対象および方法

(1) 対 象 者

対象者は某株式会社の神奈川県内にあるA事業所および東京都内にあるB事業所に勤務する男性社員で，BMI 24 kg/m^{2}以上の者を対象に行われたウェルネススクール参加者である。スクール参加者は36名であり，そのうち女性4名と研究への同意が得られなかった5名を除く27名を対象者とした。

ウェルネススクールは体重減少を目標とした6ヶ月間のプログラムで，社員食堂を受託する給食会社の管理栄養士によるカフェテリアでの料理の組み合わせ方などを中心とした食事に関する講義（60分×3回）と保健師による運動の講義（60分×1回）を受け，各自が目標設定を決め取り組むものである。各自で1ヶ月に減らす体重を設定し6ヵ月後の目標を立て，それを目指すための1日のエネルギー摂取量の設定を行った。昼食は各自が設定した1日のエネルギー摂取量の35%を目標値とした。また対象者はFeliCaシステムに登録することで，食堂での昼食選択状況のセルフモニタリングが可能となっている。

FeliCaシステムは，非接触ICカードFeliCaを用いたデータ自動収集システムである。これは，社員食堂の支払い清算システムで利用されているプリペイド型電子マネーに搭載されている識別IDを利用し，個別に購買履歴を集計するものである。このシステムの利点は，利用者の手を煩わせることなく喫食履歴の情報（料理名及び料理ごとのエネルギー，三大栄養素，食物繊維，食塩相当量，1食の合計値，PFC比率）の収集と蓄積を完全に自動で行うことができ，それによって継続的な昼食の選択状況の観察ができることである。

(2) 調査期間および調査内容

調査はウェルネススクールによる介入期間である2007年5月から11月の6ヵ月間である。昼食選択状況は，FeliCaシステムを用いて，調査期間中6ヵ月間観察した。さらに，同期間に提供された社員食堂の料理のエネルギー量を解析した。また，保健師により，介入前（スクール第1回目開講前），3ヵ月後，6ヵ月後に身体測定（身長，体重，体組成，血圧，腹囲）および血圧測定を行った。

活動量を確認するため，介入前10日間と介入後から3ヵ月までの間，メモリー機能を持つ加速度計付き歩数計（生活習慣記録機 Lifecorder PLUS：株式会社スズケン）を装着してもらった。解析にあたり，加速度計から得られた総消費量から基礎代謝量，身体活動レベル（PAL）を算出した。

(3) 統計解析

体重の減少・維持増加の2群間の平均値の差の検定は対応のないt検定，Mann-Whitney U検定，3群間以上の平均値の差の検定は繰り返しのある一元配置分散分析後，有意差が認められたものについてはBonferroniの多重比較を行った。有意水準は0.05とし，表中の数値は平均値±標準偏差で表した。統計学的処理は統計ソフトSPSS（ver. 11.0 SPSS Inc.）を用いた。

結　　果

(1) 各食堂の概要

それぞれの食堂の概要を表1に示す。いずれも給食の運営は全面委託しており，カフェテリア方式で食事が提供されている。

表1　対象施設の概要

	A事業所		B事業所	
業務	技術開発		事務	
勤務時間	フレックス		フレックス	
従業員数（人）	6,300		4,100	
食堂の運営	委託		委託	
対象食堂	1	2	3	4
受託業者	a	b	a	c
提供方式	カフェテリア		カフェテリア	
昼食の提供料理種類数	22	24	50	50
予定利用者数（人）	2,161	1,700	1,858	1,516

A事業所は社員食堂が4つ設置されており，運営は2社の給食会社（a，b）に2ヵ所ずつ委託している。2社が受託しているうち，各1ヵ所，計2ヵ所の施設を対象とした。両食堂において，小鉢①は70 kcal以下，小鉢②は71 kcal以上の2区分にして提供している。また，b社受託の食堂では主食の大盛りが2－3品提供されている。

B事業所では社員食堂が2つ設置されており，運営は2社の給食会社（a，c）に委託している。その2ヵ所の施設を対象とした。それぞれの食堂において，量を調節している料理（スモールサイズ）が4－5品ある。

すべての食堂において，プライスカードにエネルギー，たんぱく質，食塩相当量の記載がされている。また，Web上で1週間分のメニューおよびエネルギー量を確認することができる。また，対象者はFeliCaシステムによって，選択した食事の栄養成分の履歴を閲覧することができる。

(2) 社員食堂の提供メニューの特性

対象施設の介入期間中（6ヵ月）の提供メニューのうち，主菜のサイズ別のエネルギー量と副菜のエネルギー量を表2に示す。なお，本研究における主菜，副菜の区分は，各食堂で主菜，副菜として提供されていた料理を指す。

レギュラーサイズの主菜の平均エネルギー量は374±

表2　介入期間中に提供された料理のエネルギー量

(kcal)

	平均値±SD
主　菜 レギュラーサイズ	$n=2,519$ 374 ± 140 (99－959)
主　菜 スモールサイズ	$n=1,170$ 259 ± 87 (70－651)
副　菜	$n=$ 804 90 ± 49 (4－339)

