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The Affects of Long-term Oral Vitamin D Treatment on Abdominal Aortic Calcification and Vertebral Bone Mineral Content in the Elderly Patients with Osteoporosis

Nobuyuki Sugihara[1], *Masunori Matsuzaki*[1], *Kaneto Furutani*[2], *Yukio Kato*[3] *and Muneo Miyawaki*[4]

[1]The Second Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan.
[2]Department of Radiology, [3]Department of Internal Medicine, [4]Department of Surgery, Ube-First Hospital, Ube, Yamaguchi 755, Japan. (Received July 20, revised October 2, 1992)

Abstract We investigated the affects of long-term oral vitamin D treatment on aortic wall and vertebral bone in the elderly patients with osteoporosis by computed tomography. We calculated the aortic calcification index (ACI) of calcification volume to aortic volume within 10 slices in the lower abdominal aorta, and measured the bone mineral content (BMC) of three lumbar vertebral bodies (the 2nd, 3rd, 4th) using a calibration phantom. We compared the ACIs and BMCs between 15 patients under no vitamin D therapy (10 men, 5 women, 79±7 yrs)(Group-I) and 17 patients with osteoporosis under vitamin D therapy (4 men, 13 women, 75±6 yrs)(Group-II). The patients in Group-II received vitamin D everyday to sum up the duration into 849±333 days and the dosage into 604±323μg. ACI slightly increased from 9.2±7.2 to 11.0±8.5% in Group-I (NS), and similarly increased from 9.3±10.6 to 12.2±12.9% in Group-II(NS). Percent change of ACI was higher in Group-II (58±73%) than in Group-I (32±52%)(NS). BMC slightly decreased from 63.2±40.6 to 62.4±45.4mg/cm^3 in Group-I (NS), but increased a little from 57.8±51.4 to 63.3±44.6mg/cm^3 in Group-II (NS). These results suggest that there is a tendency to increase in both aortic calcification and bone mineral content under an even small dosage but long-term treatment of oral vitamin D.

Key Words : Abdominal aortic calcification, Vitamin D, Atherosclerosis, Osteoporosis, Bone mineral content.

Introduction

Vitamin D is recently used for a prophylatic medicine against the bone fractures and lumbar pains in the elderly patients with osteoporosis in Japan and Europe[1,2]. The clinical pharmacological effectiveness of vitamin D is due to the increase or maintenance in bone mineral content by the increase in intestinal calcium absorption[3,4] and the stimulation of osteoblast osteophytic activation[5-7]. According to the previous experimental reports[8-10], the hypercholesterol feeding with an execessive vitamin D induces to massive calcium deposition combined with the atheromatous plaque in aortic wall. However, it is unkown whether the clinical dosage administration of oral vitamin D to the patients with osteoporosis aggravates or prevents the calcium deposition on

aortic wall.

In the present study, we investigated the affects to the small dosage, long-term therapy of oral vitamin D on the calcium metabolism of abdominal aorta and lumbar vertebral bone in the elderly osteoporotic patients by using a computed tomography.

Subjects and Methods

Subjects: Thirty-two elderly patients of in- and outpatients consulted to Ube-First Hospital from November 1987 to March 1992 were studied (14 men, 18 women, [mean age 77±7 yrs]) and devided into age-matched two groups: Group-I was consisted of 15 patients under no vitamin D therapy (10 men and 5 women, age ranged from 61 to 89 yrs [mean age 79±7]). Group-II was comprised of 17 patients under the oral administration of vitamin D (4 men and 13 women, age ranged from 65 to 83 yrs [mean age 75±6]). All of patients were simultaneously examined in both abdominal aorta and lumbar vertebal bones by computed tomography twice at an interval over a year and a half: 856±247 days in Group-I (ranged from 546 to 1325 days) and 881±338 days in Group-II (ranged from 526 to 1519 days). In Group-II, 11 patients received the oral vitamin D (1, α(OH)D_3) everyday with the dose of 0.5μg/day, and similarly 6 patients received 1.0μg/day. The duration and dosage of vitamin D therapy summed into 849±333 days (ranged from 490 to 1437 days) and 604±323μg (ranged from 245 to 1372μg), respectively. The patients with a low risk of bone fracture in men had no indication of vitamin D treatment. The patients who had no symptom of osteoporosis had also no vitamin D treatment. The indication of vitamin D treatment against osteoporosis was determined by the requirement for preventing the bone fracture and improving the symptoms of lumbago or neuralgia.

