The aim of this study was to examine the secretory capacity of endocrine pancreas beta cells in healthy cows and cows suffering from left displaced abomasum (LDA) by determination of glucose and insulin concentrations in the peripheral circulation during glucose tolerance test (GTT). A total of twenty healthy cows (Control group) and twenty cows suffering from left abomasal displacement (Experimental group) were chosen for this study. Cows in the control group were exposed to GTT once, while cows in the experimental group were exposed on the day, day 3 and day 6 after the diagnosis of LDA, as well as 3 days after surgical treatment and reposition of the abomasum. Initial blood glucose concentrations in healthy cows were significantly lower than in the cows suffering from LDA only on day 3 (P<0.05) and day 6 (P<0.01) after the disease was diagnosed. All the glucose values determined both in healthy and diseased cows at 30 minutes after the start of glucose infusion were much higher than physiological values. Regardless to the decline of glucose levels from 60 to 180 minutes after start of glucose infusion, hyperglycemia maintained in both healthy cows and cows suffering from LDA. Compared to healthy cows the rate of decline was slower in cows suffering from LDA at the day of diagnosis, as well as 3 and 6 days after the diagnosis. At the end of the experiment, 240 minutes after the start of the infusion, glucose concentrations were significantly higher in diseased cows on the day of diagnosis and day 3 and 6 after diagnosis than in the control group (P<0.001, respectively) and in diseased cows 3 days after surgery (P<0.001, respectively). Mean initial concentrations of serum insulin were significantly higher in healthy cows than in cows suffering from LDA only on day 6 after diagnosis. From 30 to 240 minutes after the start of glucose infusion, insulinemic responses to intravenous glucose administration were statistically higher in healthy cows than in experimental cows on the day of diagnosis, day 3 and day 6 after diagnosis, as well as 3 days after surgery. On the day of diagnosis and three days after, insulinemia decreased to initial levels 180 minutes after the start of the infusion. Six days after diagnosis, insulinemia decreased to initial values at 120 minutes after the start of the glucose infusion.
Three days after surgery, insulinemia decreased to values similar to initial at 240 minutes after the start of the infusion. Glucose tolerance test results showed that the best validation of pancreas endocrine function was between 60 and 120 minutes, as well as 180 and 240 minutes after the glucose infusion started. Our results also confirmed that cows suffering from LDA are in the stage of pancreatic endocrine dysfunction and that the rate of this dysfunction was higher when the disease lasted longer. The highest rate of pancreatic endocrine dysfunction was observed 6 days after diagnosis. On day 3 after surgical treatment and reposition of the abomasum, glucose tolerance test results showed that the pancreatic endocrine function recovered.

Key words: cow, displacement of abomasum, glucose tolerance test

INTRODUCTION

Displacement of the abomasum is a common problem of dairy cattle in early lactation and it is a disorder that occurs mainly in high producing dairy cows (Geishauser, 1995). Eighty to ninety percent of all abomasal displacements are located on the left-side (Shaver, 1997). The cause of left displaced abomasum (LDA) in cattle is multifactorial (Van Winden and Kuiper, 2003). A prerequisite for the development of LDA is abomasal atony or hypotony, with subsequent gas distension of the abomasal fundus preceding displacement. Abomasal atony can result from hypocalcaemia (Madison and Troutt, 1988), endotoxemia (Vlaminck et al., 1985), alkalaeica (Constable et al. 1992), hypergastrinemia (Sen et al., 2002) and hyperinsulinemia (Van Winden and Kuiper, 2003). There is evidence that hypoinsulinemia caused by hypocalcaemia may provoke abomasal atony (Bloom et al., 1975; Hove, 1978). Metabolic alkalosis can be a cause of hypocalcaemia via reduced sensibility of parathyroid hormone receptors (Van Winden and Kuiper, 2003) and is therefore also considered a risk factor for abomasal displacement.

Changes of blood biochemical parameters during displacement of abomasum, may indicate dysfunctions of different organs which can be in some cases irreversible. A higher concentration of glucose was found in cattle affected by LDA compared to the control group and this was explained by the possibility that endocrine pancreas function may be affected during this disease (Van Meirhaege et al., 1988; Itoh et al., 1998; Pravettoni et al., 2004). Endocrine pancreas dysfunction is commonly the crucial factor that determines the final outcome of the disease. If pancreatic insufficiency occurs due to impaired pancreatic vascularisation and juice elimination, a lethal outcome is possible in a very short period of time. Namely, extracellular pancreatic enzymes cause pancreatic tissue autodigestion. Thereafter, part of the tissue enters in the general circulation and histamine concentration increases. Cattle die due to intoxication and cardiovascular collapse (Šamanc and Damnjanović, 1994). Hyperglycemia in cows suffering from abomasal displacement may be a consequence of insensitivity
of body tissues to insulin for glucose clearance (Pravettoni et al., 2004). Factors that might stimulate insulin insensitivity have not been clearly defined. It is assumed that butyrate and other insulinogenic fatty acids may play a role in the pathogenesis of insulin resistance by reducing cellular sensitivity to insulin and causing high plasma concentrations of glucose and insulin (Breukink et al., 1976). Different degrees of insulin resistance in dairy cows affected with LDA were found in one study (Holtenius et al., 1990).

