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The Effect of Dietary Protein and Calcium on Urinary Calcium in Young Men*

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食餌蛋白質과 칼슘이 人體의 尿中 칼슘양에 미치는 影響

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要 約

食餌蛋白質과 칼슘의 攝取量이 尿中 칼슘 排泄量에 미치는 영향을 검토하기 위하여 7명의 白人成人男子를 硏究對象者로 하여 실험하였다. 연구대상자가 7일동안 섭취한 食餌의 測量記錄에 의거하여 蛋白質(動物性 및 植物性), 칼슘과 燐의 1日平均 섭취량을 분석하고 24시간의 尿中 칼슘양을 분석하였다.

蛋白質과 칼슘의 1日平均 섭취량은 각각 103g과 1,237mg이었다. 뇨중 칼슘의 24시간 배설량은 食餌에 따라 다양하여 121mg부터 258mg에 달하였다. 모든 실험대상자의 蛋白質攝取量을 4단계로 나누었을 때 : 低(53g), 中(87g), 上(117g)과 高(153g)이었으며, 이때 뇨중 칼슘배설량은 179mg, 189mg, 184mg과 264mg이었다. 蛋白質 섭취수준이 高로 증가함에 따라 뇨중칼슘양은 현저히 증가되었다. 특히 동물성단백질 섭취량이 뇨중칼슘양에 영향을 주는 것으로 나타났으며 식물성단백질 섭취량은 유의한 영향을 미치지 않았다.

한편 食餌칼슘의 1日平均 섭취량을 4단계로 나누었을 때 : 低(544mg), 中(842mg), 上(1,232mg)과 高(1,834mg)이었으며, 이때 뇨중칼슘 배설량은 169mg, 196mg, 222mg과 197mg이었다. 식이칼슘 섭취량이 低水準에서 上으로 증가되었을 때 뇨중칼슘양에 유의적 차이가 있었으나 上에서 高水準으로 증가되었을 때는 유의적 차이가 없었다.

이상의 결과에서 뇨중칼슘 배설량은 食餌칼슘 섭취량의 변화보다 蛋白質 섭취량의 변화에 더 영향을 받는 것으로 나타났으며 특히 高蛋白食에 의해서 크게 영향을 받는 것으로 나타났다. 그러므로 上水準(117g) 이상의 단백질 과잉섭취는 바람직하지 못하다고 볼 수 있다.

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INTRODUCTION

In the past decade, many investigators have given great attention to the effect of protein intake on calcium metabolism. Research reported by Johnson et al[1]. in 1970 first highlighted the relationship of protein intake to calcium metabolism. It was concluded that urinary calcium excretion was more closely correlated to protein intake than to calcium intake. The higher the dietary protein, the greater the urinary calcium excretion. This finding was further corroborated by Walker and Linkswiler[2], and others[3]~[15]. Many concluded in general that urinary calcium was positively correlated with protein intake, regardless of calcium intake. These results may have important implications on the pathogenesis of osteoporosis. Urinary calcium would be a simple reflection of bone demineralization, taking into consideration the effects of protein on calcium absorption.

To extend the validity of the hypothesis that urinary calcium excretion is significantly increased by protein intake, it is necessary to discuss experimental conditions. Most studies were carried out using controlled subjects fed purified protein. Little information is available on the effect of natural food (protein) sources on urinary calcium excretion. From this account, the purpose of this study is to further examine the effects of protein intake on urinary calcium excretion in the free living individual. This study focuses on the analysis of daily food intake of young males and correlations with urinary calcium excretion.

METHODOLOGY

Subjects

Seven young male subjects were selected by non-random, purposive sampling. Because sex, age, race, health and disease conditions would affect levels of urinary calcium, a homogeneous group was necessary. Subjects were all healthy caucasians between 22 and 30 years of age. Their initial weight ranged from 61.2 to 78.4 kg and their height from 167.6 to 182.9 cm (Table 1). They had no known metabolic disorders.

Method of Data Collection

The subjects were free living and consumed a selfselected diet for a period of 7 days. Each subject weighed and recorded food intake and completed a 24-hour urine sample each day.

Dietary Records

Dietary records completed by the subjects were used to assess each individual's nutrient intake including total protein, animal and plant protein, calcium, phosphorus, and methionine. All substances consumed were identified and weighed. Food composition was based on Nutritive Value of American Foods in Common Units[16] and Food Values of Portions Commonly Used[17].