In Group-I, we selected the patients of the following basal diseases: 7 patients with arrhythmia and ischemic heart disease; 6 with hypertension; 5 with cerebrovascular disease; 1 with acute pyelonephritis; and 2 with osteoporotic spondylosis deformans. In Group-II, all patients had osteoporosis associated with spondylosis deformans or osteoarthrosis. This group patients had other some concomitant diseases in 8 patients with hypertension; 5 with arrythmia and valvular or ischemic heart disease; 4 with cerebrovascular disease or senile demenz; and 1 with bronchial asthma. In this study, we excluded the following secondary osteoporosis due to the renal failure, endocrine disease, post-oophorectomy and hysterectomy. In addition, we excluded the following atherosclerotic disease: hyperlipidemia, diabetes mellitus, rheumatic arthritis, and chronic collagen disease. Furthermore, aortic aneurysm or dissection and the marked distortion of aorta were excluded. We also excluded the patients with the long-term bedridden state due to cerebrovascular disease or osteoarthrosis; the patients with the malnutrition after gastrectomy or suffering from mental anorexia; and the patients under the treatment of hormonal drug.

The patients in Group-I received continuously or irregularly the medical therapy against their basal diseases by the drug of calcium antagonist (nifidepine in 3 patients, and nicardipine in 2, respectively); anticoagulant (aspirin 1, dilazep 1, ticlopidine 4, cuwamarine 1); diuretic (furosemide 6); coronary vasodilator (isosorbite-dinitrate 2, nicorandil 3); antihypertensive (enalapril 2, prazosin 2); antiarrhythmic (digitalis 3, disopyramide 1, inderal 1); and brain metabolic activator or vasodilator (bifemelan 2, indeloxazine 2, and lisuride 1). Similarly, Group-II patients received the medical therapy by calcium antagonist (nicardipine in 7 patients, nifedipine in 4, diltiazem in 2, manidipine in 1, and verapamil in 1, respectively); diuretic (furosemide 6); anticoagulant (ticlopidine 5, dilazep 2); coronary vasodilator (nitroglycerin 1, isosorbite-dinitrate 1); antihypertensive (enalapril 1); antiarrhythmic (digitalis 6, disopyramide 2); bronchodilator (theophyllin 1); and brain drug (bifemelan 2, indeloxazine 2, lisuride 1, and amantazine 1).

Measurement of aortic calcification index (ACI) in abdominal aorta: Using single-energy CT equipment (Hitachi Medico, CT-W400-20), we scanned the abdominal aorta and produced the images under the following conditions: X-ray voltage 120 KV; current

