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Effects of Dietary Protein Levels and Sources on Calcium and Phosphorus Metabolism in Young Korean Women*

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한국성인 여성의 단백질 섭취수준과 동·식물성 급원이 칼슘 및 인대사에 미치는 영향

구재옥 · 곽충실** · 최혜미**

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식이 단백질 섭취수준 및 동·식물성 단백질 급원이 칼슘 및 인대사에 미치는 영향을 검토하기 위하여, 10명의 한국성인 여성을 대상으로 26일간의 통제 식이실험을 실시하였다.

대사실험 연구는 6일간의 적응기간과 10일간의 중단백식이(60g 단백질, 545mg Ca)와 10일간의 고단백질 식이(90g 단백질, 575mg Ca)로 구성되었다. 중·고단백 식이기간 동안 동물성 단백질(75% 동물성 단백질)과 식물성 단백질(75% 식물성 단백질) 식이군으로 나누어, 같은 식이에서 중단백식이 후 고단백식이를 섭취토록 하였고, 실험식이 마지막 4일간은 각 2명에게 300mg 칼슘을 보충시켰다.

칼슘 흡수율은 단백질 섭취량의 증가시 동물성 단백질 식이군에서 유의적으로 증가하였다. 칼슘 보충은 급원에 관계없이 칼슘 흡수를 증가시켰다. 중단백 식이기간 동안 동물성과 단백질 식이에서 칼슘흡수율은 약 30%이었으며, 고단백질식이 기간 동안 동물성 단백질에서 46%, 식물성 단백질에서 37%이었다. 칼슘을 보충시켰을 때, 중단백 식이기간 동안 칼슘 흡수율은 약 46%이었으며, 고단백 식이군에서 53%이었다. 뇨중 칼슘배설은 단백질 섭취수준에는 영향을 받지 않았으나, 동물성 단백질 식이에서 식물성 단백질 식이보다 칼슘배설양이 많았다. 칼슘 균형은 단백질 섭취증가와 칼슘 보충으로 호전되었다.

인의 흡수는 단백질 섭취수준에는 영향을 받지 않았으며 인 섭취량에 따라 증가하였다. 인 흡수율은 동물성 단백질 식이군에서 약 77~81%로 식물성 단백질 식이군에서의 55~65%보다 높았다.

이상의 결과로 보아 단백질 섭취수준 60g에서 90g으로의 증가와 칼슘보충은 뇨중 칼슘 배설에는 영향을 주지 않으며, 칼슘 흡수를 증진시켜서 칼슘 균형을 호전시키는 것으로

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Introduction

Protein is one of dietary components known to affect calcium metabolism in human. Several investigators reported an increase in dietary protein was related to increase urinary losses of calcium, resulting in negative calcium balance[1)-8)]. Linkswiler and associates[1)2)5)6)] found that the increase in urinary calcium was not accompanied by a comparable increase in apparent calcium absorption, resulting impaired calcium balance, when dietary protein increased from 47 to 142g /day. Many reports indicate that urinary calcium excretion increased markedly when the meat protein intake increased. However, Spencer and colleagues[9)10)] observed that a high meat protein diet has little effect on urinary calcium excretion. They attributed this to the phosphorus content of the diet[9)10)]. Hegsted et al.[11)] found that calcium balance was improved with higher protein intake, but the beneficial effects appeared only when initial protein intake was low level.

It is generally known that the intakes of protein and of calcium by Koreans are relatively lower than Americans and Europeans. In Korea, 70% of dietary protein intake are from plant origin, contrasting to the fact that Westerners largely rely on animal origin protein. This difference needs to reexamine the effect of protein intake on calcium metabolism. Our previous study[12)] showed that urinary calcium excretion was not apparently related with dietary protein when protein intake was increased from 45g to 85g/day.

The main purpose of this study was to examine the effects of levels and sources(animal and plant) on fecal and uninary calcium excretion, and also on calcium balance. The daily calcium intake in the subjects was maintained at approximately 560mg daily.

Materials and Methods

1. Subjects

The subjects were ten healthy female volunteers between 20 and 24 years of age. Their initial weights ranged from 44.9 to 59.7kg and their heights from 151.4 to 161.9cm.