Urinary Calcium Analysis

Urinary calcium was determined by the *o*-cresolphthalein complexon method : Sixty Second Calcium Reagents[18], using a Turner Model 350 Spectrophotometer*.

Preceding procedures of the method were as follows :

1. Bring the urine specimen to pH 4.0 with concentrated HCl and mix well.

Table 1. Age weight and height of seven subjects

Subject	Age yr	Weight kg	Height cm
1	22	74.8	177.8
2	27	61.2	167.6
3	22	68.0	177.8
4	23	67.1	180.3
5	26	77.1	182.9
6	30	74.8	182.9
7	20	78.4	175.3
Mean ± SD.	24.3 ± 3.5	71.6 ± 6.3	177.8 ± 4.9

2. Dilute an aliquot of this acidified specimen 1:1 with deionized water. Use this diluted specimen as a sample in the method.

Statistical Analysis

The data from each subject were used for internal comparison. For all subjects, the dietary intakes of nutrients Ca : P ratio and urine volume were individually plotted against urinary calcium excretion. Data were treated statistically by analysis of variance and by one-tailed t-test[19].

RESULTS AND DISCUSSION

There was a wide variation in urinary calcium excretion between subjects and from day to day (Fig. 1). The mean daily urinary calcium, protein, calcium and phosphorus intake for each subject is shown in Table 2. Mean daily urinary calcium ranged from 121 ± 40 mg to 258 ± 104mg across all subjects. Subject 7 excreted less calcium than any other subject at all levels of protein intake, while subject 1 and 5 excreted more calcium than the others. The average daily protein intake ranged from 92 ± 32 to 112 ± 40g or from 164 to 200 percent of the Recommended Dietary Allowance. Animal protein intake was 66 percent of total protein intake. Mean daily animal protein ranged from 40 ± 26 to 88 ± 27g.

*Manufactured by Ohaus Scale Corp., Union, N. J. Serial no. 1201

Total Protein

The range and average daily nutrient intakes are shown in Table 3, when the nutrient intakes of all subjects are divided into four levels ; low,

