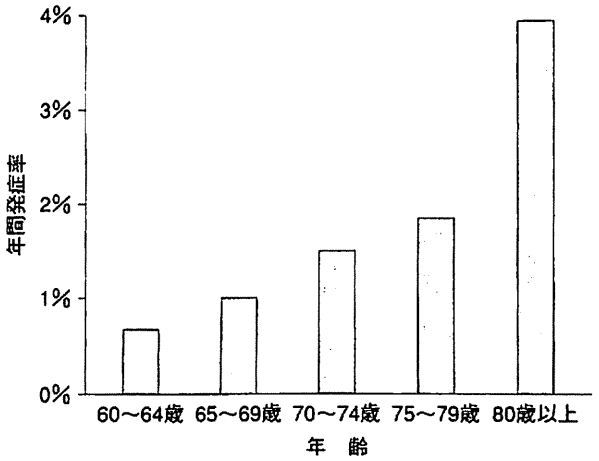

図2　認知症の年間発症率
「国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）」の8年間の縦断的観察から．

率と，2008年度の65歳以上の全国人口2,822万人から，認知症患者数は406万人と推定された．しかし，施設入所者などを加えれば，これよりも患者数はさらに多い可能性がある．従来の方法での患者数推計は，認知症自立度Ⅱ以上を認知症として208万人との推計が出されていたが，患者数は少なくともその約2倍存在することになる．しかし，今回の調査は主として地方の市町村で実施されたので，今後は都市部における同様の調査を行い，検証を行う必要があろう．

認知症の発症率

発症率を推定するためには，同一対象集団について複数年にわたっての繰り返しの調査が必要であり，有病率の推定よりも難しく，わが国の疫学調査の結果では認知症の発症率の推定はほとんど行われていない．われわれは，無作為抽出された地域住民を長期にわたって追跡した「国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）」[4]のデータを用いて，8年間の縦断的な検討から認知症の発症率の推定を行った（図2）．その結果では，60歳以上の地域住民の1.5%が毎年認知症となっていた．年齢が高くなるほど発症率は上昇し，80歳以上では毎年4.0%が認知症となっていたという結果であった．

認知症患者数の将来推計

5歳ごとの性別・年齢別の認知症有病率が今後も大きく変わらないものとして，人口の高齢化に伴う認知症患者数の将来推計を行ってみた．性別・年齢別の認知症有病率は今回の全国調査の結果を用い，人口推計は国立社会保障・人口問題研究所の2006年度12月推計を用いた．2010年度の65歳以上の認知症推定患者数は全体として458万人で，有病率は約15.6%であると推定される．今後，高齢者人口，特に後期高齢者の人口が急増し，表1に示したように患者数は2020年度に574万人，2030年度には666万人と，これからの20年間に認知症の患者数はさらに大きく増加すると予測される．予防や治療法の開発など，早急な対策によって患者数削減を達成しないと，患者の介護や医療に関わる費用は大きく上昇し，それによって国民経済が破綻してしまうことにもなりかねない状況にある．

表1　認知症患者数および有病率推定値

年　度	推定患者数	65歳以上推定人口	有病率
2010	458万人	2,941万人	15.6%
2015	529万人	3,378万人	15.7%
2020	574万人	3,590万人	16.0%
2025	617万人	3,635万人	17.0%
2030	666万人	3,667万人	18.2%
2035	656万人	3,725万人	17.6%
2040	605万人	3,853万人	15.7%
2045	601万人	3,841万人	15.6%
2050	634万人	3,764万人	16.8%
2055	659万人	3,646万人	18.1%

おわりに

世界有数の長寿の国であるわが国は急速に高齢化が進み，それとともに認知症患者の数も増大していく．今後15年間で認知症に関わる介護費用は大きく増加し，年間10兆円に達するとも予想される[5]．高齢化が進む一方で，少子化も進み，介護に関わることのできる労働人口は激減する．このままでは認知症によって日本の社会が崩壊するといっても過言ではない．しかし，認知症の発症を2年遅らせることができれば，それだけで年間1兆円もの介護費用，医療費が削減できる可能性がある[5]．最近は，認知症の進行を緩徐化する作用をもつ薬物が次々に開発され，またアルツハイマー病に対するワクチンの開発なども進められている．認知症は生活習慣病でもあり，生活習慣の改善である程度の予防が可能である．認知症の素因としての遺伝子多型の研究も進み始めている．このような研究の推進により認知症を克服して，高齢者の知的機能を守り，高

齢者の社会参画を可能にしていくことが，今後の日本の長寿社会を守っていくためにはぜひとも必要である．

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Epidemiological View of Dementia in Japan

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The methods of prevention and medical cure of dementia are still unclear. Disease situations chronically develop to severe stage over a long period of time. The burden for care and medical treatment of dementia is huge. The incidence of dementia increases with age. Therefore, number of dementia patients and cost for care and treatment will increase rapidly with aging in Japanese society. The epidemiological studies of dementia including prevalence and incidence statistics are very important for estimation of medical cost and policymaking of care and welfare in the elderly. The first national survey of dementia prevalence was tried in 7 areas in Japan. The prevalence rate of dementia was estimated to be 14.4 percent. Number of the elderly population 65 years and over is about 30 million in 2010 and number of dementia patients is estimated 4.5 million which was more than double of the previous estimation. Number of dementia patients will increase to 6.7 million in 2030. Researches on prevention and therapy for reduction of dementia are tasks of pressing urgency.

