu a anorganického fosforu po intravenózním podání roztoku propionátu u krav zdravých a krav v ketóze

Cílem studie bylo určit míru využití glukózy periferními tkáněmi na základě změn v koncentracích glukózy, insulinu a anorganického fosforu u krav zdravých (n = 10) a ketotických (n = 10) po i.v. infuzi propionátu. Vzorky krve byly odebrány oběma skupinám krav v následujících časových intervalech: těsně před (v čase 0) a 8, 30, 60, 120, 180, 240 a 480 min po i.v. podání 1.84 $mol \cdot l^{-1}$ roztoku propionátu v dávce 1 $ml \cdot kg^{-1}$ živé hmotnosti. Koncentrace glukózy a insulinu v krvi krav z obou skupin se významně zvýšily během 120 minut testu ($p < 0,05$). Ve srovnání se zdravými zvířaty byly v krvi krav s ketózou významně nižší koncentrace glukózy během 30, 60, 120 a 240 min experimentu v důsledku snížené glukoneogeneze v játrech krav v ketóze. Během 240 a 480 minuty testu byly zaznamenány u krav v ketóze ve srovnání se zdravými signifikantně nižší koncentrace ($p < 0,05$) insulinu v krvi. Tento nález ukazuje, že schopnost betabuněk endokrinního pankreatu produkovat insulin je u ketotických krav snížena. Po i.v. podání propionátu klesla po 8, 30, 60, 120, 240 a 480 min koncentrace anorganického fosforu u krav z obou skupin. Po 480 min testu byl zaznamenán významný pokles koncentrace anorganického fosforu u krav v ketóze ve srovnání se zdravými ($p < 0,05$). Svědčí to o aktivním vstupu glukózy do glykolýzy periferních tkání. Naše výsledky naznačují, že u krav v ketóze dochází ke zvýšené utilizaci glukózy v periferních tkáních.

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