Original Article

Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly

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There have been methodological problems for studying hypovitaminosis D and K in the elderly. First, studies were done either by evaluating food intake or measuring their circulating levels, but rarely by both in Japan. In this paper, vitamin D and K intakes and their circulating levels were simultaneously determined. Second issue is whether hypovitaminosis D and K are independent of general malnutrition, prevalent in the elderly. We tried to statistically discriminate them by principal component analysis (PCA). Fifty institutionalized elderly were evaluated for their circulating 25 hydroxy-vitamin D (25OH-D), intact parathyroid hormone (PTH), phylloquinone (PK), menaquinone-7 (MK-7) levels, and their food intake. Although average vitamin D intake (7.0 µg/day) exceeded the Japanese Adequate Intake (AI) of 5.0 µg/day, average serum 25OH-D concentration was in the hypovitaminosis D range (11.1 ng/mL). Median vitamin K intake was 168 µg/day, approximately 2.5 times as high as AI for vitamin K. Nevertheless, plasma PK and MK-7 concentrations were far lower than those of healthy Japanese elderly over 70 years old. PCA yielded four components; each representing overall nutritional, vitamin K_2, vitamin D, and vitamin K_1 status, respectively. Since these components are independent of each other, vitamin D- and K-deficiency in these subjects could not be explained by overall malnutrition alone. In summary, institutionalized elderly had a high prevalence of hypovitaminosis D and K, and the simultaneous determination of their circulating level and dietary intake is mandatory in such studies. PCA would yield fruitful results for eliminating the interference by confounders in a cross-sectional study.

Key Words: hypovitaminosis D, hypovitaminosis K, principal component analysis, adequate intake, institutionalized elderly

INTRODUCTION

Vitamin D is of utmost importance in enhancing the intestinal absorption of calcium and phosphorus,[1,2] with its deficiency causing skeletal mineralization defect; rickets and osteomalacia. Recently, it has come to the general attention that inadequate supply of vitamin D, even in its milder form (vitamin D insufficiency), is associated with increased risk of fracture through negative calcium balance, hence secondary hyperparathyroidism.[1,2] Vitamin D insufficiency is also reported to be associated with muscle weakness. Recent clinical studies have indicated that intervention with vitamin D supplementation reduced the incidence of falling in elderly subjects.[3] Clinically important non-vertebral fractures, such as hip and wrist fractures are triggered by falling. Thus, vitamin D insufficiency would render the elderly subjects more prone to fracture through its effects both on the skeleton and muscle. Recently, lower serum level of 25 hydroxy-vitamin D (25OH-D) was reported to be a significant risk factor even for mortality.[4]

Vitamin D insufficiency is quite common in the elderly population,[5,6] and institutionalized elderly are at even higher risk for vitamin D insufficiency.[7-10] Factors hitherto postulated to be responsible include low dietary vitamin D intake,[7,9] reduced dermal capacity to produce vitamin D with aging and minimal sun exposure.[11,12]

In contrast to vitamin D, the skeletal action of vitamin K has called our attention only quite recently. The only biological action of vitamin K has been considered to be its role as the coenzyme of γ-glutamyl carboxylase (GGCX) in the liver, by which additional carboxyl group is introduced into the glutamic acid residue in four of the

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Table 1. Background profiles and results from blood tests of the study subjects