コラム COLUMN

高齢者のうつと栄養

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KEY WORD
うつ，高齢者，n-3系多価不飽和脂肪酸，魚類脂肪，コレステロール，アミノ酸

うつとは

うつ病とは気分がひどく落ち込み，なにごとにも興味や喜びが感じられなくなり，そのことに著しい苦痛を感じ，日常生活に支障を生じるようになった状態で，正式には大うつ病（major depression disease）ともいい，気分障害（mood disorder）の一形態である[1,2]．

うつ病は先進諸国ではもっとも頻度の高い精神疾患の1つであり[2]，2004年のWHO reportによれば[3]，うつ病は下部呼吸器疾患，下痢性疾患についで「疾病による世界全体の負担」の第3位で，先進国を中心とした高収入国だけで推定すると，社会全体でもっとも負担となっている病気である．

うつ病は自殺とも関連し，自殺者の30～70%がうつ病の診断に当てはまるとも，またうつ病患者の自殺率は一般人の10倍以上ともいわれている．わが国は先進諸国のなかでも自殺率が高いことが知られており，十数年来，自殺死亡者数は年間3万人前後で社会問題となっている．

昨今，「うつ（状態）」あるいは「抑うつ（状態）」という言葉は，大うつ病の診断基準を満たさない場合も含めて，より広い意味で用いられている．疫学調査では精神科医の診断を必要としない，自記式質問票を用いてうつの頻度や有病率の調査を行うことが多い．筆者らが1997年から行っている「国立長寿医療研究センター・老化に関する長期縦断疫学研究[4]」ではCES-D（Center for epidemiology studies depression scale）というスクリーニング検査を用いて一般地域住民のうつを調査している．直近の第6次調査（2008～2010）では，40歳から80歳代までのうつの頻度は13.1%（男性12.3%，女性13.9%）で女性にやや多かった[5]（**表1**）．国内の他の文献でもわが国の高齢者のうつの頻度はおおよそ10～20%で，女性に多いことが知られている．

うつの関連要因（栄養要因以外）

うつ病には遺伝的要因が40～50%，後天的要因が50～60%関与すると考えられている．うつ病は単一遺伝子ではなく，多因子遺伝疾患である．後天的な関連要因としては，大脳成長期のストレスや精神的トラウマ，身体疾患や障害（副腎皮質ホルモンや甲状腺ホルモンの上昇・低下，膠原病，パーキンソン病，糖尿病，脳血管疾患，動脈硬化症，頭部外傷，あ

表1　地域在住中高年者のうつの頻度（%）

	40～49歳	50～59歳	60～69歳	70～79歳	80～89歳	全体
男性	12.1	10.2	8.7	17.4	15.1	12.3
女性	11.1	14.1	13.7	15.3	17.7	13.9
全体	11.6	12.0	11.2	16.4	16.4	13.1

国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）．第6次調査（2008～2010）でのCES-D検査結果による（16点以上を「うつ」と定義）

る種のがん，気管支喘息など）の関与が報告されている．

ストレスも，うつやうつ病の大きな誘因である．慢性的なストレスや制御しがたいストレスに対して，人は不安や怒り，無気力感や抑うつ感，認知障害などの不適応的反応を示す[2]．高齢者ではとくに家族，知人との死別，役割・仕事からの離脱，健康・ADLの障害などがうつの契機となるといわれている．しかし同じようなストレス下にあっても，個人のストレスに対する対応パターン（ストレスコーピング）によってうつの発症は影響される．

このようにうつの発症にかかわる要因は多岐にわたり，相互にも関連していて非常に複雑である．

うつと栄養とのかかわり

食事をとることそのものが満足感，充足感につながることは誰もが実感している．うつや感情には脳内のセロトニンやノルエピネフリンが関連しているが，脳内伝達物質の前駆物質の多くは食物から供給される[6]．これまでにうつとの関連が検討された栄養学的要因は，セロトニンやノルエピネフリンの前駆物質であるトリプトファン，チロシン，フェニルアラニン等のアミノ酸，神経細胞膜の主成分である多価不飽和脂肪酸やコレステロール，動脈硬化との関連も報告されている葉酸・ビタミンB_{12}・ホモシステイン，さらにはグルタミン，タウリン，テアニン等のアミノ酸，ヨードや鉛，ビタミンD，炭水化物や糖，アルコール摂取や低栄養など，きわめて多岐にわたっている．ここでは多価不飽和脂肪酸，コレステロールと脳内伝達物質やアミノ酸についてまとめる．

多価不飽和脂肪酸とうつ

魚に多く含まれるn-3系脂肪酸〔ドコサヘキサエン酸（DHA），エイコサペンタエン酸（EPA）等〕の摂取，あるいは魚摂取とうつとの関連を検討した報告は多い．Maesら[7]は，うつ病患者で血漿リン脂質中のn-3系多価脂肪酸の欠乏がみられた，と報告している．若年女性に関しては妊娠中の魚摂取が出産後うつ病を減らした[8]という報告やEPA，DHA摂取が若年成人女性の2年後のうつを抑制した[9]という報告がある．また，うつの高齢者では血清EPAが低く，うつ病の重症度と血清EPAが逆相関していた[10]．さらに地域住民での大規模調査でEPAやEPA/AA比が高い群ではwell-beingの指標が高かった[11]．Hibbelnらは，各国の魚摂取量とうつの頻度の間に負の関連があることを示している[12]．しかし，n-3系脂肪酸とうつとの関係を否定する報告も多数あり，一定の結論には達していない．肯定的研究，否定的研究ともに横断的な研究が多く，対象が限られているのも難点である．

筆者らは前述したNILS-LSAの詳細な栄養調査の結果を用いて，第1次調査で抑うつのなかった65歳以上の高齢者を対象として2年後の抑うつの有無と食品群・栄養素摂取との関係を報告している[13]．医学的・社会的交絡要因を調整したステップワイズ多重ロジスティック解析の結果，女性では有意な項目は認められなかったが，男性では，魚類脂肪，獣肉類，ビタミンD，アラキジン酸が有意となった（表2）．魚介類脂肪に関しては摂取量が1標準偏差（2.5 g/日）増えるごとに抑うつの危険率が約1/3に減少することが示された．また魚介類脂肪を1日4.8 g以上摂取している群ではそれ未満の群よりも有意に2年後のCES-D得点が低く，うつ傾向が小さいと考えられた．これはサバなら30 g，アジなら70 gから摂取される魚類由来脂肪量とほぼ同等であった（図1）．n-3系脂肪酸とうつとの関係は横断的検討では有意であったものの，縦断的検討で多くの交絡要因を調整すると有意ではなくなった．

n-3系脂肪酸の抑うつとの関係の作用機序については，Hibbelnらは中枢神経系の細胞膜のn-3/n-6比の低下が神経内分泌や受容体の性状に影響を与える可能性を指摘している[12]．またEPAやDHAは脳卒中や脳血管の動脈硬化を抑制することが知られているが，動脈硬化はうつと関連していることから，これらのn-3系脂肪酸が抗動脈硬化作用を介してうつ発症に抑制的に作用している可能性もある．

