Insulin secretion and glucose uptake in hypomagnesemic sheep fed a low magnesium, high potassium diet

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Hyperglycemic and euglycemic clamp experiments were conducted to evaluate insulin secretion and glucose uptake in the hypomagnesemic sheep fed a low magnesium (Mg), high potassium (K) diet. Five mature sheep were fed a semipurified diet containing 0.24% Mg and 0.56% K (control diet) and five were fed 0.04% Mg and 3.78% K (low Mg/high K diet) for at least 2 weeks. In the hyperglycemic clamp experiment, plasma glucose concentrations were raised and maintained at a hyperglycemic steady-state (approximately 130 mg/100 ml) by variable rates of glucose infusion during the experimental period (120 minutes). The insulin response in the sheep fed the low Mg/high K diet (31.0 μU/ml) were significantly ($P < 0.01$) lower than those (111.7 μU/ml) of the sheep fed the control diet. In the euglycemic clamp experiment, insulin was infused at rates of 5, 10, 15, or 20 mU/kg/min, each followed by variable rates of glucose infusion to maintain a euglycemic steady-state (basal fasting levels). Hypomagnesemic sheep fed the low Mg/high K diet had significantly ($P < 0.01$) lower mean glucose disposal (3.72 mg/kg/min) across the insulin infusion rates compared with those of the sheep fed the control diet (5.37 mg/kg/min). These results suggest that glucose-induced insulin secretion and insulin-induced glucose uptake would be depressed in hypomagnesemic sheep and are caused by feeding the low Mg/high K diet.

Keywords: Glucose; insulin; hypomagnesemia; magnesium; potassium; ruminants

Introduction

The incidence of hypomagnesemic tetany (grass tetany) in ruminants, characterized by a decrease in magnesium (Mg) levels in extracellular fluid, is often associated with a low Mg and high potassium (K) content in the early spring pasture.[1] Magnesium has been shown to be necessary for several enzyme activities related to glucose metabolism and to play an important role in some endocrine secretions.[2-5] Therefore, hypomagnesemia may disturb intermediary carbohydrate metabolism, although some reports have shown no or a contrary effect of Mg deficiency on glucose metabolism in sheep.[6,7] Several experiments[8-10] have suggested that intravenous or intraruminal infusion of potassium chloride in Mg-deficient animals might depress insulin-induced glucose uptake by all the tissues in ruminants. Terashima et al.[11] reported that plasma insulin responses to glucose infusion and feeding might decrease in wethers on a high K diet. These data suggest that the result of hypomagnesemic animals fed a low Mg, high K diet may be a disorder in glucose metabolism. However, this correlation still remains unclear. The objectives of the present study were to evaluate glucose-induced insulin secretion and insulin-induced glucose uptake in hypomagnesemic sheep fed a low Mg, high K diet.

Materials and Methods

Animals and diet

Five mature sheep weighing about 47.0 kg were kept in metabolism crates and were provided experimental semipurified diets. The normal Mg/normal K diet (control) and the low Mg/high K diet were prepared by

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Table 1 Ingredient and chemical composition of basal semipurified diet

Item	Percentage of diet
Ingredient	
Corn starch	30.0
Corn cobs (ground)	20.5
Dextrose	20.5
Cellulose powder	16.7
Soybean protein	8.2
Vegetable oil	0.9
Feed additive[a]	0.8
Dicalcium phosphate	1.2
Sodium chloride	0.8
Potassium bicarbonate	0.4
Chemical composition[b,c]	
Crude protein	5.03
Magnesium	0.05
Potassium	0.52
Calcium	0.35

[a] Contained per 100 g feed additive: vitamin A, 100,000 IU; vitamin D, 15,000 IU; thiamine hydrochloride, 30 mg; riboflavin, 100 mg; pyridoxine hydrochloride, 60 mg; cyanocobalamine, 6 μg; menadione, 8 mg; calcium pantothenate, 100 mg; nicotinamide, 400 mg; ascorbic acid, 50 mg; choline chloride, 6,000 mg; folic acid, 2 mg; DL-carnitine chloride, 30 mg; DL-α-tocopherol, 500 mg; chlorophyllin sodium copper salt, 100 mg; DL-methionine, 1,000 mg; lysine monohydrochloride, 100 mg; potassium and magnesium asparaginate, 100 mg; yeast extract powder, 10,000 mg; magnesium chloride, 3,600 mg; copper sulfate, 157 mg; zinc sulfide, 250 mg; iron sulfide, 2,000 mg; calcium carbonate, 175 mg
[b] By determination
[c] Calculated metabolizable energy: 2.45 Mcal/kg diet

adding 0.3% MgO and 5.4% KCl to the basal semipurified diets (*Table 1*), respectively. During a 7-day preliminary period, all sheep were fed the control diet containing 0.24% Mg and 0.56% K. They were randomly assigned to the control diet group or the low Mg/high K diet (0.04% Mg and 3.78% K) group. Each animal was provided the diet for at least 2 weeks based on metabolic size for body weight (BW $kg^{0.75}$) to meet the daily metabolizable energy requirements for maintenance.[12] Deionized water was provided ad libitum.