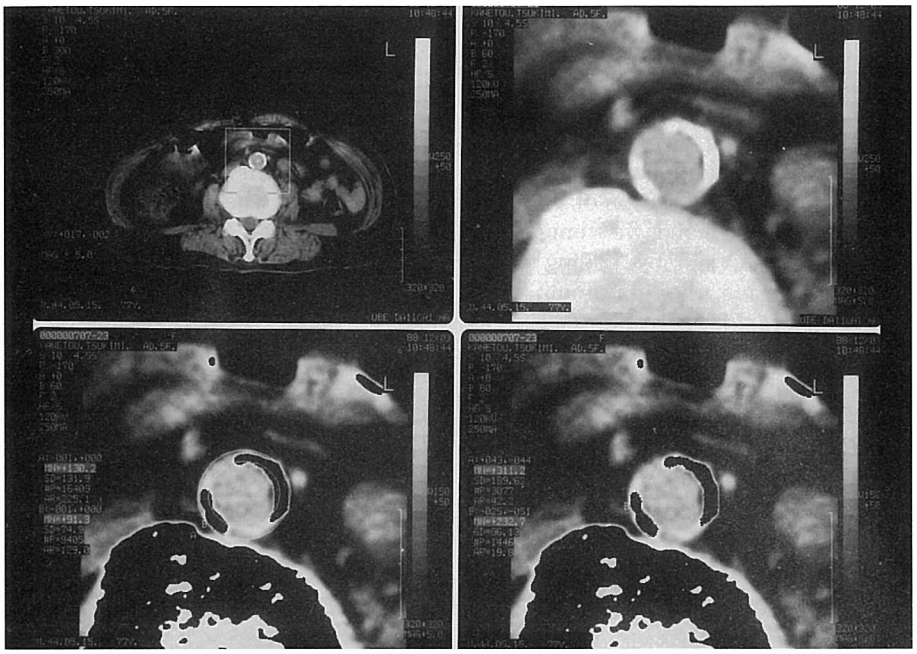

Fig 1. Measurement method of abdominal aortic calcification by computed tomography (CT).
Upper left : The image of abdominal aorta is set at the region of interest (ROI) by the rectangular square for zooming. Upper right : Abdominal aorta is magnified by zooming five folds. Calcification sites in aorta wall appear as a white image. Lower left : Aortic calcification over an optimal CT threshold number represent as a black image. Lower right : The area of calcification is hand-manually traced along the outline of calcification.

amplitude 250 mA; slice thickness 1cm; slice interval 0cm; scanning field 30cm; scanning time 4.5 sec; window level +50; window width 250; calculation matrix size 320×320 pixels[11].

As shown as Fig. 1, we scanned the relevant abdomen of each patient in a supine position, and set the region of interest (ROI) around abdominal aorta by the rectangular square (Fig. 1, Upper left). For clarifying the outline of aortic calcification, we magnified an image of abdominal aorta on the display by zooming five folds (Fig. 1, Upper right), and established an optimal CT threshold number of 115 Hounsfield Unit. This CT number was determined to produce an adequate calcification image along the internal or external margin of aortic wall by visual inspection. In order to discriminate more clearly the outline of aortic calcification, we developed the binary image over this CT threshold number in a black color (Fig. 1, Lower left). By means of handmanually

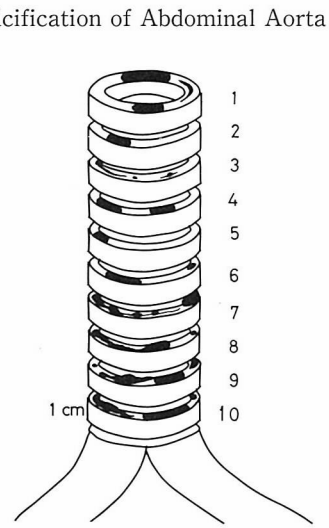

Fig 2. Measurement method for aortic calcification index (ACI ; %) of calcification volume (CV ; cm³) to aortic volume (AV ; cm³) within 10 slices upward over the bifurcation of abdominal aorta.

tracing the outline individual calcification sites using a track ball, we calculated their areas of aortic calcification (Fig. 1, Lower right). As shown in Fig. 2, we summed up the calcification volume (CV;cm^3) by adding the individual area of calcified sites within 10 slices upward over the bifurcation of abdominal aorta. On the other hand, we summed up the aortic volume (AV;cm^3) by adding each ellipsoidal circle using the maximum and minimum diameters of external margin of aortic wall within 10 slices. Subsequently, we calculate the ACI (%) of CV to AV[11].

Measurement of bone mineral content (BMC) in lumbar vertebral body: As shown in Fig. 3, we measured the BMC of vertebral bodies with the lumbar CT using a calibration phantom (Chugai Pharmaceutical, B-MAS) according to the method of Fujii et al[12]. We examined under the same CT scanning conditions as the abdominal aorta[11]. The phantom was attached closely to the lumbar back of the patient in a supine position, and set at an optimal angle of gantry of CT equipment for scanning a slice as perpendicular as possible to the midplane of the vertebral body. Thus, both the vertebral body and the phantom system were scanned simultaneously (Fig. 3, Upper left). Subsequently, we set the ROI in the spongious bone of the vertebral body using the largest ellipsoidal circle excluding a cortical bone and a

