Original Article

High prevalence of hypovitaminosis D and K in patients with hip fracture

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髖部骨折病患維生素 D 與 K 不足之高盛行率

過去研究顯示髖部骨折與維生素 D 及維生素 K 不足有關，但較少研究將兩者共同納入探討。本研究之對象為 99 位有髖部骨折的病患，檢測其整體營養及體內維生素 D 與維生素 K 的狀態。女性患者血清 25-羥化維生素 D(25OH-D)濃度平均只有約 9 ng/mL，顯示女性患者有嚴重維生素 D 缺乏。男女性患者血清中副甲狀腺素及男性血清 25OH-D 平均濃度與對照組皆沒有顯著差異。然而在男女性髖部骨折患者，其血漿維生素 K_1 及維生素 K_2 濃度都顯著較對照組低。以羅吉斯回歸分析發現，體內白蛋白、維生素 K_1 及 25OH-D 濃度皆為骨折發生風險之顯著獨立預測因子，具呈負相關。最後以主成份分析進行臨床參數統整後，獲得三項代表參數，分別代表整體營養狀態、維生素 D 營養狀態及維生素 K 狀態。總而言之，本研究顯示髖部骨折患者易出現維生素 D 及維生素 K 缺乏，且與整體營養不良無關。

關鍵字：維生素 D 缺乏、維生素 K 缺乏、髖部骨折病患、整體營養不良、主成分分析

Original Article

Bone is more susceptible to vitamin K deficiency than liver in the institutionalized elderly

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In Japan, γ-carboxylation of blood coagulation factors is the basis for determining adequate intake (AI) for vitamin K in Dietary Reference Intakes (DRIs) issued in 2010. Recently, vitamin K is also known to be essential for preventing fracture. In this study, relative susceptibility of liver and bone to vitamin K deficiency was studied. Thirty-seven elderly institutionalized subjects were evaluated for vitamin K status by measuring serum PIVKA (protein induced by vitamin K absence) –II and ucOC (undercarboxylated osteocalcin) levels, as sensitive markers for hepatic and skeletal vitamin K deficiency, respectively. Serum PIVKA-II and ucOC levels, with their cut-off values in the parentheses, were 20.2±8.9 mAUmL (28 mAU/mL) and 4.7±3.0 ng/mL (4.5 ng/mL), respectively. Median vitamin K intake was approximately 200 μg/day, which is more than 3 times higher than the current Japanese AI. Vitamin K intake was significantly correlated with serum PIVKA-II and ucOC/OC levels, but not with serum ucOC level. Although serum ucOC level is generally a good indicator for vitamin K status, multiple regression analysis revealed that elevated bone turnover marker significantly contributed to serum ucOC level. All subjects had vitamin K intake exceeding AI for vitamin K. Nevertheless, serum PIVKA-II and ucOC concentrations exceeded the cut-off value in 14% and 43% of subjects, respectively. The present findings suggest that vitamin K intake greater than the current AI is required for the skeletal health in the institutionalized elderly.

Key Words: vitamin K, adequate intake, γ-carboxylation, ucOC, PIVKA-II

INTRODUCTION

Gamma-glutamyl carboxylase (GGCX) catalyzes the conversion of glutamyl (Glu) residue into γ-carboxyglutamyl (Gla) residue in certain proteins. The most fundamental role of vitamin K is the one as a cofactor of GGCX.[1] Although GGCX is present in various tissues, its role in the liver has received most attention until recently. In the liver, conversion of Glu residue to Gla residue takes place in four of the blood coagulation factors (II, VII, IX, and X), by which they acquire calcium-binding ability and are activated.[1] Recently, attention have been focused on the physiological roles of vitamin K-dependent proteins in extrahepatic tissues such as bone and blood vessel.[2,3] Osteocalcin is produced by osteoblasts, the most abundant non-collagenous protein in the bone matrix. Through γ-carboxylation, osteocalcin gains hydroxyapatite-binding ability, and regulates bone mineralization.[2] Recent evidences strongly suggest that skeletal vitamin K deficiency increases the risk of hip fracture.[4] Matrix Gla protein (MGP); another vitamin K-dependent protein, is an inhibitor of vascular calcification.[5-7]

In the current Japanese Dietary Reference Intakes (DRIs) issued in 2010, Adequate Intake (AI) for vitamin K in the adult is uniformly 75 μg/day for men and 65 μg/day for women. These values however, carries some problems when applied to the study population.[8] First, they are based on data from America or Europe. Since nutrients intake is greatly dependent on nationality or dietary patterns, vitamin K status in the Japanese must be studied. Second, they are from healthy young volunteers, not from the elderly who are likely to have nutrients malabsorption. This is especially the case with fat-soluble vitamins including vitamin K due to various factors such as decreased secretion of bile acids and pancreatic juice, and reduced dietary fat intake.[8] Finally, AI for vitamin K was determined as the dose sufficient to maintain normal blood coagulation with little mentioning to bone.[8] Serum levels of protein induced by vitamin K absence-II (PIVKA-II) and undercarboxylated osteocalcin (ucOC) are sensitive markers for vitamin K deficiency in the liver and bone, respectively. Vitamin K status in the liver and bone

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can be separately evaluated by measuring these markers. By employing such methodology, previous studies have shown that much higher doses of vitamin K are needed for the γ-carboxylation of osteocalcin than for that of blood coagulation factors.[9,10]

Thus it is possible that an elderly judged to be vitamin K sufficient based on the current AI has skeletal vitamin K deficiency and increased fracture risk. In this paper, we have measured serum PIVKA-II and ucOC levels, assessed vitamin K intake, and studied the prevalence of vitamin K deficiency in the liver and bone in the institutionalized elderly.

MATERIALS AND METHODS

Subjects

The study subjects were 37 institutionalized elderly (male 8, female 29) in a nursing home, Kayu-Shirakawa. Exclusion criteria were routine medication that has potential interference with bone metabolism and vitamin K status such as warfarin. None had history of hepatic diseases. 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 PIVKA-II and ucOC levels were measured by electro chemiluminescence immunoassay (ECLIA) (Sanko Junyaku, Co, Ltd, Tokyo, Japan) as the markers of hepatic and skeletal vitamin K deficiency, respectively. Serum intact osteocalcin (intact OC) was measured by enzyme immunoassay (EIA) (Mitsubishi Yuka, Tokyo, Japan). The ucOC/OC was calculated as the ratio of ucOC to intact OC. Serum levels of tartrate-resistant acid phosphatase-5b (TRACP-5b) and bone specific alkaline phosphatase (BAP) were measured by EIA (DS Pharma Biomedical, Osaka, Japan) and chemiluminescence enzyme immunoassay (CLEIA) (Beckman Coulter Inc, Tokyo, Japan), respectively. TRACP-5b and BAP are markers of bone resorption and bone formation, respectively. The reference range of serum TRACP-5b was 170-590 mU/dL in male and 120-420 mU/dL in female, and that of serum BAP was 3.7-20.9 μg/L in male and 3.8-22.6 μg/L in female.

Nutrition intake study

Nutrient intake was assessed by food record method. The intake of vitamin K was calculated by multiplying the amount of vitamin K supplied from the institution with the average percentage intake. Based on these records, their intake of vitamin K was calculated using the software (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan). Vitamin K intake/kg body weight was also calculated, since 1 μg/kg of vitamin K is considered to be sufficient for maintaining normal coagulation in the adult according to the Japanese DRI 2010.[8]

Statistical analyses

Statistical analyses were performed using the SPSS 17.0 J for Windows (SPSS. Japan Inc, Tokyo, Japan). Association between variables was analyzed by Pearson's or Spearman rank correlation coefficient. Multiple regression analyses with stepwise method were performed to determine independent determinants for serum ucOC and ucOC/OC. Chi-square test was employed for categorical data.

RESULTS

Background profiles of the study subjects

The background profiles and biochemical data are shown in Table 1. Care level is a 5-grade score which is commonly used in the long-term care insurance in Japan with higher number indicating more intensive care needed. It was higher than grade 3 in 78% of subjects, indicating that they had low physical activity level. For example, most of the present subjects required wheelchair for transportation. In 27% of subjects, serum albumin level was lower than 3.5 g/dL, which is a generally accepted cut-off for malnutrition. Overall, nutritional parameters including the biochemical indicators and body mass index (BMI) remained within the reference range for most of the subjects. Thus, despite the elderly population and high level of care needed, the subjects' nutritional status was considered to be generally preserved. Although average serum TRACP-5b and BAP levels were within the reference range as a whole, 20% and 32% of subjects had serum BAP and TRACP-5b level above upper reference range, respectively. Serum PIVKA-II and ucOC levels were 20.2±8.9 mAU/mL and 4.7±3.0 ng/mL, respectively. All subjects were on orally consumed their meals. Although energy intakes were lower than estimated energy requirement (EER) of DRI in all men and 93% of women, the intake of macronutrients such as protein, fat and carbohydrates appeared appropriate for their age and sex. Average vitamin K intake was 194±51 (median; 197)

Table 1. Baseline data of the study subjects

	(M/F; 8/29, n=37)
Age (y)	85.1±8.2 (87.0)
Care level	Median; 3 (min-max; 1-5)
Body weight (kg)	45.9±6.1 (46.1)
Height (cm)	149.3±9.7 (145.3)
BMI (kg/m^2)	20.6±2.5 (20.0)
Serum Albumin (g/dL)	3.7±0.3 (3.8)
Serum triglyceride (mg/dL)	119±41 (118)
Serum total cholesterol (mg/dL)	198±49 (191)
eGFR (ml/min./1.73m^2)	65.4±15.8 (63.3)
Serum BAP (μg/L)	18.4±9.6 (17.6)
Serum TRACP-5b (mU/dL)	365.2±124.9 (372.0)
Serum ucOC (ng/mL)	4.7±3.0 (3.8)
Serum total OC (ng/mL)	6.1±3.1 (5.4)
ucOC / intact OC	0.81±0.36 (0.80)
Serum PIVKA-II (mAU/mL)	20.2±8.9 (18.0)
Energy intake (kcal)	1346±129 (1401)
Protein intake (g)	53.2±5.2 (55.4)
Fat intake (g)	35.6±3.6 (36.9)
Carbohydrates intake (g)	193.8±18.7 (199.4)
Vitamin K intake (μg/day)	194±51 (197)
Vitamin K intake/BW (μg/BW kg/day)	3.5±1.1 (3.4)

Data are expressed as mean±SD with the values in parentheses showing the median.