	Total	Male	Female	*p* value
n	50	15	35	-
Age (y)	87.6±8.0 (88.5)	84.9±7.9 (83.0)	88.7±7.8 (90.0)	0.133
Level of care needed	3.6±1.1 (4.0)	3.3±1.0 (3.0)	3.7±1.2 (4.0)	0.228
Body height (cm)	144.0±11.6 (142.0)	157.0±7.8 (159.0)	138.4±7.8 (139.0)	<0.01
Body weight (kg)	43.6±9.3 (43.2)	50.3±7.9 (49.9)	40.7±8.3 (38.1)	0.001
Body mass index (kg/m2)	21.0±3.8 (20.1)	20.5±3.4 (19.6)	21.3±4.0 (20.2)	0.476
Serum albumin (g/dL)	3.7±0.4 (3.7)	3.8±0.4 (3.9)	3.6±0.4 (3.6)	0.136
Serum total cholesterol (mg/dL)	184±37 (184)	186±26 (195)	183±41 (183)	0.828
Serum triglyceride (mg/dL)	98±41 (92)	96±47 (75)	98±39 (93)	0.403
Serum aminotransferase (U/L)	22±11 (19)	20±7 (17)	22±12 (19)	0.603
Serum alanine aminotransferase (U/L)	16±10 (13)	16±7 (13)	16±12 (12)	0.235
eGFR (mL/min./1.73m2)	61±20 (60)	67±19 (67)	59±21 (57)	0.208
Serum 25-hydroxyvitamin D (ng/mL)	11.1±3.1 (11.2)	10.3±3.5 (9.3)	11.5±3.0 (11.6)	0.274
Serum parathyroid hormone (pg/mL)	30.8±11.8 (30.0)	29.9±11.1 (31.0)	31.3±12.2 (30.0)	0.736
Plasma phylloquinone (ng/mL)	0.73±0.70 (0.58)	0.62±0.29 (0.60)	0.77±0.82 (0.53)	0.992
Plasma menaquinone-7 (ng/mL)	0.53±0.37 (0.45)	0.59±0.47 (0.47)	0.51±0.32 (0.44)	0.849

Data are expressed as mean±SD with the values in parentheses showing the median.
Comparison of indices between males and females were done by unpaired t test or Mann-Whitney test depending on normality.
eGFR; estimated Glomerular Filtration Rate.

Table 2. Daily dietary intakes of the study subjects

	Total	Male	Female	*p* value
Energy (kcal)	1322±159 (1387)	1374±96 (1416)	1300±175 (1386)	0.160
Protein (g)	51.0±5.8 (53.3)	53.1±3.6 (54.6)	50.2±6.3 (53.5)	0.091
Fat (g)	32.8±3.9 (34.6)	34.2±2.4 (35.3)	32.2±4.3 (34.5)	0.095
Carbohydrates (g)	178±20 (186)	185±12 (189.7)	175±21 (186)	0.093
Calcium (mg)	494±53 (504)	503±50 (506)	490±54 (502)	0.157
Vitamin D (μg)	7.0±1.4 (7.7)	7.4±0.9 (7.8)	6.9±1.5 (7.6)	0.107
Vitamin K (μg)	155±30 (168)	164±19 (172)	151±33 (168)	0.107

Data are expressed as mean±SD with the values in parentheses showing the median. Comparison of indices between male and women were done by unpaired t test or Mann-Whitney test depending on normality.

blood coagulation factors (II, VII, IX, X) to yield γ-glutamic carboxyl (Gla) residue.[13] Other extrahepatic proteins are also γ-carboxylated by GGCX, such as osteocalcin (bone Gla protein; BGP) and matrix gla protein (MGP).[14] Recent evidences suggest that vitamin K deficiency is associated with increased risk of fracture. When subjects were categorized into quartiles according to their vitamin K intake, fracture risk in the lowest quartile was twice as high as that in the highest quartile.[15] The age-adjusted incidence of vertebral fracture was significantly higher in subjects with low plasma phylloquinone levels than those with high plasma levels in Japanese women.[16] In addition, the association of circulating vitamin K level and bone mineral density (BMD) has also been reported. For example, low plasma phylloquinone concentration was associated with low BMD at the femoral neck in men, and lower spine BMD in postmenopausal women without estrogen replacements.[17] High serum concentration of undercarboxyled osteocalcin (ucOC), which is a sensitive indicator of skeletal vitamin K insufficiency, was a significant risk factor of hip fracture independent of BMD.[18,19]

Plasma phylloquinone level is subject to alteration by aging,[20,21] and elderly subjects have been reported to have low plasma phylloquinone concentrations.[22] Of note is the report that elderly nursing home residents generally had a poor dietary vitamin K intake compared to the ambulatory elderly.[23]

Studies on the role of hypovitaminosis D and K in the elderly, especially the institutionalized ones are greatly hampered by the fact that they are also generally malnourished. Arguments against the significance of these vitamins have been made that decreased serum concentrations of these vitamins is merely a reflection of overall malnutrition. In this paper, we have tried to statistically discriminate hypovitaminosis D and K from general malnutrition by using principal component analysis (PCA), which has been employed in clinical nutrition for the analyses of dietary pattern.[24,25]

MATERIALS AND METHODS

Subjects

The study subjects were 50 institutionalized elderly (male 15, female 35) in a nursing home, Kayu-Shirakawa. Exclusion criteria were routine medication that has potential interference with vitamin D or vitamin K status. Detailed information about this study was given and written consent was obtained from the subject or the proxy. The study protocol was approved by the ethical committee in Kyoto Women's University.