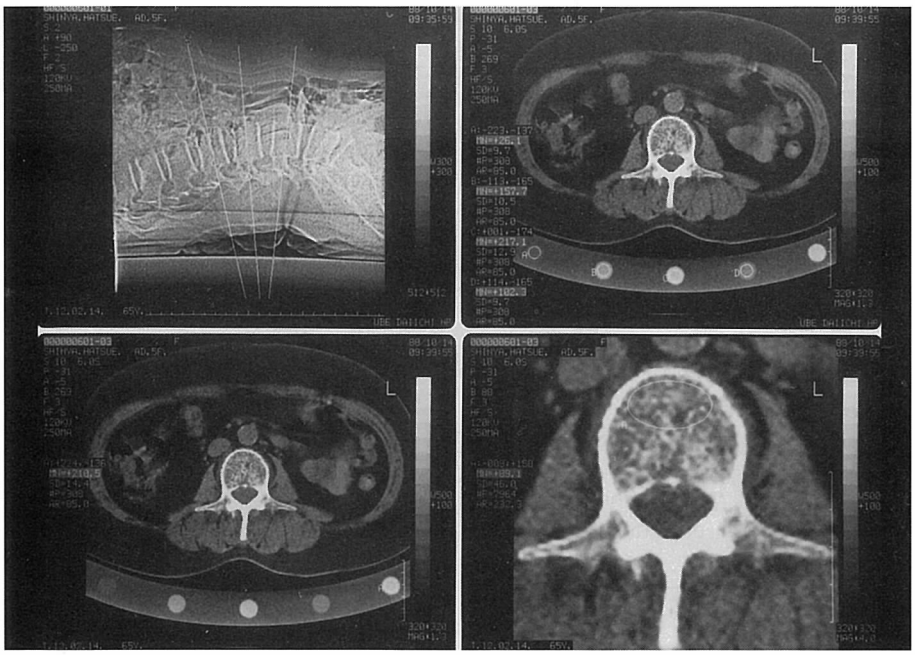

Fig 3. Measurement method for bone mineral content (BMC) in lumbar vertebral bodies by quantitative computed tomography (CT) using a calibration phantom.

Upper left : Scanning method on the 2nd, 3rd and 4th vertebral bodies in a scout view.

Upper right and lower left : Simultaneous scanning method of both the vertebral body and the calibration phantom attached closely to the lumbar back. After setting the five regions of interest (ROIs) on five standard substances of the phantom, each mean CT value was calculated in each ROI.

Lower right : After setting the ROI on spongious bone in the vertebral body by the largest ellipsoidal circle, excluding the cortical bone and foramen of nutrition, the mean CT value was calculated in each ROI from the 2nd to 4th body.

nutritional foramen on the display. Then, we calculated the mean CT number in the ROI of vertebral body (Fig. 3, Lower right). Similarly, we set the five ROIs on five standard substances in the phantom by using round circles, and calculated the mean CT numbers within the five ROIs (Fig. 3, Upper right and lower left). Five standard substances were composed of the following concentrations of $CaCO_3$: 32.31, 80.17, 133.47, 177.03, and 233.66mg/cm³, in this order[12]. The first regression line was calculated by the least squares method using both the mean CT number and the corresponding $CaCO_3$ concentration of each standard substance. By applying the mean CT number of the vertebral body to the first regression line, BMC (mg/cm³) was obtained. In all patients, we simultaneously scanned the 2nd, 3rd and 4th lumbar vertebral bodies (Fig. 3, Upper left). Results from the 3rd body were used as a representative BMC for most patients. In patient with compression fractures or marked calluses formation complicated of trabecular microfracture in the 3rd vertebral body, results of the 2nd or 4th body were used as a representative BMC. The changes in BMCs were studied by results of the same vertebral body between the initial and final examination. In this study, patients with severe compression fractures or marked scoliosis throughout the three vertebral bodies were excluded.

Thus, we studied the differences of the ACIs and BMCs between before and after the vitamin D therapy in Group-II patients, in comparison with the these differences in Group-I patients.

Statistics analysis: The statistical significance of differences between two groups and between the initial and final examination was used by the paired and non-paired Student's t-test. Comparison of categorical variables in frequency was performed by chi-square test. P value lower than 0.05 was significant. The mean value represent as mean± standard deviation (SD).

Results

ACI: In shown as Table 1, men were significantly more and less than woman in Groups-I and -II, respectively ($p<0.05$). Table 1 and Fig. 4 showed the differences of ACIs between the initial and final examination and between Groups-I and II. ACI in Group-I slightly increased at the mean difference of +1.8% from 9.2±7.2% to 11.0± 8.5%, but showed no statistical difference. Similarly, ACI in Group-II slightly increased at the mean difference of +2.9% from 9.3± 10.6% to 12.2±12.9%, but showed no significant difference between before and after the vitamin D therapy. Table 1 and Fig. 5 showed the percent changes in ACIs from the initial to the final examination in each

Table 1. Number of men and women, age, aortic calcification index (ACI), percent change of ACI, bone mineral content (BMC), and number of patients whose the 2nd (L2), 3rd (L3) and 4th (L4) vertebral body was used for a representative BMC, in Groups -I and -II