2. Experimental design

The 26-day period of metabolic balance study was divided into three part ; a 6-day adaptation period, 10-day moderate level of protein diet(10g N) period and another 10-day high level of protein diet(14g N) period. During the moderate protein period, subjects D, E, G, H and J recieved animal protein diet which consisted of 75% of animal protein and 25% of plant protein, while subjects A, B, C, F and K recieved diets which include 75% of plant protein. During the high protein period, subjects D, E, G, H, and J received high level of animal protein diet and subjects A, B, C, F and K received high level of plant protein diet. Main sources of animal protein were meat, egg and milk.

3. Diet

All subjects received 10g N, 530mg Ca and 1020 mg P per day during the adaptation period. Calcium and phosphorus intakes during the following two periods were similar ; each subject consumed about 2000kcal, 560mg Ca and 1190mg P

in the moderate protein period and 2000kcal, 570 mg Ca and 1490mg P for high protein diet. Subjects A, B, D and E were supplemented with 300mg Ca(Calcium gluconate) for last 4 days of each diet period.

The composition of these four diets has been previously published[13]. Meals were served at 9 : 30, 12 : 30 and 17 : 30 during the experimentation. The subjects were required to consume only the meals supplied by the research unit. Demineralized water was provided ad libitum for drinking and was used in all food preparation.

All of the urine and stool specimens were collected on the 5th and the 6th days and the last two days of each diet period. Each of the periods was demarcated by giving brilliant blue markers throughout the experimentation. Fasting blood smaples were drawn on the first and the 7th days of each metabolic period.

Analysis : Composite samples of an entire day's intake were analyzed to calculate the total daily intake. Meal samples and 2-day pooled stool were homogenized. Aliquots of meal, stool and 2-day pooled urine were kept frozen until analyzed. Nitrogen was determined by the micro-Kjeldahl method[14]. Calcium and phophorus in the diet, plasma, urine and stool were measured by Inductively Coupled Plasma Quantorecorder(Shimadzu ICPQ 1000)[15][16].

The data of the calcium and phosphorus absorption and balance were statistically analyzed by analysis of variance, t-test and Pearson's correlation.

Results

Table 1 shows the means of daily fecal & urinary calcium, calcium balance and calcium absorption during the experimental periods. There was a wide variation in fecal and urinary calcium excretion among subjects. The levels of protein intakes affected fecal calcium contents, calcium absorption and calcium balance. Fecal calcium decreased as the protein intake was increased from 60g to 90g daily. The mean fecal calcium contents were 378mg and 299mg, respectively($p<0.05$) when animal protein diet was consumed. Result showed apparent absorption of calcium 161mg(30

Table 1. Effects of dietary protein levels and sources on fecal & urinary calcium, Ca balance and absorption rate

Group	Period	Intake	Fecal Ca	Urinary Ca	Balance	Ca absorption	
		mg/d	mg/d	mg/d	mg/d	mg/d	%
Animal protein diet	MP I	538.9	377.9^{a}± 123.1	143.2 ± 46.0	17.9^{a}± 86.2	161.1^{1}± 123.1	29.9^{a}± 22.8
	HP I	557.3	298.5^{b}± 54.8	134.4^{a}± 37.5	124.3^{b}± 47.6	258.8^{b}± 54.8	46.4^{b}± 9.9
Plant protein diet	MP I	552.5	395.0 ± 118.0	110.0 ± 42.2	47.5^{a}± 92.1	157.5 ± 172.0	28.5 ± 21.3
	HP I	591.2	374.6 ± 77.0	83.5^{b}± 6.0	133.2^{b}± 72.6	216.6 ± 77.0	36.7 ± 13.0

Mean± S.D.
Absorption=Ca Intake−Fecal Ca
Balance=Ca Intake−(Fecal Ca+Urinary Ca)
MP : Moderate Protein
HP : High Protein
I : The data from 5, 6th days pooled samples
Values with different alphabet within the row are significant different at $p<0.05$ level

Table 2. Effects of calcium supplement on fecal & urinary calcium, Ca balance and absorption rate