μg/day in the study population, 166±50 (median; 159) μg/day in males and 202±49 (median; 224) μg/day in females. It was approximately 220% and 310% of the AI in DRI in male and female subjects, respectively. All subjects had vitamin K intake exceeding AI. In addition, the vitamin K intake/kg body weight was 3.5±1.1 μg/day in the present study subjects, far exceeding 1μg/kg.

Correlations among vitamin K intake and serum PIVKA-II, OCs

Table 2 shows that vitamin K intake was significantly correlated with serum PIVKA-II and ucOC/OC levels, but not with serum ucOC concentrations. (Table 2)

Correlations among serum OCs and bone turnover markers

Serum TRACP-5b and BAP levels were significantly correlated with serum ucOC concentration, but not with ucOC/OC ratio. (Table 3)

Multiple regression analyses for serum OCs levels

Multiple regression analyses revealed that serum TRACP-5b level was a significant determinant of serum ucOC concentration. Vitamin K intake was a significant predictor for ucOC/OC. (Table 4)

Relative susceptibility of liver and bone to vitamin K deficiency

Serum PIVKA-II level exceeded the cut-off level (28 mAU/mL) in only 14% of the subjects, whereas serum ucOC concentration was above the cut-off value (4.5 ng/mL) in 43% of subjects, which was significantly different by chi-square test (p<0.001). (Table 5)

DISCUSSION

Vitamin status could be evaluated by several ways such as measuring its blood concentration or measuring the markers representing the vitamin status. Recently, we have reported that the prevalence of vitamin D- and K-deficiency is quite high in the institutionalized elderly by measuring plasma levels of 25 hydroxy-vitamin D concentration which is the best indicator of vitamin D status, and plasma vitamin K concentration.[11] Plasma vitamin K concentrations, however, only reflect the vitamin K status as a whole, and do not provide us with information regarding the vitamin K status in various tissues individually. Thus, in this study, we have evaluated the subjects' vitamin K status by measuring their serum levels of PIVKA- II and ucOC rather than their plasma vitamin K levels.

First, we have studied the association between serum levels of PIVKA-II and ucOC, and vitamin K intake. Vitamin K intake was significantly correlated with PIVKA-II and ucOC/OC, but not with ucOC. Similar findings were also reported by Booth *et al* that circulating levels of PIVKA-II and ucOC/OC ratio reflected dietary vitamin K intake, whereas serum ucOC levels did not.[9] Two mechanisms were considered to be responsible for these find-

Table 2. The correlation between vitamin K intake and serum levels of PIVKA-II and ucOC

	ucOC		ucOC/OC		PIVKA-II	
	r	p-value	r	p-value	r	p-value
Vitamin K intake	0.092	0.588	-0.416	0.010	-0.362	0.028

Correlations of vitamin K intake with markers for vitamin K deficiency were analyzed by Spearman rank correlation.

Table 3. The correlation of serum ucOC and uc/OC ration and bone turnover markers

	ucOC		ucOC/OC	
	r	p-value	r	p-value
Serum TRACP-5b	0.425	0.009	0.014	0.935
Serum BAP	0.517	0.001	0.243	0.147

Correlations of serum OCs with bone turnover markers were analyzed by Spearman rank correlation.

Table 4. Multiple regression analyses for serum ucOC level and ucOC/OC ratio

Dependent variable	R^2	Independent variable	β	p-value
ucOC	0.206**	Serum TRACP-5b	0.454	0.005
ucOC/OC	0.134*	Vitamin K	-0.366	0.026

The abbreviations are β for β coefficient. Independent predictor(s) for serum OCs levels were analyzed by multiple regression analyses with stepwise method. Sex, serum TRACP-5b, and vitamin K intake (μg) were included in all analyses.
*; p< 0.05, **; p< 0.01

Table 5. Number of subjects with vitamin K sufficiency and deficiency in the liver and bone

	Vitamin K sufficiency	Vitamin K deficiency
In the bone (serum ucOC concentration)	21 (57%)	16 (43%)
In the liver (serum PIVKA-II concentration)	32 (86%)	5 (14%)

Values represent number of subjects, with percentage of subjects in the parentheses. Vitamin K status in the bone and that in the liver were significantly different by chi-square test (p<0.001).

ings. The first is the different bioavailability of phylloquinone (PK; vitamin K_1) and menaquinones (MKs; vitamin K_2). In the present study, PK was the major form of vitamin K taken as in America or Europe,[12,13] since the subjects had no intake of natto which contains large amount of MK-7 during the study.[14] Recent studies have shown that PK can be utilized for γ-carboxylation in the liver, but can only be utilized in extrahepatic tissues after conversion into MK-4.[15,16]

Second issue is the association of serum ucOC level with bone turnover. Serum levels of BAP and TRACP-5b reflect osteoblastic bone formation and osteoclastic bone resorption, respectively, and are elevated in the high turnover state. Since osteocalcin is produced in osteoblasts,[17] it is conceivable that serum concentration of osteocalcin as well as its subfraction, ucOC level is increased with high turnover. Thus, it is currently under debate whether ucOC alone is satisfactory or measurement of ucOC as well as ucOC/OC is a better indicator of vitamin K status. In the present study, vitamin K intake was a significant predictor for ucOC/OC, but not with ucOC. Therefore, there is a possibility that ucOC/OC is a better index for vitamin K status than serum ucOC concentration. Unfortunately, however, there is no cut-off value published regarding ucOC/OC ratio, while the clinical usefulness of serum ucOC measurement is increasingly acknowledged. Thus, analysis using ucOC/OC could not be done as serum ucOC level in Table 5.

The cut-off value of 4.5 ng/mL for serum ucOC was validated by Shiraki by simultaneously evaluating the subjects' dietary intake of vitamin K, blood levels of vitamin K and ucOC.[18] They also reported that serum ucOC concentration exceeding 5.5 ng/mL was associated with increased risk of fracture. The clinical usefulness of ucOC measurement was previously reported, although with different assay procedure of hydroxy-appatite binding assay. In the European epidemiological study, Vergnaud *et al* reported that subjects in the lowest quartile of femoral neck bone mineral density (BMD) and those in the highest quartile of ucOC had increased hip fracture risk with an odds ratio of 2.4 and 1.9, respectively. These two risk factors were independent of each other, and those with both conditions had a even higher odds ratio of 5.5.[19] Thus, serum ucOC concentration is shown to be a good indicator of skeletal vitamin K deficiency, and a predictor of fracture risk.

In the current study subjects with vitamin K intake far exceeding AI, serum concentration of PIVKA-II and ucOC were within the reference range in 86% and 57% of the subjects respectively, which was significantly different. Thus, their vitamin K intake is sufficient for γ-carboxylation in the liver, but not in the bone, and bone is much more susceptible to vitamin K deficiency than liver. Such difference is likely to arise from the anatomical basis that vitamin K absorbed from the intestine is first transported to liver and preferentially used there, then utilized in extrahepatic organs.[9,10]

Booth *et al* in their depletion-repletion studies, reported that the γ-carboxylation of prothrombin was restored at 200 μg/day of PK, whereas that of osteocalcin was not even at 450 μg/day of PK.[9] Schurgers *et al* also reported that undercarboxylated prothrombin concentration was significantly decreased at supplementary intake of 100 μg/day of PK, whereas ucOC level did not decrease below 300 μg/day of PK.[10] Furthermore, Binkley *et al* reported that supplementation with 1,000 μg/day of vitamin K was optimal for the maximal γ-carboxylation of osteocalcin.[20] These results suggest that at least 300-500 μg g/day of vitamin K intake is required for the sufficient γ-carboxylation in the bone. Our results in the Japanese elderly are compatible with these results from Caucasians, and have additionally provided data on the prevalence of hepatic and skeletal vitamin K deficiency.

We believe that this paper is of importance in considering the AI for vitamin K. The current DRI states that the AI for vitamin K was determined based on its requirement for the γ-carboxylation of blood coagulation factors. The present findings suggest that vitamin K intake greater than the current AI is required for the skeletal health in the institutionalized elderly. Further studies with lager number of subjects and intervention studies are necessary to define the amount of vitamin K necessary for the elderly.

AUTHOR DISCLOSURES

None of the authors have any conflicts of interest.

