

The Change of Calcium, Phosphorus and Bone Metabolic Markers in Serum and Urine during the Calcium Supplementation and Restriction

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The present study was designed to investigate changes in the levels of calcium and inorganic phosphorus (IP) in serum and urine when calcium was supplemented or restricted. And also serum bone specific alkaline phosphatase (b-ALP), mid-portion parathyroid hormone (PTH), 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}_3$), osteocalcin (BGP) and urinary deoxypyridinoline (D-Pyr) were investigated. During the adjustment period (4d), healthy male subjects were given the same diet that satisfied recommended dietary allowance (RDA) for Japanese (650mg calcium). Then, during the 7-day experimental period, some groups received supplemental calcium to a 300mg base as follows: S group (n=4) 1800 mg/d; C group (n=3) 350 mg/d; R group (n=4) none. Blood samples were collected from each subject before lunch on days 1, 5 and 8 (see Fig.1). Also, samples from the second urination, day 1, until the first urination, day 2 repeated for days 5 and 8, were collected for 24-h. The results showed that there was no significant difference in serum Ca^{2+} and urine calcium levels among the groups. There was no significant difference in serum IP levels too, but the S group's IP level was always the lowest of all groups. However, in the urination samples for days 1 and 8, the C group was higher than the S group, and on day 5, the R group was higher than the S group in IP levels. As for b-ALP activity, day 1 showed that the R group was lower than the S group ($R < S$), and on day 8 the S group was lower ($R > S$). In fact, on day 8 there was a 40% rise for the R group while the S group had a drop of 50%. Even so, on day 5 there was no statistical difference among the groups, though the S group was the highest and the R group was the lowest. As for D-Pyr: there was no significant difference among the groups, but the C group was the highest. The PTH levels of R and S groups were lower than the C group, but not significant. On day 8, the S group's PTH level was lower than the R group's. Throughout the experiment, the S group's $1,25(\text{OH})_2\text{D}_3$ was always lower than the R group's, but the S group's BGP was always highest. These results suggest that the calcium overdose in their diets prearrange bone formation superior to bone resorption the level of serum or urine calcium, phosphate, and serum b-ALP, BGP, PTH and $1,25(\text{OH})_2\text{D}_3$, and high calcium supplementation in a short period is effective treatment for osteoporosis.

Key words : calcium supplementation, calcium restriction, calcium metabolic hormone, biochemical markers, osteoporosis
カルシウム強化、カルシウム制限、骨代謝ホルモン、骨代謝マーカー、骨粗鬆症

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Introduction

Fractures among Japanese elderly are a major health problem because osteoporosis makes the bones fragile. The quantity of dietary calcium affects peak bone mass¹⁾; and, bone is an active tissue undergoing continual replacement. Generally, a low calcium diet over a long period of time leads to negative calcium balance, secondary hyper-parathyroidism, increased bone loss, and fractures^{2) 3)}. But short-term (e. g. 7 days) investigations on the effects of a low or high calcium diet in serum or urine calcium and IP, and hormones, BGP, and b-ALP in humans have not been reported previously. Lene and Peder reported on a four-day study, but they didn't investigate bone metabolic markers⁴⁾. Calcium supplementation was effective in the prevention of osteoporosis⁵⁾, but was not effective for postmenopausal women⁶⁾. When the intake of calcium is low, the risk of osteoporosis is higher⁷⁾. Other reports conversely, have shown calcium intake has no relation to osteoporosis⁸⁾. Therefore, it is not clear whether an overdose of calcium is an effective treatment for osteoporosis. The purpose of this study is to measure acute effects of calcium intake in a short period on bone metabolism in healthy young men through the observation of bone metabolic markers, calcium metabolic hormones, calcium and IP concentrations. Besides we examine that overdose of calcium is effective treatment for osteoporosis.

Materials and Methods

Subjects

Eleven healthy male subjects between the ages of 19 and 20 years participated in the experiment after written consent. Average weight was 62.1kg (S.D.=3.1) and

average height was 169.0cm (S.D.=3.7). All subjects had no medical history of nephrolithiasis, bone disease, peptic ulcer, intestinal resection, malabsorption, cirrhosis or renal disease.