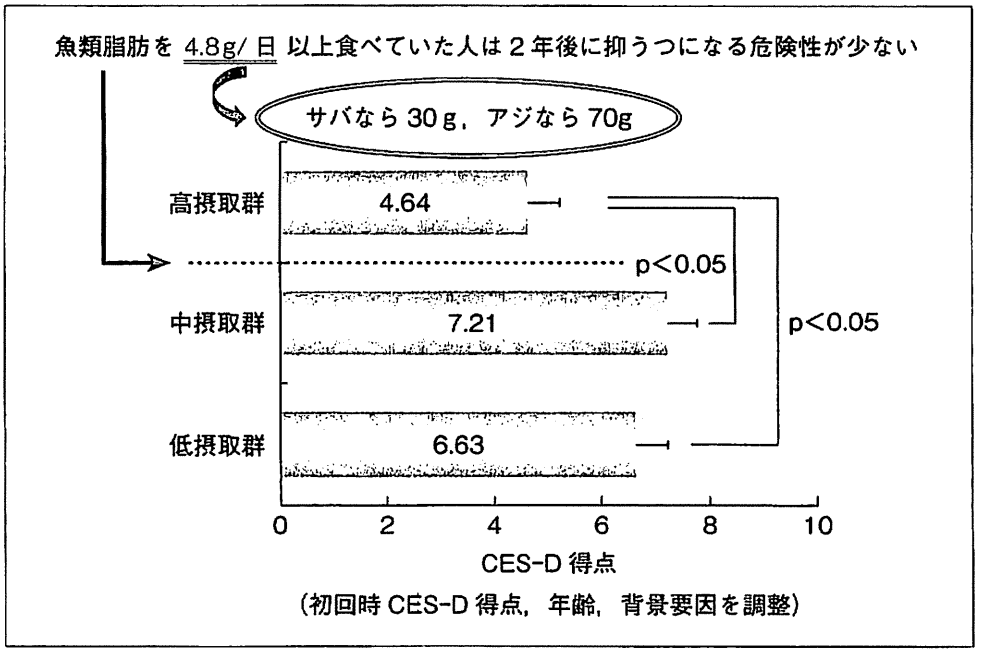
表2　抑うつと関連があった食品群・栄養素（縦断的検討，ステップワイズロジスティック回帰分析）

初回調査時に抑うつがなかった者を対象とし，年齢，初回時CES-D得点，老研式生活活動度指標，喫煙，自覚的健康度，就業，家庭内収入，学歴，HDLコレステロール，アルカリフォスファターゼ，遊離T3，甲状腺刺激ホルモン，血小板数，BMIを調整した．

	Odds比（1 s.d. あたり）	95%信頼区間
男性		
魚類脂肪	0.308	0.105～0.908
ビタミンD	0.361	0.137～0.950
獣鳥肉類	2.261	1.154～4.431
アラキジン酸	1.660	1.016～2.712
女性		
（有意な項目なし）		

国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）．第1次調査（1997～2000），第2次調査（2000～2002）調査結果による

図1　魚類脂肪摂取量3分位別の2年後の抑うつ得点（男性）



国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）．第1次調査（1997～2000）．第2次調査（2000～2002）調査結果による

コレステロールとうつ

コレステロールは生体の細胞膜の必須成分であり，血液中や胆汁中に多く含まれるが，これら以外に体内に蓄積されているコレステロールの約30%は脳神経系に分布しているといわれている．コレステロールとうつとの関係の研究でいままでにもっとも注目を集めたのは，高脂血症治療薬による血清コレステロール低下と自殺や事故・暴力死との関係を報告した研究であろう[14]．24,187人の男性を対象とした無作為割付臨床試験の結果として報告されたこの研究の後しばらく，この結果に肯定的な研究と否定的な研究がつぎつぎと発表された．一方，観察的な研究として1993年にLancetに掲載されたMorganらの報告によると，地域在住高齢男性で低コレステロール血症者では高コレステロール血症者と比較して，10年以上後での抑うつの危険性が約3倍で

あった[15]．カナダの国民栄養調査に基づいた研究では，血中コレステロール4分位でもっとも低い群ではいちばん高い群と比較して20年間の自殺率が6倍高かった[16]．その一方で，地域高齢者の抑うつと低コレステロール血症との見かけ上の関連は，関連要因を調整すると消失する，という報告もある[17]．

前述のMuldoonらはその後，高脂血症治療薬の大規模無作為試験のメタアナリシスを行い，高脂血症治療薬による血清コレステロールの低下と自殺死等の間には有意な関連が認められなかった，と報告している[18]．

チロシン，トリプトファン等のアミノ酸とうつ

うつに関連する脳内神経伝達物質であるセロトニンはトリプトファンから，ノルアドレナリンはチロシンやフェニルアラニンから生合成される．このようなアミノ酸の摂取がうつと関連する可能性は当然考えられるが，アミノ酸の食品成分に関するデータベース構築が不十分であるため，ヒトでの観察研究は限られている．

McTavishら[19]はラットにチロシン欠乏食を与えると脳内のカテコラミン放出やドーパミンの集積が低下した，と報告している．ヒトにおいてもトリプトファンの摂取や血中セロトニン濃度と抑うつとの関連が報告されている[20,21]．ヒトにおける介入研究ではEllenbogenらが低トリプトファン食を用いた介入研究を行い，血清中トリプトファン濃度が80～90%低下した女性では対照群よりもうつ症状や疲労感などが強かったと報告している[22]．

最近筆者らはアミノ酸食品成分表を整備し，日常摂取されるたんぱく質の90%以上をアミノ酸に置き換えることに成功した[23]．この成分表を用いて，18種類のアミノ酸と抑うつとの関連を横断的・縦断的に検討したが，チロシンやトリプトファンとうつとの間に有意な関連は認められなかった[24]．

まとめ

食事は高齢者の心身の健康に影響を与える要因としてきわめて重要である．うつと栄養との関係についての研究もおびただしい数があるが，一定の結論は得られていない．このことは栄養がうつと無関係であることを意味するのではなく，栄養と健康事象との関連における疫学研究の難しさを示しているものである．

