Effect of a High Protein Diet on Calcium Metabolism in Sheep

Masayuki Funaba*, Hajime Nabeta, Hideo Yano and Ryoji Kawashima

Department of Animal Science, Faculty of Agriculture, Kyoto University, Sakyo-ku Kyoto-shi 606

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Abstract

The effects of a high protein diet on calcium (Ca) handling in the kidney and Ca mobilization were evaluated in sheep. Utilizing a cross-over design, a clearance study and a EDTA infusion study were conducted in six adult wethers fed a control diet (CP: 9.9%, ME: 2.02 Mcal/kg) or a high protein diet (CP: 19.6%, ME: 2.02 Mcal/kg). Urinary Ca excretion was increased and plasma Ca concentrations were decreased by the high protein diet. An increase in glomerular filtration rate would be responsible for an increase in filtered Ca across glomeruli and an increase in urinary Ca excretion. The high protein diet also elevated plasma free hydroxyproline level. Although plasma ionized Ca concentrations linearly decreased in both dietary treatments during intravenous EDTA infusion, the rate of reduction in ionized Ca concentrations tended to be smaller in sheep fed the high protein diet than in sheep fed the control diet. Before EDTA infusion, plasma immunoreactive parathyroid hormone (iPTH) level was increased in sheep fed the high protein diet. However, the increase in iPTH level was smaller in sheep fed the high protein diet than that in those fed the control diet. Ca mobilization from available Ca pool was stimulated by the high protein diet. This study indicates that a high protein diet increases urinary Ca excretion and depresses plasma Ca levels, leading to activate bone Ca resorption in sheep.


Key words: calcium metabolism, high protein diet, sheep

It is well known that a high protein diet increases urinary calcium (Ca) excretion in men and an increased Ca loss has been suggested to involve in the etiology of osteoporosis. DRAPER and co-workers indicated that a high protein diet did not increase bone resorption but femoral radioautographs of rats fed high sulfur-containing amino acids showed a reduction in metaphyseal bone, suggesting depressed bone formation in rats. Although men usually excrete more than 10% of dietary Ca intake via the kidney, urinary Ca excretion in ruminants such as sheep and cattle is less than 5% of dietary intake.

Little attention has been paid to the Ca metabolism of ruminants fed a high protein diet. Diets are likely to become protein-rich when brewery's by-products born from beer and whiskey industry are given to ruminants. This study examined the effect of a high protein diet on Ca handling in the kidney and the ability of Ca mobilization in sheep.

Materials and Methods

Six adult Corriedale wethers (58 kg average body weight) were kept in metabolic cages.
urine collection. Experimental diets shown in Table 1 were given at 8:00 and 15:00 daily at a level of 1% of body weight for each meal and water was provided ad libitum except for EDTA infusion period. A control diet adequately contains crude protein for maintenance in sheep22) and the crude protein content of the high protein diet is two times as much as that of the control diet. According to calculation, metabolizable energy contents of the control diet and the high protein diet are 2.02 Mcal/kg, which meets the requirement of sheep for maintenance22).

The study was divided into two metabolic trials. In the first trial, half of wethers were fed the control diet, and the other half were fed the high protein diet. In the second trial, the treatments were reversed. Each trial consisted of a 14-day adjustment period followed by a 5-day clearance study and a 1-day EDTA infusion study. During the clearance study, urine was collected daily into glass bottle containing 2 ml of toluene, and stored at -20°C till analysis. Blood samples were taken from the jugular vein just before (T0) and 6 hours after feeding in the morning feed (T6), and blood plasma samples were obtained by centrifugation. Glomerular filtration rate (GFR) during the clearance study was estimated by endogenous creatinine clearance28), and the renal tubule reabsorption of Ca was calculated as the difference between urine Ca and filtered Ca across the glomeruli, where filtered Ca equals the product of GFR and plasma UF-Ca concentration. The study of EDTA infusion was conducted on a day after the clearance study of each trial. Two % (w/v) disodium ethylene diamine tetraacetate (Wako Pure Chemical Industries, Osaka) (EDTA-Na2-2H2O), and 0.08 % (w/v) procaine (Wako Pure Chemical Industries, Osaka) which lessens a pain of the blood vessel were infused through an indwelling jugular vein catheter. EDTA infusion of 0.30 mmol/kg body weight was administrated to wethers for 60 minutes by a constant infusion pump. Four blood samples were collected before the start of EDTA infusion and blood samples were taken every 15 minutes for 90 minutes after the start of infusion. Thereafter, blood samples were taken at 120, 150, 180 and 240 minutes.