Protocol of Study

The study consisted of both a 4-day adjustment period (Figure 1), and a 7-day experimental period. The subjects were separated into three groups by body weight and body length. Three groups were not statistically different in body weight and length. For 11 days all subjects were given the same diet which satisfied Recommended Dietary Allowances (RDAs) for Japanese. But during the last 7 days, calcium was supplemented to a 300mg base as follows: S group, 1800mg/d; R group, none; and C group, 350mg/d (see Fig.2). No drugs, cigarettes or alcohol were taken before or during the experiment. Experimental diets contained 1198 ± 35 (1158-1266) mg/d of phosphorus and 561 ± 384 (1175-82) I.U./d of vitamin D. The first day's experimental diets were given after an overnight fast, between 7 am and 8 am. After 4h (before lunch), day 1 blood samples were collected, and repeated for days 5 and 8 (see Fig.1). One day's urine samples were collected from the second urination of day 1 to day-2 first urine and repeated for days 5 and 8. Blood samples and complete 24-hour urine samples were collected on days 1, 5 and 8 during the experimental diet period. After blood samples were obtained, serum was stored at -80°C and later analyzed for calcium, IP, b-ALP, BGP, PTH and $1,25(\text{OH})_2\text{D}_3$. Urine was collected in plastic containers and samples were acidified with 1 ml 6 mol HCL/L per 100 ml urine before being analyzed, then analyzed for calcium, IP and D-Pyr. Consistency was examined during the 24-h urinary creatinine excretion period. Body weight was measured each morning.

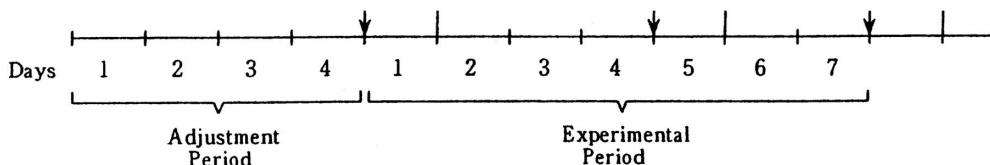


Fig 1. Blood and Urine Collection

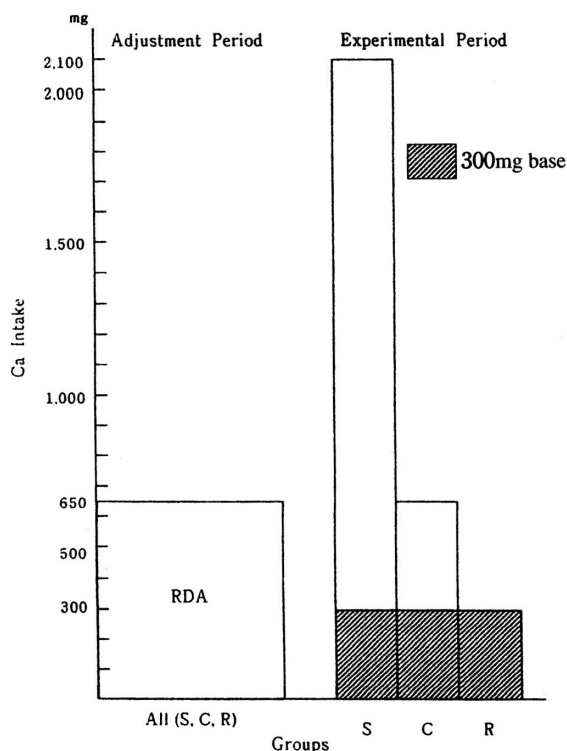


Fig 2. Calcium Intake

Biochemical Analysis

A biochemical analysis was done as follows: Serum and urine calcium: o-cresolphthalein complexone method (be used kit, Wako Pure Chemical Industries, Ltd., Tokyo); Serum and urine IP: *p*-methyl-aminophenol method (Wako Pure Chemical Industries, Ltd., Tokyo); b-ALP: polyacrylamide gel disc electrophoresis⁹⁾; Intact BGP: immunoradiometric assay (Mitsubishi Chemical Industries, Ltd., Tokyo)¹⁰⁾; mid-portion PTH: Radio immuno assay method (Yamasa Soy Sauce C., Inc. Tokyo)¹¹⁾; Ca²⁺: Ion-selective electrode method¹²⁾; 1-25(OH)₂D₃: radioreceptor assay (Yamasa Soy Sauce C., Inc. Tokyo)¹³⁾; D-Pyr: HPLC method; and, Urine creatinin: alkaline picric acid method (be used kit, Wako Pure Chemical Industries, Ltd., Tokyo).