Laboratory data

Blood was obtained after overnight fasting. After centrifugation, serum was kept frozen at –30°C until analysis. Serum concentration of 25OH-D was measured by radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA).

Circulating level of intact parathyroid hormone (PTH) was measured by electro chemiluminescent immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany). Plasma vitamin K_1 (phylloquinone; PK), and menaquinone-7 (MK-7) levels were determined by high-performance liquid chromatography-tandem mass-mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS) using a HPLC system (Shimadzu, Kyoto, Japan) and API3000 LC-MS/MS System (Applied Biosystems, Foster City, CA) with ^{18}O-labeled vitamin K as the internal standard.[26]

Nutrition intake study

Since the subjects were institutionalized and their diet was supplied from the institution, their nutrients and energy intake were calculated by multiplying the supplied nutrients on the basis of the Standard Tables of Food Composition in Japan, 5th ed. with the average percentage intake in a preceding month by the staff.[27] Percentage intake was assessed for each subject at every meal, and the monthly average percentage intake was calculated. Based on these records, their intake of energy and nutrients was calculated using software (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan).

Statistical analyses

Statistical analyses were performed with SPSS 15.0J (SPSS Japan Inc., Tokyo, Japan). Comparison of two independent groups was made with Student's t-test or Mann-Whitney test depending on normality. Multiple regression analyses by stepwise method were performed to determine independent factors for circulating levels of vitamin D and K levels. The relationship between various nutritional indices and circulating vitamin D- and K- levels was analyzed with principal component analysis (PCA), which is a statistical method to summarize the various parameters into a small number of summary factors (components). These components are obtained in such a way that the first component is extracted from the initial raw data with the maximal amount of information (eigenvalue), and the second one is extracted from the remaining information. Therefore, each component is mutually independent. Components with the eigenvalue greater than 1 were adopted, as in usual practice.

RESULTS

Biochemical markers and circulating concentrations of vitamin D and K

Baseline characteristics and data from blood examination are shown in Table 1. There was no gender difference in the age and level of care needed, which is a 5-grade score in the long-term care insurance in Japan with a higher number indicating the need for more intensive care. The level of care needed was higher than grade 3 in 78% of subjects. Most of the present subjects required wheelchair for transportation. Body height and body weight were significantly higher in males than in females. Body mass index (BMI), or serum albumin, total cholesterol and triglyceride concentrations did not significantly differ between the two groups. Generally, serum albumin level less than 3.5 g/dL is considered to indicate malnutrition. Serum albumin level was below this value in 26% of subjects. Inasmuch as the advanced age and high level of care needed, nutritional parameters remained within the reference range in most of the subjects. None of the study subjects had severe hepatic or renal dysfunction. There is a general consensus that a serum 25OH-D concentration less than 20 ng/mL indicates hypovitaminosis D.[2] Serum 25OH-D concentration was <10 ng/mL in 40% of subjects, 10-20 ng/mL in 58%, and ≥20 ng/mL in only one subject. None of the subjects had a serum PTH level above the cut-off value (65 pg/mL). Plasma PK and MK-7 concentrations in all of the subjects were 0.73±0.70 ng/mL and 0.53±0.37 ng/mL, respectively. In the present study, serum PK was less than 1 ng/ml and serum MK-7 was less than 1 ng/ml, in 85% and 90% of the subjects, respectively. The interpretation for these values will be given in the "Discussion" section. There were no gender differences in plasma vitamin K levels, serum 25OH-D or PTH.