すなわち，食習慣とうつの双方に影響を与える個人のライフスタイルや社会経済的な要因を完全に調整することは困難であり，また食品とそれに含まれる栄養素との間には強い関連があることから，1つの栄養素と健康事象との間に関連が認められても，それが真にその栄養素による効果なのか，あるいはその栄養素を多く含有する食品に含まれている他の栄養素によるのか，あるいはそれらの栄養素の複合作用によるのか，明らかではない．ヒトでの介入研究で長期間にわたって食事を完全に制御することは困難であり，さらに栄養素の吸収，代謝，作用や脳内伝達物質の受容体などには遺伝的要因がかかわっており，因果関係をより複雑にしている．

今後は遺伝的要因や栄養素間の相互作用を考慮した，より詳細な研究が必要と考えられる．

しかしながら，従来の報告を俯瞰すると，高齢者において，n-3系脂肪酸やコレステロールを十分に含む，アミノ酸バランスのとれた良質な食生活を維持し，低栄養やビタミン類・微量元素の不足を防ぐことは，そのほかの心身の疾患にとって望ましいことであるだけでなく，うつの予防にも好ましいことであると考えられる．

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5 病院・施設・在宅を結ぶ 高齢者の栄養ケア

CONTENTS

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A Significant Relationship between Plasma Vitamin C Concentration and Physical Performance among Japanese Elderly Women

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Background. Maintenance of physical performance could improve the quality of life in old age. Recent studies suggested a beneficial relationship between antioxidant vitamin (eg, vitamin C) intake and physical performance in elderly people. The purpose of this study was to examine the relationship between plasma vitamin C concentration and physical performance among Japanese community-dwelling elderly women.

Methods. This is a cross-sectional study involving elderly females residing in an urban area in Tokyo, Japan, in October 2006. We examined anthropometric measurements, physical performance, lifestyles, and plasma vitamin C concentration of participants.

Results. A total of 655 subjects who did not take supplements were analyzed. The mean age (±standard deviation) of participants was 75.7 ± 4.1 years in this study. The geometric mean (geometric standard deviation) of plasma vitamin C concentration was 8.9 (1.5) μg/mL. The plasma vitamin C concentration was positively correlated with handgrip strength, length of time standing on one leg with eyes open and walking speed, and inversely correlated with body mass index. After adjusting for the confounding factors, the quartile plasma vitamin C level was significantly correlated with the subject's handgrip strength (p for trend = .0004) and ability to stand on one leg with eyes open (p for trend = .049).

Conclusions. In community-dwelling elderly women, the concentration of plasma vitamin C related well to their muscle strength and physical performance.

Key Words: Plasma vitamin C—Physical performance—Elderly women—Japanese.

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PHYSICAL performance and physical ability are the most important indicators of health status in elderly people and are also closely related to the quality of life. Declines in physical performance and physical activity, whether from specific disease, fall, fracture, poor nutrition, or aging itself, are associated with future disability, morbidity, and death (1,2).

In recent years, many studies have examined the roles of diet, protein, and vitamins in physical performance and physical activity(3–5). Several studies have associated low serum albumin concentration with deteriorated muscle strength and function (6,7). Some other studies have examined the relationship between serum vitamin D level and physical performance such as muscle mass, muscle strength, handgrip, walking speed, and functional capacity (8,9). Cesari et al. (3) examined the relationship between antioxidant vitamin intake (vitamin C, vitamin E, β-carotene, and retinol) and physical performance in elderly people and showed significant positive correlations between most antioxidants, especially vitamin C, and higher skeletal muscular strength in this group of people.

There are a number of mechanistic hypotheses about the potential beneficial effects of antioxidant vitamins(10–12). Vitamin C, vitamin E, β-carotene, and retinol are important antioxidants that are not synthesized by humans and, therefore, are mainly supplied via dietary intake. Vitamin C

(ascorbic acid) is a water-soluble antioxidant present in the cytosol and extracellular fluid and can directly react with free radicals such as superoxide ($O_2\cdot^-$) and hydroxyl radicals (·OH) (13,14). Each one of these oxygen-derived intermediates is considered highly reactive because of their unstable electron configurations, which could attract electrons from other molecules, resulting in another free radical that is capable of reacting with yet another molecule. This chain reaction is thought to contribute to lipid peroxidation, DNA damage, and protein degradation during oxidative stress. Oxidative damage is thought to play an important role in the age-related decline of functional activity in human skeletal muscle (15). Concentration of plasma vitamin C, which has potent antioxidant activity, is known to increase after exercise (4).

An increase in the amount of blood vitamin C content has been used as an indicator of increased oxidative reaction (11). Previous studies have examined the effects of vitamin C supplementation on physical performance and exercise (4,11). Although findings from some of the previous studies do not support any beneficial effect of increased antioxidant intake on physical performance, other studies have shown improved recovery from exercise with antioxidant intake and have also shown a preventive role of antioxidant supplementation against oxidative damage. These studies were carried out on athletes after heavy exercise. So far, however, there has been no study examining the relationship between physical performance and blood levels of vitamin C, which may be a more direct marker of the antioxidative ability of the human body.

The present study, to the best of our knowledge, is the first report that examines the relationship between plasma vitamin C concentration and physical performance in Japanese community-dwelling elderly women.

Subjects and Methods

Study Subjects

The present cross-sectional study was carried out as part of a project involving mass health examination of community-dwelling people ("Otasha-kenshin" in Japanese) aged 70 years and older living in Itabashi-ku, Tokyo. "Otasha-kenshin," which literally means "health examination for successful aging," is a comprehensive health examination program for community-dwelling older adults aimed at preventing geriatric syndromes including falls and fractures, incontinence, mild cognitive impairment, depression, and undernutrition (16).

The eligible subjects were all female residents, aged between 70 and 84 years, living in the Itabashi area, an urban part of Itabashi-ku, Tokyo, Japan in October 2006. The population of women belonging to this age range and residing in the Itabashi area was 5937, and they were recruited by invitation through postal mail. Of them, 1,112 women applied for admission and 957 women ultimately participated in this study. The participants who were taking vitamin C supplements (n = 238) were excluded from the primary analyses for examination of the relationship between plasma vitamin C and physical performance because intake of supplements could strongly influence the plasma vitamin C level. Thus, data from 655 subjects were ultimately used for the primary analysis. However, data from the 238 supplement users were also used for subanalysis to determine whether any relationship exists between vitamin C supplementation and physical performance.