Period	Intake	Fecal Ca	Urinary Ca	Balance	Ca absorption	
	mg/d	mg/d	mg/d	mg/d	mg/d	%
MP II 1	545.8	372.2± 138.5	102.2± 34.2	72.1^{a}± 127.8	174.2^{a}± 133.5	32.6± 23.5
MP II+Ca 2	845.8	457.6± 272.3	124.1± 25.1	264.9± 281.0	388.2± 272.7	45.9± 32.1
HP II 1	574.3	416.9± 125.0	109.4± 48.1	48.8^{a}± 70.6	158.2^{a}± 107.5	28.7^{a}± 18.0
HP II+Ca 2	874.3	410.5± 134.7	122.9± 34.2	381.8^{b}± 125.8	422.8^{b}± 157.3	53.1^{b}± 15.0

Mean± S.D.
II : The data from 9, 10th days pooled samples
1 : The data of subjects C, F, G, H, J, K
2 : The data of subjects A, B, D, E
Values with different alphabet are significant different at $p<0.05$ level within row

%) and 259mg(46%)($p<0.05$). There was apparent improvement in the absorption of calcium when the subjects were fed the high level of protein rather than moderate level. But the effect was statistically significant in animal protein diet.

The levels of protein did not affect urinary calcium excretion. However, there was significantly higher urinary calcium excretion(134mg) in animal protein diet than that(83mg) in plant protein diet($p<0.01$) during the high protein period. Urinary calcium was positively correlated with animal protein level($r=0.526$, $p<0.0001$) but negatively correlated with plant level($r=-0.579$, $p<0.0001$).

Calcium balance was improved as the protein intake was increased from 60g to 90g in both animal and plant protein diet. The calcium balance in the animal protein diet was 18mg and 124mg, respectively, and the calcium balance in the plant protein diet was 47mg and 133mg, respectively when the moderate and high protein was consumed($p<0.05$).

Calcium supplement(300mg) increased calcium absorptions 214mg and 265mg during the moderate and high protein period respectively ($p<0.05$). However, urinary calcium was not affected by the calcium supplement. Calcium supplement improved calcium balance of protein intake (Table 2).

Table 3. Serum concentration of calcium (mg/100ml)

Period	Dietary group	
	Animal protein	Plant protein
Adaptation	9.43± 0.29^{b}	9.09± 0.48^{ab}
MP I	9.87± 0.18^{a}	9.60± 0.50^{a}
HP I	8.51± 0.29^{c}	8.59± 0.29^{b}

Mean ± S.D.
Values with different alphabet within the row are significantly different at $p<0.05$ level

Table 3 shows the means of serum concentration of calcium for each diet period. Serum concentration of calcium was significantly decreased when the subjects consumed high protein than when they consumed moderate protein for both animal and plant protein diet($p<0.05$).

Table 4 shows the means of daily fecal, urinary phosphorus, phosphrus balance and its absorption. The amount of phosphorus excreted in feces was not affected by the level of protein intake, but affected source of protein intake. Fecal phosphorus excreted markedly higher amounts when given the high plant protein diet than when given high animal protein diet($p<0.05$). Phosphorus absorption was more efficient when given the high animal diet(77−81%) than when given the high plant diet(55−65%). Urinary phosphorus excre-

Table 4. Effects of dietary protein levels and sources on fecal & urinary phosphorus, phosphorus balance and absorption

