ABSTRACT

Concentrations in blood of glucose, acetoacetate, 3-hydroxybutyrate, free fatty acids, insulin, calcium, magnesium, sodium, and potassium of 19 spontaneously ketotic cows and 16 normal lactating cows were compared. Acetoacetate, 3-hydroxybutyrate, and free fatty acids were higher and glucose and magnesium were lower in ketotic cows than in normal cows. At 4 h after intravenous administration of 250 g glucose, 3-hydroxybutyrate was decreased and calcium increased. At 4 h after intraruminal administration of 400 ml of 1,2-propanediol, acetoacetate, 3-hydroxybutyrate, and magnesium were decreased and glucose increased. Intravenous administration of 100 g of xylitol or intraruminal administration of 188 g of magnesium propionate appeared effective to decrease ketones and to increase glucose in blood.

INTRODUCTION

Ketosis is still a problem in dairy herds of many countries. Ketotic cows show increased concentrations of ketone bodies and free fatty acids (FFA) and decreased concentration of glucose in blood (3, 31, 38). Insulin secretion of ketotic cows can be depressed (15, 32). According to Krebs (18), ketosis is related to the shortage of oxaloacetate in the hepatic mitochondria because of an increased demand of oxaloacetate for gluconeogenesis. Studies of Baird et al. (1, 2, 3) have supported this hypothesis. However, the study of gluconeogenic enzymes in ketotic cows can not prove Krebs's hypothesis clearly (5). Compared with rats, the ruminant has a peculiarity in that the activity of 3-hydroxybutyrate dehydrogenase in the hepatic mitochondria is exceedingly low (28), although the ratio of 3-hydroxybutyrate to acetoacetate in blood is higher (3). As we can not produce spontaneously ketotic cows experimentally, progress in this research field is limited. Glucose, 1,2-propanediol (propylene glycol), sodium propionate, or glucocorticoid administration have been practiced for treatments (9, 30, 33). In our work we compared several blood metabolites of spontaneously ketotic cows with those of normal lactating cows and estimated the short-period effects of administration of glucose, xylitol, 1,2-propanediol, or Mg propionate on blood components of ketotic cows to attempt to understand characteristics of ketosis and to establish a treatment.
intraruminal administration of 400 ml of DL-1,2,-propanediol or 750 ml of 25% Mg propionate (pH 6.5). These doses were considered to be near maximum doses to be administered without difficulty.

About 10 ml of jugular blood sample was drawn into the vacuum tube containing 143 units heparin natrium before and 4 h after the treatment and stored in the ice-box. On the same day, plasma was separated by centrifugation at 5°C in the laboratory.

Plasma Analysis

Glucose, acetoacetate, and 3-hydroxybutyrate were analyzed immediately. Three milliliters of plasma were deproteinized by addition of 3 ml of 1.0 N HClO4 and centrifuged. Three milliliters of the supernatant were neutralized with 1.5 ml of .7 M K3PO4 and centrifuged. With this supernatant, glucose was analyzed by enzymatic method using hexokinase and glucose-6-phosphate dehydrogenase (Boehringer) and ketone bodies by 3-hydroxybutyrate dehydrogenase (Boehringer). Final assay mixture for glucose analysis contained 260 mM triethanolamine buffer (pH 7.6), 6.1 mM MgCl2, .8 mM ATP, .42 mM NADP+, .3 units/ml hexokinase, and .15 units/ml glucose-6-phosphate dehydrogenase. Glucose concentration was determined by measuring amounts of NADPH generated (7). Acetoacetate and 3-hydroxybutyrate concentrations were measured by the method of Williamson et al. (37) with the reaction times of 30 and 60 min at 37°C by Gilford spectrophotometer model 240.

Other components were analyzed on frozen samples stored at about -20°C. Free fatty acids were analyzed by the kit of NEFA-test Wako (Wako Pure Chemical Ind., Osaka) by quantifying the formation of copper-bathocuproine-complex at 480 nm. Insulin was measured by the radioimmunoassay method with Insulin-kit Daiichi (Phadebas insulin test) containing Sephadex-anti-insulin complex (Pharmacia Fine Chemicals). Calcium and Mg were analyzed by atomic absorption spectrophotometry with Perkin-Elmer model 303 by diluting plasma with 1000 ppm strontium solution containing strontium chloride and water. Sodium and K were analyzed by flame spectrophotometry with Hiranuma FPF-3A by diluting plasma with 100 ppm lithium solution containing lithium chloride.

RESULTS AND DISCUSSION

In Table 1, concentrations of plasma components of ketotic cows are shown with those of normal cows. Acetoacetate, 3-hydroxybutyrate, and FFA of ketotic cows were significantly higher than of normal cows, and glucose of ketotic cows was significantly less than of normal cows. These are the same tendencies as for (3, 5, 31, 38). Magnesium of ketotic cows was significantly less than of

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<tr>
<th>TABLE 1. Concentrations of blood plasma components of 19 spontaneously ketotic cows and 16 normal lactating cows.1</th>
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<tbody>
<tr>
<td>Ketotic cows</td>
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<tr>
<td>X      SD</td>
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<tr>
<td>Glucose (mg/dl)</td>
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<tr>
<td>Acetoacetate (mg/dl)</td>
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<tr>
<td>3-Hydroxybutyrate (mg/dl)</td>
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<td>Free fatty acids (meq/liter)</td>
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<td>Insulin (µU/ml)</td>
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<td>Na (meq/liter)</td>
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<td>K (meq/liter)</td>
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1 Data are shown as means (X) ± SD.
2 p, Significance of difference between ketotic cows and normal cows.
3 NS, Not significant.
normal cows. However, these amounts were not in the range of Mg-deficient state (21, 29). Insulin, Ca, Na, and K were not different between ketotic and normal cows. In contrast, low basal insulin of plasma and depression of blood Ca in ketotic cows are reported by Hove (15) and Halse et al. (10), respectively.