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Original Article

Bone is more susceptible to vitamin K deficiency than liver in the institutionalized elderly

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居住機構老人骨骼比肝臟易受維生素 K 缺乏影響

日本 2010 年發佈的膳食營養素參考攝取量(DRI)中，維生素 K 的足夠攝取量是根據凝血因子的 γ-羧化作用而訂定的。近來，維生素 K 也被視為預防骨折不可或缺的角色。本研究在於比較肝和骨骼對維生素 K 缺乏的敏感性。評估 37 位居住機構的老人之維生素 K 狀況—測量血清 PIVKA–II (因維生素 K 缺乏所產生的蛋白質)和 ucOC (未羧化的骨鈣素)濃度，兩者分別為肝和骨骼在維生素 K 缺乏時的敏感指標。受試者血清 PIVKA-II 和 ucOC 濃度分別為 20.2±8.9 mAU/mL (臨界值 28 mAU/mL)和 4.7±3.0 ng/mL (臨界值 4.5 ng/mL)。維生素 K 攝取量中位數約為 200 μg/day，超過了日本目前所建議的足夠攝取量 3 倍。維生素 K 攝取量與血清 PIVKA-II 和 ucOC/OC 濃度顯著相關，但與血清 ucOC 濃度無相關。雖然血清 ucOC 濃度是體內維生素 K 狀況很好的指標，但複迴歸分析顯示骨骼轉換標記增加，也會影響血清 ucOC 濃度。所有的受試者維生素 K 攝取量皆超過足夠攝取量。然而，分別有 14%和 43%受試者的血清 PIVKA-II 和 ucOC 濃度超過臨界值。本研究結果建議，對於住在機構的老人，為維持骨骼健康，維生素 K 攝取量應超過目前建議的足夠攝取量。

關鍵字：維生素 K、足夠攝取量、γ-羧化作用、未羧化骨鈣素、PIVKA-II

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Body weight divided by squared knee height as an alternative to body mass index

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ABSTRACT

Weight/height^2 (Quetelet's index) is the basis for defining both underweight and obesity. Height, however, is often not precisely measurable in the elderly due to involutional changes such as spinal deformity. Body volume or body surface area are not proportionately decreased even with height loss. Previous reports have shown that Quetelet's index is overestimated in the elderly with height loss. Then we have made a hypothesis described below.

Maximal height or height at youth would better represent the subjects' nutritional or clinical status. The distinction of these two heights has not been mentioned before. There have been many publications showing the equations to estimate height from the surrogate parameter(s) such as knee height (KH). Most equations published so far are expressed as estimated height = $a + b \times \mathrm{KH} - c \times$ age, where a, b, and c are constants. Negative correction by age is unexceptionally far greater in women than in men. Apparently, previous researchers have estimated current height by their equations.

Maximal height cannot be measurable. It, however, is unaffected by age by its definition. Therefore, maximal height does not have to be corrected by age, and would be almost proportional to KH. Then weight/KH^2 could be a better alternative to the most commonly used weight-height ratio; weight/height^2; the Quetelet's index.

Height is the basis for various clinically important indices such as body surface area (BSA) and energy requirement. Employing current height could lead to the underestimation of BSA or energy requirement in the elderly with height loss. Our hypothesis described here would yield a novel and better indices for the clinical assessment of the elderly.

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Introduction

Body weight is one of the most fundamental indices in the clinical evaluation of the subjects. Longitudinal and serial observation of body weight provides us with valuable information concerning the alteration in the subjects' clinical status. In the cross-sectional settings, however, the usefulness of body weight for the clinical evaluation is seriously limited by the fact that it is influenced by the body size. Therefore, weight must be corrected by some parameter(s) representing the body size. For such purpose, body weight divided by squared height (weight/height^2) is commonly employed and called body mass index (BMI) [1]. High BMI and low BMI are the basis for the diagnosis of obesity and emaciation, respectively.

Why is the weight/height^2 the standard weight-height ratio? Since human body is three-dimensionally structured, body volume may be proportional to height^3. In that case, body weight would be proportional to height^3. Then one could argue for weight/height^3 as another weight-height ratio. Indeed, weight/height^2 is not the only weight-height ratio, and might be more properly called Quetelet's index (hereafter abbreviated as QI) [1,2].

Other weight-height ratios have been reported, such as weight/height ratio (weight/height), Khosla-Lowe index (weight/height^3), Ponderal index (weight/height$^{1/3}$), Benn's idex (weight/height^p) where p is a population-specific exponent [3]. Of these, QI is considered to be the most appropriate weight-height ratio, since it fulfills the following requirements.

Thus, a preferred weight-height ratio must be maximally correlated with body mass and minimally correlated with stature [4]. Hereafter in this paper, weight/height^2 will be designated as QI rather than BMI for clarity. The idea of body weight divided by squared height (weight/height^2) as the weight-height ratio was originally developed by a Belgian mathematician, Adolphe Quetelet in the 19th century [2]. World Health Organization (WHO) defined overweight as well as underweight based on QI [5]. In other words, QI is not the theoretically derived standard weight-height ratio *a priori*, but has become the *de facto* standard because of its clinical usefulness.

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Quetelet's index in the elderly

The curve showing the relationship between QI and mortality are considered to be U-shaped. Thus, both overweight or obesity, and underweight are associated with increased mortality. There have been numerous publications, however, to cast doubt that the above theory holds true in the elderly [4,6–8]. Two examples will be given below.

First, many studies have indicated that the optimal QI associated with the lowest mortality differs in the elderly and in the younger generations. Andres reported that QI with the lowest mortality was 22.9 kg/m^2, 25.8 kg/m^2, and, 26.6 kg/m^2 in men aged 40–49 years, 50–59 years, and 60–69 years, respectively. In women, it was 23.2 kg/m^2, 25.2 kg/m^2, and 27.3 kg/m^2, respectively [4]. Matsuo also reported that QI with the lowest mortality was 22.5 kg/m^2 and 24.8 kg/m^2 in men aged 40–59 years and 60–79 years old, and it was 21.9 kg/m^2 and 23.3 kg/m^2 in women aged 40–59 years and 60–79 years old [6]. Thus, it has consistently been demonstrated that QI with the lowest mortality is higher than in the younger generations.

Second, the association between the underweight and the all-cause mortality is much more debated. Excess mortality associated with underweight has been reported to be lower, higher, or unaffected by aging [6].

Height loss in the elderly

Aging is almost inevitably associated with height loss. Various factors contribute to the involutional height loss, the most important cause of which would be the vertebral compression fracture caused by osteoporosis. Osteoporosis is a condition that renders the patients susceptible to fragility fractures, such as spinal, hip, and wrist fractures [9]. Spinal fracture, which is the most common osteoporosis-related fracture, is a compression one in its nature. Thus it causes spinal deformity and height loss. Since post-menopausal estrogen deficiency is the most important cause of osteoporosis, it is quite conceivable that women are at much greater risk for height loss than men. Prospective study with repeated height measurement has confirmed that women indeed lost more height than men [10]. Sorkin et al. have reported that average cumulative height loss from age 30 to 70 was 3 cm for men and 5 cm for women, and that from age 30 to 80 was 5 cm for men and 8 cm for women. Such involutional height loss poses serious problem upon the validity of QI as the weight-height ratio in the elderly. It is already pointed out that there will be an overestimation of QI in the elderly because of shortened height [10]. As described above, an ideal weight-height ratio should be minimally correlated with stature. QI is generally considered to fulfill this requirement, which, however, may not always hold true in the elderly.

Estimation of height using surrogate measurement

Height cannot be exactly measured in the elderly too often, due to various reasons such as vertebral fracture, disc degeneration and frailty. Then height is estimated from the surrogate parameters such as arm span and knee height (KH) [1]. Of these, KH is the most frequently used, since it could be easily measured even in the elderly and minimally affected by the involutional changes. Many equations have been so far published to predict height from KH, the most well known of which is Chumlea's one; height = 64.19 + 2.02 × KH − 0.04 × age for men, and 84.88 + 1.83 × KH − 0.24 × age for women [11]. In most equations hitherto published, negative height correction by age is much greater in females than in males, the reason of which, however, has not been described in the previous publications.

Hypotheses

Height generally means the distance between the top and bottom of the body. Height loss means the shortening of this distance. We believe that two types of height should be distinguished in the clinical evaluation of the elderly; height A and height B in Fig. 1. Height A is the current height. Height B would be quite close to the height at youth or maximal height. A woman in Fig. 1 has spinal deformity and height loss, but her body volume or body surface area is not proportionally diminished. Then, it is obvious that calculating QI using the current height (height A in Fig. 1) would lead to the significant overestimation.

Although previous authors on establishing the equation to predict height from KH do not seem to have considered the distinction of these two heights, but much greater negative correction by age in women strongly indicate that they meant current height in their equations.

Then we have come to an idea that employing maximal height would yield a better estimate of weight–height ratio in the elderly. Since maximal height cannot be measured in the elderly, it must be estimated. Unlike the current height, maximal height is independent of age by its definition. Therefore, the correction by age would be unnecessary for the estimation of maximal height. KH is little affected by age and would be almost proportional to maximal height.

Then we have made a hypothesis that weight divided by squared KH (BMI–KH) could be a good alternative to usual QI in the clinical evaluation of the elderly. Malnutrition in the elderly is a major health problem, and the significance of nutritional assessment has been stressed [12,13]. Despite many parameters currently available, such as anthropometric and laboratory ones, there is no consensus on what parameter would best predict the nutritional status of the elderly. We believe that BMI–KH could be a promising alternative to usual BMI; Quetelet's index in the elderly.

Clinical implications of BMI–KH

We believe that taking the distinction of two heights could yield a solution to the above-mentioned apparent paradox regarding the QI in the elderly.

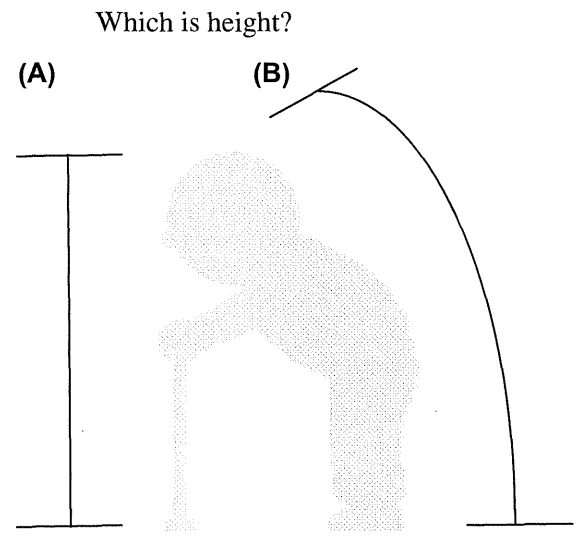

Fig. 1. Two heights are shown. Height (A) is the distance between the ground and the top of the body, and the current height. Height (B) corresponds to the maximal height or height at youth.