Nutritional intake in the study subjects

The nutrients intake in the males and females were not statistically different as shown in Table 2. During the preparation of this paper, Dietary Reference Intake (DRI) for Japanese 2010 (DRI 2010) was released on May 29, 2009.[28] Since this work was done in 2006, however, consideration is made basically according to DRI 2005.[29] The intake of macronutrients such as protein, fat and carbohydrates appeared appropriate for their age and sex. The adequate intakes (AI) for calcium in Japan are 750 mg for men and 650 mg for women over 70 years. The AI for vitamin D is 5 μg/day, and that for vitamin K is 75 μg/day for men and 65 μg/day for women respectively. Although average calcium intakes in both groups were lower than the AI in DRI 2005, the average daily vitamin D intake was 7.0 μg, which is 140% of the AI in DRI 2005. The average daily intake of vitamin K in whole subjects was 155 μg, which is more than twice the AI for each gender. Thus, apparently these subjects had sufficient intakes of vitamin D and K based on AI in DRI 2005.

Multiple regression analyses for the determination of independent factor for circulating vitamin D, K concentrations.

In multiple regression analyses, vitamin D intake was a significant determinant of serum 25OH-D level, although the R^2 was low. Serum triglyceride level was the only significant predictor for plasma MK-7 concentration, and vitamin K intake and serum triglyceride concentrations significantly contributed to plasma PK level (Table 3).

Principal component analysis (PCA)

Since institutionalized elderly are generally malnourished, it is quite important to determine whether the low vitamin D - and K -status is independent of overall malnutrition or not. Then PCA was performed with the parameters included for analysis being serum albumin, triglyceride, cholesterol, 25OH-D, PTH levels and plasma PK, MK-7 concentrations. Four components were obtained and explained 82% of the variance. The first component was composite of high albumin, total cholesterol and 25OH-D, and second component consisted of high triglyceride, low

Table 3. Multiple regression analyses for the determination of independent factors for circulating vitamin D, K concentrations

	R^2	*p* value	Variable	β	*p* value
Serum 25OH-D	0.095	0.033	Vitamin D intake	0.309	0.033
Plasma PK	0.181	0.011	Vitamin K intake	0.290	0.042
			Triglyceride	0.380	0.009
Plasma MK-7	0.255	<0.001	Triglyceride	0.505	<0.001

Only significant predictors are shown. The abbreviations are β for β coefficient, and *p* for *p* value. Independent predictor for serum 25OH-D or plasma PK, MK-7 concentrations was analyzed by multivariate analysis with stepwise regression. Age, level of care needed and serum triglyceride and total cholesterol concentrations were included in all analyses. Vitamin D intake was additionally included in the analysis for plasma 25OH-D concentration. For plasma PK and MK-7, vitamin K intake was additionally included.

Table 4. Principal component analysis of nutrition indices

	Component 1	Component 2	Component 3	Component 4
Serum Albumin	0.880	0.004	0.047	0.059
Serum triglyceride	0.229	0.734	0.119	0.380
Serum total cholesterol	0.800	0.320	-0.046	-0.060
Serum 25OH-D	0.434	-0.457	-0.658	-0.033
Serum PTH	0.156	-0.273	0.877	-0.090
Plasma PK	-0.014	0.030	-0.071	0.986
Plasma MK-7	0.117	0.832	-0.238	-0.152

Factor loadings to four components after varimax rotation are shown. Loadings greater than 0.35 are shown in bold
Four components thus obtained were considered to represent the following nutritional status; component 1: overall nutritional status, component 2: vitamin K_2 status, component 3: vitamin D status, and component 4: vitamin K_1 status.

25OH-D, and high MK-7. The third component was composite of low 25OH-D and high PTH, and the fourth component was composed of high triglyceride and high PK. The interpretation of each component was made as follows; the first component representing overall nutritional status, the second component, vitamin K_2 status, the third component, vitamin D status, and the fourth component representing vitamin K_1 status (Table 4).

DISCUSSION

Nutritional status would be adequately assessed by both evaluating the subjects' food intake and measuring their circulating or urinary markers. This principle would hold true especially in the elderly, since they are at high risk for malabsorption or utilization defects of nutrients. Unfortunately in Japan, vitamin D and K status in the elderly has been studied either by evaluating their food intake, as in the annual National Nutrition Survey Japan (NNS-J) or by measuring circulating level of these vitamins,[21,30-33] but rarely by both.[12,34]

Institutionalized elderly have been our special concern, since they are much more susceptible to hypovitaminosis D and K deficiency than the healthy elderly. The NNS-J in 2006 showed that subjects over 70 years of age, including both genders, had the following daily nutrients intakes: energy 1761 kcal, calcium 551 mg, vitamin D 9.0 μg, vitamin K 273 μg,[35] which were higher than those of the subjects in the present study. Gastrointestinal absorption of nutrients in the present study subjects would be impaired also. These considerations led us to simultaneously evaluate both vitamin D and K intakes and its circulating levels in the present study.