( ) は提供エネルギーの範囲

140 kcal であり，99 kcal－959 kcal までの料理が提供されていた。また，100 kcal 単位で分布を確認したところ，300 kcal 以上 400 kcal 未満の料理が最も多く提供されていた。スモールサイズの主菜の平均エネルギー量は 259 ±87 kcal であり，70 kcal－651 kcal までの料理が提供されていた。スモールサイズの主菜では，100 kcal 以上 200 kcal 未満の料理が最も多く提供されていた。また，スモールサイズで，レギュラーサイズと同じような 600 kcal 以上 700 kcal 未満の料理が提供されていた。副菜の平均エネルギー量は 90±49 kcal であり，4 kcal－339 kcal までの料理が提供されていた。副菜は 50 kcal 単位で分布を確認したところ，50 kcal 以上 100 kcal 未満の料理が最も多く提供されていた。副菜として 300 kcal を超える料理も提供されており，その中にはコロッケ・フライ等の揚げ物，脂質の多いサラダがあり，日替わりで毎日提供されていた。

(3) 対象者の特性

1）社員食堂の利用状況と解析対象者

対象者27名のうち，継続的に社員食堂を利用していた者は19名であった。夕食のみの利用，単品のみの利用，自宅からお弁当を持参している者は「食堂利用なし」とした。観察期間中の食堂の営業日数は両事業所とも112日であり，「食堂利用者」の平均食堂利用率は69.5%，「食堂利用なし」の者では17.3%であった。

解析にあたり「食堂利用者」について6ヵ月後（スクール終了時）に体重減少が見られた者と維持または増加した者との間で，昼食の選択状況を比較した。なお，6ヵ月後の身体測定結果がない1名を除き，最終の解析対象者は18名となった。解析対象者を体重減少群，体重維持・増加群の2群にわけ，体重減少群11名，体重維持・増加群は7名となった。平均食堂利用率は体重減少群で72.2%，体重維持・増加群で64.8%であった。

2）身体状況

対象者の身体状況について表3に示す。介入前の身長，体重に両群に有意な差は認められなかった。体重減少群では介入前と3ヵ月後で体重，BMI，腹囲，体脂肪率（$p<0.001$）が有意に減少した。また，3ヵ月後から6ヵ月後では有意差は認められなかったものの，若干の減少が見られた。体重維持・増加群では，体重，BMI で介入前から3ヵ月後ではほぼ横ばいで推移したが，3ヵ月後から6ヵ月後で有意に増加していた（それぞれ3ヵ月後と6ヵ月後 $p<0.01$）。また，体脂肪率は介入前から3ヵ月後には有意に減少したものの，6ヵ月後には3ヵ月後より有意に高くなっていた（$p<0.01$）。

3）活動量

平均総エネルギー消費量，平均歩数，平均 PAL はいずれも体重減少群，体重維持・増加群で有意な差は認められなかった（表4）。

表3　体重変化別の介入期間中の身体状況の変化

	体重減少群　$n=11$			repeated-measure ANOVA	体重維持・増加群　$n=7$			repeated-measure ANOVA
	介入前	3ヵ月後	6ヵ月後	p 値	介入前	3ヵ月後	6ヵ月後	p 値
年齢	39.5 ± 7.0				37.1 ± 6.9			
身長 (cm)	168.3 ± 5.2				172.5 ± 9.5			
体重 (kg)	74.6 ± 10.7[a]	71.1 ± 10.2[b]	70.6 ± 10.3[b]	<0.001	79.9 ± 9.7	79.2 ± 9.3[a]	80.2 ± 9.6[b]	0.003
BMI (kg/m^2)	26.3 ± 2.8[a]	25.0 ± 2.6[b]	24.8 ± 2.6[b]	<0.001	26.8 ± 2.7	26.6 ± 2.6[a]	27.0 ± 2.7[b]	0.004
体脂肪率 (%)	23.2 ± 3.5[a]	21.2 ± 3.9[b]	21.0 ± 3.8[b]	<0.001	23.0 ± 2.9[b]	21.8 ± 2.5[a]	23.8 ± 2.2[b]	0.002[1)]
腹囲 (cm)	91.3 ± 6.9[a]	86.3 ± 7.3[b]	85.9 ± 7.7[b]	<0.001	93.4 ± 12.6	91.4 ± 8.4	92.1 ± 8.9	0.037
収縮期血圧 (mmHg)	127 ± 12	122 ± 7	125 ± 15	0.415	126 ± 13	128 ± 8	133 ± 11	0.195
拡張期血圧 (mmHg)	79 ± 5	79 ± 6	78 ± 8	0.746	86 ± 9	81 ± 6	83 ± 7	0.346

1) $n=6$

repeated-measureANOVA　介入前，3ヵ月後，6ヵ月後の3群間での比較

異なる上付きの符号間で有意差あり（Bonferroni 多重比較：$p<0.05$）

値は平均値±SD

表4 体重変化別の介入後3ヵ月間の活動量

	体重減少群	体重維持・増加群	対応のない t 検定
	$n=11$	$n=7$	p 値
総消費量（kcal）	2,332 ± 191	2,415 ± 264	0.488
歩数（歩）	10,603 ± 2,180	9,408 ± 2,537	0.216
PAL	1.50 ± 0.06	1.47 ± 0.10	0.422