Samples in the clearance study were analyzed as follows; Ca was by an atomic absorption spectrophotometry (AA-782, Nippon Jarrell-Ash, Kyoto), inorganic phosphorus (Pi) was by the method of CHEN et al.10) and creatinine was by alkaline picric acid method24). Also, plasma concentrations of ultrafilterable Ca (UF-Ca)23), free hydroxyproline (Free-Hyp)5) and urea nitrogen (BUN) (by the method of urease-indophenol) were measured. Plasma insulin (IRI) and parathyroid hormone (iPTH) were measured by radioimmunoassay (Insulin RIA kit, Eiken Immunocochemical Laboratory, Tokyo and INS-PTH radioimmunoassay kit, Nichols Institute, U.S.A.). Urinary net acid excretion (NAE) was measured by the method of CHAN9) and YANG et al.33) using a pH autotitrator (Potentiometric Automatic Titrator, AT-118, Kyoto Electronics, Kyoto). This method has been shown to be applicable to sheep urine and to reflect acid–base conditions in an animal body13).

Blood plasma samples during EDTA infusion were analyzed for total Ca, ionized Ca (Sera-
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The rates of Ca mobilized from available sources were estimated by the modification of the method by CONTRERAS et al.\textsuperscript{11) as follows.

\[ R = 1 - V \times (\Delta \text{Ca}) / I, \]

where \( R \) is the rate of Ca mobilization per an infused amount of EDTA (mmol/mmol EDTA), \( I \) is the amount of EDTA (mmol) during the infusion period, \( \Delta \text{Ca} \) is a decrease in ionized Ca concentration (mmol/l) during the infusion period and \( V \) is volume of a compartment (l) within which there is rapid equilibration of ionized Ca. An independent measurement of \( V \) can be obtained from the infused amount of EDTA and the changes in the plasma concentration of Ca bound to EDTA during and after the infusion period, as described by CONTRERAS et al.\textsuperscript{11).}

Data were analyzed by analysis of variance using the GLM procedure of SAS program\textsuperscript{25). Factors considered were feed, animal and error.

Results

Clearance study

Plasma concentrations of Ca, Pi, Free-Hyp, BUN, IRI and iPTH are presented in Table 2. Plasma Ca concentrations were significantly lower (\( p<0.05 \)) and plasma Free-Hyp concentrations and iPTH levels were significantly higher in sheep fed the high protein diet, compared to those in sheep fed the control diet. The high protein diet elevated BUN concentrations and plasma IRI concentrations to 170.5% and 143.8% of control, respectively. Plasma Pi concentrations were not affected by the dietary treatment.

As shown in Table 3, urinary volume and urinary excretion of Ca and Pi were increased by the high protein diet. Both sheep fed the control diet and the high protein diet showed the values of negative acids excretion, i.e. net bases excretion. More alkaline-rich urine was excreted in sheep fed the high protein diet than

| Table 2. Effects of the high protein diet on plasma components |
|-------------------|------|--------|-----|-----|-------|
|                  | Ca   | Pi    | Free-Hyp | BUN | IRI   | iPTH  |
|                  | mg/100 ml | mg/100 ml | µU/ml | µU/ml | pg/ml |
| Control          | 10.02  | 4.27  | 0.26  | 16.6 | 17.7  | 30.0  |
| High-protein     | 9.96*  | 4.41  | 0.28* | 29.4** | 25.4** | 34.6* |
| SEM              | 0.03  | 0.07  | 0.01  | 0.2  | 1.1  | 1.4  |

*: significantly differ from the control group at the level of \( p<0.05 \) and \( p<0.01 \), respectively.

SEM = standard error of the mean.

| Table 3. Effects of the high protein diet on urinary excretions of Ca, P and acids |
|-----------------------------|-----|-----|-------|
| Volume                      | Ca  | Pi  | NAE   |
| ml/day                      | mg/day | mg/day | mEq/day |
| Control                     | 1452 | 30.6 | 2.7 | -156.7 |
| High-protein                | 1838** | 42.7* | 10.4** | -239.0** |
| SEM                         | 58  | 3.7  | 1.5  | 9.7  |

*: significantly differ from the control group at the level of \( p<0.05 \) and \( p<0.01 \), respectively.