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As the possible causes of inconsistency in the association between obesity and underweight and mortality in the elderly, Nagai et al. have mentioned various possibilities, such as history of cancer and cardiovascular diseases, inadequate adjustment for several confounders including smoking, alcohol consumption, physical activity, and socioeconomic status [8]. Another factor of importance not mentioned by them is the validity of height measurement. Given the QI calculated with current height, thus overestimated, the relationship between QI and mortality would be obscured. Although higher QI has been reported to be associated with the lowest mortality in the elderly [4,6], "true" QI may not be high.

In a large-scale and long-term cohort study in Japan, multivariate-adjusted relative risk (RR) for all-cause mortality was greatly dependent on the age of the study subjects in women [6]. In those 40–59 years old, RR in the group with QI exceeding 30 kg/m^2 was 2.23 (95% confidence interval; CI 1.46–3.42) compared to that with QI 21.0–22.9 kg/m^2, whereas it was only 1.39 (95% CI 1.14–1.69) 60–79 years old. In contrast, RR in men was not different between age groups. Although the reason for this gender difference is not discussed by the authors, we believe that our hypothesis could yield a possible explanation. Since women are much more likely to lose their height, their QI is quite prone to be overestimated. Thus it is possible that the subjects with QI higher than 30 kg/m^2 in the above-mentioned study is actually not obese, thus was not associated with higher mortality.

Other clinical implications

Height is an essential anthropometric parameter in the patients' evaluation. In addition to BMI, It is also a prerequisite for the calculation of such important indices as body surface area (BSA), and resting energy expenditure (REE) [14,15]. BSA is considered to be superior to body weight as an index of metabolically active mass, since it is less affected by adiposity. BSA could be calculated by various equations such the one by Dubois and Dubois; BSA (m^2) = 0.007184 × weight (kg)$^{0.425}$ × height (cm)$^{0.725}$ [14]. BSA has various clinical applications with some examples given below. Glomerular filtration rate (GFR) is corrected by BSA [16]. Furthermore, dosage of some therapeutic drugs is determined in terms of BSA [17].

Furthermore, height is needed for determining the patients' energy expenditure. REE is usually calculated using various equations, the well-known of which is the Harris-Benedict equation; BMR = 66 + 13.7 × weight (kg) + 5 × height (cm)−6.76 × age (years) for men and BMR = 655 + 9.6 × weight (kg) + 1.8 × height (cm)−4.7 × age (years) for women [15]. Patients' total energy expenditure (TEE) is then calculated as TEE = REE × activity factor × stress factor. Thus height is necessary in deciding how much energy the patients' need, and essential in the medical nutritional therapy. Then, estimating height using the above-mentioned equations can be problematic. For example, if a dietitian estimates height from KH using Chumlea's equation, calculate REE with Harris-Benedict equation using the height estimated from KH, and determine the TEE, i.e. the energy intake necessary for the elderly subjects, it could be a substantial underestimate. Then the elderly subjects may experience malnutrition due to insufficient energy supplied. Epidemiological studies, favorably the cohort ones, with mortality and morbidity as the clinical outcomes, are to be performed for the comparison of QI and BMI–KH as the clinical useful weight-height ratio in the elderly.

Although detailed consideration on the possible roles of KH in estimating BSA or BEE is beyond the scope of this manuscript, we believe that there is a possibility that these parameters are better predicted by maximal height or KH than the current height.

Conflict of interest

None of the authors have any conflict of interest.

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Clinical Study

Fat Restriction Is Associated with Impaired Quality of Life in Patients with Ulcerative Colitis and Crohn's Disease

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Inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease, is reported to be associated with impaired health-related quality of life (QOL). Although decreased QOL in these subjects has been reported to be associated with various factors, the effect of nutritional therapy, especially nutrients intake on QOL has received less attention. In this study, we evaluated the various factors including nutrients intake on QOL using SF-8 in 64 patients with IBD. Patients with IBD seem to have decreased QOL especially in the mental aspects. The percentage energy intake from fat of total energy fat intake (% energy) of the whole subjects, was lower than those of the annual National Nutrition Survey in Japan. Multiple regression analyses revealed that fat intake (% energy) was a significant predictor for mental component summary. In conclusion, fat restriction contributes to impaired QOL especially in the mental aspects in IBD patients.

1. Introduction

Inflammatory bowel disease (IBD); ulcerative colitis (UC) and Crohn's disease, is reported to be associated with impaired health-related quality of life (HR-QOL). In this paper, HR-QOL will be simply designated as QOL. Decreased QOL in these subjects has been reported to be related to various factors such as age, gender [1, 2], treatment effects [3], disease activity, and social environment [4]. However, the effect of nutritional therapy on the QOL of IBD patients has received less attention, most of which is devoted to the parenteral nutrition therapy, not the nutritional therapy in general [5, 6].

Since excessive fat intake is considered to worsen the inflammation in the intestine, its restriction has traditionally been employed in Japan as the oral nutritional therapy for IBD patients, especially for those with CD, which, however, has its own pros and cons.

Recently, we have studied the possible involvement of hypovitaminosis D and K in the development of osteoporosis in IBD patients [7]. In face of apparently sufficient intake of these vitamins, their plasma levels were quite low in these patients. Paradoxically, plasma concentrations of vitamin D and K were correlated with the fat intake but not with their intake of these vitamins. These results were more prominent in patients with CD than those with UC. Then it was concluded that fat-soluble substances such as vitamin D and K were not effectively absorbed from the intestine without concomitant intake of enough fat.

Through this paper, we were interested in what fat restriction means from the patients' perspectives and studied

the effect of fat restriction on the QOL of IBD subjects in this paper.

2. Subjects and Methods

2.1. Subjects. Study subjects were 64 patients with IBD attending the gastroenterology clinic at the Kyoto University Hospital; 33 with CD (19 men/14 women) and 31 with UC (20 men/11 women). Detailed information was given and written consent was obtained. The study protocol was approved by the ethical committee of the Kyoto Women's University. Almost all patients (27/33 in CD and 28/33 in UC) were receiving 5-aminosalicylic acid. Glucocorticoid therapy was given to four and two patients with CD and UC, respectively. Immunosuppressive drug therapy was performed in 25 and 4 patients with CD and UC, respectively. Eight patients with CD, but none with UC, were on combined therapy of infliximab, synthetic glucocorticoid, and immunosuppressive drug. Fifteen patients with CD and one with UC were on enteral or total parenteral nutrition therapy, respectively.

2.2. Methods

2.2.1. Dietary Information. Dietary information was obtained from food intake records in 2 weekdays by the patients. By calculating these records, their energy and nutrients intakes were obtained by computer software program (Healthy Maker Pro 501, Mushroom soft Corp.).

2.2.2. QOL Measurement. QOL was assessed using the Japanese Short Form Health Survey (SF-8), a widely used generic questionnaire [8]. Eight subscales are obtained; physical function (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social function (SF), role emotional (RE), and mental health (MH). RP and RE refer to the limitations due to physical or emotional reasons, respectively. They are also summarized into two summary scores: physical component summary (PCS) and mental component summary (MCS). Data are transformed to deviation scores based on Japanese norms [8]. Higher scores indicate better QOL, with 50 corresponding to the national norms.

2.2.3. Statistical Analyses. Statistical analyses were performed using SPSS 17.0J for Windows (SPSS. Japan Inc., Tokyo, Japan). Comparison of data from IBD patients with Japanese norms was done by one-sample t test. The difference between two independent groups was analyzed by unpaired t test or Mann-Whitney test depending on normality. Correlations between two independent variables were analyzed by Pearson's or Spearman's correlations. Multiple regression analysis was performed to determine independent factors for QOL scores in IBD patients.

3. Result

3.1. Background Profiles and Biochemical Indices. The baseline characteristics of the patients are shown in Table 1.

Table 1: Background profiles and results from blood tests in patients with CD and UC.

	CD	UC	P value
Age (y)	35.6 ± 7.3	41.7 ± 17.3	.343[a]
Sex (F/M)	19/14	20/11	—
Disease duration (y)	13.7 ± 7.4	6.8 ± 4.8	<.001[b]
Body mass index (kg/m^2)	19.5 ± 2.3	21.1 ± 3.3	.025[b]
Disease location (involving small bowel/not involving small bowel)	30/2	0/31	—
Glucocorticoid therpy	4	2	—
Immunosuppressive therapy	25	4	—
Immunopotentiating therapy (TNF-α)	8	0	—
Enteral or total parenteral nutrition therapy	15	1	—
C-reactive protein (g/dl)	0.6 ± 1.0	0.3 ± 0.6	.135[b]
Albumin (g/dl)	3.9 ± 0.4	4.3 ± 0.3	<.001[b]
Total cholesterol (mg/dl)	126.9 ± 25.0	177.1 ± 40.3	<.001[b]

Values represent mean ± SD. Comparison of indices between patients with CD and those with UC was done by unpaired t test[a] or Mann-Whitney test[b] depending on normality.

CD patients had significantly longer disease duration and lower BMI than UC patients. While nutritional indices such as serum albumin and total cholesterol were lower in CD subjects, there was no significant difference in C-reactive protein which is an inflammatory parameter between these groups. Most of patients were in remission.

3.2. Energy and Nutrients Intake in CD and UC Patients. Food intake could be evaluated in 62 patients (31 with CD and 31 with UC). Energy and nutrients intake in these patients is shown in Table 2. Fourteen patients with CD were on enteral nutrition, and each one of subjects with CD and UC was on total parental nutrition. Although the energy intake was not significantly different between the two groups, fat intake was significantly lower in CD patients than UC subjects. The annual National Nutrition Survey in Japan (NNS-J) in 2008 showed that in subjects of 30–39 or 40–49, years of age including both genders [9], the daily fat intake (% energy) was 26.5% or 25.6%, respectively. These were significantly higher than those of IBD subjects in this study (P = .001; data not shown). Subjects with enteral or parental nutrition had fat intake only approximately half of that in subjects with oral intake (data not shown). The percentage energy intake from protein, fat, and carbohydrates was significantly different between CD and UC subjects.