値は平均値±SD

表5 体重変化別の給食でのエネルギーおよび栄養素選択量

	体重減少群	体重維持・増加群	Mann-Whitney U 検定
	$n=11$	$n=7$	p 値
エネルギー（kcal）	676 ± 73	709 ± 64	0.011
たんぱく質（g）	27.4 ± 3.0	29.5 ± 3.3	0.002
脂質（g）	19.7 ± 4.5	23.4 ± 5.0	<0.001
炭水化物（g）	93.9 ± 10.0	91.6 ± 9.4	0.276
食物繊維（g）	5.9 ± 1.2	5.8 ± 1.6	0.643
ナトリウム（食塩相当量）（g）	4.0 ± 0.9	4.3 ± 1.2	0.038
たんぱく質エネルギー比率（%）	16.4 ± 1.4	16.8 ± 1.8	0.358
脂質エネルギー比率（%）	25.1 ± 3.5	28.4 ± 4.3	<0.001
炭水化物エネルギー比率（%）	56.5 ± 4.0	52.8 ± 4.6	<0.001
食堂利用率（%）[1]	72.2 ± 10.9	64.8 ± 15.8	0.25

[1] 食堂利用率＝昼食利用回数／営業日数×100
値は平均値±SD

(4) 食事選択状況

1）6ヶ月間のエネルギーおよび栄養素選択量

介入期間中の給食（昼食）選択状況を表5に示す。体重減少群の平均エネルギー選択量が676±73 kcalであったのに対し，体重維持・増加群では709±64 kcalと有意に多かった（$p<0.05$）。なお，介入前2週間の平均選択量では800 kcal以上の選択であった者も見られたが，介入中は500 kcal以上800 kcal未満の範囲の選択であった。介入中の最頻値は体重減少群で600 kcal以上700 kcal未満，体重維持・増加群では700 kcal以上800 kcal未満であった。

また，たんぱく質，脂質，脂質エネルギー比率，食塩相当量も体重維持・増加群で有意に多かった。炭水化物エネルギー比率は体重減少群で有意に高かった（$p<0.001$）。

考　　察

食堂利用者を体重減少群と体重維持・増加群に分けて給食（昼食）で選択したエネルギー量の違いを検討したところ，体重減少群の平均エネルギー選択量は676±73 kcalであり，体重維持・増加群の709±64 kcalより有意に少なかった。また，選択エネルギー量の分布を見ると，最頻値は体重減少群で600 kcal以上700 kcal未満，体重維持・増加群では700 kcal以上800 kcal未満であった。これらのことから，適正体重を目標に減量の必要がある場合において600－700 kcal程度の昼食を継続的に摂取することは効果がある可能性があると考える。また，体重減少群の脂質エネルギー比率は25.1±3.5%と目標量に近く，また，体重維持・増加群の28.4±4.3%よりも有意に低かった。食物から摂取する脂肪の量が増加すると体重増加につながることは明らかであり[4] 大幅な減量を長期間維持している者はそうでない者よりも脂質エネルギー比率が低いことを示す報告[5] もある。このことから，エネルギー給与量のほかに脂質給与量の設定も重要であり，脂質エネルギー比率の目標量である20%以上25%未満の基準を1食給食においても目標とすることが望ましいと言える。

今回，対象者の身体活動量の変化の有無を継続的にモニタリングするために加速度計法を用いた。対象者の状況から，簡便な方法であることが重要な要素であった。実際の栄養管理業務としての活用を考えれば費用的な面も考慮しなければならない。加速度計法は，エネルギー代謝測定室や二重標識水法を妥当基準とすると誤差を有するとされているが，日常のおおよその身体活動を評価するには妥当であるという報告が多く，歩行，走行を中心とした日常生活・職場での身体活動による消費エネル

ギーの推定に利用できるとされている[6~10]。今回の対象者の身体活動量は3ヶ月目までは変化がなく，また，体重減少群と体重維持・増加群にも違いは認められなかった。

