The Rates of Oxidation of Primary Nutrients in Hypomagnesemic Sheep Fed a Low Magnesium and High Potassium Diet

Yoshiaki TERASHIMA, Yoshio HAMANO, Yasuyuki ISHIKAWA
and Hiroaki SANO*

Faculty of Animal Science, Kitasato University,
Towada-shi 034

(Received September 9, 1992)

Abstract The experiment was conducted to determine the effect of hypomagnesemia induced by feeding a low magnesium (Mg) and high potassium (K) diet on lipid concentrations in plasma and rates of oxidation of primary nutrients in sheep. Sheep were fed either a control or low Mg/high K diet containing 0.38 and 0.05% magnesium on dry matter basis, respectively for over 10 days. Insulin-induced glucose uptake was measured using a euglycemic clamp technique, and respiratory gas analysis was performed to estimate oxidation rates of carbohydrate, fat and protein in the 24-hr starved state and during the euglycemic clamp experiment. Sheep fed the low magnesium (Mg)/high potassium (K) diet had lower ($P < .01$) plasma Mg levels than the control diet-fed group. Plasma phospholipids elevated ($P < .10$), and triglycerides and free fatty acids tended to increase in the hypomagnesemic sheep fed the low Mg/high K diet. In the euglycemic clamp experiment, insulin-induced glucose uptake tended to decrease in the hypomagnesemic group, but not significantly. The diet treatment and euglycemic clamp experiment did not affect energy expenditure (heat production), but the low Mg/high K diet-fed sheep had slightly reduced ($P < .13$) non-protein respiratory quotient (RQ). The low Mg/high K diet feeding increased ($P < .06$) fat oxidation rate, and tended to decrease carbohydrate oxidation rate, though the rates of oxidation were unaffected by performing the euglycemic clamp experiment. These results indicated that hypomagnesemia induced by a low Mg/high K diet feeding stimulated lipid metabolism, and increased the fat oxidation rate in sheep.

Anim. Sci. Technol. (Jpn.) 64 (4) : 357-363, 1993.

Key words : hypomagnesemic sheep, nutrient oxidation, blood lipids

Hypomagnesemia in ruminants has been shown to be associated with hypoglycemia and ketosis^{1,13)}. Magnesium deficiency has also been reported to produce hyperlipemia, and affect lipid metabolism in rats^{2,7)}. It appears, therefore, that intermediate metabolism of carbohydrate and fat may be altered by unknown mechanism when animals become hypo-

magnesemia. In our previous report, hypomagnesemic sheep induced by the low magnesium (Mg)/high potassium (K) diet feeding had significantly lower insulin responsiveness to glucose and insulin-induced glucose uptake of whole body compared with those of the control sheep⁴⁾. These results suggest that hypomagnesemia in ruminants affects insulin secretory

* Present address : Faculty of Agriculture, Iwate University, Morioka-shi 020

response and insulin action, and may result in altered metabolic rates of nutrients, including the rates of oxidation of primary nutrients. However, there was no available information on this matter in hypomagnesemia in ruminants. The objective of the present experiment was to determine rates of oxidation of carbohydrate, fat and protein in hypomagnesemic sheep induced by the low Mg/high K diet feeding.

Materials and Methods

Animals and diets: Six ewes weighing about 45 kg were divided at random into control and low Mg/high K diet groups of three sheep each. Animals were kept in metabolic crates in a controlled environment room at 20°C during the whole experimental period. The basal semi-purified diet was formulated as reported previously^{4,11)}, and the ingredient and chemical compositions of the diet are shown in Table 1. The control and low Mg/high K diets were prepared by adding 0.33% MgO and 5.42% KCl to the basal diet, respectively. Mg and K contents on dry matter basis were 0.38 and 0.52%, and 0.05 and 5.94% in the control and low Mg/high

K diets, respectively. Animals were fed either the control or low Mg/high K diet to meet the daily metabolizable energy (ME) requirements for over 10 days. Water was available at all times except for periods of respiratory gas analysis and euglycemic clamp experiment. Blood samples were taken in the 24-h starved state to determine the dietary effect on plasma concentrations of minerals (calcium, magnesium and potassium) and lipids (triglyceride, phospholipid and free fatty acid). The condition of 24-h starved state could avoid any effects of diurnal variation on nutrient absorption from the gastrointestinal tract, and provide low CO₂ production in the rumen fermentation.