		Group-I	Group-II	P value
Men, Women	(n=)	(10, 5)	(4, 13)	P<0.05
Age	(years old)	79.1± 7.1	75.2± 6.5	NS
Initial ACI	(%)	9.2± 7.2	9.3±10.6	NS
Final ACI	(%)	11.0± 8.5	12.2±12.9	NS
Percent change of ACI	(%)	32.2±47.3	57.8±73.3	NS
Vertebral body	(L2,L3,L4) (n=)	(1, 13, 1)	(2, 10, 5)	NS
Initial BMC	(mg/cm³)	63.2±40.6	57.8±51.4	NS
Final BMC	(mg/cm³)	62.4±45.4	63.3±44.6	NS
P value of				
Initial vs Final ACI		NS	NS	
Initial vs Final BMC		NS	NS	

Values : Mean±SD ; n=Number of patients ; NS=Not significant.

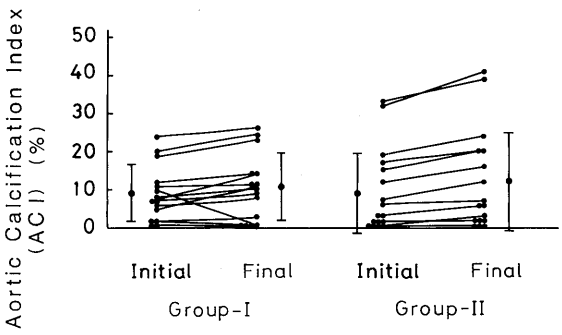

Fig 4. Comparison of changes in aortic calcification index (ACI) between the initial and final examination in each Group.

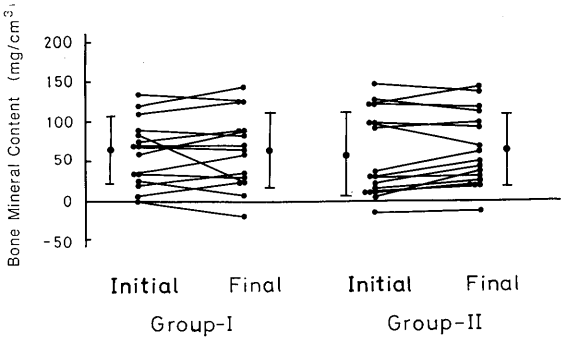

Fig 6. Comparison of changes in bone mineral content (BMC) between the initial and final examination in each Group.

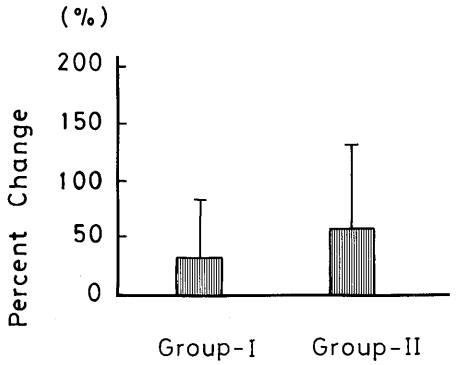

Fig 5. Comparison of percent changes in aortic calcification index (ACI) between two Groups.

patient of Groups- I and II. This percent change was larger in Group- II (57.9±73.3%) than in Group- I (32.2±51.7%), but showed no significant difference between two Groups.

BMC: Table 1 and Fig. 6 showed the differences of BMCs between the initial and final examination and between Groups- I and -II. As a representative BMC by the same vertebral body between the initial and final examination, 1 patient was used by the 2nd lumbar vertebral body, 13 were by the 3rd, and 1 was by the 4th respectively in Group- I. Correspondingly, 2 by the 2nd, 10 by the 3rd, and 5 by the 4th respectively in Group- II (Table 1).

In Group- I, BMC slightly decreased at the mean difference of -0.8mg/cm³ from 63.2± 40.6mg/cm³ to 62.4±45.4mg/cm³, but showed no statistical difference. On the other hand, BMC in Group- II slightly increased at the mean difference of +5.5mg/cm³ from 57.8± 51.4mg/cm³ to 63.3±44.6mg/cm³, but showed no significant difference.