本研究の対象者はPALが1.48でありレベルⅠ（低い）と推定された。この活動量の推定から目標のエネルギー摂取量を設定し，この値に対する昼食の選択量の比率を検討した。体重減少群と体重維持・増加群では推定エネルギー必要量（EER）に占める昼食の割合に有意差は認められず，体重減少群で31.0±3.2%，体重維持・増加群で31.9±3.4%であった。しかし，体重減少が見られた者の昼食のエネルギー量は700 kcal以下であり，維持・増加群よりも有意に少なかった。今回は昼食以外の食事内容を把握していないため，1日の総エネルギー摂取量から食事ごとのエネルギーの配分比率を算出することはできなかった。1日の総エネルギー摂取量の把握ができない条件下で体重減少を目標として昼食のエネルギー量を設定する場合，EERに占める割合で示すよりも600－700 kcal程度として設定した方が適切かもしれない。また，日本人の食事摂取基準のエネルギーの策定において，成人における推定エネルギー必要量の推定誤差は1日当たり概ね±200 kcal－最大300 kcal程度と考えられている。従って，給食の献立作成基準として，推定エネルギー必要量の付近を目指すこととし，幅を設けてエネルギー給与量を設定する必要があると考える。その点から考えると，エネルギー給与量の計画を立てる上で，600－700 kcal程度と幅を持たせ，かつその代表値として650 kcalという基準を設定することが給食の献立計画としては現実的と考える。主菜，副菜を選択して650 kcal程度に抑えるためには，食堂で提供されているごはんを主食として選択すると想定すると302 kcalである（主食からとれる穀物エネルギー比率46%）。従って主菜は200 kcal－300 kcal，副菜は50 kcal－150 kcalで提供されることが望ましい。本研究の対象施設で提供されている主菜の最頻値は315 kcalであり，スモールサイズの主菜の提供もあることから，比較的組み合わせやすいといえる。しかし，1品で600 kcalを超える主菜が提供されていたことは食環境を整備する点からの課題である。減量を目標としたヘルシーメニューを提供する場合，利用者が継続的に望ましい食事を選択できるような食環境の整備が必要と考えられる。

カフェテリア方式による食事の提供は，お客様の満足度も考慮することが経営的に求められるため，嗜好性を満足させるために料理の多様性が必要である。しかし，健康増進法において「献立の作成にあたり，喫食者の給与栄養量が確保できるよう，施設における献立作成基準を作成するよう努めること」とされており，献立作成基準として，提供する料理区分ごとの種類数とそれに応じたおよそのエネルギーおよび栄養素量の範囲が示されることが望まれている[11]。利用者の栄養管理を目指し，エネルギーの適正範囲を献立作成基準として作成しておくことは必要である。また，副菜については，毎日コロッケのような揚げ物が提供されていたり，200 kcalを超える副菜が提供されていた。脂質エネルギー比率を25%程度の適正な範囲に保つためには，主菜のみならず副菜への配慮も必要である。

Heymsfieldらは体重減少がうまくいかない理由として，被験者が指示通りできていないことを挙げており[12]，森らはDHAや大豆イソフラボンを添加したお弁当の提供による効果ではあるが，1日1食でもバランスの良い食事をすることが生活習慣病の予備群に効果があることを支持している[13]。カフェテリア方式は自分で選択できるところに利点がある。多種類多品目が提供されているため，食事内容が多様で豊かになる反面，個人の嗜好に頼った選択がなされていれば健康管理上問題になる[14,15]。カフェテリア方式による提供においても体重管理のための主食，主菜，副菜がそろう食事を毎日1種類でも提供することが望まれる。

また，有意差は見られなかったが，体重減少群の食堂利用率が72.2%であったのに対し，体重維持・増加群では64.8%であり，体重減少群の方が食堂利用率は高かった。Roosらは，健康的な食事が提供される場合，社員食堂で昼食を食べることが食事の質に関係し，社員食堂を利用している者はそうでない者よりもBMIが低い傾向が見られたとしている。さらに，労働時間中に野菜を含む料理を提供することは，勤労者の食事を改善する効率的な方法かもしれないとしており，社員食堂で昼食を食べる機会を増加させることに重点を置くべきとしている[16]。また，健康増進法施行規則第9条に規定される栄養管理基準を実施している項目が多い施設で給食提供を受けることは，昼食1食であっても利用者の健康や食事について良好な態度形成につながる可能性を示唆する報告もある[17]。健康的な食事にアクセスできることを前提としたときに，社員食堂の利用が健康管理につながると考えられるため，社員食堂の利用を促す手立てが勤労者の健康管理の一つになることが考えられる。

本研究の限界は，1企業の男性従業員を対象とした事例的検討の結果であることである。また，社員食堂における昼食の選択状況データを収集するにあたり利用したシステム上，摂取量ではなく提供栄養量からの検討であるため，4ヵ所の食堂で提供されたメニューの違いによる影響を否定できない。さらに，体重減少については，対象者に同一内容の介入をしていることから，昼食以外の食事を含め，望ましい食事選択のために必要なスキル

や知識が高まり，全体的には望ましい結果が得られている可能性があることである。従って，今後，介入研究を含めた研究の積み重ねが必要である。

ま と め

本研究は，特定給食施設である社員食堂において，適切な栄養管理を行うために，社員食堂利用者の料理選択のアセスメントから得られる情報に基づき，勤労男性の体重管理を目標としたヘルシーメニューのエネルギー給与量の検討を行った。

1）介入期間中6ヵ月の平均エネルギー選択量は体重減少群では676±73 kcal，体重維持・増加群では709±64 kcalであり，体重減少群で有意に少なかった。