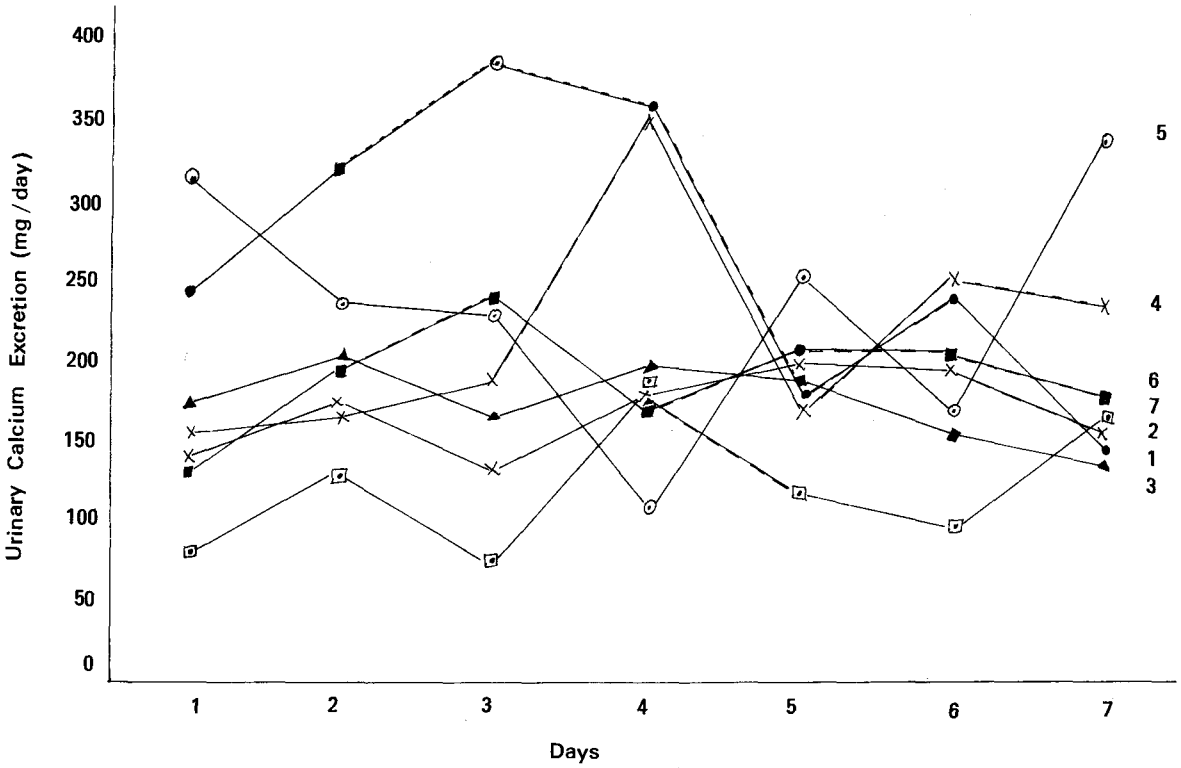

Fig. 1. Urinary calcium for each of seven subjects for seven days.

Table 2. Daily mean of seven day nutrient intakes

Human subject	Animal protein g	Plant protein g	Total protein g	Calcium mg	Phosphorus mg
1	84 ± 19	28 ± 13	112 ± 26	1635 ± 919	1763 ± 731
2	60 ± 13	43 ± 21	103 ± 32	831 ± 443	1855 ± 534
3	61 ± 22	37 ± 7	98 ± 24	1026 ± 458	1679 ± 334
4	58 ± 21	37 ± 21	95 ± 38	936 ± 283	1875 ± 1133
5	88 ± 27	24 ± 7	112 ± 28	1420 ± 386	1891 ± 506
6	40 ± 26	52 ± 22	92 ± 32	1294 ± 449	1532 ± 543
7	84 ± 35	28 ± 17	112 ± 40	1514 ± 700	1894 ± 816
Mean ± S.D.	68 ± 28	35 ± 18	103 ± 31	1237 ± 594	1784 ± 667

Table 3. Mean dietary protein and calcium intakes, at various levels for human subjects

Nutrient	Low	Medium	Inter-mediate	High
Total protein g*	53	87	117	153
Animal protein g	35	63	86	117
Plant protein g	19	27	36	59
Calcium mg**	544	842	1232	1843

* Levels adapted from walker and linkswiler who divided protein intake into three levels : 47 (low), 95 (medium) and 142 g (high). J. Nutr. 102 : 1297 –1302, 1972.

** Levels adapted from spencer et al. who divided calcium intake into four levels : 200 (low), 800 (normal), 1100 (intermediate) and 2000 mg (high). Am. J. Clin. Nutr. 31 : 2167 –2180, 1978.

medium, intermediate and high. Table 4 show mean urinary calcium for the various dietary levels shown in Table 3. When high protein diets were consumed, the mean urinary calcium was 264 ± 84 mg. There was a significant increase in calcium excretion in the urine when the subjects consumed the highest level of protein than when they consumed low levels ($P < 0.05$), medium levels ($P < 0.025$), or intermediate levels ($P < 0.01$). However, there was no statistical difference in urinary calcium excretion between medium and intermediate levels of protein intake.

Results from this study indicate that high protein intakes increase urinary calcium excretion in young males. The data agree with those from previous studies in which dietary protein has been shown to increase urinary calcium excretion[1)3)–6)8)11)–13)20)]. When the protein intake increased from low to high, the increase urinary calcium was 85 mg. In previous studies[1)2)4)8)9)], the increase ranged from 163 to 209 mg when protein intake increased the same amount. The moderate increase in urinary calcium observed in some of the present cases contrasts with large increase reported by other investigators who used purified proteins. The 85 mg increase of urinary calcium was still rather high for self-selected diets when considering the counterpart of the cited studies in which the protein ranged widely from o to 562 g per day with formular diet.