Besides the determinations of b-ALP, BGP, PTH, ionized calcium, deoxypyridinoline and 1,25(OH)₂D₃ were done by Otsuka Assay Laboratories (Tokushima) and BML C., Inc. (Tokyo).

Materials

A refined eggshell powder used as the calcium supplement (580mg/ 10ml when diluted with water as calcium carbonate, Kewpie Co., Tokyo Japan). The supplement was mixed into three meals.

Table1. Changes in Serum and Urinary Calcium

Days		1	5	8
Serum Ca ²⁺ (mg/dl)	R(n=4)	4.4±0.0	4.5±0.1	4.5±0.1
	C(n=3)	4.1±1.1	4.5±0.2	4.5±0.1
	S(n=4)	4.4±0.4	4.6±0.1	4.7±0.1
Urinary Ca (mg/dl)	R(n=4)	120±71	163±58	180±69
	C(n=3)	170±78	177±126	217±91
	S(n=4)	270±127	285±184	295±64

Values : Mean±S.D.

R, C and S show restricted group, control group and supplemental group, respectively.

Table2. Changes in Serum and Urinary Inorganic Phosphorus(IP)

Days		1	5	8
Serum IP (mg/dl)	R(n=4)	3.55±0.08	3.04±0.34	2.40±0.82
	C(n=3)	3.60±1.36	3.43±1.08	2.53±0.21
	S(n=4)	2.43±0.25	2.43±0.57	2.03±0.25
Urinary IP (g/day)	R(n=4)	1.09±0.27	0.82±0.19	1.09±0.40
	C(n=3)	1.80±1.00	1.70±0.56	2.75±1.36
	S(n=4)	0.58±0.27	0.32±0.30	0.62±0.42

Values : Mean±S.D.

R, C and S show restricted group, control group and supplemental group, respectively.

Table3. Changes in b-ALP Activities

(KAU)

Days	1	5	8
R(n=4)	4.8±0.8	4.7±1.9	6.6±0.6
C(n=3)	4.9±0.9	6.9±3.0	5.2±1.3
S(n=4)	8.0±3.8	8.1±2.3	4.2±2.3

Values : Mean±S.D.

R, C and S show restricted group, control group and supplemental group, respectively.

Table4. Changes in Intact BGP Concentration

(ng /ml)

Days	1	5	8
R(n=4)	9.4±2.7	10.1±2.4	11.4±2.0
C(n=3)	9.8±3.7	8.0±2.1	11.0±3.1
S(n=4)	12.3±6.5	13.5±3.0	13.7±7.7

Values : Mean±S.D.

R, C and S show restricted group, control group and supplemental group, respectively.

Table5. Changes in Urinary Deoxypyridinoline

(μ mol/mol Cr)

Days	1	5	8
R(n=4)	4.3±1.3	4.3±1.0	4.5±0.6
C(n=3)	4.7±1.5	5.0±1.7	4.7±1.5
S(n=4)	4.5±1.9	4.8±1.5	4.5±1.7

Values : Mean±S.D.

R, C and S show restricted group, control group and supplemental group, respectively.

Table6. Changes in 1,25(OH)₂D₃

(pg/ ml)

Days	1	5	8
R(n=4)	43.6±10.7	57.0±12.8	49.4±12.9
C(n=3)	34.9±6.3	37.3±8.1	44.2±5.3
S(n=4)	39.2±13.2	35.2±4.7	43.0±7.0

Values : Mean±S.D.

R, C and S show restricted group, control group and supplemental group, respectively.

Table7. Changes of Serum Mid-potion PTH

(pg/ ml)

Days	1	5	8
R(n=4)	218±85	160±61	207±65
C(n=3)	246±71	224±69	228±73
S(n=4)	220±66	177±41	146±58

Values : Mean±S.D.

R, C and S show restricted group, control group and supplemental group, respectively.

Results

1. Calcium and Inorganic Phosphorus

The changes in ionized serum calcium (Ca^{2+}) and urine calcium concentration are shown in Table 1. In all groups, Ca^{2+} concentration was steady during the experimental diet period. While the urinary calcium concentration of the S group was the highest, no significant difference was found. Average inorganic phosphorus of serum and urinary concentration is shown in Table 2. Serum phosphate concentration on days 1, 5, and 8 was lowest to highest, $\text{S} < \text{R} < \text{C}$. Always, urinary concentration was also lowest to highest, $\text{S} < \text{R} < \text{C}$.