2）6ヵ月間の平均脂質エネルギー比率は，体重減少群では25.1±3.5%，体重維持・増加群では28.4±4.3%であり，体重減少群が有意に低かった。

3）昼食の選択エネルギー量の最頻値は体重減少群で600 kcal以上700 kcal未満，体重維持・増加群では700 kcal以上800 kcal未満であった。

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文　献

1）平澤マキ：「事業所給食」における食育，保健の科学，**48**, 735–739（2006）
2）労務研究所：2007年版　民間企業150事業所対象 職場給食の経営指標と価格，旬刊　福利厚生，**1957**, 5–12（2007）
3）労務研究所：「職場給食の経営指標と価格」付帯調査 社員食堂の栄養，健康管理をみる，旬刊　福利厚生，**1960**, 26–31（2007）
4）Garrow, J.S., James, W.P.T., Ralph, A. 編　渡邊令子訳：ヒューマンニュートリション　第10版，147–158（2004）医歯薬出版，東京
5）Shick, S.M., Wing, R.R., Klem, M.L., McGuire, M.T., Hill, J.O. and Seagle, H.: Persons successful at long-term weight loss and maintenance continue to consume a low calorie, low fat diet, *J. Am. Diet. Assoc.*, **98**, 408–413（1998）
6）田中茂穂：間接熱量測定による1日のエネルギー消費量の評価，体力科学，**55**, 527–532（2006）
7）山田誠二，馬場快彦：運動強度を加味したカロリーカウンターによる運動時消費エネルギー量の測定，産業医科大学雑誌，**12**, 77–82（1990）
8）海老根直之，島田美恵子，田中宏暁，西牟田守，吉武裕，斎藤慎一，Jones, P.J.H.：二重標識水法を用いた簡易エネルギー消費量推定法の評価—生活時間調査法，心拍数法，加速度計法について—，体力科学，**51**, 151–164（2002）
9）横地正裕，新実光朗：糖尿病患者の歩行時にカロリーカウンターによって測定されたエネルギー量の妥当性，理学療法学，**22**, 178–180（1995）
10）横山有見子，川村　孝，玉腰暁子，野田明子，平井真理：加速度計による身体活動量の測定の妥当性，スポーツ医・科学，**12**, 23–27（1999）
11）石田裕美，村山伸子，由田克士：特定給食施設における栄養管理の高度化ガイド・事例集，38–43（2007）第一出版，東京
12）Steven, B.H., Joyce, B.H., Joel, W.B., Dale, A.S., Ngozi, E. and Angelo, P.: Why do obese patients not lose more weight when treated with low-calorie diets? A mechanistic perspective, *Am. J. Clin. Nutr.*, **85**, 346–354（2007）
13）森　真理：ヘルシーランチによる生活習慣病のリスク軽減効果，食品工業，**49**, 40–48（2006）
14）高橋道子，鹿嶋康子，渋谷久恵，鈴木和雄，稲葉佳代子，山本妙子，飯田　稔：カフェテリア方式による事業所給食の実態（第1報）提供されている料理の内容，神奈川県立栄養短期大学紀要，**18**, 53–62（1986）
15）三松永典：社員食堂における栄養管理の実際—栄養展示会—，保健の科学，**42**, 989–993（2000）
16）Eva, R., Sirpa, S.L. and Tea, L.: Having lunch at a staff canteen is associated with recommended food habits, *Public Health Nutr.*, **7**, 53–61（2003）
17）平田亜古（分担研究者）：分担研究報告書　事業所給食における栄養管理の実施状況と利用者の健康と食に対する知識・態度の関係　平成17年度厚生労働科学研究「特定給食施設における栄養管理の実施状況とその基準に関する研究」報告書，87–103（2006）
18）石田裕美：分担研究報告書　従業員食堂を活用したハイリスク者の栄養教育プログラムの検討　平成19年度厚生労働科学研究費補助金　循環器疾患等生活習慣病対策総合研究事業　勤労者の健康づくりのための給食を活用した集団及びハイリスク者への対策に関する研究報告書，45–74（2008）

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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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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.

REFERENCES

1. Bilezikian JP, Silverberg SJ. The role of parathyroid hormone and vitamin D in the pathogenesis of osteoporosis. In: Marcus R, Feldman D, Kelsey J, editors. Osteoporosis 2nd ed. San Diego CA: Academic Press; 2001. pp.71-84.
2. Lips P. How to define normal values for serum concentrations of 25-hydroxyvitamin D? An overview. In: Feldman D, Pike JW, Glorieux FG, editors. Vitamin D 2nd ed. San Diego CA: Academic Press; 2005. pp. 1019-28.
3. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, et al. Effect of Vitamin D on falls: a meta-analysis. JAMA. 2004;291:1999-2006.
4. Kuroda T, Shiraki M, Tanaka S, Ohta H. Contributions of 25-hydroxyvitamin D, co-morbidities and bone mass to mortality in Japanese postmenopausal women. Bone. 2009; 44:168-72.