All participants were examined at the Tokyo Metropolitan Institute of Gerontology's hall. Physical performance, blood examinations, lifestyle assessments, and anthropometric measurements were performed as described below (9).

The present study was approved by the ethics review committee of the Tokyo Metropolitan Institute of Gerontology. All subjects gave written informed consent.

Anthropometric Measurements

Height and weight of each participant were measured, and body mass index was defined as weight/height^2 (kg/m^2). Body composition measurements (percent body fat) were obtained by segmental bioelectrical impedance using eight tactile electrodes according to the manufacturer's instructions (In Body 3.0; Biospace, Seoul, Korea). Measurements for the triceps surae muscles were taken between the knee and the ankle, at the level of maximum circumference of the medial and anterior calf of the left leg of each participant at sitting position.

Physical Performance

Physical performance was assessed by muscle strength (handgrip strength), balance capability, and usual and maximal walking speeds, without prior practice before the actual measurements. These assessments are routinely conducted for the elderly community as described previously (9). Handgrip strength (kg) was measured once for the dominant hand with the subjects in a standing position using a Smedley's Hand Dynamometer (Yagami, Tokyo, Japan). Grip devices were calibrated with known weights. Subjects held the dynamometer at thigh level and were encouraged to exert the strongest possible force. Balance capability was measured in terms of the length of time standing on one leg, that is, we asked the subjects to look straight ahead at a dot 1 m in front of them and to stand on the preferred leg with their eyes open and hands down alongside the trunk. The time until balance was lost (or maximum 60 seconds) was recorded. We used the better of two trials in the analysis. To determine the walking speed, participants were asked to walk on a flat surface at their "usual and maximum walking speeds." Two marks were used to delineate the start and end of a 5-m path. The start mark was preceded by a 3-m approach to ensure that the participants achieved their pace of usual or maximum before entering the test path. The participants were also instructed to continue walking past the end of the 5-m path for a further 3 m to ensure that their walking pace was maintained

throughout the test path. The time taken to complete the 5-m walk was measured by an investigator and used for analysis. Walking test at maximum speed was repeated twice, and the faster speed was recorded for the test.

All physical performance tests were performed between 9 AM and 4 PM during the day. We have no data on the reproducibility of the measurements. To reduce interexaminer variation, each test was conducted by the same staff member specifically trained for this study.

Blood Examinations

Blood samples (nonfasting) were collected from the subjects between 9 am and 4 pm during the day. There was no difference in mean plasma vitamin C concentration with regard to the time of collection (data not shown). Venous blood samples were drawn into Ethylene diamine tetraacetic acid tubes. Plasma was then obtained by centrifugation at 3,000 rpm for 15 min at 4°C and subsequently used for biochemical assays. Plasma was treated with Ethylene diamine tetraacetic acid to prevent the spontaneous vitamin C degradation. Next, 100 μl of the plasma was dispensed into storage tubes, to which 450 μl of 3% metaphosphoric acid solution was added, and the mixture was stored at −80°C until further use. Vitamin C concentration was determined by an High performance liquid chromatography-electrochemical detection–based method (17). The analysis was carried out centrally in our laboratory. Serum albumin concentration was measured by the Bromocresol Green method (Special Reference Laboratories Inc., Tokyo, Japan). The coefficient of variation for serum albumin found using this method was less than 1% (9).

Lifestyle Assessment

Information regarding the participants' general health (such as medical history, smoking habits, alcohol drinking habits, regular exercise habits, vegetable intake, fruit intake and use of vitamin C supplement) was collected by interview, and history of medical conditions including hypertension, stroke, heart attack, diabetes mellitus, and hyperlipidemia was self-reported.

Alcohol drinking habits of the subjects were classified as nondrinker, current drinker, or ex-drinker. Smoking habits of the subjects were classified using three categories: never smokers, current smokers, and ex-smokers. The frequency of vegetable and fruit intake was asked using four categories: almost every day, once every two days, once or twice per week, and almost never. Subsequently, for analysis, the categories were summarized as almost every day and others.

Statistical Analysis

Data were summarized as mean and standard deviation or percentage values. The data of plasma vitamin C concentration was logarithmically transformed to approximate a normal distribution and was summarized as the geometric mean and geometric standard deviation.

Table 1. Characteristics of Study Subjects ($N = 655$)

Characteristic	Mean (*SD*)
Age (y)	75.7 (4.1)
Height (cm)	149.1 (5.7)
Weight (kg)	51.0 (8.3)
Body mass index (kg/m^2)	22.9 (3.4)
Triceps surae muscle (cm)	33.1 (2.8)
Plasma vitamin C (μg/ml)*	8.9 (1.5)
Serum albumin (mg/dL)	4.3 (0.2)
Body composition	
Percent body fat (%)	32.2 (7.0)
Physical performance tests	
Handgrip strength (kg)	18.7 (4.4)
One leg standing with eyes open (s)	35.2 (23.5)
Usual walking speed (m/s)	1.2 (0.3)
Maximal walking speed (m/s)	1.8 (0.4)
	%
Medical history	
Hypertension	50.7
Stroke	6.6
Heart attack	21.2
Diabetes mellitus	9.0
Hyperlipidemia	34.7
Alcohol drinking habit	
Current	25.3
Former	5.0
Never	69.6
Smoking habit	
Current	3.7
Former	5.7
Never	90.7
Regular exercise habit	
Yes	69.2
No	30.8
Vegetable intake	
Everyday	84.2
Others†	15.8
Fruit intake	
Everyday	81.8
Others†	18.2

Notes: Data of vitamin C supplement users were excluded.
*The geometric mean and geometric *SD*.
†Including participants taking vegetables/fruits not everyday or almost never.