Table 2: Comparison of nutrient intakes in CD and UC patients.

		IBD ($n = 62$)	CD ($n = 31$)	UC ($n = 31$)	P value
Energy	Intake (kcal)	1816 ± 465 (1804)	1847 ± 392 (1842)	1785 ± 533 (1764)	NS
Protein	Intake (g)	66.0 ± 21.8 (63.5)	71.0 ± 20.6 (67.2)	60.9 ± 22.0 (61.6)	NS
Fat	Intake (g)	44.7 ± 21.6 (43.0)	38.7 ± 17.6 (37.4)	50.6 ± 23.6 (48.1)	$P < .05$
Carbohydrates	Intake (g)	275.4 ± 91.6 (268.6)	298.3 ± 93.1 (275.7)	252.4 ± 85.4 (254.9)	$P < .05$
Protein (% energy)		14.4 ± 2.7 (14.2)	15.0 ± 2.2 (15.6)	13.5 ± 2.9 (13.6)	$P < .001$
Fat (% energy)		22.4 ± 9.6 (24.6)	19.5 ± 8.9 (18.9)	25.2 ± 9.5 (26.8)	$P < .001$
Carbohydrates (% energy)		63.2 ± 9.6 (62.4)	65.2 ± 8.6 (64.0)	56.5±9.5 (60.5)	$P < .001$

Data are expressed as mean ± SD with the values in parentheses showing the median. Comparison of indices between patients with CD and those with UC was done by unpaired t test

Table 3: Dimensional SF-8 scores in patients with CD and UC.

	IBD ($n = 64$)	CD ($n = 33$)	UC ($n = 31$)
PF	50.1 ± 4.7 (53.6)	50.1 ± 4.5 (53.6)	50.0 ± 5.0 (53.6)
RP	*48.2 ± 6.8 (48.5)	48.7 ± 5.3 (48.5)	47.7 ± 8.1 (48.5)
BP	50.8 ± 7.6 (51.8)	50.5 ± 6.8 (51.8)	51.2 ± 8.5 (51.8)
GH	*47.8 ± 7.5 (50.7)	*47.7 ± 6.5 (50.7)	47.8 ± 8.5 (50.7)
VT	49.6 ± 6.5 (54.5)	48.4 ± 5.7 (45.3)	51.0 ± 7.1 (54.5)
SF	**46.2 ± 8.3 (45.2)	*46.9 ± 7.2 (45.2)	*45.5 ± 9.4 (45.2)
RE	*48.3 ± 6.4 (49.1)	48.0 ± 6.5 (49.1)	48.6 ± 6.5 (49.1)
MH	**47.3 ± 6.5 (45.0)	*46.8 ± 7.5 (45.0)	*47.8 ± 5.4 (50.3)
PCS	49.0 ± 6.7 (49.1)	49.2 ± 5.4 (49.0)	48.9 ± 7.9 (50.0)
MCS	***46.1 ± 6.6 (46.5)	**45.7 ± 7.1 (46.6)	**46.6 ± 6.0 (46.5)

Data are expressed as mean ±SD with median in the parentheses. One-sample t test was used for comparison between Japanese norms and scores of CD or UC patients. The asterisk denotes the significant difference (*$P >$ .05; **$P >$ .01; ***$P >$ .001).

3.3. QOL Assessment. In Table 3 is shown the eight subscales and two summary scores of SF-8 in subjects with IBD patients. Since data are expressed as the deviation values normalized by the Japanese normative values, the value "50" correponds to Japanese norm. Subscales such as RP, GH, SF, MH, and MCS were significantly lower than the Japanese norms.

Table 3 shows the comparison between CD and UC subjects. There were no significant differences in the eight subscales and two summary scores except for lower VT in CD patients than in those with UC.

3.4. Correlations between PCS/MCS Scores and Clinical Characteristics, Biochemical Markers, and Nutritients Intakes. We analyzed the correlation between these summary scores and biochemical indices, fat intake expressed as the percentage energy intake from fat of total energy, fat intake (% energy) (Table 4). Fat intake (% energy) was significantly correlated with MCS in CD patients. There was significant but weak, correlation between PCS and serum albumin and MCS and BMI in UC patients. In the whole subjects, BMI was significantly correlated with PCS, and fat intake (% energy) was associated with MCS.

3.5. Multiple Regression Analysis for Variable Associated with PCS/MCS Scores. Then multiple regression analyses were done to study the determinant(s) of the subjects' PCS and MCS (Table 5). Variables included in the analysis were types of disease (CD/UC), BMI, serum concentrations of Alb, and fat intake (% energy). BMI was the significant predictor of PCS score (βcoefficient 0.29, $P = .023$) whereas fat intake was the only significant determinant of MCS score (βcoefficient 0.29, $P = .027$).

4. Discussion

Recently, various questionnaires have been developed for QOL evaluation, both generic and disease targeted [10]. Generic ones, by their definition, only consist of questions related to the subjects' general status and do not include the questions related to the features which are specific to a certain disease. Therefore, they are applicable to such studies as comparing the impact on QOL by various diseases or even to the evaluation of healthy subjects. In contrast, disease-targeted ones include items specific to a certain disease. They can be more sensitive than the generic ones in detecting the QOL impairment closely related to a certain disease state but are not applicable to the evaluation of patients with other diseases. Various disease-targeted questionnaires have been developed for IBD subjects; the most well known of which would be IBDQ (inflammatory bowel disease questionnaire) including many items related to the patients' gastroenterological problems [11]. Since the purpose of our current work was to study the effects of nutritional therapy on the patients' QOL, we considered it more appropriate to evaluate the patients' QOL using the generic questionnaire.

SF-36 is one of the most commonly used generic questionnaires, and SF-8, used in this study, is the shortened one. Eight subscales, two summary scores are obtained, and expressed as the deviation values, which are normalized by the nations' normative value. Many previous papers on the QOL of IBD patients using SF-36 seem to have handled the data improperly [2, 4]. For example, Bernklev and Andersson expressed their data as the 0–100 scale scores [2, 4], which

Table 4: Correlations between PCS/MCS scale scores and clinical characteristics, biochemical markers, and fat intake as proportion of total energy intake.

		IBD ($n = 64$)		CD ($n = 33$)		UC ($n = 31$)	
		PCS	MCS	PCS	MCS	PCS	MCS
Diseaae duration (y)	r	0.012	−0.175	0.070	−0.221	−0.085	−0.066
Body mass index (kg/m^2)	r	0.261*	0.088	0.144	−0.075	0.248	0.415*
C-reactive protein (g/dl)	r	−0.083	0.075	−0.058	0.196	−0.116	−0.045
Albumin (g/dl)	r	0.235	0.082	0.092	0.064	0.424*	0.059
Total cholesterol (mg/dl)	r	0.033	0.196	−0.132	0.169	0.174	0.249
Fat intake(% energy)	r	0.175	0.287*	0.146	0.458***	0.238	0.109

The asterisk denotes the value is significant correlation (*$P < .05$, **$P < .01$, ***$P < .001$) by Pearson's correlation or Spearman's correlation.

Table 5: Multiple regression analyses for the predictor(s) of PCS and MCS scores in IBD patients.

	PCS score		MCS score	
	$r^2 = 0.086$	$P = .023$	$r^2 = 0.081$	$P = .027$
	β	P	β	P
CD/UC (1;CD, 2;UC)	−0.141	.283	−0.059	.657
BMI	0.293	.023	0.069	.594
Alb	0.141	.309	0.024	.855
Fat intake (% total energy)	0.121	.347	0.285	.027

Abbreviations are as follow: β for β coefficient and P for P value. Determinants of independent predictors for PCS/MCS scores were analyzed by multivariate analysis with stepwise method. Variables included were CD/UC, BMI, serum albumin concentration, and fat intake (% total energy)

can be misleading [12]. In the present paper, data were analyzed according to the authorized instruction.

In this study, subscales such as RP, GH, SF, RE, MH, and MCS were significantly lower than the Japanese norms. Decreased RP in face of normal PF is conceivable considering that the patients do not have severe physical impairment but have some limitation in their daily activities by reasons such as the bowel habit problem. Impaired SF would be also conceivable from the similar viewpoint. As a whole, patients with IBD seem to have decreased QOL especially in the mental aspects.

Then, we have analyzed variables associated with PCS and MCS. There were substantial differences in the objective clinical features of patients with CD and UC. For example, CD patients had longer disease duration and lower nutritional status than those of UC subjects. Nevertheless, there were no significant differences in 7 out of 8 dimensions between the two conditions. Namely, QOL which represents the patients' subjective evaluation of their health states seems to be impaired in both CD and UC patients.

Then, we have studied the determinants for PCS and MCS. PCS score was correlated with indices representing their nutritional status such as BMI ($r = 0.261$, $P < .05$) and albumin with marginal significance ($r = 0.235$, $P = .066$). In contrast, none of these factors were significantly correlated with MCS. Thus, it was considered unlikely that disease activities or other clinical features alone could account for the impaired mental aspects of QOL in these subjects. The association of QOL with mental aspects of the subjects has been previously reported. Boye et al. reported that neuroticism was a significant predictor for mental and vitality subscales of SF-36 in IBD patients using multiple regression analyses controlled for gender, age, and clinical disease activity [13]. Martin also reported that QOL was not closely correlated with the clinical features in CD patients [14]. These results, together with our current findings, suggest that mental aspects can more strongly affect QOL than clinical ones in IBD patients.