5. Eastell R, Lawrence Riggs B. Vitamin D and osteoporosis. In: Feldman D, Pike JW, Glorieux FG, editors. Vitamin D 2nd ed. San Diego CA: Academic Press; 2005. pp.1101-20.
6. Nakamura K, Nishiwaki T, Ueno K, Yamamoto M. Age-related decrease in serum 25-hydroxyvitamin D concentrations in the frail elderly: a longitudinal study. J Bone Miner Metab. 2007;25:232-6.
7. Lips P, van Ginkel FC, Jongen MJ, Rubertus F, van der Vijgh WJ, Netelenbos JC. Determinants of vitamin D status in patients with hip fracture and in elderly control subjects. Am J Clin Nutr. 1987;46:1005-10.
8. Lips P, Wiersinga A, van Ginkel FC, Jongen MJ, Netelenbos JC, Hackeng WH, et al. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. J Clin Endocrinol Metab. 1988;67:644-50.
9. Suominem M, Laine T, Routasalo P, Pitkala KH, Rasanen L. Nutrient content of served food, nutrient intake and nutritional status of residents with dementia in a finnish nursing home. J Nutr Health Aging. 2004;8:234-8.
10. Przybelski R, Agrawal S, Krueger D, Engelke JA, Walbrun F, Binkley N. Rapid correction of low vitamin D status in nursing home residents. Osteoporos Int. 2008;19:1621-8.
11. Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet. Lancet. 1989;2:1104-5.
12. Nashimoto M, Nakamura K, Matsuyama S, Hatakeyama M, Yamamoto M. Hypovitaminosis D and hyperparathyroidism in physically inactive elderly Japanese living in nursing homes: relationship with age, sunlight exposure and activities of daily living. Aging Clin Exp Res. 2002;14:5-12.
13. Vermeer C. Gamma-carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. Biochem J. 1990; 266:625-36.
14. Booth SL. Skeletal functions of vitamin K-dependent proteins: not just for clotting anymore. Nutr Rev. 1997;55:282-4.
15. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr. 2000;71:1201-8.
16. Tsugawa N, Shiraki M, Suhara Y, Kamao M, Ozaki R, Tanaka K, et al. Low plasma phylloquinone concentration is associated with high incidence of vertebral fracture in Japanese women. J Bone Miner Metab. 2008;26:79-85.
17. Booth SL, Broe KE, Peterson JW, Cheng DM, Dawson-Hughes B, Gundberg CM, et al. Associations between vitamin K biochemical measures and bone mineral density in men and women. J Clin Endocrinol Metab. 2004;89:4904-9.
18. Seibel MJ, Robins SP, Bilezikian JP. Serum undercarboxylated osteocalcin and the risk of hip fracture. J Clin Endocrinol Metab. 1997;82:717-8.
19. Vergnaud P, Garnero P, Meunier PJ, Bréart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab. 1997; 82:719-24.
20. Sokoll LJ, Sadowski JA. Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population. Am J Clin Nutr. 1996;63:566-73.
21. Tsugawa N, Shiraki M, Suhara Y, Kamao M, Tanaka K, Okano T. Vitamin K status of healthy Japanese women: age-related vitamin K requirement for gamma-carboxylation of osteocalcin. Am J Clin Nutr. 2006;83:380-6.
22. Sadowski JA, Hood SJ, Dallal GE, Garry PJ. Phylloquinone in plasma from elderly and young adults: factors influencing its concentration. Am J Clin Nutr. 1989;50:100-8.
23. Tse SL, Chan TY, Wu DM, Cheung AY, Kwok TC. Deficient dietary vitamin K intake among elderly nursing home residents in Hong Kong. Asia Pac J Clin Nutr. 2002;11:62-5.
24. Northstone K, Ness AR, Emmett PM, Rogers IS. Adjusting for energy intake in dietary pattern investigations using principal components analysis. Eur J Clin Nutr. 2008;62: 931-8.
25. Kesse-Guyot E, Bertrais S, Péneau S, Estaquio C, Dauchet L, Vergnaud AC, et al. Dietary patterns and their sociodemographic and behavioural correlates in French middle-aged adults from the SU.VI.MAX cohort. Eur J Clin Nutr. 2009;63:521-9.
26. Suhara Y, Kamao M, Tsugawa N, Okano T. Suhara Y, Kamao M, et al. Method for the determination of vitamin K homologues in human plasma using high-performance liquid chromatography-tandem mass spectrometry. Anal Chem. 2005;77:757-63.
27. Resources Council, Science and Technology Agency, Japan. Standard Tables of Food Composition in Japan, 5th ed. Tokyo: Printing Bureau, Ministry of Finance; 2000. 2009/11/4 [cited 2000/11/22]; Available from: http://www.mext.go.jp/english/news/2000/11/001150.htm#top. (in Japanese).
28. Ministry of Health, Labour, and Welfare, Japan. Dietary reference intakes for Japanese 2010. Tokyo: Daiichi-Shuppan; 2010. 2009/11/4 [cited 2009/5/29]; Available from: http://www-bm.mhlw.go.jp/shingi/2009/05/s0529-4.html. (in Japanese).
29. Ministry of Health, Labour, and Welfare, Japan. Dietary reference intakes for Japanese 2005. Tokyo: Daiichi-Shuppan; 2005. 2009/11/4 [cited 2004/11/22]; Available from: http://www.mhlw.go.jp/houdou/2004/11/h1122-2.html. (in Japanese).
30. Nakamura K, Nashimoto M, Yamamoto M. Summer/winter differences in the serum 25-hydroxyvitamin D3 and parathyroid hormone levels of Japanese women. Int J Biometeorol. 2000;44:186-9.
31. Nakamura K, Nashimoto M, Hori Y, Yamamoto M. Serum 25-hydroxyvitamin D concentrations and related dietary factors in peri- and postmenopausal Japanese women. Am J Clin Nutr. 2000;71:1161-5.
32. Nakamura K, Nashimoto M, Yamamoto M. Are the serum 25-hydroxyvitamin D concentrations in winter associated with forearm bone mineral density in healthy elderly Japanese women? Int J Vit Nutr Res. 2001;71:25-9.
33. Kaneki M, Hodges SJ, Hosoi T, Fujiwara S, Lyons A, Crean SJ, et al. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. Nutrition. 2001;17:315-21.
34. Nakamura K, Tsugawa N, Saito T, Ishikawa M, Tsuchiya Y, Hyodo K, et al. Vitamin D status, bone mass, and bone metabolism in home-dwelling postmenopausal Japanese women: Yokogoshi Study. Bone.2008;42:271-7.
35. Ministry of Health, Labour, and Welfare, Japan. The National Nutrition Survey 2006. Tokyo: Daiichi-Shuppan; 2009. 2009/11/4 [cited 2008/04]; Available from: http://www.mhlw.go.jp/houdou/2008/04/h0430-2.html. (in Japanese).
36. McKeown NM, Jacques PF, Gundberg CM, Peterson JW, Tucker KL, Kiel DP, et al. Dietary and nondietary determinants of vitamin K biochemical measures in men and women. J Nutr. 2002;132:1329-34.
37. Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino RB Sr, et al. Genetic and non-genetic correlates of vitamins K and D. Eur J Clin Nutr. 2009;63:458-64.
38. Ikeda H, Doi Y. A vitamin-K2-binding factor secreted from Bacillus subtilis. Eur J Biochem. 1990;28:219-24.
39. Nakamura K, Nishiwaki T, Ueno K, Yamamoto M. Serum 25-hydroxyvitamin D levels and activities of daily living in