To determine the pathophysiology of the endocrine pancreas, the intravenous glucose tolerance test (GTT) is commonly carried out in cattle (Bigger et al., 1996; Samanc et al., 1996), as well as other animals (Curry et al., 1968; Kaneko et al., 1977). In ruminants, the blood glucose concentration is maintained at a stable level, as it is regulated by fermentation in the rumen and by gluconeogenesis from volatile fatty acids in the liver.

The aim of the present investigation was to assess the secretory function of beta-cells on the endocrine pancreas to release insulin after GTT and, on the basis of the results obtained in the test, to evaluate their functional state in healthy dairy cows and cows suffering from left abomasal displacement in the same phase of lactation before and after surgical correction of LDA.

MATERIAL AND METHODS

Animals

A total of twenty healthy cows (Control group) and twenty cows suffering from left abomasal displacement (Experimental group) were chosen from a Holstein-Friesian herd. The diagnosis of LDA was made on the basis of clinical symptoms. Percussion and ballottement with simultaneous auscultation of the left abdominal wall were used to identify a high-pitched resonant echo i.e. a ping and fluid-splachinc sounds associated with and left abomasal displacement. The cows included in the experiment were of similar body weight (650 kg), 4 to 6 years old, had an average of 2.8 lactations with a mean yield of 7625 L (calculated over 305 days) in the previous lactation and were all in the earliest stage of lactation (7 to 14 days post partum).

Surgery

Surgery was performed in cows with LDA on day 7 after the displacement was diagnosed. The abomasum was repositioned followed by omentopexy. Omentopexy was performed in accordance with the surgical procedure described by Jennings (1984).

Experimental procedure

All cows, from both the control and experimental group, were fitted with a dual jugular catheter before the start of glucose infusion. Glucose tolerance test (GTT) was carried out in the morning at 09 h and 3 h after feeding in accordance with the procedure described by Samanc et al. (1996). Cows in the control group were exposed to GTT once, while cows in the experimental group were exposed
on day 0, day 3 and day 6 after the diagnosis of LDA, as well as 3 days after surgery.

**Blood sampling**

Blood samples (10 mL) were collected from the contralateral vein immediately before glucose administration and at 30, 60, 120, 180 and 240 minutes after the start of infusion. Portions of the blood samples were allowed to coagulate spontaneously (approximately 15 minutes) on ice. The serum was decanted, centrifuged at 1,000 x g for 20 min at 4°C and preserved at -18°C until used for insulin analyses. Sodium fluoride, as anticoagulant, was used to obtained plasma samples for glucose analysis. These portions of blood were set immediately on ice. Within 30 to 60 min the tubes were centrifuged at 1,000 x g for 20 min at 4°C and plasma was preserved at -18°C until analyzed.

**Assays**

Plasma glucose concentrations were measured using commercial kits (GOD/PAP test, Dialab Austria). 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 value, standard deviations (SD), standard error (SE) and CV for each group of cows were calculated at each time interval. Probability and the statistical significance of differences between the mean values were calculated using Student's t-test.