Before the interpretation of our data, determination procedure for vitamin K deserves some discussion. There have been discrepancies on the plasma concentration of vitamin K in the previous literature, which is at least partly due to the different determination procedure employed. Recently we have developed a novel procedure for the determination of vitamin K analogs with high sensitivity and specificity, based on high-performance liquid chromatography-tandem mass-mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS).[26] With this procedure, plasma concentrations of PK and MK-7 were 0.73±0.70 ng/mL (median 0.58 ng/mL) and 0.53±0.37 ng/mL (median 0.45 ng/mL), respectively in the current study. In our recent study, plasma concentrations for PK and MK-7 were 1.29±1.09 ng/mL (median 0.94 ng/mL) and 4.21±6.81 ng/mL (median 2.14ng/mL), respectively in the healthy Japanese elderly over 70 years old using the same assay procedure.[21] In the same study, lowest concentration of plasma vitamin K level to avoid the elevation of serum ucOC concentration was 2.5 ng/ml for PK and 6.4 ng/ml for MK-7.[21] Since serum ucOC level is a sensitive indicator of skeletal vitamin K insufficiency, these figures can yield a rough estimate of circulating vitamin K levels needed by the skeleton.

The median intake of vitamin K in the current subjects was 168 μg, which was more than twice the AI in DRI 2005. The AI for vitamin K was not altered in DRI 2010. Dietary vitamin K intake has been identified as an important determinant of plasma phylloquinone concentration in previous studies.[36,37] In the present study, vitamin K intake was also significantly associated with plasma PK, but not with plasma MK-7. Since they were not supplied with fermented soybean; natto, which contains exraordinary amount of MK-7,[38] phylloquinone from green vegetables is likely to be the major contributors to the total vitamin K intake in our subjects. Thus plasma PK alone correlated with total vitamin K intake, adjusted by serum triglyceride. These data strongly suggest that these subjects are vitamin K-deficient in spite of the fact that their dietary intake is far above the AI in according to DRI 2005, and increased vitamin K intake would be effective in improving plasma PK levels in institutionalized elderly in present study.

As in the case of vitamin K, average dietary intake of vitamin D was around 7 μg/day, which is approximately 140% of the AI in subjects in the present study. Nevertheless, the average serum 25OH-D concentration was only 11.1 ng/mL. Thus, most subjects in the present study had hypovitaminosis D in spite of apparently sufficient vitamin D intake.

Although the multiple regression analysis has identified vitamin D intake as the significant contributor to serum 25OH-D concentration, the R^2 value was low, which indicates that the current model could explain only a small portion of variation. Several factors could be responsible for the above results. First, because of walking disability and other physical dysfunction, the chance of sun exposure was minimal in most of the current study subjects, but it was not null. Thus, sun exposure may also partly explain the above results. Unfortunately, however, detailed information about sun exposure was unavailable. Furthermore, ADL itself has been reported to be related to serum 25OH-D levels,[39] on which detailed information is not available in the current study. Secondly, the intestinal absorption of vitamin D is likely to decrease due to factors such as compromised intestinal ability for nutrients absorption and limited fat intake.[40] Nevertheless, oral vitamin D intake seems to be of value in the institutionalized elderly for improving their vitamin D status. Cashman *et al.* reported dose-dependent increase in serum 25OH-D concentration after incremental supplementation with vitamin D_3 in free-living adults over 64 years of age.[41] Although AI for vitamin D slightly increased to 5.5 μg/day in recently issued DRI 2010, the elderly subjects are likely to require much more vitamin D intake to avoid hypovitaminosis D considering the various problems to interfere with absorption and utilization as discussed above. A second issue with regard to the above discussion; disturbed intestinal absorption and limited fat intake, will also apply to the discrepant intake and circulating level of vitamin K.