Glucose clamp techniques

The hyperglycemic and euglycemic clamp techniques were used to evaluate insulin secretion and glucose uptake in hypomagnesemic sheep. The aim of the glucose clamp experiment was to maintain plasma glucose levels at a hyperglycemic or a euglycemic steady-state for 120 minutes. These techniques were basically similar to those described for human and sheep studies.[13,14] Catheters were inserted into bilateral jugular veins and maintained by filling with a sterile solution of heparin sodium (heparin, 100 U/ml). A catheter was used for blood sampling, and another was used for glucose and insulin infusion. The glucose clamp experiments were conducted at 4- to 6-day intervals after 2 weeks of experimental diet feeding.

In the hyperglycemic clamp experiment, blood glucose levels were acutely raised to the desired hyperglycemia (100 mg/100 ml) and were maintained at that plateau by variable rates of glucose infusion (20% dextrose). Blood glucose levels were measured at 5-minute intervals throughout the experimental period, and the glucose infusion rate was empirically determined.

In the euglycemic clamp experiment, crystalline porcine insulin (Insulin Novo Actrapid MC, Novo Industri, Denmark) was diluted with isotonic saline. Concurrent with the continuous insulin infusion at rates of 5, 10, 15, or 20 mU/kg/min, glucose was infused to maintain the euglycemic plateau (basal fasting levels). Glucose and insulin infusions were achieved by using a multichannel peristaltic pump (Perista Bio-minipump AC-2120, Atto Co., Ltd., Japan).

Under the steady-state plasma glucose conditions, the amount of infused glucose equals the amount of glucose uptake from the glucose space into the tissues, assuming that endogenous glucose production is completely suppressed.[13] In the present study, the glucose space was assumed to be 179 ml/kg body weight[14] and to not be changed by the diet treatments. The glucose disposal was calculated from mean values of 20-minute periods[13] during the fixed hyperglycemia from 60 to 120 minutes and the euglycemia from 40 to 120 minutes after the start of the glucose clamp experiments.

Analytical methods

Blood samples (5 ml) were taken in heparinized tubes. After measuring the blood glucose, the remaining samples were centrifuged at 2500 × *g* for 15 minutes. Blood and plasma glucose were measured by the glucose analyzer (Glucose Analzyer GLU-1, Erma Optical Works, Ltd., Japan) using the glucose oxidase method. The plasma samples were assayed for insulin by double binding radioimmunoassay (IRI Radioimmunoassay Kit, Eiken Chemical Co., Ltd., Japan). Plasma Mg, K, and calcium (Ca) were determined by atomic absorption spectrophotometry.[15]

Statistical calculations

All parameters were expressed as a mean value ± standard error. The significance of differences between diet treatments on plasma glucose, insulin, and mineral levels were analyzed by *t* test. In the hyperglycemic clamp experiment, one-way analysis of variance was used for significant differences in insulin response and glucose disposal between diet treatments. In the euglycemic clamp experiment, two-way design was used for the glucose disposal to test diet treatments and the insulin infusion rates.

Results

Plasma minerals

All animals completely consumed the daily allowance of rations, and no changes in body weights were observed during the entire experimental period. After 2 weeks of diet treatment, plasma Mg levels in the sheep

Table 2 Effect of diet treatment on plasma magnesium, potassium, and calcium levels in sheep[a]

	Diet treatment[b] (mg/100 ml)	
	Control	Low Mg/high K
Magnesium	3.51 ± 0.25	1.45 ± 0.36[c]
Potassium	25.41 ± 0.51	23.35 ± 1.03
Calcium	8.06 ± 0.31	7.50 ± 0.20

[a] Each value represents a mean of five animals ± SEM
[b] See text
[c] Significantly different from the control ($P < 0.01$)

Table 3 Plasma insulin response and glucose disposal in sheep fed the control and low Mg/high K diets in a hyperglycemic clamp experiment[a]

	Diet treatment[b]	
	Control	Low Mg/high K
Insulin response (μU/ml)	111.7 ± 36.3	30.1 ± 1.8[c]
Glucose disposal (mg/kg/min)	3.79 ± 0.79	2.25 ± 0.15

[a] Each value represents a mean of four animals ± SEM
[b] See text
[c] Significantly different from the control ($P < 0.01$)