The increase in urinary calcium caused by a high protein diet seems to reflect, in part, the loss of calcium from bone, probably as a result of added acid load[8)11)]. It appears, in part, to be an increase in the filtered load of calcium by the glomeruli and a decrease in calcium reabsorption by the renal tubules[7)–9)11)12)13)]. Our study supports this explanation. The urine volume data are the

Table 4. Mean daily urinary calcium for the various dietary levels shown in table 3 (Mean ± SD mg)

Nutrients	Low	Medium	Intermediate	High
Total protein	179 ± 53 **	189 ± 73	184 ± 55****	264 ± 84 ***
Animal protein	176 ± 44 *	196 ± 74	220 ± 80****	226 ± 102
Plant protein	199 ± 91	209 ± 76	193 ± 65	197 ± 44
Calcium	169 ± 46	196 ± 71	222 ± 21**	197 ± 44

* .10 > P > .05 ** P < .05 *** P < .025 **** P < .01
Total protein : low vs high** medium vs high*** intermediate vs high****
Animal protein : low vs intermediate* medium vs intermediate****
Calcium : low vs intermediate**

most critical in this argument.

Animal Protein

The level of animal protein intake had a positive effect on urinary calcium. There was a significant difference in urinary calcium excretion between low and intermediate level of animal protein intakes ($0.10 > p > 0.05$) and between medium and intermediate levels ($P < 0.025$). However, no significant difference was observed between intermediate and high intakes. On the other hand, the level of plant protein intake did not affect urinary calcium excretion.

The data also indicate that animal protein enhances urinary calcium excretion. These results confirm previous reports that an increased intake of animal protein (34 g / day) significantly increased urinary calcium[10]. The results of Spencer et al.[23] when meat was added to the diet there by resulting in a simultaneous increase in both protein and phosphorus indicate that the phosphorus content of protein foods protects against high protein diets.

The increase in urinary calcium on a high animal protein diet may be explained by the production of acids such as ammonium ion, free organic acid, sulfate, phosphate, and oxalate[10][11]. Animal protein in the present study includes more methionine and phosphorus than plant protein. The increase of methionine intake from medium (1727 mg) to high (3272 mg) significantly increased calcium excretion from 177 ± 57 to 228 ± 88 mg.

Methionine produces sulfate in the body which seems to induce urinary calcium excretion.

Calcium

In the present study, increases in calcium intake from low to intermediate levels lead to greater urinary calcium loss, and the increase from intermediate to high calcium intake suppresses calcium excretion. This finding confirms other reports that urinary calcium excretion is affected by the level of calcium intake[21][22]. This finding needs to be confirmed by covarient analysis and by varying calcium intakes while keeping protein intake constant. There is much variation in urinary calcium among subjects ingesting a similar diet. There are always some individuals who excrete much more than the average individual ; hence, in order to determine the effect of dietary calcium on urinary calcium excretion, the same subjects should be studied at several levels of calcium and of protein intake.

The overall results indicate that the magnitude of urinary calcium excretion depends on the level of protein intake rather than calcium in the diet. Statistical justification for this agrees with previous literature.

An interesting point in this study is that increases in urine volume induce increases in urinary calcium excretion. This is supporting evidence that protein increases urinary calcium excretion by inducing an increase in urine volume. The results of the present study also indicate that high protein diets are potentially important in the pathogenesis of osteoporosis over prolonged periods of time.

ABSTRACT

Studies were carried out on seven young, adult caucasian males to determine the short-term effects of protein (animal or plant) and calcium intakes on the excretion of urinary calcium. The subjects were studied on a self-selected diet for a period of seven days. Mean daily protein and calcium intakes were 103±31 g and 1237±594 mg, respectively. Variation among subjects in the mean urinary calcium excretion per 24 hour was from 121±40 to 258±104 mg.

When the protein intake of all subjects was divided into four levels : low (× = 53 g), medium (87 g), intermediate (117g) and high (153 g), The mean urinary calcium was 179±53, 189±73, 184 ±55 and 264±84 mg, respectively. Urinary calcium increased significantly with an increase in protein intake. The calcium excretion was seriously increased with the protein intake above the intermediate level. Animal protein intake was more closely related to urinary calcium excretion than plant protein. There was a significant difference in the urinary calcium excretion when calcium intakes increased from low (× = 544 mg) to intermediate levels (1232 mg). However, the difference between intermediate and high levels (1834 mg) was not significant. Urinary calcium was 169±46 mg on the low calcium diet, 196±71 mg on the medium, and 222±21 mg on the intermediate calcium intake. Calcium excretion was more closely related to changes in protein intake than to changes in calcium intake.

Some nutritional implications can be drawn from this research. Protein intakes above the intermediate level (117 g) are not recommendable. An over intake of calcium, however, may not be a serious problem for the calcium balance.

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