Respiratory gas analysis and euglycemic clamp technique: After animals were fed each experimental diet for over 10 days, they were subjected to indirect open-circuit calorimetry in the 24-h starved state, and followed by a euglycemic clamp technique combined with respiratory gas analysis. Polyethylene catheters were inserted into the carotid artery of skin loop for blood sampling and the jugular vein for glucose and insulin infusions as previously described^{4,8)}. Animals were also fitted with a Foley bladder catheter on the day of the experiments performed to collect urine.

To estimate oxygen (O₂) consumption and carbon dioxide (CO₂) production, respiratory gas analyses were performed in the 24-h fasted state for 60 min and during the latter half of the 2-h euglycemic clamp experiment for 60 min, respectively. The open-circuit ventilated hood (head cage) made from polyvinyl was used in the experiment. The head cage with 89 cm of height, 47 cm of width and 40 cm of depth was installed inside with 2 fans for better air circulation. The air flow rate at the outlet of head cage was set to be 35 l/min during the respiratory gas analysis period. In the steady-state conditions, O₂ and CO₂ concentrations in inlet and outlet air were measured using O₂ (Model 501-DS, P.K.Morgan

Table 1. Ingredient and chemical composition of semi-purified basal diet

Item	% of DM
Ingredient	
Corn starch	30.0
Corn cobs (gr.)	20.5
Dextrose	20.5
Cellulose	16.7
Soy protein	8.2
Vegetable oil	0.8
Feed additive	1.6
Dicalcium phosphate	1.2
Potassium bicarbonate	0.5
Chemical composition	
Crude protein	6.07
Calcium	0.35
Magnesium	0.05
Potassium	0.52

Ltd., England) and CO₂ (Type D/6U/54/A, The Anatical Development Co. Ltd., England) analyzers. Standard gasses of O₂ and CO₂ were used for calibration.

Urinary excretion was obtained every one hour intervals during the whole respiratory gas analysis period, and urinary nitrogen excretion was determined for estimating rate of protein oxidation. The nonprotein respiratory quotient (NPRQ) and metabolic rate was calculated by using equations, based on the measurement of O₂ consumption, CO₂ production and urinary N excretion³⁾. Rates of oxidation of carbohydrate (CHO) and fat were estimated using an equation of ZUNTZ and SCHUMBURG⁵⁾.

In the euglycemic clamp technique, crystalline porcine insulin diluted with isotonic saline was continuously infused (8 mU/kg BW/min) following by variable glucose infusion rates to maintain the euglycemic plateau (basal level). The procedure used in the present experiment was almost the same as described previously^{4,8,10)}.

Mineral (calcium, magnesium and potassium) contents in plasma and diets were measured by atomic absorption spectrophotometry. Plasma triglyceride, phospholipid and free fatty acid (FFA) concentrations were determined by each Test-Wako kit (Wako Pure Chem. Indus. Ltd.), respectively. The plasma glucose concentration

was measured by using an automated glucose analyzer (Model GLU-1, Toa Electronics Ltd.) and plasma insulin was assayed by a RIA kit (Eiken Chemical Co. Ltd.).

Differences in plasma minerals and lipids between the diet treatment were analyzed in a one way layout design. The parameters obtained from the euglycemic clamp experiment were tested to determine the diet effect. All data from the indirect calorimetry in the 24-h starved state and during euglycemic clamp technique were analyzed by a two-way analysis of variance. Main factors were period (in the 24-h fasted state and during euglycemic clamp experiment) and diet treatment.

Results and Discussion

The mean plasma Mg concentration was lower ($P < .01$) in the Mg/high K diet-fed sheep than in the control diet-fed sheep (Table 2). Plasma K was apt to be higher ($P < .10$) in the low Mg/high K diet compared to the control group. The calcium concentration was unaffected by the diet treatment. These results confirm early finding that the low Mg/high K diet feeding induces hypomagnesemia in sheep^{4,11)}.