**RESULTS**

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

Initial blood glucose concentrations in healthy cows (2.64 ± 0.44 mmol/L) were significantly lower than in cows suffering from LDA on day 3 (3.02 ± 0.61 mmol/L; P<0.05) and day 6 (3.40 ± 0.62 mmol/L; P<0.01) (Figure 1). There was no significant difference in initial blood glucose concentrations between the control group and the group suffering from LDA on the day of diagnosis (2.45 ± 0.62 mmol/L) and day 3 after surgery (2.69 ± 0.62 mmol/L). At 30 minutes after the start of glucose infusion, glucose concentrations in the blood plasma of healthy cows (9.81 ± 1.82 mmol/L) were statistically higher than in cows suffering from LDA on the day of diagnosis (8.63 ± 1.51 mmol/L; P<0.05) and 3 days after surgery (7.84 ± 1.11 mmol/L; P<0.01), but not statistically different than in cows suffering from LDA on day 3 (9.86 ± 2.03 mmol/L) and day 6 (10.02 ± 1.87 mmol/L) after diagnosis. However, all the glucose values determined both in the control and experimental groups at 30 minutes after the start of glucose infusion were much higher than physiological values. Regardless the decline of glucose levels from 60 to 180 minutes after the onset of glucose infusion, hyperglicemia was determined in healthy cows and cows suffering from LDA.
Compared to healthy cows the rate of decline was slower in cows suffering from LDA at the day of diagnosis, as well as 3 and 6 days after diagnosis. Thus, 120 minutes after the start of glucose infusion the concentration of glucose in the control group (4.24 ± 0.69 mmol/L) was significantly lower than in the experimental group on the day of diagnosis (6.08 ± 1.72 mmol/L; P<0.001), day 3 (7.33 ± 1.43 mmol/L; P<0.001) and day 6 (8.09 ± 1.81 mmol/L; P<0.001) after diagnosis.

Glucose mean values at the end of the experiment were significantly higher in the experimental group on the day of diagnosis (3.26 ± 0.62 mmol/L), day 3 (3.67 ± 0.96 mmol/L) and day 6 (3.80 ± 1.14 mmol/L) after diagnosis than in the control group (2.60 ± 0.33 mmol/L; P<0.001, respectively) and cows with LDA 3 days after surgery (2.78 ± 0.45 mmol/L; P<0.001, respectively).

Mean initial concentrations of serum insulin (Figure 2) were significantly higher in healthy cows (12.38 ± 4.17 mIU/L) than in cows suffering from LDA on day 6 (8.65 ± 3.75 mIU/L; P<0.01), but was not significantly different from blood insulin concentrations in cows suffering from LDA on the day of diagnosis (11.85 ± 3.61 mIU/L) 3 days after diagnosis (10.97 ± 3.25 mIU/L) and 3 days after surgery (14.98 ± 4.66 mIU/L). After the start of glucose infusion, insulin concentrations in healthy cows were significantly higher, compared to initial levels, 30 minutes (70.13 ± 21.92 mIU/L; P<0.01), 60 minutes (62.61 ± 26.15 mIU/L; P<0.01), 120 minutes (31.72 ± 17.12 mIU/L; P<0.01) and 180 minutes (20.02 ± 11.16 mIU/L; P<0.01) after GTT was started. At 240 minutes after the start of glucose infusion, insulinemia in healthy cows (17.35 ± 11.72 mIU/L) was not statistically different than the initial value. Additionally, during the period from 30 to 240 minutes after the start of glucose infusion, insulinenic responses to intravenous glucose
administration were statistically higher in healthy cows than in diseased cows on the day of diagnosis, day 3 and day 6 after diagnosis, as well as 3 days after surgery (except at the 180th minute for cows with abomasal displacement 3 days after surgery). On the day of diagnosis, values for insulinenia at 30, 60 and 120 minutes after the start of infusion (35.54 ± 13.69 mIU/L; 29.32 ± 11.76 mIU/L; 18.34 ± 6.42 mIU/L) were significantly higher (P<0.001 respectively) than initial levels (11.85 ± 3.61 mIU/L). Insulinenia at 180 minutes (12.50 ± 3.72 mIU/L) and 240 minutes after the start of glucose infusion (10.61 ± 4.20 mIU/L) were similar to initial levels determined at the day of diagnosis of LDA. Three days after diagnosis, values for insulinenia at 30 minutes (31.30 ± 10.91 mIU/L), 60 minutes (22.32 ± 6.69 mIU/L), and 120 minutes after the start of infusion (13.90 ± 5.38 mIU/L) were significantly higher (P<0.001; P<0.001; P<0.05, respectively) than initial levels (10.97 ± 3.25 mIU/L). Three days after LDA was diagnosed, plasma insulinenia concentrations at 180 minutes after the start of infusion (11.27 ± 3.17 mIU/L) were similar to the initial levels, and the insulinenia determined 240 minutes after the start of infusion (8.62 ± 3.12 mIU/L) was significantly lower than initial levels (P<0.05). Six days after diagnosis, values for insulinenia determined 30 minutes (25.50 ± 9.50 mIU/L) and 60 minutes (15.01 ± 6.64 mIU/L) after the start of glucose infusion were significantly higher (P<0.001) than initial levels (8.65 ± 3.75 mIU/L), while there was no significant difference between initial values and values determined at the 120th minute (10.57 ± 4.52 mIU/L), 180th minute (7.65 ± 3.28 mIU/L) and 240th minute (6.64 ± 2.90 mIU/L) after the start of glucose infusion. Three days after surgery, insulinenia was significantly higher than initial values (14.98 ± 4.66 mIU/L) at 30 minutes (52.02 ± 17.83 mIU/L; P<0.001), 60 minutes (43.46 ± 13.78 mIU/L; P<0.001), 120 minutes (23.33 ± 6.19 mIU/L; P<0.001) and 180 minutes (17.98 ± 4.31 mIU/L; P<0.05) after the