Although serum 25OH-D level was extremely low, average serum PTH level was within the reference range. Circulating 25OH-D concentrations showed significant negative correlation with serum PTH levels ($r = -0.293$, $p = 0.041$; data not shown), which suggests that the negative feedback regulation of PTH secretion by vitamin D is not impaired in the current population. Kuchuk *et al*. reported that the elevation of serum PTH concentration by vitamin D deficiency is moderate in its magnitude, and usually fell into the reference range.[42] Thus they stressed the importance of serum 25OH-D level, and argued that for bone health maintenance and physical performance in the elderly, serum 25OH-D concentration above 50-60 nmol/L (20-24 ng/mL) was required.

Although the institutionalized elderly are considered to be generally malnourished,[43-45] nutritional status appeared rather satisfactory in the present study subjects in face of hypovitaminosis D and K. Then we analyzed the relationship between the overall nutrition and circulating levels of vitamin D and K by PCA. The PCA have yielded four components representing: overall nutritional status, vitamin D status, vitamin K_2 status, and vitamin K_1 status respectively. Serum 25OH-D also exhibited some association with the first component, representing the overall nutritional status. One of the reasons for the above results would be that 25OH-D is bound to vitamin D-binding protein (DBP) and albumin during its transport in circulation.[46] Since these components are independent of each other by their definition, these results suggest that hypovitaminosis D and K in the institutionalized elderly do not merely reflect general malnutrition, and have their own role. Confounders are serious challenge in the clinical studies. In the intervention studies, randomization would eliminate the interference by the confounders. It would be less problematic in the case of cohort studies. Adjustment for confounders is quite difficult in the cross-sectional studies like the current one. Multivariate analyses such as PCA would be of help in eliminating the interference by confounders in this type of studies.

In conclusion, institutionalized elderly had high prevalence of hypovitaminosis D and K in spite of their dietary intake exceeding the AI in DRI 2005 in Japan, which suggests that the requirement for these vitamins would be higher in these subjects. Additionally, hypovitaminosis D and K were shown to be independent of general malnutrition by PCA, which would be a useful analytical procedure for eliminating the interference by confounders in cross sectional studies.

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AUTHOR DISCLOSURES

None of the authors have any conflicts of interest.

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Original Article

Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly

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居住機構中的老年人有高盛行率的維生素 D 及維生素 K 缺乏症且與整體的營養不良無相關

研究老年人的維生素 D 及維生素 K 缺乏症有許多方法學上的問題。首先，大多研究是藉由評估食物的攝取或是測量血中的濃度來進行的，但在日本很少同時利用這兩種方法。在本篇文章中，維生素 D 及維生素 K 的攝取以及老年人的血中濃度是同步測量的。第二個議題是維生素 D 及維生素 K 缺乏症是否與盛行於老年人的一般營養不良情形相關。我們試著藉由統計的主成份分析方法去分辨。評估 50 位機構中的老年人血中的 25-羥化維生素 D、副甲狀腺素、維生素 K_1、維生素 K_2 濃度，以及食物攝取。雖然平均維生素 D 攝取量(每天 7 克)超過日本所訂定的足夠攝取量(每天 5 克)，但平均血清中 25-羥化維生素 D 濃度(11.1 ng/mL)卻屬維生素 D 缺乏的範圍。維生素 K 攝取量的中位數為每天 168 克，這幾乎是維生素 K 的足夠攝取量的 2.5 倍。但是，血漿中維生素 K_1 及維生素 K_2 濃度是遠低於 70 歲以上健康的日本老人。應用主成份分析法，結果產生 4 個成份，分別代表整體營養狀況、維生素 K_2、維生素 D 及維生素 K_1 的營養狀況。既然每個成份都各自獨立，則這些老人的維生素 D 及維生素 K 缺乏不能用整體營養不良加以解釋。總之，在這些機構中的老年人具有高盛行率的維生素 D 及維生素 K 缺乏；爾後這類研究應該同時測量血中濃度及飲食攝取。主成份分析法，可排除橫斷性研究中其他干擾因子的作用，而得到有效的結果。

關鍵字：維生素 D 缺乏、維生素 K 缺乏、主成份分析、足夠攝取量、機構中的老年人