The low Mg/high K diet group had higher ($P < .10$) phospholipids in plasma compared to the control group (Table 2). Concentrations of

Table 2. Concentrations of plasma minerals and lipids in sheep fed a control or low Mg/high K diet

	Diet	
	Control	Low Mg/High K
Mineral (mg/dl)		
Calcium	7.96 ± 0.35	8.14 ± 0.31
Magnesium	2.62 ± 0.04	1.40 ± 0.32**
Potassium	15.3 ± 2.0	18.3 ± 1.2*
Lipid		
Triglyceride (mg/dl)	21.8 ± 5.3	24.9 ± 2.1
Phospholipid (mg/dl)	76.3 ± 4.2	94.0 ± 11.4*
FFA (μEq/l)	273 ± 92	437 ± 216

Values represent means of three animals with SD.

* $P < .10$, ** $P < .05$,; Significantly different from the control.

Table 3. Basal plasma glucose and insulin concentrations, glucose infusion rate (GIR) and mean plasma insulin increment (MPII) in the euglycemic clamp experiment in sheep fed a control or low Mg/high K diet

	Diet	
	Control	Low Mg/High K
Plasma glucose (mg/dl)	60.7±11.6	60.0±9.5
Plasma insulin (μ U/ml)	9.3± 3.1	11.0±7.2
GIR (mg/kg BW/min) ¹⁾	5.9± 1.1	4.9±1.1
MPII (mU/ml)	3.2± 0.9	3.1±0.8

Values represent means of three animals with SD.

¹⁾ During the latter half of the 2-h euglycemic clamp period.

plasma triglycerides and FFA tended to be higher when animals were fed the low Mg/high K diet, though not significantly.

Acute magnesium deficiency in weanling rats increased triglyceride and phospholipid levels in plasma, but did not affect FFA, and this defect was associated with modifications of lipoprotein metabolism^{2,7)}. On the other hand, high levels of FFA in magnesium-deficient rats after fasting have been reported, though the concentrations of triglycerides and phospholipids were unchanged⁹⁾. The discrepancy between these results may depend upon the feeding state of animals⁷⁾. In the present experiment, the tendency to increase lipids in plasma in hypomagnesemic sheep could have arisen as a consequence of increased hepatic lipogenesis and fatty acid mobilization, decreased removal of lipids from the blood or a combination of those by some unknown mechanism.

In the euglycemic clamp technique, basal glucose and insulin concentrations were not influenced by the diet treatment (Table 3). The blood glucose concentration was almost clamped at the basal levels during the 2-h period of insulin-glucose infusion (Fig. 1). There was no significant difference in glucose infusion rates (GIR) during the last half of the infusion period between the diet treatment, though GIR in the low Mg/high K diet-fed sheep was a little lower than the control sheep (Table 3 and Fig. 1). In our previous report, the hypomagnesemic sheep had a decreased

Fig. 1. Changes in plasma insulin and glucose concentrations, and glucose infusion rate (GIR) in sheep fed the control (open) and low Mg/high K (solid) diets during the euglycemic clamp experiment. Values represent means of three animals with SD.

GIR, suggesting that insulin-induced glucose uptake of whole body could be depressed by feeding the low Mg/high K diet to the ruminants⁴⁾. The present result was similar to those of the previous experiment.

There was no significant difference in heat

Nutrient Oxidation Rate in Hypomagnesemic Sheep

Table 4. Nonprotein respiratory quotient(NPRQ), heat production and rates of oxidation of carbohydrate, fat and protein in the 24-h starved state and during euglycemic clamp experiment in sheep fed a control or low Mg/high K (L-Mg/H-K) diet