Theoretically, it is well known that the QOL scores in subjects with disabilities are higher than those anticipated from their objective physical impairment (disability paradox) [15]. This phenomenon is because subjects with long-term disabilities change their internal standard and make the adaptation to their actual status (response shift) [16].

Next, we have made a hypothesis that nutrients intake such as fat restriction may contribute to the impairment of mental aspects of QOL in these subjects. Although CD patients had lower fat intake than UC subjects, fat intake (% energy) of the whole subjects was significantly lower than those of the NNS-J.

Then, we have analyzed the association between these summary scores and their fat intake (% energy). Fat intake (% energy) was significantly associated with MCS, but not with PCS in patients with IBD. When CD and UC patients were separately analyzed, the correlation coefficients was almost the same, but not statistically significant anymore, probably due to the smaller number of study subjects. We then have performed the multivariate analysis. Of the various factors included for analysis types of disease (CD/UC), BMI, serum albumin, fat intake (% energy), BMI, and fat intake (% energy) were the only significant determinants of PCS and MCS, respectively. Since many IBD patients are young, they are quite likely to favor foods rich in fat. Nevertheless, fat

restriction is the common practice in the nutritional therapy for IBD. It is quite conceivable that fat restriction impairs the mental and social aspects of QOL, and enteral nutrition will make the matter even worse. Of interest, but not apparently compatible with our findings, is the report by Kuriyama et al. They reported that enteral nutrition improved the health-related quality of life of Crohn's disease patients with long-term disease duration, and enteral nutrition was an independent factor for bowel symptoms and systemic symptoms [17]. In their study, IBDQ was employed for the assessment of QOL, which is an IBD-targeted questionnaire with many items related to the patients' gastroenterological problems. Thus it is likely that only the physical aspects of QOL were detected, and mental aspects were overlooked in their study.

Two additional considerations might be added to the current finding: decreased QOL in IBD patients and its association with fat restriction. First, considering the response shift, actual detrimental effect of fat restriction on the mental aspects of QOL might be even greater. Second, the adaptation process seems to be only partial. Chronic pain is known to be associated with response shift [18]. However, the association of fat restriction with impaired mental aspects of QOL was obvious in the current study. Since food intake is one of the most fundamental requirements, it is likely that subjects with fat restriction cannot easily adapt to a situation with long-term fat-restricted diet.

In conclusion, fat restriction exerts undesirable effects on IBD patients in two different ways: decreased intestinal absorption of fat-soluble substances such as vitamin D and K and impaired QOL especially in the mental aspects.

Conflict of interests

None of the authors have any conflict of interests.

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Relationship of homocysteine and homocysteine-related vitamins to bone mineral density in Japanese patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: To estimate nutritional risk factors for osteoporosis in patients with type 2 diabetes, bone mineral density, homocysteine level, and intakes and levels of Hcy-related vitamins including folate, vitamin B_6 and vitamin B_{12} were analyzed in a cross-sectional study.

Materials and Methods: Lumbar spine and femoral neck bone mineral density, serum concentrations of vitamin B_6, vitamin B_{12}, and folate and plasma homocysteine levels were measured in 125 Japanese patients with type 2 diabetes. Nutrient intake values were evaluated using a food frequency questionnaire.

Results: Homocysteine was inversely correlated with bone mineral density, and with both dietary intake and serum concentration of folate. Intake of green vegetables was correlated with intake and level of folate and homocysteine levels. When the population was analyzed across the quartiles, bone mineral density, serum folate concentration, folate intake and intake of green vegetables were lowest in the highest homocysteine group.

Conclusions: In patients with type 2 diabetes, the nutritional status of folate might affect the homocysteine level, a putative risk factor for osteoporosis. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00088.x, 2011)

KEY WORDS: Osteoporosis, Homocysteine, Folate

INTRODUCTION

Diabetes is becoming increasingly recognized as a risk factor for osteoporotic fracture. Although fracture risk in patients with type 2 diabetes is increased compared with normal subjects, not only in those with low bone mineral density (BMD) but also in those with normal or high BMD[1–3], decreased BMD is a major determinant of fragility fracture.

Patients with type 2 diabetes often follow a calorie-restricted diet, but few studies have investigated the sufficiency of these nutrients for the maintenance of skeletal health. Generally, nutrient intake increases along with energy intake. *Ad libitum* food intake values obtained from a longitudinal study in institutionalized elderly found that intake values of vitamins increased along with increased energy intake[4]. In contrast, implementation of a low-fat, low-energy diet (1000 or 1500 kcal/day) in patients with overweight and hyperlipidemia has been shown to result in a decrease of the intake of certain nutrients, including B-vitamins[5].

Folate, vitamin B_6 and vitamin B_{12} are important enzymatic cofactors in the synthesis of methionine from homocysteine (Hcy), and an elevation of Hcy can be caused by insufficiency of folate, vitamin B_6 or vitamin B_{12}. Numerous studies have linked high circulating Hcy levels and low concentrations of folate or vitamin B_{12} with increased risk of low BMD in non-diabetic subjects[6–14]. The possibility that elevated Hcy is a risk factor for osteoporosis is suggested by studies of patients with homocystinuria, a rare autosomal recessive disease characterized by markedly elevated levels of plasma Hcy, in which early onset of generalized osteoporosis has occurred[15,16]. The underlying pathophysiological mechanism of osteoporosis in patients with elevated Hcy is not completely understood. Hcy has been reported to interfere with cross-links of newly formed collagen[17,18], and consequently with bone mineralization and strength[19], as well as to stimulate osteoclast formation and activity[20,21]. However, there has been no report on the association of Hcy and Hcy-related vitamins with osteoporosis in patients with diabetes. Furthermore, vitamin insufficiency was evaluated only by serum vitamin concentrations in most of these studies, and there has been no comprehensive investigation of the relationship of dietary intake of nutrients and

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serum vitamin concentrations with Hcy and BMD among subjects in the same study.

In the present study, to evaluate nutritional risk factors for osteoporosis in patients with type 2 diabetes, BMD, Hcy level, and intakes and levels of Hcy-related vitamins including folate, vitamin B_6 and vitamin B_{12} were analyzed.

MATERIALS AND METHODS

Study Population

A total of 125 Japanese patients with type 2 diabetes admitted between December 2008 and June 2009 to Kyoto University Hospital were sequentially enrolled in the study. Lateral lumbar X-ray was carried out to exclude those with scoliosis, compression fractures and ectopic calcifications. Subjects with bilateral hip fractures or prosthesis and other diseases that might influence bone metabolism including liver disease, renal dysfunction (serum creatinine above 2 mg/dL), hyperthyroidism, hyperparathyroidism, hypercorticoidism, and hypogonadism were excluded. All subjects were free of drugs that influence bone and calcium metabolism including glucocorticoids, bisphosphonates, calcitonin injection, estrogens, selective estrogen receptor modulators, vitamin D, vitamin K, thiazide diuretics, heparin and anticonvulsants. The number of patients treated with thiazolidinedione and metformin was 7 and 28, respectively. The present study was cross-sectional in design, and was approved by The Ethical Committee of Kyoto University Hospital and complies with the Helsinki Declaration. Written informed consent was obtained from all participants.

Measurement of Bone Mineral Density

BMD was measured by dual-energy X-ray absorptiometry (DXA; Discovery; Hologic, Waltham, MA, USA) at the lumbar spine (L1-L4) and femoral neck. The coefficient of variation of the measurements of BMD was 0.39%. BMD (g/cm^2) was expressed as *Z*-score calculated on the basis of the normal reference values of the age- and sex-matched Japanese group provided by the DXA system manufacturer. Because male and female patients of different ages were included in the study, comparison of BMD was made based on *Z*-scores. Fat mass and lean body mass (without bone mineral content) were measured by DXA (Hologic Discovery; Hologic) using whole-body absorptiometry software, and each value was expressed in kilograms.

Biochemical Measurements

Blood samples were obtained after overnight fasting immediately after admission. Glycosylated hemoglobin (HbA_{1c}) was measured by high performance liquid chromatography (HPLC). The value for HbA_{1c} (%) is estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA_{1c} (%) = HbA_{1c} [Japan Diabetes Society (JDS); %] +0.4%, considering the relational expression of HbA_{1c} (JDS; %) measured by the previous Japanese standard substance and measurement methods and HbA_{1c} (NGSP)[22]. Fasting serum C-peptide was measured by ELISA (ST AIA-PACK C-Peptide; Toso Corporation, Tokyo, Japan). Bone-specific alkaline phosphatase (BAP) was measured by enzyme immunoassay (Osteolinks BAP; DS Pharma Biomedical, Suita, Japan), and urine N-terminal cross-linked telopeptide of type-I collagen (uNTx) was measured by ELISA (Osteomark NTx ELISA Urine; Inverness Medical, Waltham, MA, USA). Plasma Hcy levels were determined by HPLC using a thiol-specific fluorogenic reagent, ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfate[23], and the upper limit of Hcy was 13.5 nmol/L. As pyridoxal 5′-phosphate (PLP) is the predominant circulating form of vitamin B_6, serum PLP concentrations were measured by HPLC[24,25] for evaluation of vitamin B_6 status. For vitamin B_{12} measurement, 0.2 mmol/L acetate buffer (pH 4.8) was added to the serum samples, and the vitamin B_{12} was converted to cyanocobalamin by boiling with 0.0006% potassium cyanide at acidic pH. Cyanocobalamin was determined by the microbioassay method using *Lactobacillus leichmanii*, ATCC 7830[24,25]. Serum folate was determined by the microbioassay method using *Lactobacillus casei* ATCC 2733[24,25].