noninstitutionalized elderly Japanese requiring care. J Bone Miner Metab.2005;23:488-94.
40. Kuwabara A, Tanaka K, Tsugawa N, Nakase H, Tsuji H, Shide K, et al. High prevalence of vitamin K and D deficiency and decreased BMD in inflammatory bowel disease. Osteoporos Int. 2009;20:935-42.
41. Cashman KD, Wallace JM, Horigan G, Hill TR, Barnes MS, Lucey AJ, et al. Estimation of the dietary requirement for vitamin D in free-living adults >=64 y of age. Am J Clin Nutr. 2009;89:1366-74.
42. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P. Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. J Clin Endocrinol Metab.2009;94:1244-50.
43. Visvanathan R. Under-nutrition in older people: a serious and growing global problem!Visvanathan R. J Postgrad Med. 2003;49:352-60.
44. Kagansky N, Berner Y, Koren-Morag N, Perelman L, Knobler H, Levy S. Poor nutritional habits are predictors of poor outcome in very old hospitalized patients. Am J Clin Nutr. 2005;82:784-91.
45. Vellas B, Lauque S, Andrieu S, Nourhashemi F, Rolland Y, Baumgartner R, et al. Nutrition assessment in the elderly. Curr Opin Clin Nutr Metab Care. 2001;4:5-8.
46. Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab. 1986;63:954-9.

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

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.

REFERENCES

1. Vermeer C. γ-Carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. Biochem J. 1990;266: 625-36.
2. Booth SL. Skeletal functions of vitamin K-dependent proteins: not just for clotting anymore. Nutr Rev. 1997;55:282-4.
3. Vermeer C, Shearer MJ, Zittermann A, Bolton-Smith C, Szulc P, Hodges S, Walter P, Rambeck W, Stöcklin E, Weber P. Beyond deficiency: potential benefits of increased intakes of vitamin K for bone and vascular health. Eur J Nutr. 2004;43:325-35.
4. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr. 2000;71:1201-8.
5. Danziger J. Vitamin K-deoendent proteins, warfarin, and vascular calcification. Clin J Am Soc Nephrol. 2008;3:1504-10.
6. Proudfot D, ShanahanCM. Molecular mechanisms mediating vascular calcification: role of matrix Gla protein. Nephrology. 2006;11:455-61.
7. Shanahan CM, Proudfoot D, Farzaneh-Far A, Weissberg PL. The role of Gla proteins in vascular calcification. Crit Rev Eukaryot Gene Expr. 1998;8: 357-75.
8. Ministry of Health, Labour, and Welfare, Japan. Dietary reference intakes for Japanese 2010. Tokyo: Daiichi-Shuppan; 2010 (in Japanese).
9. Booth SL, Martini L, Peterson JW, Saltzman E, Dallal GE, Wood RJ. Dietary phylloquinone depletion and repletion in older women. J Nutr. 2003;133:2565-9.
10. Schurgers LJ, Shearer MJ, Hamulyák K, Stöcklin E, Vermeer C. Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose-response relationships in healthy subjects. Blood. 2004;104:2682-9.
11. Kuwabara A, Himeno M, Tsugawa N, Kamao M, Fujii M, Kawai N et al. Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly. Asia Pac J Clin Nutr. 2010;19:49-56.