SEM = standard error of the mean.
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Table 4. Effects of the high protein diet on glomerular filtration load and fractional reabsorption rate of Ca and P

<table>
<thead>
<tr>
<th>Filtered load</th>
<th>Reabsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>GFR (l/day)</td>
<td>Ca (g/day)</td>
</tr>
<tr>
<td>Control</td>
<td>131.17</td>
</tr>
<tr>
<td>High-protein</td>
<td>150.33**</td>
</tr>
<tr>
<td>SEM</td>
<td>4.43</td>
</tr>
</tbody>
</table>

**: significantly differ from the control group at the level of p<0.01.
SEM=standard error of the mean.

Fig. 1. The changes of plasma ionized Ca concentrations (%) during and after EDTA infusion. Points are means with their standard errors represented by vertical bars. Initial values are set at 100%.

Fig. 2. The changes of plasma iPTH concentrations during and after EDTA infusion. Each value is expressed as a percentage of the initial values. During EDTA infusion, ionized Ca concentrations linearly decreased in both dietary treatments. Ionized Ca concentrations increased and tended to recover after the completion of EDTA infusion, however, these values would not reach the initial value at the end of study. The rate of decrease in ionized Ca concentrations tended to be smaller in sheep fed the high protein diet than that in sheep fed the control diet.

The changes in plasma iPTH concentrations induced by EDTA infusion are shown in Fig. 2. The infusion of EDTA stimulated the secretion of PTH in all sheep. Although the initial level
Table 5. Effects of the high protein diet on Ca mobilization from available sources

<table>
<thead>
<tr>
<th></th>
<th>Infused</th>
<th>Mobilized</th>
<th>ΔCa</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol</td>
<td>mmol/mmol</td>
<td>mmol/l</td>
<td>l</td>
</tr>
<tr>
<td>Control</td>
<td>17.73</td>
<td>0.44</td>
<td>0.88</td>
<td>11.37</td>
</tr>
<tr>
<td>High-protein</td>
<td>16.45</td>
<td>0.53*</td>
<td>0.78*</td>
<td>9.92*</td>
</tr>
<tr>
<td>SEM</td>
<td>0.51</td>
<td>0.01</td>
<td>0.02</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*: significantly differ from the control group at the level of p<0.05.
SEM = standard error of the mean.

Discussion

The cause of increased urinary Ca excretion by a high protein diet is still controversial. Although Massry and Kleeman\(^{21}\) stated that a rise in GFR and a consequent increase in filtered Ca across the glomeruli were closely correlated with protein-induced hypercalciuria in dogs, some reports showed that depressed Ca reabsorption by renal tubule cells was responsible for the hypercalciuria in men.\(^{3,17}\) Furthermore, Linkswiler and co-workers observed that both an increase in filtered loads and depressed reabsorption caused the stimulation of Ca excretion via urine in men fed a high protein diet.\(^{15,16,19,27}\)

As observed in our previous study, the high protein diet with normal energy induced the increase in GFR, resulting in a tendency to augment of filtered Ca loads.\(^{14}\) The high protein diet did not affect the reabsorption of Ca by renal tubular cells. The combination of the increase in filtered Ca and no effect on Ca reabsorption led to the increase in urinary Ca excretion.\(^{14}\) Since the high protein diet elevated BUN level, the increased osmotic diuresis was thought to cause the increase in GFR and filtered Ca loads. However, only the osmotic diuresis cannot account for the increased urinary Ca excretion, since the infusion of arginine in diabetic rats increased urinary volume but did not influence urinary Ca excretion.\(^{32}\)

This study showed that a two fold increase in dietary protein caused a 39% increase in urinary Ca excretion in sheep. In men a 100% increase in protein intake has been reported to elevate urinary Ca excretion at the amount of 50%.\(^{4,16}\) The difference of protein sources could be responsible for the difference of urinary Ca excretion between in sheep and in men. Protein in feeds originated from plants such as soybean meal contains a lower level of sulfur-containing amino acids. Some reports suggested that urinary sulfate which was
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derived from the catabolism of sulfur-containing amino acids partly involved in protein-induced hypercalciuria\(^{27,30,31}\). WHITING and DRAPER\(^{30}\) indicated that the hypercalciuria was correlated with a dietary amount of their sulfur-containing amino acids. Additionally, a high level of soy protein did not increase urinary Ca excretion in some reports\(^{8,35}\), suggesting that no increment in urinary Ca excretion was due to a low level of dietary sulfur-containing amino acids\(^{35}\). Since soybean meal was used as protein source, the relative small degree of hypercalciuria might be induced in this study.