In Table 2 several effects of administration are shown. The intravenous administration of glucose significantly decreased 3-hydroxybutyrate and increased Ca. The intraruminal administration of 1,2-propanediol significantly decreased acetoacetate, 3-hydroxybutyrate, and Mg and increased glucose. In administration of xylitol or Mg propionate, no significant differences of blood components were detected. Animal variations were large. Xylitol or Mg propionate administration seemed to be effective as well as glucose administration because these treatments tended to decrease ketones and to increase glucose. In our experimental conditions, 1,2-propanediol administration was the most effective in decreasing ketones and in increasing glucose, which might be caused by its largest dose. In all treatments insulin, FFA, Na, and K showed no significant postadministration changes. However, in some treatments insulin and FFA concentrations might have changed immediately after administration. According to Kronfeld (19) and Treacher et al. (35), an immediate response in insulin secretion and a rapid decline in FFA concentration occur after glucose administration.

In ketotic cows, overproduction of ketones may be caused primarily by oxidation of the greater supply of FFA in the liver, because ketone production from the alimentary source of butyrate at the ruminal wall may be depressed by loss of appetite usually associated with spontaneous ketosis. According to Lomax et al. (23), in the dairy cow insulin secretion is diminished during lactation in response to an insulinotropic agent. Especially in ketotic cows, responses of insulin secretion following glucose infusions are poor (15). A glucagon-like hormone produced by the gastrointestinal tract may promote lipolysis when coupled with hypoglycemia and reduced plasma insulin concentrations (26). According to our unpublished results, 1,2-propanediol administration does not stimulate insulin secretion in the dairy cow, whereas propionate administration does. Xylitol is a gluconeogenic substrate (16).
and an insulinotropic agent (20). Administration of glucose, xylitol, or propionate may change blood insulin and FFA in less than an hour after administration (19), whereas administration of 1,2-propanediol may not. As shown in Table 2, 4-h postadministration observation showed that ketone concentrations were decreased significantly in spite of high FFA concentrations by administration of glucose or 1,2-propanediol. A decline in ketone body production while blood FFA concentrations remain elevated strongly suggests that factors working to reduce ketogenesis must operate within the liver (1).

According to Katz and Bergman (17), a change in hepatic FFA metabolism may be a factor in development of ketosis. Recent studies of McGarry et al. (24, 25) show that malonyl-CoA becomes a competitive inhibitor of carnitine acyltransferase 1 and that hepatic concentrations of malonyl-CoA influenced by the ratio of glucagon to insulin in blood can play an important role to regulate hepatic fatty acid oxidation and ketogenesis in the rat, so the regulatory role of malonyl-CoA in the ketogenesis of spontaneously ketotic cows merits further investigation. According to studies of metabolite concentrations in the liver of ketotic cows or lactating cows deprived of food (1, 2, 3, 35), variation in concentrations of oxaloacetate and its precursors is important in regulating ketogenesis. Propionate is the most available substrate for gluconeogenesis of the ruminant (22), and propionate conversion to lactate at the ruminal wall is a minor pathway (36). In contrast to propionate, 1,2-propanediol can be metabolized via lactate and pyruvate for the gluconeogenic pathway in the lactating cow (8). Propionate and 1,2-propanediol require different enzymes and effectors for their metabolism. In the dairy cow, in vivo competition between propionate, on the one hand, and lactate and pyruvate, on the other, for uptake by the liver is shown (4).

We used Mg propionate instead of Na propionate because according to our unpublished result, intraruminal administration of Mg propionate exhibits more powerful antiketogenic effect than that of Na propionate, and according to Yoshida and Yamatari (39), administration of Mg salt with glucose is effective for treatment of ketosis. According to our studies, 1,2-propanediol can be utilized as an energy source when goats are fed several other types of diets (14, 27). Intraruminal administration of 500 ml of 1,2-propanediol to normal lactating cows during the morning feeding can cause such unusual responses as increased respiration rates and cessation of eating by some cows (12). Also, 1,2-propanediol may become an active agent in vivo to stimulate rumen papillary development of milk-fed kids (11, 13). The mechanisms of these miscellaneous effects of 1,2-propanediol remain to be solved.

Intravenous administration of glucose has been a routine practice for treatment of spontaneous ketosis. According to Bartley and Black (6), when glucose is supplied exogenously to cows, there is a decrease in endogenous glucose production. According to Thompson et al. (34), glucose loading can reduce glucose output from the liver and stimulate utilization of gluconeogenic substrates for lipogenesis, which may not be a favored condition for treatment of ketosis. A larger dose of Mg propionate is not recommended, because our unpublished observation showed that compared with Na propionate, Mg propionate might cause lethargy in some animals, probably by its depressing effect on the nervous system. It seems justifiable to suggest tentatively that the combination of intravenous administration of xylitol and intraruminal administration of 1,2-propanediol may be an effective method for treatment of ketosis.

REFERENCES