2. Bone Metabolic Markers and Hormones

Table 3 shows the changes in serum b-ALP activity throughout the study. On the first day, the b-ALP activity of the S group was the highest compared with the other groups. On the 5th day, the R group's b-ALP was not as high as C and S groups, but the difference was not significant. In contrast, on the 8th day, activity of R group's b-ALP became higher than the other two groups'. Serum b-ALP activity was $\text{S} < \text{C} < \text{R}$ on day 8—the reverse of the first day.

Table 4 shows the changes of intact BGP concentration. Individually, there was little difference shown in intact BGP level during the experimental periods. But the S group's intact BGP level was always the highest compared to both the other groups.

Table 5 shows changes of urinary D-Pyr excretion as bone resorption marker. The C group's D-Pyr excretion was the highest compared with the other groups, but the difference was not significant.

Table 6 shows how $1,25(\text{OH})_2\text{D}_3$ concentration changed during the experimental period. $1,25(\text{OH})_2\text{D}_3$ concentration level was higher in the R group compared to the S group all days. However, on day 8, C and S group's levels rose, while R group's level fell.

Table 7 shows how PTH changed during the experiment. The C group was the highest of all other groups throughout the study. S group's PTH level decreased consistently during this study; however, the R group's PTH level increased from days 5 to 8, but not significantly.

Discussion and Conclusion

Serum ionized calcium, which makes up 50% of the calcium in plasma, is the physiologically active form⁽¹⁴⁾. Thus, ionized calcium was measured (see Table 1).

There was no significant difference among the three groups in serum Ca^{2+} concentration.

However, the S group's urinary calcium excretion was the highest. It appears that the calcium supplementation of over-RDA induced a high calcium excretion in urine, and the subjects of the S group were hypercalciuria during the experiment. Conversely, the R group had the lowest calcium excretion. This shows that urinary calcium excretion is positively related to dietary calcium^{(15) (16) (17) (18)}. And variations in daily calcium excretion reflect a wide range not only in the calcium content of food, but also in calcium requirements^{(19) (20)}.

Plasma calcium concentration prior to urinary calcium excretion is adjusted by PTH and $1,25(\text{OH})_2\text{D}_3$ ⁽²¹⁾. In the S group, PTH concentration fell slowly during the experimental period (Table 7). It appeared that as soon as calcium intake occurred, the diet contained an overabundance of which calcium, ionized to a very high concentration. It then controlled the PTH secretion. D.R.Fraser (1988) reported that when Ca^{2+} concentration falls, as in the case of the R group, there is an increase in the secretion of PTH which acts to enhance the activity of the 1-hydroxylase in the kidney. In fact, the R group's $1,25(\text{OH})_2\text{D}_3$ was always the highest. This study supports Fraser's in that the effect of the extra $1,25(\text{OH})_2\text{D}_3$, by stimulating target cells, increased the extra-cellular Ca^{2+} concentration leading to a fall in PTH secretion⁽²²⁾. As in the Fraser study, PTH secretion fell in both S and R groups. During this time PTH was more highly concentrated in C group than the other two groups.

The R group's $1,25(\text{OH})_2\text{D}_3$ concentration was the highest of the other two groups during the experimental days. But serum ionized calcium level was always the same as the other groups. It appears that the $1,25(\text{OH})_2\text{D}_3$ endocrine system had been stimulated by calcium concentration. During the calcium restricted period, there may have been a small quantity of calcium influx in the blood, which was hormonally regulated by PTH and $1,25(\text{OH})_2\text{D}_3$ ⁽²³⁾. At the same time the $1,25(\text{OH})_2\text{D}_3$ promoted calcium absorption by the small intestines.

Rosalind (1990) said that there is usually a wider range of serum phosphorus concentration than serum calcium concentration⁽¹⁴⁾. Table 2 shows the changes in serum and urine phosphate. The S group's serum phosphate concentration was the lowest of the other groups during the experimental period. One possible reason for this was that calcium from the diet was very high. Perhaps excessive

calcium, combined with phosphate in the intestinal tube, became an insoluble product. Therefore, phosphate absorption was restrained, while formation of hydroxyapatite with excessive calcium was promoted. Urinary S group's phosphorus excretion was lower than the other group's. This may be one reason for the low level intestinal absorption of phosphate. The other reason may be that the secretion of PTH and $1,25(\text{OH})_2\text{D}_3$ were controlled by low serum phosphate concentration which decreased the reabsorption of phosphate from the distal portions of the nephron.