Evaluation of Dietary Nutrient Intake

A food frequency questionnaire (FFQ) validated by Takahashi *et al.*[26,27] was used to calculate nutrient intakes. The FFQ used in the present study included questions on the consumption of various food items over the previous 1 or 2 months. Daily nutrient intake was calculated by multiplying the frequency of consumption of each food by the nutrient content of the portion size and summing the products for all food items. The FFQ is validated against 7-day dietary records and the FFQ-estimated nutrient intake values are 72–121% of those of 7-day dietary records[26]. The reproducibility of the FFQ at intervals of 1–2 months is 93–119% for each nutrient[26]. Correlations of dietary folate intake, serum folate concentration, and plasma Hcy level with intakes of various food groups including grain/rice, potato, green vegetables, other vegetables, fruits, seaweeds, beans/soy products, seafood, meats, egg, milk products and oil/fat were evaluated.

Statistical Analysis

Data were expressed as mean ± SD. SPSS statistical software (version 13.0; SPSS, Chicago, IL, USA) was used for all statistical analyses. Pearson's correlation coefficient was calculated as a measure of association by adjusting for age and sex where appropriate. Stepwise multiple linear regression analyses were carried out to determine independent factors for plasma Hcy levels including (i) dietary vitamin B_6, vitamin B_{12} and folate intake values; and (ii) serum PLP, vitamin B_{12} and folate concentrations as independent variables. The relationship between BMD with Hcy and Hcy-related vitamins was further explored using a quartile-based analysis. Statistical differences among the groups were evaluated using analysis of covariance (ANCOVA) adjusted for age and sex, and Dunnett's multiple comparison tests by comparison with the highest Hcy group. $P < 0.05$ was considered significant.

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RESULTS

Clinical characteristics, laboratory data and nutrient intake of subjects are shown in Table 1. The average serum vitamin B_{12} concentration was 1.45 ± 0.45 pmol/mL (Table 1) and there was no difference between patients taking metformin (1.52 ± 0.49 pmol/mL, n = 97) and those without (1.43 ± 0.49 pmol/mL, n = 28). Nutrient intake values were significantly positively correlated with total energy intake (Table 2). Dietary vitamin B_6, vitamin B_{12} and folate intake values were positively correlated with serum vitamin B_6, vitamin B_{12} and folate levels, respectively (Table 2). Plasma Hcy levels were negatively correlated with both dietary intake and serum concentration of folate (Table 2). Only vitamin B_6 intake and not vitamin B_6 concentration showed a weak negative correlation with Hcy; the influence of vitamin B_{12} on Hcy elevation was unclear (Table 2). Stepwise multiple linear regression analyses were carried out to determine independent factors for plasma Hcy levels. Dietary folate intake was a significant predictor of Hcy when dietary vitamin B_6, vitamin B_{12} and folate intake values were included as independent variables (R^2 = 0.088, β-coefficient = −0.297, P < 0.001), and serum folate concentration also was a significant predictor of Hcy when serum PLP, vitamin B_{12} and folate concentrations were included as independent variables (R^2 = 0.121, β-coefficient = −0.347, P < 0.001). We then evaluated the correlations of folate intake and the concentrations of folate and Hcy with intake of the various food groups determined by FFQ. Dietary folate intake and serum folate concentration were significantly associated with intakes of certain food groups including potato, green vegetables, other vegetables and fruits. Only intake of green vegetables was significantly correlated with the plasma Hcy level (Table 3).

Bone mineral density of lumbar spine (SP-BMD) and femoral neck (FN-BMD) were positively correlated with body mass index (BMI) and fat mass, although no significant correlations were found in diabetes-related parameters including fasting plasma glucose, HbA_{1c} and diabetes duration (Table 4). Both SP-BMD and FN-BMD were positively correlated with fasting serum C-peptide, but these correlations were cancelled when adjusted for BMI. Urinary NTx, a marker of bone resorption, was negatively correlated with FN-BMD. As nutrient intake significantly increases with energy intake, nutrition intakes were also evaluated by adjusting for calories. As a result, calorie-adjusted folate intake was positively correlated with SP-BMD, although the association between calorie-adjusted folate and FN-BMD did not reach statistical significance. There were no significant associations between BMD of both sites and serum concentrations of vitamin B_6, vitamin B_{12} and folate. The plasma Hcy concentration was negatively correlated with both

Table 1 | Background characteristics of the study subjects

Characteristic	
No. subjects	125
Male/female	79 (63.2%)/46 (36.8%)
Age (years)	61.2 ± 12.4
Duration of diabetes (years)	11.2 ± 9.4
Diabetes treatment (diet/OHA/Ins/Ins + OHA)	27 (21.6%)/62 (49.6%)/28 (22.4%)/8 (6.4%)
BMI (kg/m^2)	24.9 ± 4.9
Fat mass (kg)	16.5 ± 9.8
Lean body mass (kg)	45.9 ± 9.3
Fasting plasma glucose (mg/dL)	160.2 ± 48.6
HbA_{1c} (%)	9.6 ± 2.2
Fasting serum C-peptide (ng/mL)	1.71 ± 0.89
Serum BAP (U/L)	23.5 ± 8.7
uNTx (nMBCE/mmol Cr)	35.6 ± 19.8
Energy intake (kcal/day)	2073.2 ± 582.5
Protein/fat/carbohydrate intake (g/day)	73.6 ± 19.7/64.4 ± 23.7/278.7 ± 80.2
Calcium intake (mg/day)	596.0 ± 213.6
Vitamin D intake (μg/day)	9.21 ± 4.48
Vitamin B_6 intake (mg/day)	1.22 ± 0.34
Vitamin B_{12} intake (μg/day)	8.81 ± 4.65
Folate intake (μg/day)	287.4 ± 100.5
Serum PLP concentration (pmol/mL)	61.3 ± 29.1
Serum vitamin B_{12} concentration (pmol/mL)	1.45 ± 0.45
Serum folate concentration (pmol/mL)	27.5 ± 10.3
Plasma homocysteine concentration (nmol/mL)	11.2 ± 5.1

Data are number of patients (categorized data) or mean ± SD (quantitative data).
BAP, bone-specific alkaline phosphatase; BMI, body mass index; Ins, insulin; OHA, oral hypoglycemic agents; PLP, pyridoxal 5′-phosphate; uNTX, urine N-terminal cross-linked telopeptide of type-I collagen.

Table 2 | Correlations among dietary nutrient intake values, serum concentrations and plasma homocysteine levels adjusted for age and sex

	r	P
Correlations of total energy intake with various nutrient intakes		
Vitamin B_6 (mg)	0.521	<0.001
Vitamin B_{12} (μg)	0.253	0.005
Folate (μg)	0.331	<0.001
Correlations of intake values with serum concentrations		
Vitamin B_6	0.192	0.034
Vitamin B_{12}	0.336	<0.001
Folate	0.400	<0.001
Correlations of plasma Hcy levels with B vitamins		
Vitamin B_6 intake (mg)	−0.207	0.022
Vitamin B_{12} intake (μg)	−0.001	0.988
Folate intake (μg)	−0.328	<0.001
Serum PLP concentration (pmol/mL)	0.002	0.982
Serum B_{12} concentration (pmol/mL)	0.001	0.993
Serum folate concentration (pmol/mL)	−0.369	<0.001

Hcy, homocysteine; PLP, pyridoxal 5′-phosphate.

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Table 3 | Correlations of dietary folate intake, serum folate concentration and plasma homocysteine level with various food groups

	Dietary folate intake		Serum folate concentration		Plasma Hcy level	
	r	*P*	*r*	*P*	*r*	*P*
Grain/rice	−0.076	0.399	−0.086	0.341	−0.056	0.538
Potato	0.470	<0.001	0.220	0.014	0.012	0.895
Green vegetables	0.843	<0.001	0.361	<0.001	−0.207	0.020
Other vegetables	0.620	<0.001	0.197	0.027	0.077	0.390
Fruits	0.338	<0.001	0.206	0.021	0.018	0.839
Seaweeds	0.322	<0.001	0.072	0.426	0.071	0.435
Beans/soy products	0.390	<0.001	0.156	0.083	0.016	0.856
Seafood	0.313	<0.001	0.075	0.407	−0.017	0.848
Meats	0.065	0.474	0.042	0.643	−0.070	0.435
Egg	0.278	0.002	0.068	0.450	−0.056	0.538
Milk products	0.108	0.230	0.113	0.208	−0.035	0.698
Oil/fat	0.145	0.107	0.161	0.073	−0.112	0.214

Hcy, homocysteine.

Table 4 | Correlations of bone mineral density of lumbar spine and femoral neck with diabetes-related parameters, bone turnover markers and B vitamin status

	SP-BMD		FN-BMD	
	r	*P*	*r*	*P*
BMI (kg/m^2)	0.288	0.001	0.463	<0.001
Fasting plasma glucose (mg/dL)	−0.149	0.098	−0.113	0.210
HbA_{1c} (%)	0.098	0.194	0.053	0.556
Diabetes duration (years)	0.082	0.366	0.057	0.528
Fasting serum C-peptide (ng/mL)	0.182	0.045	0.285	0.001
BAP (U/L)	0.112	0.218	−0.061	0.499
uNTx (nMBCE/mmol Cr)	−0.138	0.084	−0.183	0.042
Vitamin B_6 intake (mg)	−0.032	0.727	−0.053	0.559
Vitamin B_6 intake (mg/100 kcal)	0.113	0.211	0.005	0.959
Vitamin B_{12} intake (μg)	0.012	0.899	0.166	0.065
Vitamin B_{12} intake (μg/1000 kcal)	0.054	0.554	0.148	0.102
Folate intake (μg)	0.103	0.256	0.112	0.216
Folate intake (μg/1000 kcal)	0.198	0.027	0.153	0.090
Serum PLP concentration (pmol/mL)	−0.062	0.497	−0.007	0.936
Serum B_{12} concentration (pmol/mL)	0.023	0.799	0.058	0.524
Serum folate concentration (pmol/mL)	0.104	0.248	0.114	0.205
Plasma Hcy concentration (nmol/mL)	−0.278	0.002	−0.201	0.025

BAP, bone-specific alkaline phosphatase; BMI, body mass index; FN-BMD bone mineral density of femoral neck; Hcy, homocysteine; PLP, pyridoxal 5′-phosphate; SP-BMD, bone mineral density of lumbar spine; uNTX, urine N-terminal cross-linked telopeptide of type-I collagen.