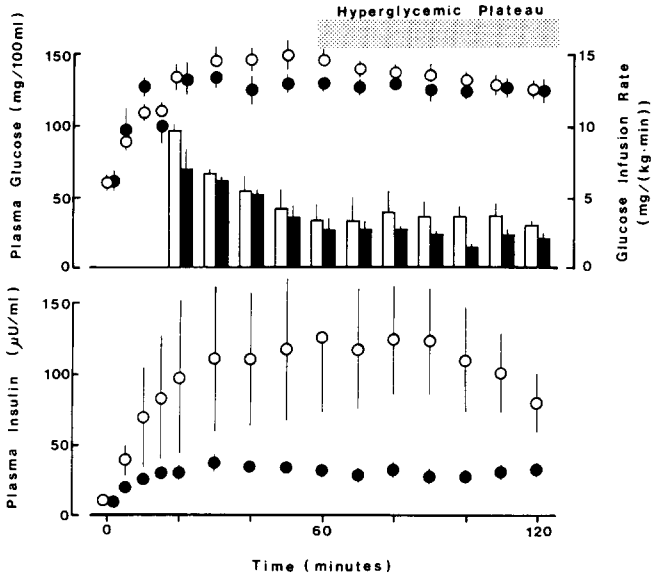

Figure 1 Summary of plasma glucose, insulin levels (circles), and glucose infusion rate (bars) in sheep fed the control (open) and low Mg/high K (solid) diets during a hyperglycemic clamp experiment; each value represents a mean of four animals ± SEM

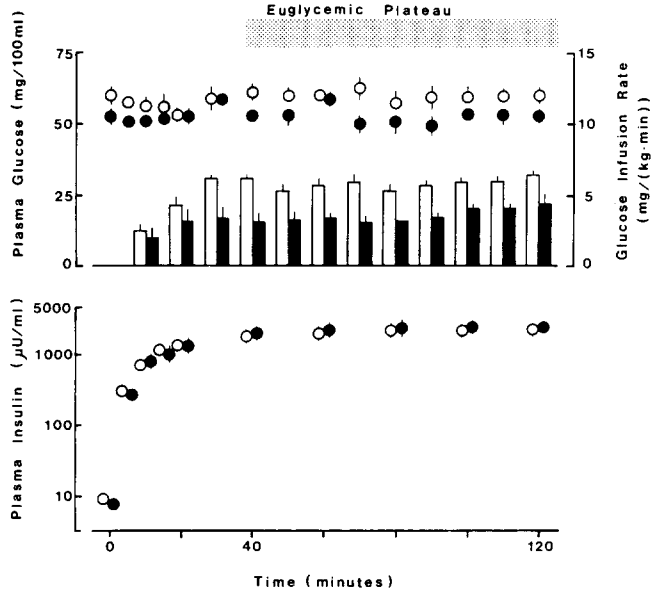

Figure 2 Summary of plasma glucose, insulin levels (circles), and glucose infusion rate (bars) in sheep fed the control (open) and low Mg/high K (solid) diets during a euglycemic clamp experiment using a 10 mU/kg/min insulin infusion rate; each value represents a mean of five animals ± SEM

fed the low Mg/high K diet (1.45 mg/100 ml) were significantly lower ($P < 0.01$) than those of the sheep fed the control diet (3.51 mg/100 ml) (*Table 2*). Plasma K and Ca concentrations were slightly lower in the low Mg/high K diet-fed sheep than in the control diet-fed sheep, but not significantly.

Hyperglycemic clamp technique

One of the five sheep being used in the hyperglycemic clamp study was excluded from the results because a stable hyperglycemic condition could not be achieved. Changes in plasma glucose and insulin concentrations and the glucose infusion rate during the hyperglycemic clamp experiment are shown in *Figure 1*. The preinfusion plasma glucose and insulin levels for the sheep fed the control diet and the low Mg/high K diet were 57.8 versus 62.8 mg/100 ml and 11.4 versus 9.7 μU/ml, respectively. Thus, diet treatments did not affect plasma glucose and insulin concentrations. Plasma glucose was raised to the desired hyperglycemic states 20 minutes after the initiation of glucose infusion, and that plateau was maintained during the experimental period. The mean plasma glucose concentrations in the steady-state were 135.0 and 129.3 mg/100 ml, respectively, when sheep were fed the control and low Mg/high K diets. Plasma insulin concentrations rapidly increased after the initiation of the glucose infusion in both groups, and retained high levels during the experimental period. The mean plasma insulin levels in the hyperglycemic steady-state (insulin response) were significantly lower ($P < 0.01$) in the low Mg/high K diet-fed sheep (31.0 μU/ml) than those of the control diet-fed sheep (111.7 μU/ml) (*Table 3*). The glucose disposal in the sheep fed the low Mg/high K diet tended to be smaller compared with those of the control diet-fed sheep, but there was no significant difference between diet treatments.