The age-adjusted Pearson's correlation coefficient between the plasma vitamin C concentration and other factors were calculated. The least square means and SEs adjusted for potential confounders were calculated and compared between categories by analysis of covariance. To examine the relationship between plasma vitamin C concentration and physical performance, statistical adjustment was done by analysis of covariance for variables (except for other physical performance variables) that were correlated to plasma vitamin C concentration with $p < .20$. The same analyses were repeated for the 238 users of vitamin C supplement. All statistical analyses were performed using the SAS (version 9.0; SAS Institute Inc., NC).

RESULTS

Table 1 summarizes the basic characteristics of the subjects. As shown, the mean age (±standard deviation) of the

Table 2. Correlation between Plasma Vitamin C Concentration and Selected Factors ($N = 655$)

Factor	Correlation*	
	r	p
Age	−0.004	.91
Height	0.04	.27
Weight	−0.05	.19
Body mass index	−0.08	.054
Triceps surae muscle	0.001	.98
Serum albumin	−0.04	.33
Percent body fat	−0.12	.002
Handgrip strength	0.16	<.001
One leg standing with eyes open	0.15	<.001
Usual walking speed	0.14	<.001
Maximal walking speed	0.09	.036

Notes: Number of subjects is slightly different for the selected factors because of missing values.

*Age-adjusted Pearson's correlation coefficient between logarithm of vitamin C concentration and each factor.

subjects was 75.7 ± 4.1 years. The geometric mean (geometric standard deviation) of plasma vitamin C concentration was 8.9 (1.5) μg/mL. The prevalence of women eating vegetables everyday was 84.2% and those eating fruits everyday was 81.8%.

The age-adjusted geometric mean of plasma vitamin C concentration was significantly lower in subjects who had a medical history of hypertension (8.53 vs 9.22, $p = .0015$) and diabetes mellitus (7.59 vs 9.00, $p = .002$) as compared with those who did not. A history of stroke, heart attack, or hyperlipidemia was not associated with plasma vitamin C concentration. Subjects who took fruits every day had a significantly higher concentration of vitamin C than those who did not (9.14 vs 7.78, $p < .0001$). Vegetable intake, alcohol drinking habit and smoking habit were not related to plasma vitamin C concentration (not shown in table).

Table 2 shows the age-adjusted correlations between the plasma vitamin C concentration and selected factors. As shown, the plasma vitamin C concentration was positively but modestly correlated with handgrip strength, length of time standing on one leg with eyes open, as well as usual walking speed and maximal walking speed, and modestly inversely correlated with body mass index and percent body fat of the subjects.

Table 3 shows the relationship between plasma vitamin C concentration and each physical performance after adjusting for confounding factors. Results obtained after the adjustment for potential confounders confirmed that the plasma vitamin C concentration was correlated with the handgrip strength independently from the other factors (eg, p for trend = .0004 after adjusting for age, body mass index, percent body fat, hypertension, diabetes mellitus, and fruit intake; Table 3). There was also a significant relationship between the plasma vitamin C level and the subject's length of time standing on one leg with eyes open after adjustments for age, body mass index, percent body fat, hypertension, diabetes mellitus, and fruit intake (Table 3; p for trend = .049). We did not observe any significant association between the plasma vitamin C level and the usual or the maximal walking speed of the subjects.

A subanalysis using data from the 238 vitamin C supplement users showed almost null relationship between handgrip strength and plasma vitamin C concentration (data not shown).

Discussion

A previous study has shown an association between higher daily dietary intake of vitamin C and skeletal muscle strength in elderly people (3). Results described in the present study indicated that plasma vitamin C concentration was positively related with muscle and physical performance in community-dwelling elderly women. To the best of our knowledge, this is the first study showing a significant

Table 3. Relationship between Plasma Vitamin C Concentration and Physical Performance Adjusted for Potential Confounder

	Quartile of plasma vitamin C level				
	Q1	Q2	Q3	Q4	
Physical performance	Mean ± *SE*	Mean ± *SE*	Mean ± *SE*	Mean ± *SE*	p for trend
Handgrip strength (kg), N	154	159	154	152	
Age adjusted	17.70 ± 0.34	18.75 ± 0.33	18.75 ± 0.34	19.60 ± 0.34	.0001
Multivariate adjusted*	17.83 ± 0.34	18.83 ± 0.32	18.89 ± 0.33	19.60 ± 0.33	.0004
One leg standing with eyes open† (s), N	162	163	164	161	
Age adjusted	31.44 ± 1.71	33.98 ± 1.70	37.70 ± 1.70	37.83 ± 1.71	.003
Multivariate adjusted*	33.39 ± 1.74	34.08 ± 1.67	37.63 ± 1.67	37.50 ± 1.70	.049
Usual walking speed (m/s), N	146	154	145	147	
Age adjusted	1.13 ± 0.02	1.19 ± 0.02	1.23 ± 0.02	1.21 ± 0.02	.008
Multivariate adjusted*	1.18 ± 0.02	1.19 ± 0.02	1.22 ± 0.02	1.21 ± 0.02	.23
Maximal walking speed (m/s), N	146	154	154	147	
Age adjusted	1.70 ± 0.03	1.76 ± 0.03	1.82 ± 0.03	1.76 ± 0.03	.15
Multivariate adjusted*	1.76 ± 0.03	1.77 ± 0.03	1.80 ± 0.03	1.75 ± 0.03	.94

Notes: Values are least squares mean and *SE* adjusted for the factors by analysis of covariance. Q1–Q4: first to fourth quartile groups of plasma vitamin C concentration, respectively.

*Adjusted for age, body mass index, percent body fat, hypertension, diabetes mellitus and **fruit intake**.

†Length of time standing on one leg with eyes open.

correlation between plasma vitamin C concentration and handgrip strength and ability to stand on one leg with eyes open. We, however, were unable to find any relationship between skeletal muscle mass and plasma vitamin C concentration. Handgrip strength has been found to correlate well with the strength of other muscle groups and is thus a good indicator of overall strength (18). Consistent with this idea, handgrip strength was found to be a strong and consistent predictor of all-cause mortality and morbidity of Activities of Daily Living in middle-aged people (19). The handgrip test is considered an easy and inexpensive screening tool to identify elderly people at risk of disability. Handgrip strength, an indicator of overall muscle strength, is thought to predict mortality through mechanisms other than underlying disease that could cause muscle impairment (18,19). The one leg standing test is one of the balance tests (20). The test is a clinical tool to assess postural steadiness in a static position by quantitative measurement. Many studies have shown that the decreased one leg standing time is associated with declines in Activities of Daily Living and increases in other morbidities including osteoporosis and fall (20).