Group	Period	Intake	Fecal P	Urinary P	Balance	P absorption	
		mg/d	mg/d	mg/d	mg/d	mg/d	%
Animal	MP I	1181.5	$260.1^{a}\pm 24.9$	$803.5^{a}\times 84.3$	$117.8^{a}\pm 97.0$	$921.4^{a}\pm 24.9$	$78.0^{a}\pm 2.1$
protein	MP II	1181.5	$276.3^{a}\pm 101.7$	$734.2^{b}\times 90.8$	172.2 ± 108.7	$905.2^{a}\pm 101.7$	$76.6^{a}\pm 9.6$
diet	HP I	1396.4	$261.1^{a}\pm 82.7$	$970.8^{c}\times 90.7$	185.0 ± 140.0	$1135.8^{b}\pm 82.9$	$81.3^{a}\pm 5.9$
	HP II	1396.4	$267.3^{a}\pm 44.3$	$920.7^{c}\times 48.8$	$208.4^{b}\pm 92.7$	$1129.1^{b}\pm 44.3$	$80.8^{a}\pm 3.6$
Plant	MP I	1195.5	$415.4^{b}\pm 115.5$	$616.8^{d}\pm 110.6$	163.2 ± 193.5	$780.05^{c}\pm 155.5$	$65.2^{b}\pm 13.0$
protein	MP II	1195.5	$510.7^{b}\pm 202.4$	$575.9^{d}\pm 31.8$	57.8 ± 233.1	$684.8^{c}\pm 202.4$	$57.3^{b}\pm 18.9$
diet	HP I	1577.8	$692.8^{c}\pm 271.4$	$694.7^{b}\pm 100.7$	190.3 ± 263.9	$885.0^{c}\pm 271.4$	$56.1^{b}\pm 17.2$
	HP II	1577.8	$711.1^{c}\pm 261.8$	$705.3^{b}\pm 107.9$	161.4 ± 310.9	$732.4^{c}\pm 263.3$	$54.9^{b}\pm 16.6$

Mean± S.D.

Absorption=P Intake−Fecal P, Balance=P Intake−(Fecal P+Urinary P)

MP : Moderate level of protein

HP : High level of protein

Values with different alphabet within the row are significantly different at $p<0.05$ level

tion was higher when given the animal protein diet than when given plant protein diet. However, phosphorus balance was not affected by the level and the source of protein.

Discussion

The results indicate that animal protein enhanced calcium absorption during the high protein diet period. Our data are in line with previous studies that an increased intake of animal protein improved calcium absorption[2)-4)17)18)]. Most studies observed that fecal calcium was significantly decreased as the increase of protein intake from 47g to 95g daily. But the decrease of fecal calcium did not occur when its increase was in the range between 95g and 142g daily. However, this finding is not entirely consistant with many investigators' observations that extremely high protein intake did not significantly affect the increase of the absorption of dietary calcium[5)6)9)10)]. In the present study, the calcium absorption rates were 29−30% during the moderate protein period, and 46% in high animal protein diet. Our results of the calcium absorption rates were similar to that of other sutides. Choi and Ju[29)] reported the calcium absorption rates of soy protein(30%) were significantly lower than those of the meat protein diet(50%) when given 87g protein. Another study also reported that the calcium absorption rates in meat and soy protein diet were 34% and 30% respectively when given 530mg Ca and 90g protein.

In the present study the increase of urinary calcium excretion was not found in the high level of protein diet. Several reports demonstrated an opposing view in this regard[1)-7)17)18)], while present result is supported by several studies[12)19)20)]. The failure in increase of urinary calcium excretion is most likely due to the amount of protein intake increased. The studies with the oppsoing view reported that the urinary calcium increase was 100−200mg when the protein intake was expanded approximately three times. But the difference of the protein intake level was only 30g in the present study. The amount of the urinary calcium excretion was found to be affected by the sources of dietary protein. The urinary calcium excretion was significantly higher in the

amount(51mg) of animal protein diet than that of the plant protein during the high protein period. The difference of the urinary calcium excretion between the aminal and plant protein diet was not found during the moderate protein level. It occured only when the increase of the animal protein intake was relatively large amount(50g) during the high protein diet. Animal protein diet generally tends to include phosphorus as well. In regard to the fact that phosphorus restrain urinary calcium excretion, our research design provided a balanced diet in which both animal protein and plant protein contained almost same amount of phosphorus. Many other reports support our finding that an increased animal protein intake was significantly related with urinary calcium excretion[17)18)21)22)].

The calcium balance was affected by the level of protein rather than the source of protein in the present study. The calcium balance improved 106mg in animal protein diet, and 85mg in plant protein as the level of protein intake increased from moderate to high level. The data clearly indicate that protein intake enhanced calcium balance. This can be explained by the beneficial effect of protein on calcium absorption. The amounts of calcium absorbed were increased 98mg in the animal protein diet and 5.9mg in the plant protein as the level of protein intake increased. On the other hand, the amount of urinary calcium excretion did not differ between the two levels of protein intake. This finding is not completely consistant with the reports[1)-7)17)18)] that an increased intake of protein has a detrimental effect on calcium balance. Our finding is attributable to the absence of increase in urinary calcium excretion since the protein intake was increased only a little amount(30g) in the study.