Discussion

Vitamin D: Elderly persons often have poor dietary nutrition, and they are less likely to have sun exposure with a housebound lifestyle[13-15]. They also have the decreased intestinal absoption of vitamin D and the decreased capacity for dermal biosynthesis of vitamin D by sunlight ultraviolet exposure[13-15]. In addition, the reduced activity of converting enzyme in kidney and liver with aging causes to the lowered serum level of biological active form of vitamin D, $1,25(OH)_2D_3$[13-15]. The consequence of vitamin D deficiency with age by these mechanisms contributes to the decreasing in intestinal calcium absorption, and the high incidence of general osteopenia with abnormal bone mineralization, muscle weakness and bone fracture. Virtually, the serum level of vitamin D is significantly less in senile or postmenopausal osteoporotic patients than in normal subjects[16,17]. The elderly patients admitted in a hospital or institution also have the lowered level of serum vitamin D in proportion to the term of bedridding, even during short period[18].

Osteoporosis occurs more frequently in Japan than in Europe and America[19]. A low

intake of calcium-rich milk product and a low level of serum vitamin D are major causes of the Japanese osteoporosis[19]. Supplementations of both vitamin D and calcium are an appropriate therapy in Japan. In the present study, vitamin D therapy showed no significant increase in BMC. This result was compatible to the report of Geusens et al[2]. However, the treatment of vitamin D is clinically effective for preventing the pathological and non-traumatic bone fracture of senile or postmenopausal osteoporosis in Japan[2].

Vitamin D and atherosclerosis: Excess supplementation of vitamin D experimentally increases the serum cholesterol concentrations and the atherosclerotic lesions under certain condition. Previous reports in mammalian animals demostrated that the atherosclerotic lesions associated with aortic calcification induced easily in aortic wall by feeding the cholesterol rich diet with excessive amounts of vitamin D[8-10]. However, the influence of vitamin D under the small dosage of clinical treatment on aortic wall is unkown in human. Tissue distribution of vitamin D receptor in rat varies with organs, such as much in intestine and colon, many in kidney, skin, or bone, and then a little in stomach, heart or spleen[20]. There is a small number of vitamin D receptor in cardivascular system[20-23]. In the experimental reports using cultured cells, vitamin D exerts the growth of aortic endothelial cell[23]. On the other hand, the effect of vitamin D on cell proliferation of vascular smooth muscle cell is contraversary. The promoting results were shown in some reports[24,25], but the inhibiting results were demonstrated in other report[26]. After all, it remains unkown in human whether the pharmacological effect of small dosage oral vitamin D for clinical treatment involves in preventing or exaggerating the atherosclerosis.

Aortic calcification: The present study showed that there is a tendency to increase in aortic calcification under vitamin D therapy Group more than under no vitamin D therapy Group. Some problems about this result were considered as follows: 1) The interval between the initial and final examination of this study may be too short to detect the change in calcium deposition on aortic wall. More longer interval for evaluation of atherosclerosis will be required. 2) There was sex difference between two Groups. In the elderly, women have a higher incidence of osteoporosis and a lower incidence of atherosclerotic disease than men. It will be required to match the sex between two Groups, but will be very difficult. 3) Many patients received some kinds of calcium antagonist, which have both an anti-hypertensive and anti-atherosclerotic pharmacological effects[27], but have no significant affect on calcium regulating hormone and calcium homeostasis[28]. Calcium antagonists may prevent the increasing calcification induced by vitamin D. However, we could not withdraw the calcium antagonist for the basal disease. 4) The direct hemodynamic effect of vitamin D on cardiovascular system should be considered. The clinical hypotensive effect of vitamin D was reported in human[29], and the reduction effect on arterial resistence and aortic wall thickening by vitamin D was showed in rat[30]. Vitamin D receptor in rat heart is involving in the secretion of atrial natriuric peptide and the reduction of systemic blood pressure[31]. However, other report demonstrated that there was no relationship between the serum level of vitamin D and blood pressue in hypertension[32]. The reduction of blood pressure by vitamin D was not statistically detectable in this study. 5) The patients are exposed to X-ray irradiations by CT examination more than by the plain film or gamma-ray scintigram. The major factor of exposing to much irradiation is the longer scanning time by CT than by other examinations. CT examination is the most powerful method to discriminate the organic calcification or stone from other in the clinical usage equipments. Notwithstandingly, frequent CT examination within short period should be avoided.

The results of this study suggest that an even small dosage but long-term treatment of vitamin D may aggravate the atherosclerosis. The clinical pharmacological affects of oral vitamin D on cardiovascular system will be required to evaluate at a longer interval of at least five years than at an interval of this study.