The mechanisms of depressed Ca reabsorption by renal tubular cells in men fed a high protein diet has been suggested in several reports. ALLEN \textit{et al.}\(^{3}\) reported that insulin might be related with the increase in urinary Ca excretion by a high protein diet. DEFRONZO \textit{et al.}\(^{12}\) showed that insulin infusion increased urinary Ca excretion under conditions of euglycemia in men. SCHUETTE \textit{et al.}\(^{27}\) observed a positive correlation between urinary Ca excretion and net acid excretion in men and mild metabolic acidosis has been suggested to cause an increase in urinary Ca excretion\(^{20,29}\).

In this study, although a plasma IRI level was elevated by the high protein diet which was coincident with a study by ALLEN \textit{et al.}\(^{3}\), a poor correlation between plasma IRI levels and urinary Ca excretion was observed \((r = -0.01, p>0.92)\). ALLEN \textit{et al.}\(^{3}\) studied continuous insulin secretion after the consumption of a single diet, however, a blood sample was taken only 6 hours after the high protein diet feeding in this study. Since insulin secretion has been known to have a considerable variation in a day and to be stimulated after an ingestion in sheep\(^{26}\), a detailed observation on relation between continuous postprandial plasma IRI levels and urinary Ca excretion would be needed.

Also, the high protein diet did not increase urinary net acid excretion but adversely decreased it, indicating the augmentation of urinary net base excretion and mild metabolic alkalotic status.

A decrease in plasma Ca concentrations by the high protein diet, which have been thought to result from the augmentation of Ca excretion via urine at least in part, caused the stimulation of PTH secretion and led to the elevation of plasma Free-Hyp level, which indicated an increase in bone resorption. The stimulation of bone resorption would implies the activation of bone metabolism which involves in both bone resorption and bone formation\(^{17}\). Sheep fed the high protein diet could mobilize more Ca from Ca storage places such as the skeleton and extracellular fluid etc, during the EDTA infusion. The high protein diet might promote a sharp response to PTH because the increased rate of plasma iPTH during and after EDTA infusion was smaller in sheep fed the high protein diet than those fed the control diet.

Acknowledgments

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28) Tomas, F.M., Phosphorus homeostasis in
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高タンパク質飼料の摂取がめん羊のカルシウム代謝に及ぼす影響

舟場正幸*・鍋田 肇・矢野秀雄・川島良治

京都大学農学部，京都市左京区 606

高タンパク質飼料を摂取しためん羊の腎におけるカルシウム (Ca) の動態および EDTA 負荷時の血中への Ca 動員量を検討した。対照飼料 (CP: 9.9%, ME: 2.02 Mcal/kg) と高タンパク質飼料 (CP: 19.6%, ME: 2.02 Mcal/kg) を 2 頭のめん羊に与え、腎クリアランス試験と EDTA 負荷試験を行なった。

高タンパク質飼料摂取により、尿中 Ca 排泄量の増加と血漿 Ca 濃度の減少が見られた。その要因として、余余血消化過の増加とそれに伴う余余体循環 Ca 量の増加が、尿中 Ca 排泄量の増加をもたらしたと考えられた。また、血漿遊離ハイドロキシプロリン濃度は、高タンパク質飼料区で高くなった。

EDTA 負荷により、血漿イオン化 Ca 濃度は血漿と割合的に減少したが、低下の程度は高タンパク質飼料の摂取により緩和される傾向が認められ、Ca 動員量は高タンパク質飼料区で有意に増加した。EDTA 負荷前の血漿上皮小体ホルモン (iPTH) 濃度は、高タンパク質飼料区で高かったが、負荷時および負荷後の血漿 iPTH 濃度の増加は、高タンパク質飼料摂取により小さくなる傾向であった。

めん羊に高タンパク質飼料を与えすると、尿中 Ca 排泄量の増加と血漿 Ca 濃度の減少がみられるが、骨吸収は促進されており、これは PTH 分泌とその反応性的増加によるものと考えられた。

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* 現所属：麻布大学獣医学部，相模原市 229

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