Period	Starved state		Euglycemic clamp		Difference		
Diet	Control	L-Mg/H-K	Control	L-Mg/H-K	Period (P)	Diet (D)	P × D
NPRQ	0.93±0.07	0.84±0.12	0.88±0.04	0.81±0.05	NS	NS	NS
Heat production (kcal/kg ^{0.75} /h)	3.27±0.59	3.11±0.07	3.04±0.26	3.45±0.09	NS	NS	NS
—Oxidized amount (mg/kg ^{0.75} /h) —							
Carbohydrate	579±244	325±293	389±133	284±149	NS	NS	NS
Fat	65± 53	159±130	112± 27	214± 57	NS	*	NS
Protein	59± 9	62± 9	85± 9	59± 1	**	**	***
—Percentage (%) of oxidation (based on energy)—							
Carbohydrate	71± 20	43± 38	53± 13	34± 17	NS	NS	NS
Fat	21± 20	48± 39	35± 11	59± 17	NS	*	NS
Protein	8± 0	9± 1	12± 2	7± 0	**	**	***

Values represent means of three animals with SD.

*P<0.10, **P<0.05, ***P<0.01

NS : No significance

production between in the 24-h starved state and during the euglycemic clamp experiment, and between the diet treatment (Table 4.). While NPRQ values were not affected by the euglycemic clamp technique, they were apt to be declined in the low Mg/high K diet-fed group compared with those of the control sheep, suggesting that the rate of oxidation of fat was increasing. Several studies have reported that glucose infusion stimulated energy expenditure in man^{6,12}). Therefore, we expected that heat production during the euglycemic clamp technique might increase, but no change in energy expenditure was observed in the present experiment.

Calculated oxidation rates of carbohydrate (CHO), fat and protein are shown in Table 4. The euglycemic clamp experiment did not influence the rate of oxidation of these nutrients across the diet treatment. The amounts of oxidation of fat increased (P<.06) from 89 in the control to 187 mg/BW^{0.75}/h in the low Mg/high K diet across period (Table 4). The percentages of oxidation rates of CHO tended to decrease (P<.14), and fat oxidation rates

increased (P<.11), when animals were fed the low Mg/high K diet. The euglycemic clamp experiment significantly increased the rate of protein oxidation in the control diet-fed sheep. However, the increased rate was very slight (Table 4).

There was no information on rates of oxidation of nutrients in hypomagnesemia in ruminants. Increases in lipid concentrations in plasma may lead to greater oxidation rate of fat, because of greater supply of lipid substrate to tissues in the body. In the present experiments, the increased oxidation rate of fat observed in the low Mg/high K diet-fed sheep may be explained by the tendency to increase lipids in plasma. The rates of oxidation of nutrients did not vary during the euglycemic clamp experiment. Therefore, glucose and insulin infusions did not change the rates of oxidation of primary nutrients under the present experimental condition.

Although the number of animals used in the present experiment was marginal, the significant increase in fat oxidation rate in the low Mg/high K diet-fed sheep was observed. These

results strongly suggest that hypomagnesemia in ruminants stimulates fat oxidation. However, the mechanism involved in this phenomenon remains unclear.

Acknowledgement

This work was supported in part by a grant in-aid (No.04660310) from the Ministry of Education, Science and Culture of Japan.