The results of calcium supplement study confirmed the importance of calcium contents of the meal on calcium absorption and balance. Calcium supplement(300mg) with 550mg Ca diet improved calcium balance during the moderate and high protein period ; 193mg and 333mg respectively. This was mostly due to the increase of the calcium absorbed during the two levels of protein intake peroid ; 214mg and 265mg respectively. Because the present study was a short term study, the large amount of calcium absorbed with calcium supplement seems to be a transient effect on calcium metabolism. The absorption rates of calcium supplement group during the moderate and high protein period were 46% and 53% respectively. This result was not totally consistent with the other studies. This may be attributable to the well utilization of calcium gluconate supplement. Spencer et al.[23)] reported that calcium absorption increased 174mg, resulting in improved calcium balance(154mg) when the calcium intake was increased 600mg with constant 85g protein intake. Our previous study also observed that the calcium balance improved 20mg as the calcium intake increased 300mg[12)].

The serum concentration of calcium was significantly affected by the level of protein intake in the study. Many other studies observed that the serum concentration of calcium was relatively constant no matter what protein levels and sources might be[1)2)12)24)]. The disagreement of serum calcium concentration in the present study seems partly due to the large difference of calcium balances(85−106mg) between the two levels of protein intake in the present study. The result was confirmed by two studies that the serum concentration of calcium decreased as the calcium balance increased[17)18)]. While the difference of calcium balance was in between 25mg and 20mg, the serum concentration of calcium was constant [9)12)]. The reason for this is yet to need a clear explanation.

The phosphorus intake was increased 200mg as the protein intake increased 30g daily in the

animal and plant protein diet. The phosphorus was absorbed more effeciently in the animal protein diet than in plant protein diet. The data are accorded with the other studies on the phosphorus absorption rate[25)-27)]. Joo and Paik[27)] reported that phosphorus absorption rates were 45.5% in soy protein diet and 65.6% in meat protein diet. There are also many other studies which reported that the absorption rates were 45−54% in soy protein diet and 80−85% in animal protein diet based on Korean general meals. The reason for this result seems to be higher content of fiber in soy or plant protein diet. The result of present study on phosphorus balance was similar to the results that of the research carried by Pie and Paik[28)]. These results indicate that the source of protein is an important factor for phosphorus absorption.

There was a wide variation in fecal and urinary phosphorus excretion between the subjects even with the same diet. Therefore, the data did not reveal effects of the protein levels and the sources on phosphorus balance.

Summary

This study was to examine the effects of dietary protein levels and the sources of protein(animal and plant) on calcium and phosphorus metabolism in 10 healthy Korean female adults. The 26-day study consisted of a 6-day adaptation period and 10-day moderate protein(10g N, 550mg Ca) and 10-day high protein(14g N, 570mg Ca) period. During the experimental period, the subjects were divided into two groups, either consuming animal protein diet(75% animal protein) or plant protein diet(75% plant protein). Calcium(300 mg) was supplemented to two subjects of each diet group for the last 4 days. Feces, urine and diet were analyzed nitrogen, calcium and phosphorus.

The apparent absorption of calcium was significantly increased as the protein intake was increased from 60g to 90g in animal protein diet. Average calcium absorption rate was 30% and 46% from high and moderate levels of animal protein diet.

Urinary calcium excretion was not affected by the amount of the protein intake in both animal and plant protein diet. There was significantly higher urinary calcium excretion(134mg) in high level of animal protein diet than that(83mg) in high level of plant protein diet. Calcium balance was improved as the protein intake increased and calcium was supplemented. Phosphorus absorption was more efficient in the high animal diet(77−81%) than in the high plant diet(55−65%).

The overall results indicate that an increase of protein and calcium supplement in moderate protein intake can improve calcium balance due to the increase of calcium absorption.

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