SP-BMD and FN-BMD, showing that hyperhomocysteinemia is clearly associated with low BMD in patients with type 2 diabetes (Figure 1).

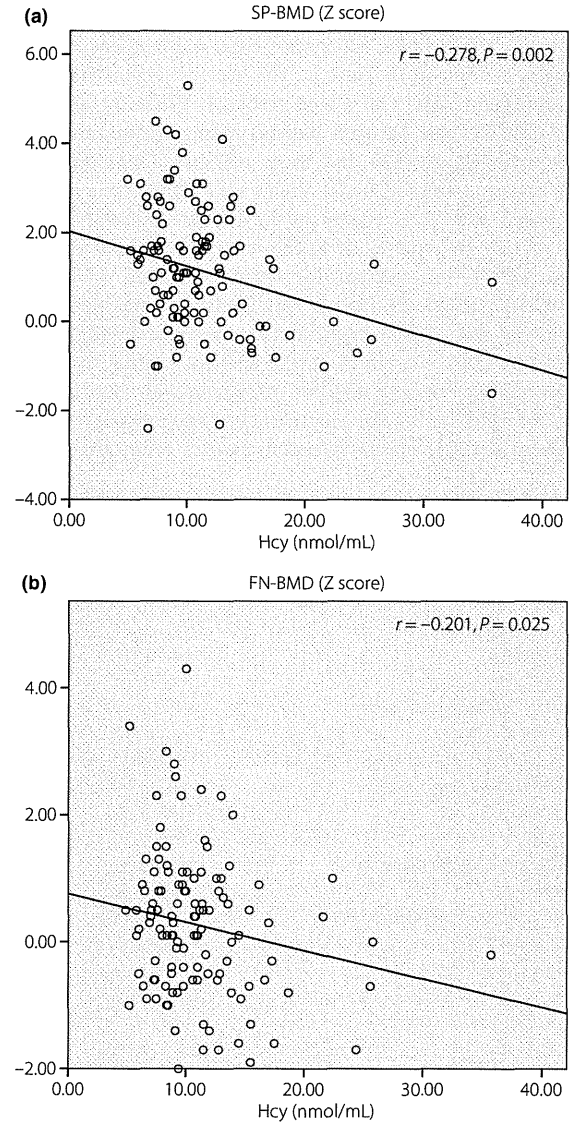

Figure 1 | The relationship between homocysteine (Hcy) and bone mineral density of lumbar spine (SP-BMD) and femoral neck (FN-BMD).

As hyperhomocysteinemia derived from folate insufficiency has been suggested to be involved in low BMD, we compared clinical characteristics of the study population across the quartiles of Hcy (quartile 1, n = 31, Hcy < 8.3 nmol/mL; quartile 2, n = 32, Hcy 8.3 to <9.9 nmol/mL; quartile 3, n = 32, Hcy 9.9 to <12.8 nmol/mL; quartile 4, n = 30, Hcy > 12.8 nmol/mL). There were no significant differences across the quartiles in general clinical characteristics including age, BMI, diabetes-related parameters, energy intake, and vitamin B_6 and vitamin B_{12} status (Table 5). However, SP-BMD and FN-BMD were significantly lower in patients in the highest quartile of Hcy than

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Table 5 | Comparison of clinical characteristics according to homocysteine quartiles adjusted for age and sex

Hcy concentration (nmol/mL)	Quartile 1 (4.9–8.0)	Quartile 2 (8.1–9.9)	Quartile 3 (10.0–12.8)	Quartile 4 (12.8–35.7)	ANCOVA *P*
Male/female	17/14	21/11	23/9	18/12	
Age (years)	59.3 ± 13.8	58.1 ± 12.6	63.9 ± 8.7	64.0 ± 13.4	0.212
BMI (kg/m^2)	25.0 ± 4.4	25.8 ± 5.0	25.0 ± 5.6	23.8 ± 4.5	0.461
Fasting plasma glucose (mg/dL)	158.8 ± 44.8	162.0 ± 45.8	155.6 ± 50.0	164.6 ± 55.4	0.721
HbA_{1c} (%)	10.1 ± 2.3	9.9 ± 2.5	9.1 ± 1.8	9.4 ± 2.1	0.378
Diabetes duration (years)	9.5 ± 8.4	10.2 ± 9.7	12.6 ± 8.6	12.4 ± 9.0	0.183
SP-BMD (Z score)	1.34 ± 1.43*	1.24 ± 1.38*	1.39 ± 1.24*	0.50 ± 1.18	0.037
FN-BMD (Z score)	0.45 ± 0.99**	0.32 ± 1.23*	0.26 ± 0.96*	−0.27 ± 1.03	<0.001
Energy intake (kcal/day)	2161 ± 543	2145 ± 565	2069 ± 563	1910 ± 650	0.260
Vitamin B_6 intake (mg)	1.31 ± 0.35	1.26 ± 0.36	1.21 ± 0.32	1.09 ± 0.29	0.136
Vitamin B_{12} intake (μg)	8.59 ± 3.44	8.86 ± 4.64	9.49 ± 5.24	8.27 ± 5.21	0.798
Folate intake (μg)	323.5 ± 92.2**	287.2 ± 108.0*	305.0 ± 91.8**	231.7 ± 89.1	0.001
Intake of green vegetables (g/day)	101.9 ± 65.3*	86.1 ± 60.6	89.3 ± 47.5	68.9 ± 49.2	0.043
Serum PLP concentration (pmol/mL)	65.0 ± 33.1	60.0 ± 24.6	58.9 ± 32.9	61.4 ± 26.2	0.943
Serum B_{12} concentration (pmol/mL)	2.39 ± 0.88	2.90 ± 1.61	2.50 ± 0.73	2.53 ± 0.92	0.419
Serum folate concentration (pmol/mL)	33.6 ± 11.5**	26.9 ± 7.6*	26.9 ± 9.0*	21.7 ± 8.7	<0.001
Plasma Hcy concentration (nmol/mL)	6.9 ± 0.9**	9.1 ± 0.5**	11.3 ± 0.8**	17.8 ± 6.1	<0.001

BMI, body mass index; FN-BMD bone mineral density of femoral neck; Hcy, homocysteine; PLP, pyridoxal 5′-phosphate; SP-BMD, bone mineral density of lumbar spine. Mean ± SD, *P < 0.05, **P < 0.01 relative to the highest homocysteine quartile group.

those in patients in the other quartiles. Furthermore, patients in the highest Hcy quartile showed significantly decreased dietary folate intake, serum folate concentration and intake of green vegetables compared with those in the lower Hcy quartiles. Because the caloric intake was similar across the quartiles, the quality of the diet might be poor in the highest Hcy group. Quartile analysis revealed that the highest Hcy group showed the lowest BMD, the lowest serum folate concentration, the lowest folate intake and the lowest intake of green vegetables.

DISCUSSION

In the present study, hyperhomocysteinemia was found to be clearly associated with low BMD in type 2 diabetes patients, as it has been reported to be in non-diabetic subjects[6–14]. Furthermore, folate insufficiency might be one of the important factors in hyperhomocysteinemia, as plasma Hcy levels were negatively correlated with both dietary intake and serum concentration of folate.

Osteoporosis is a multifactorial disease, a major health problem characterized by low BMD, deterioration of bone microarchitecture and increased risk of fracture. Elevation of Hcy is one of the important risk factors for osteoporosis[28,29], and can be caused by insufficiency of Hcy-related vitamins, such as folate, vitamin B_6 and vitamin B_{12}[6–14]. Because dietary risk factors can be improved when recognized, sufficiency of Hcy-related vitamins and its relationship to osteoporosis in patients with type 2 diabetes is of primary concern.

Elevation of Hcy can be caused by insufficiency of folate, vitamin B_6 or vitamin B_{12}, and the plasma Hcy level is considered to be a fairly sensitive index of folate metabolic status compared with that of the other factors in non-diabetic subjects. Previous studies reported hyperhomocysteinemia was observed in 86% of subjects with clinically expressed folate deficiency[30]; folate is a major determinant of Hcy levels in healthy people[31,32] and vitamin B_{12} influences Hcy levels less than folate does[33,34]. Folate, vitamin B_6 and vitamin B_{12} are water-soluble vitamins, which are in general not readily stored and consistent daily intake is important. Usually, folate and vitamin B_6 deficiency develops within a month of insufficient intake. In contrast, it is known that patients with complete loss of intrinsic factor require 3–5 years to become overtly vitamin B_{12} deficient[35]. Vitamin B_{12} is a unique water-soluble vitamin, and because 80% of the 2.5 mg average whole body stock of vitamin B_{12} is reserved in the liver and vitamin B_{12} excreted in the bile and is effectively reabsorbed in the intestine, clinical signs of vitamin B_{12} deficiency take a long time to appear and progress slowly[36]. Some patients in the present study were taking metformin, which is known to inhibit absorption of vitamin B_{12}[37], but there was no difference between the patients taking metformin and those not taking metformin. As to vitamin B_6, only a weak negative correlation between vitamin B_6 intake and Hcy was not enough to conclude that vitamin B_6 is a nutritional risk factor for osteoporosis, and there have been no other studies showing the effect of vitamin B_6 on BMD.

Leafy green vegetables, such as spinach and broccoli, are rich sources of folate. Folate is also contained in a variety of foods including fruits, beans, seaweeds, liver and egg yolk. To investigate the cause of folate insufficiency, we focused particularly on dietary sources of folate. We evaluated the association of dietary folate intake, serum folate concentration, and plasma Hcy level

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