References

- 1) GRUNES, D.L., P.R. STOUT and J.R. BROWNELL, Grass tetany of ruminants. *Adv. Agron.*, **22**: 331-374. 1970.
- 2) GUEUX, E., A. MAZUR, P. CARDOT and Y. RAYSSIGUIER, Magnesium deficiency affects plasma lipoprotein composition in rats. *J.Nutr.*, **121**: 1222-1227. 1991.
- 3) JEQUIER, E., K. ACHESON and Y. SCHUTZ, Assessment of energy expenditure and fuel utilization in man. *Ann. Rev. Nutr.*, **7**: 187-208. 1987.
- 4) MATSUNOBU, S., Y. TERASHIMA, T. SENSU, H. SANO and H. ITOH, Insulin secretion and glucose uptake in hypomagnesemic sheep fed a low magnesium, high potassium diet. *J. Nutr. Biochem.*, **1**: 167-171. 1990.
- 5) MORIMOTO, H., *Kachiku Eiyogaku*. 17th ed., 271-274. Yōkendō. Tokyo. 1989.
- 6) PITTET, P., P. CHAPPUIS, K. ACHESON, F. DE TECHTERMANN and E. JEQUIER, Thermic effect of glucose in obese subjects studied by direct and indirect calorimetry. *Br. J. Nutr.*, **35**: 281-292. 1976.
- 7) RAYSSIGUIER, Y., E. GUEUX and D. WEISER, Effect of magnesium deficiency on lipid metabolism in rats fed a high carbohydrate. *J. Nutr.*, **111**: 1879-1883. 1981.
- 8) SANO, H., M. NAKAI, T. KONDO and Y. TERASHIMA, Insulin responsiveness to glucose and tissue responsiveness to insulin in lactating, pregnant, and nonpregnant, nonlactating beef cows. *J. Anim. Sci.*, **69**: 1122-1127. 1991.
- 9) SCHMALBECK, J., W.R. WILLEMS, F. DORN and T.H. GUNTHER. *Über den lipid-stoffwechsel im magnesium-mangel. Untersuchungen an einem arteriosklerose-modell. Z. Klin. Chem. Klin. Biochem.*, **10**: 270-274. 1972.
- 10) TERASHIMA, Y., F. ISHIGAKI, T. YAMAGUCHI, H. SANO and H. ITOH, Effects of dietary energy density and feeding level on insulin secretion and glucose uptake in sheep. *Anim. Sci. and Technol. (Jpn.)*, **62**: 304-307. 1991.
- 11) TERASHIMA, Y., S. MATSUNOBU and H. ITOH, Parathyroid hormone secretion in hypomagnesemic sheep fed on a low magnesium and high potassium diet. *Asian-Australasian. J. Anim. Sci.*, **2**: 275-276. 1989.
- 12) THIEBAUD, D., Y. SCHUTZ, K. ACHESON, E. JACOT, R.A. DEFRONZO, J. FELBER and E. JEQUIER, Energy cost of glucose storage in human subjects during glucose-insulin infusions. *Am. J. Physiol.*, **244**: E216-E221. 1983.
- 13) WILCOX, G.E. and J.E. HOFF, Grass tetany: An hypothesis concerning its relationship with ammonium nutrition of spring grasses. *J. Dairy Sci.*, **57**: 1085-1089. 1974.

低マグネシウム血症めん羊における各栄養素の 酸化割合について

寺島福秋・浜野美夫・石川泰之・佐野宏明*

北里大学獣医畜産学部，十和田市 034

* 現所属：岩手大学農学部，盛岡市 020

本実験は低マグネシウム血症めん羊における各栄養素の酸化割合を明らかにするため行なった。めん羊にマグネシウム (0.38%Mg) とカリウム (0.52%K) 含量の正常な飼料 (対照飼料) または Mg 含量 (0.05%) が低く，K 含量 (5.94%) の高い飼料 (低 Mg/高 K 飼料) を 10 日間以上にわたって給与した。これらのめん羊について飼料給与後 24 時間に呼吸試験を実施し，炭水化物，脂肪および蛋白質の酸化量を求めた。更に，インスリンとグルコースを同時に注入し，注入前の血糖値を維持するグルコースクランプ実験と呼吸試験を同時に実施した。低 Mg/高 K 飼料摂取めん羊の血漿 Mg 濃度は対照区に比較して有意に低下した。血漿中トリグリセライド，リン脂質および遊離脂肪酸濃度は低 Mg/高 K 飼料の摂取により増加する傾向を示した。グルコースクランプ実験時におけるグルコース注入量は低 Mg/高 K 飼料で低下する傾向を示した。熱産生量は両区ではほぼ同じ値を示したが，非蛋白呼吸商は低 Mg/高 K 区で若干低下した。低 Mg/高 K 飼料摂取めん羊の脂肪酸化量は増加し，炭水化物の酸化量は低下する傾向を示した。これらの結果より，低 Mg/高 K 飼料給与によって誘起された低 Mg 血症めん羊の脂肪酸化は促進することが示唆された。

日畜会報，64 (4) : 357-363, 1993