Our findings suggest that vitamin C may play an important role in maintaining physical performance and thereby may help to improve healthy life expectancy in the elderly. However, the usual and maximal walking speeds did not relate to plasma vitamin C concentration. Walking speed test may be an efficient tool in screening older persons with higher risk of mortality and may easily identify high-risk groups in the community (21). Walking is a rhythmic, dynamic, and aerobic activity of the large skeletal muscles that confers multifarious benefits with minimal adverse effects. Muscles of the legs, limbs, and lower trunk are strengthened, and the flexibility of their joints are preserved (22). One of the reasons why walking speed was not related to vitamin C concentration may be because walking requires coordinated movements of arms, legs, and many parts of the body rather than a simple muscle and balance function. Previous reports showed that walking balance function did not correlate with standing balance function (23). Although we did not find any clear association between walking and plasma vitamin C concentration in this study, vitamin C may still have effects on relatively simple strength and balance functions.

One of the possible explanations for the observed relationship between vitamin C and physical performance, especially handgrip strength and the ability to stand on one leg with eyes open, may be the potential protective effects of the antioxidant vitamins against muscle damage (4,11). Vitamin C is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not in humans (12). Vitamin C is an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized (12). Thus, vitamin C readily scavenges reactive oxygen and nitrogen species, thereby effectively protects other substrates from oxidative damage (10,24). Although habitual exercise reduces systemic inflammation and oxidative stress as the production of endogenous antioxidants are enhanced, acute exercise increases the generation of oxygen-free radicals and lipid peroxidation (4,25). Strenuous physical performance can increase oxygen consumption by 10- to 15-folds over the resting state to meet the energy demands and results in muscle injury (26). Prolonged submaximal exercise was shown to increase the amount of both whole-body and skeletal muscle lipid peroxidation by-products; in the case of the former, the increase was indicated by greater exhalation of pentane but not of ethane (4,27,28). Supplementation with vitamin C was shown to decrease the exercise-induced increase in the rate of lipid peroxidation (27,28). Several studies suggested that oxidative damage may play a crucial role in the decline of functional activity in human skeletal muscle with normal aging (15). Consistent with this idea, several studies showed significantly lower plasma vitamin C level in the elderly population than in the younger adult population (29–31). Because the plasma vitamin C levels in these apparently healthy elderly persons rose markedly after an oral dose of vitamin C, their initially low plasma levels can be attributed to the low intake rather than to an age-related physiological defect.

In fact, the relationship between handgrip strength and plasma vitamin C concentration was significantly different between supplement users and nonusers, that is, an almost null relationship in the former and a positive relationship in the latter (data not shown). This finding suggested that vitamin C supplementation did not have any beneficial effect on the physical performance and muscle strength despite the increased plasma level of vitamin C. A number of studies reported that vitamin C supplement users had significantly higher blood vitamin C concentration than non-users (29, 32, 33). Several studies have examined the effects of exercise on changes in the serum vitamin C concentration (34–36). Some other experimental studies have shown that vitamin C supplementation can reduce symptoms or indicators of exercise-induced oxidative stress (37–40). However, the results regarding vitamin C supplementation are equivocal, and most well-controlled intervention studies report no beneficial effect of vitamin C supplementation on either endurance or strength performance (41,42). Likewise, vitamin C restriction studies showed that a marginal vitamin C deficiency did not affect the physical performance (43). Although evidence from a number of studies show that vitamin C is a powerful antioxidant in biological systems in vitro, its antioxidant role in humans has not been supported by currently available clinical studies.

Vitamin C is especially plentiful in fresh fruits and vegetables. Plasma vitamin C concentration may be merely a marker for intake of other nutrients that are abundant in fruits and vegetables. However, the statistical adjustment for fruit intake did not attenuate the relationship between plasma vitamin C and physical performance (Table 3), suggesting that vitamin C did have some beneficial effects

independently of other nutrients. A number of biochemical, clinical, and observational epidemiologic studies have indicated that diets rich in fruits, vegetables, and vitamin C may be of benefit for the prevention of chronic diseases such as cardiovascular disease and cancer (44,45). Several cohort studies have examined associations between plasma vitamin C concentration and mortality from stroke or coronary heart disease (30,46,47). The effects of vitamin C supplementation are, however, still unclear. A pooled study suggested reduced incidences of coronary heart disease events with higher intake of vitamin C supplement (48), while another study showed that a high intake of vitamin C supplement is associated with an increased risk of mortality due to cardiovascular diseases in postmenopausal women with diabetes (49). A randomized placebo controlled 5-year trial, however, did not show any significant reduction in the mortality from, or incidence of, any type of vascular disease or cancer (50). These studies, in fact, have failed to demonstrate any benefit from such supplementation.

There are a number of potential weaknesses in our study that should be mentioned here. The subjects used in this study were not selected randomly from the study population, and they may be relatively healthy elderly women who were able to come to the health examination hall from their homes. A previous study assessed the correlation of antioxidants with physical performance and muscular strength (3) and demonstrated that a higher daily intake of vitamin C and carotene associated with skeletal muscle strength. However, we have no data regarding the presence of other dietary antioxidants in blood such as vitamin E, retinol, and carotene. In our questionnaire, participants were asked to respond "Yes" or "No" to whether they took supplements, and not about the frequency and quantity of intake of the supplements. Thus, we were unable to examine the reason why plasma vitamin C was not related to the handgrip strength in the supplement users by considering the dose of vitamin C they took.

This study was a cross-sectional study and, therefore, does not provide cause/effect relationships, although we demonstrated a significant correlation between physical performance and concentration of plasma vitamin C. Therefore, longitudinal follow-up studies and controlled clinical trials are necessary to confirm the role of plasma vitamin C and physical performance of the elderly women. These limitations should be considered in future studies.

In conclusion, we found a strong correlation of a higher plasma vitamin C concentration with handgrip strength and one leg standing time in community-dwelling elderly women. Although the elderly are prone to vitamin C deficiency, and they appear to have a higher dietary requirement for vitamin C, the beneficial effects of vitamin C supplementation to maintain physical performance in elderly people are equivocal and thus, need further in-depth studies.