References

1) Geusens, P. and Dequeker, J.: Long-term effects of nandrolone decanocate, 1α-hydroxy-vitamin D_3 or intermittent calcium infusion therapy on bone mineral content, bone remodeling and fracture rate in symptomatic osteoporosis: a double-blind controlled study. *Bone and Mineral,* **1**: 347-357, 1986.
2) Orimo, H., Shiraki, M., Hayashi, T. and Nakamura, T.: Reduced occurrence of vertebral crush fractures in senile osteoporosis treated with 1α(OH)-vitamin D_3. *Bone and Mineral,* **3**: 47-52, 1987.
3) Norman, A.W.: 1,25-dihydroxyvitamin D_3: A kidney-produced steroid hormone essential to calcium homeostasis. *Am. J. Med.,* **57**: 21-27, 1974.
4) Goff, J.P., Reinhardt, T.A., Beckman, M.J. and Horst, R.L.: Contrasting effects of exogenous 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] versus endogenous 1,25-$(OH)_2$D, induced by dietary calcium restriction, on vitamin D receptors. *Endocrinology,* **126**: 1031-1035, 1990.
5) Beresford, J.N., Gallagher, J.A., Poser, J.W. and Russell, R.G.G.: Production of osteocalcin by human bone cells in vitro. Effects of 1,25$(OH)_2D_3$, 24,25$(OH)_2D_3$, parathyroid hormone, and glucocorticoids. *Metab. Bone. Dis. & Rel. Res.,* **5**: 229-234, 1984.
6) Lieberherr, M.: Effects of vitamin D_3 metabolites on cytosolic free calcium in confluent mouse osteoblasts. *J. Biol. Chem.,* **262**: 13168-13173, 1987.
7) DeBoland, A.R. and Norman, A.W.: Influx of extracellular calcium mediates 1,25-dihydroxyvitamin D_3-dependent transcaltachia (the rapid stimulation of duodenal Ca^{2+} transport). *Endocrinology,* **127**: 2475-2480, 1990.
8) Hass, G.M., Trueheart, R.E., Taylor, C.B. and Stumpe, M.: An experimental histologic study of hypervitaminosis D. *Am. J. Pathol.,* **38**: 395-431, 1958.
9) Gillaman, T., Grant, R.A. and Hathorn, M.: Histochemical and chemical studies of calciferol-induced vascular injuries. *Br. J. Exp. Pathol.,* **41**: 11-18, 1960.
10) Toda, T., Ito, M., Toda, Y., Smith, T. and Kummerow, F.: Angiotoxicity in swine of a moderate excess of dietary vitamin D_3. *Fd Chem. Toxic.,* **23**: 585-592, 1985.
11) Sugihara, N., Matsuzaki, M. and Kato, Y.: Ectopic calcification of the abdominal aorta and bone mineral content of vertebrae. (in Japanese). *Jpn. J. Geriatr.,* **26**: 256-264, 1989.
12) Fuji, Y., Tsunenari, T., Tsutsumi, M., Miyauchi, A., Yamada, H., Fukase, M., Yoshimoto, Y., Okuno, Y., Kusakabe, H., Miyoshi, K., Fukunaga, M., Morita, R. and Fujita, T.: Quantitative computed tomography; Comparison of two calibration phantoms. *J. Bone Mineral Metabolism,* **6**: 71-74, 1988.
13) Bullamore, J.R., Gallagher, J.C., Wilkinson, R. and Nordin, B.E.C.: Effect of age on calcium absorption. *Lancet,* ii: 535-537, 1970..
14) Glkoth, F.M., Tobin, J.D., Sherman, S.S. and Hollis, B.W.: Is the recommended daily allowance for vitamin D too low for the homebound elderly ?. *J. Am. Geriatr. Soc.,* **39**: 137-141, 1991.
15) Bouillon, R.: Clinical use of vitamin D metabolite assays (Calcidiol and calcitriol). Calcium regulating hormones, vitamin D metabolites, and cyclic AMP in Schmidt H., Armbruster F.P. and Bouillon R. (eds.), *Assays and their clinical application,* ed. by Springer-Verlag, Berlin and Heidelberg, 1990, p.24-47.
16) Gallagher, J.C., Riggs, B.L., Eisman, J., Hamstra, A. and Sara, B.: Intestinal calcium absoption and serum vitamin D metabolites in normal subjects and osteoporotic patients. *J. Clin. Invest.,* **64**: 729-736, 1979.
17) Slovik, D.M., Adams, J.S., Neer, R.M., Holick, M.F. and Potts, J.T.: Deficient production of 1,25-dihydroxyvitamin D in elderly osteoporotic patients. *N. Engl. J. Med.,* **305**: 372-374, 1981.
18) McMurtry, C.T., Young, S.E., Downs, R.W. and Adler, R.A.: Mild vitamin D deficiency and secondary hyperparathyroidism in nursing home patients receiving adequate dietary vitamin D. *J. Am. Geriatr. Soc.,* **40**: 343-347, 1992.
19) Nordin, B.E.C.: International patterns of osteoporosis. *Clin. Orthop.,* **45**: 17-30, 1966.
20) Sandgren, M.E., Bronnegrad, M. and DeLuca, H.F.: Tissue distribution of the 1,25-dihydroxyvitamin D_3 receptor in the male rat. *Biochem. Biophys. Res. Comm.,* **181**: 611-616, 1991.
21) Walters, M.R., Wicker, D.C. and Riggle, P.C.: 1,25-dihydroxyvitamin D_3 receptors identified in the rat heart. *J. Mol. Cell. Cardiol.,* **18**: 67-72, 1986.
22) Kawashima, H.: Receptor for 1,25-dihydrox-