Figure 2. Serum insulin concentrations (mIU/L) in healthy and cows suffering from left displacement of abomasums (LDA) before and after intravenous administration of glucose solution
start of glucose infusion and it decreased to values similar to the initial 240 minutes after the start of infusion (14.15 ± 4.00 mIU/L).

DISCUSSION

In accordance with many authors (Šamanc et al., 1989; Muylle et al., 1990; Itoh et al., 1998; Zadnik, 2003) cows suffering from LDA were characterized by higher plasma glucose levels than healthy cows. Our results confirm these findings, since initial glucose concentrations in the experimental group on day 3 and day 6 after LDA diagnosis were significantly higher than in healthy cows and cows suffering from LDA on the day of diagnosis. Hyperglycemia is probably due to temporarily developing pancreatic insufficiency. In affected cattle, 6 days after the beginning of LDA, initial insulin concentration was statistically lower than in healthy cows, which indicates that a prolonged duration of the disease might cause endocrine pancreatic dysfunction. It is supposed that abomasal displacement impairs the outflow of pancreatic juice and disturbed blood circulation in the pancreatic parenchyma because of changes of duodenal and omental position due to dilocation of abomasum (Šamanc and Damjanović, 1994). That is the reason for pancreatic insufficiency in general, which includes insufficiency of its endocrine part, too. Hyperglycemia in cows suffering from LDA is also likely to occur when tissues fail to respond to insulin (Pravettoni et al., 2004).

It is well known that hypoinsulinemia combined with hypocalcaemia might be very important as the etiopathogenetic factor in the appearance of left displaced abomasum. Hypocalcaemia and increased sympathetic activity can abolish the release of insulin to various stimuli (Bloom et al., 1975), and changes in these factors might have interfered with the release of insulin. Calcium in plasma was not measured in our study, but other authors confirmed that cows with LDA suffered from hypocalcaemia (Kerli and Goff, 1992; Zadnik, 2003) and that hypocalcaemia is an important pathogenic factor in the development of LDA (Madison and Troutt, 1988). In our experiment, initial insulin concentration tends to be lower in cows suffering from LDA on the day of diagnosis (11.85 ± 3.61 mIU/L) and three days after (10.97 ± 3.25 mIU/L), and was significantly lower six days after (8.65 ± 3.75 mIU/L), compared to healthy cows (12.38 ± 4.17 mIU/L). Hypoinsulinemia, combined with hypocalcaemia may inhibit acid secretion in the abomasum, which leads to increased pH value. In that case, microbial activity in the abomasum might be prolonged and may cause increased gas production and abomasal dilatation (Holtenius et al., 2000; Van Winden et al., 2002; Steven et al., 2003). Hypoinsulinemia affects the activity of n. vagus, leading to its decreased tonus, which may contribute to further development of LDA (Van Widen et al., 2002). Three days after surgery the capacity for insulin secretion in beta pancreatic cells completely recovered, since the initial insulin value (14.98 ± 4.66 mIU/L) did not differ compared to healthy cows.

In the here presented experiment, GTT was done in order to assess the endocrine pancreatic function in cows with LDA. Hyperglycemia, in general, is without importance in adult ruminants under physiological conditions (Basset,
1975). In spite of this, elevated glucose in plasma is a potent stimulus to secretion of insulin. In other species it is generally accepted that glucose stimulates the release of stored insulin and the pancreatic synthesis of insulin. In the present study, the initial peak in insulin concentration was observed 30 minutes after the start of glucose infusion. This was followed by a sustained elevation of insulin concentration. Time duration of this insulin evaluation differed in each group, which indicates that insulin response to glucose level was not the same. Our results showed that insulin response to glucose infusion was significantly lower in cows suffering from LDA than in healthy cows, especially when GTT was done 3 and 6 days after the onset of the disease. The low secretory responses of insulin after the start of glucose infusion should be compared to the findings of reduced insulin in plasma of cows suffering from LDA. Experiments in man (Littledike et al., 1968) and in cattle (Bigner et al., 1996) demonstrate that blood glucose in the period preceding an experiment is of great importance for the amount of insulin released as a response to a secretory signal. The low insulin response to glucose infusions in cows suffering from LDA, therefore, probably results from a low secretory capacity of pancreas for insulin, which developed during the days of hyperglycemia which regularly accompanies this disease.