Euglycemic clamp technique

In all euglycemic clamp experiments using insulin infusion at rates of 5, 10, 15, and 20 mU/kg/min, the desired plasma glucose levels were maintained throughout the study. The representative euglycemic clamp

Table 4 Glucose disposal in sheep fed the control and low Mg/high K diets in a euglycemic clamp experiment[a]

Insulin infusion rate (mU/kg/min)	Diet treatment[b] (mg/kg/min)	
	Control	Low Mg/high K
5	4.99 ± 0.71	3.98 ± 0.32
10	5.71 ± 0.36	3.61 ± 0.48[c]
15	6.24 ± 0.92	3.68 ± 0.36[d]
20	4.53 ± 0.84	3.62 ± 0.31
Mean values	5.37 ± 0.37	3.72 ± 0.17[c]

[a] Each value represents a mean of five animals ± SEM
[b] See text
[c] Significantly different from the control ($P < 0.01$)
[d] Significantly different from the control ($P < 0.05$)

experiment using insulin infusion at a rate of 10 mU/kg/min is shown in *Figure 2*. Plasma insulin concentrations were rapidly increased and maintained at considerably high levels during the experimental period. The mean plasma insulin levels in the steady-state were 2155.4 and 2365.9 μU/ml for the control and low Mg/high K diet-fed sheep, respectively. In the control diet-fed sheep, the glucose disposal tended to increase with elevation of the insulin infusion rate from 5 to 15 mU/kg/min, but did not increase at a rate of 20 mU/kg/min (*Table 4*). The rate of insulin infusion did not affect the glucose disposal in the low Mg/high K diet-fed sheep. There were no significant differences in the glucose disposal among the insulin infusion rates across the diet treatments. The glucose disposal in the sheep fed the low Mg/high K diet (3.72 mg/kg/min) was significantly lower ($P < 0.01$) than that of the sheep fed the control diet (5.37 mg/kg/min) across the insulin infusion rates.

Discussion

Plasma minerals

As might be expected, the low Mg/high K diet which supplied 0.25 to 0.32 g of Mg/d induced hypomagnesemia. Some investigators[16,17] have reported that dietary excess K inhibited Mg absorption from the digestive tract and decreased plasma Mg levels in ruminants. Therefore, the significantly lower plasma Mg concentrations in the sheep fed the low Mg/high K diet might be due to the reduced amount of Mg intake and the reduced absorption by high dietary K.

Glucose clamp techniques

In the present study, values of the insulin response and glucose disposal would be reliable because good hyperglycemic and euglycemic controls were achieved. The correction of excreted glucose into urine is usually needed in hyperglycemic clamp experiments. In this study, however, the correction for urinary loss of glucose was disregarded, since plasma glucose levels might be held below the threshold (160 to 220 mg/100 ml) of glucose excretion in sheep.[18]

The glucose disposal represents the amount of glucose used by all the tissues, based on the assumption that the endogenous glucose production is completely depressed.[13] While a small increment in plasma insulin would completely depress hepatic glucose production in human,[19] Weekes et al.[14] demonstrated that complete suppression of endogenous glucose output was not achieved under euglycemic conditions in sheep. Therefore, the glucose disposal calculated in the present experiment may underestimate the total amount of glucose uptake in the whole body.

The markedly lower insulin response and glucose disposal were observed in the sheep fed the low Mg/high K diet. These results indicate that pancreatic B cell sensitivity to glucose and tissue sensitivity to insulin would be depressed in the hypomagnesemic sheep. Magnesium has been shown to be related to glucose metabolism and some endocrine secretions,[2-5] and to be indispensable for maintaining the effect of insulin on glucose uptake by the tissues.[20] There may be some relationship between insulin action on glucose uptake by the tissues and hyperkalemia. Deetz et al.[10] claim that insulin-induced glucose uptake would be depressed under the influence of excess K. Zierler and Rogus[21] demonstrated that insulin-induced hyperpolarization of cells increased specific D-glucose transport. The resting membrane potential is depolarized in exact proportion to the logarithm of K ion concentration in extracellular fluid.[22] A large amount of dietary K might affect the membrane potential, although the difference between intracellular and extracellular K concentrations was not determined in the present experiment.

Although the mechanism behind the reduced insulin response and glucose disposal remains unclear, the results of the present study indicate that glucose-induced insulin secretion and insulin-induced glucose utilization would be depressed in the hypomagnesemic sheep, and are caused by feeding the low Mg/high K diet.

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