12. Kamao M, Suhara Y, Tsugawa N, Uwano M, Yamaguchi N, Uenishi K, Ishida H, Sasaki S, Okano T. Vitamin K content of foods and dietary vitamin K intake in Japanese young women. J Nutr Sci Vitaminol. 2007;53:464-70.
13. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. J Nutr. 1998;128:785-8.
14. Kaneki M, Hodges SJ, Hosoi T, Fujiwara S, Lyons A, Crean SJ et al. Japanese fermented soybean food as the major determination of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. Nutrition. 2001;17:315-21.
15. Okano T, Shimomura Y, Yamane M, Suhara Y, Kamao M, Sugiura M, Nakagawa K. Conversion of phylloquinone (Vitamin K1) into menaquinone-4 (Vitamin K2) in mice: two possible routes for menaquinone-4 accumulation in cerebra of mice. J Biol Chem. 2008;283:11270-9.
16. Thijssen HH, Vervoort LM, Schurgers LJ, Shearer MJ. Menadione is a metabolite of oral vitamin K. Br J Nutr. 2006; 95:260-6.
17. Price PA, Otsuka AA, Poser JW, Kristaponis J, Raman N. Characterization of a gamma-carboxyglutamic acid-containing protein from bone. Proc Natl Acad Sci U S A. 1976;73: 1447-51.
18. Shiraki M, Aoki C, Yamazaki N, Ito Y, Tsugawa N, Suhara Y, Okano T. Clinical assessment of undercarboxylated osteocalcin measurement in serum using an electrochemiluminescence immunoassay: Establishments of cut-off values to determine vitamin K insufficiency in bone and to predict fracture leading to clinical use of vitamin K_2. Iyaku to yakugaku. 2007;57:537-46. (in Japanese)
19. Vergnaud P, Garnero P, Meunier PJ, Bréart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab. 1997;82: 719-24.
20. Binkley NC, Krueger DC, Kawahara TN, Engelke JA, Chappell RJ, Suttie JW. A high phylloquinone intake is required to achieve maximal osteocalcin γ-carboxylation. Am J Clin Nutr. 2002;76:1055-60.

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

ビタミンK

Vitamin K treatment for osteoporosis

特集

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すべての医師のための骨粗鬆症診療ガイド2010
Key words ビタミンK　ucOC(undercarboxylated osteocalcin)　menaquinone-4

ビタミンKの同族体(図1)

ビタミンKは，ビタミンK_1(phylloquinone；PK)とビタミンK_2(menaquinones；MK)に分けられる．いずれもナフトキノン骨格に側鎖が付いた構造を持ち，骨格部分は両者で共通だが，側鎖は両者で大きく異なる．PKは1種類のみであり，緑色野菜に多く含まれる．一方，MKは動物性食品に多く含まれ，側鎖の異なる同族体が多数存在し，側鎖の長さによってMK-nのように呼ばれる．腸内細菌は側鎖の長いビタミンK_2を合成し，とくにMK-7は納豆に豊富に含まれる．骨粗鬆症治療薬として臨床で用いられるグラケー®(エーザイ)は1錠にMK-4を15mg含み，1日量45mgである．

最近，PKとMKの相違点が明らかになってきており，岡野らによって，他のビタミンK同族体は，生体内でMK-4に変換されて作用することが示されている[1]．またMK(とくにMK-7)は，生体内での半減期が長いため，PKより効力が大きい[2]．

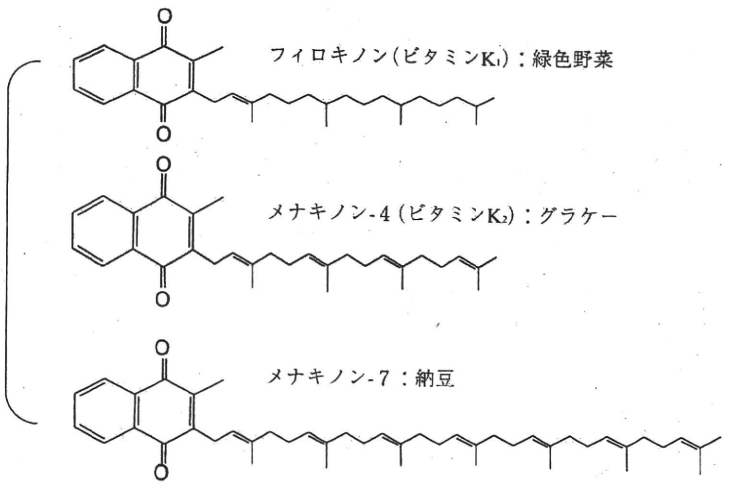

図1　ビタミンK

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