yvitamin D in a vascular smooth muscle cell line derived from rat aorta. *Biochem. Biophys. Res. Comm.*, **146**: 1-6, 1987.
23) Merke, J., Milde, P., Lewicka, S., Hugel, U., Kalus, G., Mangelsdorf, D.J., Haussler, M.R., Rauterberg, E.W. and Ritz, E.: Identification and regulation of 1,25-dihydroxyvitamin D_3 receptor activity and biosynthesis of 1,25-dihydroxyvitamin D_3: Studies in cultured bovine aortic endothelial cells and human dermal capillaries. *J. Clin. Invest.*, **83**: 1903-1915, 1989.
24) Mohtai, M. and Yamamoto, T.: Smooth muscle cell proliferation in the rat coronary artery induced by vitamin D. *Atherosclerosis,* **63**: 193-202, 1987.
25) Koh, E., Morimoto, S., Fukuo, K., Itoh, K., Hironaka, T., Shiraishi, T., Onishi, T. and Kumahara, Y.: 1,25-dihydroxyvitamin D_3 binds specifically to rat vascular smooth muscle and stimulates their proliferation in vitro. *Life Science*, **42**: 215-223, 1988.
26) MacCarthy, E.P., Yamashita, W., Hsu, A. and Ooi, B.S.: 1,25-dihydroxy-vitamin D_3 and rat vascular smooth muscle cell growth. *Hypertension*, **13**: 954-959, 1989.
27) Fleckenstein, A., Frey, M. and Fleckenstein -Grun, G.: Anthihypertensive and arterial anticalcinotic effects of calcium antagonist. *Am. J. Cardiol.*, **57**: 1D-10D, 1986.
28) Townsend, R., Dipette, D.J., Evans, R.R., Davis, W.R., Green, A., Graham, G.A., Wallace, J.M. and Holland, O.B.: Effects of calcium channel blockade on calcium homeostasis in mild to moderate essential hypertension. *Am. J. Med. Sci.*, **300**: 133-137, 1990.
29) Lind, L.A.R.S., Lithell, H., Skarfors, E., Wide, L. and Ljunghall, S.: Reduction of blood pressure by treatment with alphacalcidol. A double blind, placebo-controlled study in subjects with impaired glucose tolerance. *Acta Med. Scand.*, **223**: 211-217, 1988.
30) Xue, H., McCarron, D.A. and Bukoski, R.D.: 1,25$(OH)_2D_3$ attenuates the loss of resistence artery contractile function associated with incubation in culture media. *Biochem. Biophys. Res. Comm.*, **174**: 11-17, 1991.
31) Bidmon, H.J., Gutkowska, J., Murakami, R. and Stumpf, W.E.: Vitamin D receptors in heart: Effects on atrial antriuretic factor. *Experientia*, **47**: 958-962, 1991.
32) Youg, E.W., McCarron, D.A. and Morris, C. D.: Calcium regulating hormones in essential hypertension. Importance of gender. *Am. J. Hypertension.*, **3**: 161S-166S, 1990.