Serum b-ALP activity rose from days 5 to 8 in the R group (Table 3). However, S group's b-ALP level fell from days 5 to 8. In this research, matrix resorption may have occurred in the R group due to the low level of serum calcium concentration. Processes of reabsorption and reformation are generally supposed to be paired²⁴⁾. In order to make a place for reformation to occur, b-ALP activity had to rise in the R group. It is assumed that the response of the S group to high serum calcium concentration after high calcium intake from day 1 caused b-ALP activity to rise rapidly, and high b-ALP activity, matrix specialization and formation continued until day 5. Then surplus calcium was lost and b-ALP activity fell about 50%. These facts suggest that calcium supplement of 2100 mg was useful in bone formation and prevention of osteoporosis.

The synthesis of BGP in the osteoblasts was detected before calcification occurred.²⁵⁾ In the S group, the trend for BGP appeared to be the same as for Ca^{2+} and $1,25(\text{OH})_2\text{D}_3$. In order for excess serum calcium and b-ALP to combine, the BGP level must rise 4h after the beginning of the experiment. In childhood and adolescence, D-Pyr shows a specifically high excretion because of rapid bone growth²⁶⁾. In this research, D-Pyr excretion was not significant among the three groups. However the C group was the highest. Perhaps S and R group's bone resorption was changed by low and high calcium concentration.

In conclusion, calcium intake regulated serum and urine calcium and IP, serum PTH, $1,25(\text{OH})_2\text{D}_3$, bone specific alkaline phosphatase and BGP levels. These levels changed dramatically during the experimental period. A large quantity of calcium over-RDA decreased serum PTH, $1,25(\text{OH})_2\text{D}_3$ and D-Pyr of bone resorption marker, while b-ALP of bone formation marker and BGP secretion increased. These facts suggest that calcium

supplementation of over-RDA from oral intake preserve bone mineral content through restrained bone resorption decreasing the PTH concentration and secreting $1,25(\text{OH})_2\text{D}_3$, and calcium supplementation of over RDA would prevent osteoporosis.

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カルシウム強化時と制限時における血清および尿中の カルシウム、リン、骨代謝マーカーの変化

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カルシウム強化と制限時における血清中・尿中カルシウムとリン酸塩、骨型アルカリホスファターゼ、副甲状腺ホルモン（中間部）、 $1,25(\text{OH})_2\text{D}_3$ 、オステオカルシン、デオキシピリジノリンの変化を合わせて観察した。被験者は健康な男子で、4日間の調整期間中は日本人の栄養所要量を満たす食事を、7日間の実験期間中は3群とも同一の食事で、カルシウム以外の栄養素は栄養所要量を満たすように調整した。実験期間中はカルシウム300mg/日をベースとし、カルシウム350mg/日補充群をコントロール群（C群）、カルシウム1800mg/日補充群を強化群（S群）、カルシウム補充なしを制限群（R群）とした。血液試料は1,5,8日目の昼食前に採取し、尿試料は第2尿から翌日第1尿までの24時間尿を採取した。その結果、血清イオン化カルシウムと尿中カルシウム量は、3群間で有意な差はみられなかった。一方血清リンおよび尿中リンレベルはS群が最も低く、尿中リンは1日目、8日目はC群と、5日目はR群とに差がみられた。骨型アルカリホスファターゼ活性は1日目はR群がS群より低く、8日目はS群が低かった。5日目はS群が最も高くR群が最も低かった。R群8日目の骨型アルカリホスファターゼ活性は1日目より40%高く、S群では逆に8日目は1日目に対し50%低かった。デオキシピリジノリンには3群間に有意な差はみられなかったものの、C群が常に高かった。PTHレベルはC群が常に高く8日目にはS群がR群より低かったが有意な差はみられなかった。また実験期間中のS群の $1,25(\text{OH})_2\text{D}_3$ レベルは常にR群より低かったが、オステオカルシンはS群が常に最も高かったが、群間の有意な差はみられなかった。これらの結果より、食事中への所要量を越えたカルシウム負荷は、血清および尿中カルシウム、リン酸、血清骨型アルカリホスファターゼ活性、オステオカルシン、PTH、 $1,25(\text{OH})_2\text{D}_3$ レベルを骨吸収より骨形成優勢に整え、短期間での高カルシウム負荷は、骨粗鬆症予防に役立つことが示唆された。