At the end of the experiment (240 minutes after the start of infusion), final glucose concentrations in healthy cows were similar to initial values. On the other hand, final glucose concentrations in healthy cows were significantly higher than initial values in cows suffering from LDA on the day of diagnosis, three and six days after and were not different from the final glucose concentrations determined 3 days after surgery. These results clearly indicate that during the six days the function of the endocrine pancreas is disrupted, but insulin activity is completely recovered after surgery.

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PRIMENA TESTA OPTEREĆENJA GLUKOZOM U OCENI ENDOKRINE FUNKCIJE PANKREASA KOD KRAVA OBOLELIH OD DISLOKACIJE SIRIŠTA NA LEVO PRE I POSLE HIRURŠKOG TRETMANA

ŠAMANC H, STOJIĆ V, KIROVSKI DANIJELA, PUDLO P i VUJANAC I

SADRŽAJ

Cilj ovog rada bio je da se, određivanjem koncentracije glukoze i insulina u krvi krava tokom testa opterećenja glukozom, ispitati sekretorni kapacitet beta čelija endokrinog pankreasa kod zdravih krava i krava obolelih od dislokacije sirišta na levo. U ogled je uključeno 20 zdravih krava (kontrolna grupa) i 20 krava obolelih od dislokacije sirišta na levo (eksperimentalna grupa). Kod zdravih krava opterećenje glukozom je izvršeno jednom, dok je kod obolelih krava ovaj test izveden na dan dijagnostikovanja oboljenja, 3 i 6 dana posle, kao i 3 dana nakon hirurške intervencije i repozicije obolelog organa. Bazalna koncentracija glukoze, određena neposredno pre početka infuzije, je kod zdravih krava bila značajno niža nego kod krava obolelih od dislokacije sirišta na levo tri dana (P<0,05) i šest dana (P<0,01) nakon dijagnostikovanja bolesti. Tridesetog minuta nakon početka infuzije, hiperglikemija je utvrđena i kod zdravih i kod obolelih krava. Iako je koncentracija glukoze kod zdravih i kod obolelih krava opadala od 60. do 180. minuta posle početka infuzije, hiperglikemija se kod svih krava održala tokom ovog perioda. U poređenju sa zdravim kravama, stepen opadanja koncentracije glukoze je bio sporiji kod obolelih krava na dan dijagnostikovanja dislokacije sirišta na levo, kao i tri do šest dana posle. Na kraju eksperimenta, 240 minuta nakon početka infuzije, koncentracija glukoze je bila statistički značajno viša kod obolelih krava od normalnih na dan dijagnostikovanja bolesti, kao i tri dana kasnije u odnosu na vrednost određenu kod normalnih krava i kod bolesnih dana kasnije. Bazalna vrednost insulinemije, određena neposredno pre početka infuzije glukoze, je bila značajno viša kod zdravih u odnosu na obolele krave na dan dijagnostikovanja bolesti, kao i dana kasnije nakon postavljanja dijagnostikovanja bolesti kod normalnih krava i kod obolelih krava. U periodu od 30. do 240. minuta nakon početka infuzije glukoze, insulinski odgovor na aplikovanu glukozu je bio značajno viši kod zdravih krava na dan dijagnostikovanja bolesti, kao i 3 dana nakon dijagnostikovanja bolesti kod zdravih krava i kod obolelih krava. Na dan dijagnostikovanja bolesti, kao i tri dana kasnije, insulinemija je opala na vrednosti slične bazalnim 180. minuta nakon početka infuzije glukoze. Šest dana nakon dijagnostikovanja bolesti, koncentracija insulinina je opala na vrednosti slične bazalnim 120 minuta nakon početka infuzije. Tro dana nakon repozicije abomasuma, insulinemija je smanjena na vred-
nasti slične bazalnim 240. minuta nakon početka infuzije. Rezultati testa opterećenja glukozom daju najbolju procenu endokrine funkcije pankreasa između 60. i 120. minuta i 120. i 180. minuta posle početka infuzije glukoze. Naši rezultati ukazuju da kod krava obolelih od dislokacije sirišta na levo postoji stanje insuficijencije endokrinog pankreasa i da se to stanje pogoršava sa dužim tokom bolesti. Najveći stepen insuficijencije je ustanovljen šest dana od dijagnostikovanja oboljenja. Tri dana nakon izvođenja hirurške intervencije i repozicije dislociranog organa, rezultati testa opterećenja glukozom pokazuju da se ponovo uspostavlja normalna aktivnost endokrinog pankreasa.