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Vitamin K_1 (Phylloquinone) or Vitamin K_2 (Menaquinone-4) Induces Intestinal Alkaline Phosphatase Gene Expression

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Summary Alkaline phosphatase (ALP) hydrolyzes a variety of monophosphate esters into inorganic acid and alcohol at a high optimum pH (pH 8–10). Previously, we identified a significant increase of intestinal ALP (IAP) activity in the rat intestine on long-term dietary vitamin K supplementation. However, it was unclear whether the induction of ALP gene expression was caused by vitamin K intake. In the present study, we examined the effects of vitamin K on IAP gene expression. A total of 21 male ICR strain mice (7 wk old) were divided into three groups: control, PK, and MK groups. Mice were orally administered a 0.1-mL solution of physiological saline in the control group, phylloquinone (3 mg/kg mouse) in the PK group, and menaquinone-4 (3 mg/kg mouse) in the MK group. Four hours after administration, we determined the ALP activity of the intestinal mucosa in three areas (duodenum, jejunum, and ileum). In the MK groups, the levels of ALP activity in the jejunum increased significantly compared with the control. Moreover, reverse transcription-polymerase chain reaction (RT-PCR) analysis using specific primers revealed that IAP mRNA expression was significantly enhanced in the jejunum in both PK and MK groups. Interestingly, vitamin K administration also increased the expression of pregnane X receptor mRNA. This is the first report concerning IAP mRNA expression induced by oral administration of vitamin K. The results support the possible involvement of vitamin K in the regulation of IAP mRNA expression as a novel pharmacological effect of vitamin K.

Key Words alkaline phosphatase, phylloquinone, menaquinone, mice, intestine

Alkaline phosphatase (ALP, EC 3.1.3.1) is an enzyme containing zinc which hydrolyzes monophosphate esters into inorganic phosphoric acid and alcohol at a high optimal pH (pH 8–10). The enzyme is distributed widely throughout the living world from bacteria to animals, excluding plants, and it exists in various tissues such as the intestine, liver, kidney, bone, placenta, stomach, and leukocytes.

In humans, four kinds of ALP isozyme have been identified: tissue-nonspecific ALP (liver/bone/kidney: TNSALP), intestinal ALP (IAP), placental ALP, and germ cell ALP (*1–4*). The TNSALP gene is located on chromosome 1 and consists of 12 exons and 11 introns, with the coding sequence beginning in the second exon.

A single gene for human IAP has been isolated, and the multiple forms of mRNA encoding human IAP are due to differences in polyadenylation (*2*). Although most species express a single IAP, several kinds of IAP have been identified in three species: the mouse (*5*), rat (*6, 7*), and cow (*8*).

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In rats, ALP is classified into two types: TNSALP and IAP. IAP is present in the membrane surrounding neutral fat droplets in the microvilli of the intestinal mucosa during fat absorption, and is thought to transport dietary lipids from the intestinal tract into the circulation as a component of unilamellar membranes called surfactant-like particles (SLPs) (*9*). Two different cDNA clones, IAP-I and IAP-II, for rat IAP were isolated by Lowe et al. (*6*) and Strom et al. (*7*), respectively. Strom et al. found that the expression of IAP-II mRNA was specifically enhanced by $1\alpha,25(OH)_2D_3$ administration. The two isozymes are products of two distinct genes and their cDNA sequences show 79% homology at the amino acid level. Functional differences between IAP-I and IAP-II were suggested by the differing regulation of the expression of the two mRNAs (*10*), as well as by structural and catalytic differences (*11*).

In mice, five different ALP loci have been identified: TNSALP, IAP, embryonic ALP (EAP), *Akp6*, and *Akp-ps1*. These ALP genes code for different proteins: *Akp2* encodes TNSALP, *Akp3* encodes IAP, *Akp5* encodes EAP, *Akp6* encodes a novel IAP-like isozyme expressed globally in the gut (thus called gIAP), and *Akp-ps1* encodes

the inactive pseudo-type ALP (5, 7).

Previously, we reported the enhanced effects of vitamin K on IAP activity in rats (12). Sprague-Dawley rats (6 wk old) were divided into three groups: a control (AIN-93M diet), phylloquinone (PK: 600 mg/kg diet), and menaquinone-4 (MK-4: 600 mg/kg diet) diet group. After 3 mo of feeding of vitamin K, the animals were fasted overnight. The small intestine was removed and divided into five segments. In each segment, both PK and MK-4 increased IAP activity (12).

Vitamin K acts as a cofactor for γ-glutamyl carboxylase (GGCX), and is well-known to participate in the activation of blood coagulation factors and bone mineralization (13). All forms of vitamin K have 1,4-naphthoquinone as a common ring structure, and natural vitamin K exists in two molecular forms, vitamin K_1 (phylloquinone: PK) and vitamin K_2 (menaquinone: MK-*n*). PK is abundant in green vegetables in a compound with a phytyl side chain. Vitamin K_2 is classified into MK-1–14 due to the repeat structure of the side chain, with isopren comprising the side chain. MK-4 shows marked physiological activities as a vitamin K, and is included in many animal-based foods such as meat. Recent studies have demonstrated the possibility that vitamin K regulates the expression of bone-related genes such as ALP through steroid X receptor (SXR, also termed pregnane X receptor: PXR) (14).

In the present study, we examined whether the enhancing effect of PK or MK-4 administration on IAP activity occurs via the intestinal mucosa directly, and we revealed the effects of the oral administration of PK or MK-4 on the expression of IAPs (*Akp3* and *Akp6*) and PXR in the mouse intestine.

MATERIALS AND METHODS

Experimental animals. The care and use of mice in the present study followed the guidelines of governmental legislation in Japan on the proper use of laboratory animals, and the study protocol was approved by the Institutional Review Board of Japan Women's University. A total of 21 male ICR strain mice (7 wk old) were used (31.1±0.2 g). They were fasted overnight with free access to water. On the following day, the animals were given 0.1 mL of solution via an intragastric tube: vehicle (physiological saline) for the control group (Cont.), PK (3 mg/kg mouse) for the PK group, and MK-4 (3 mg/kg mouse) for the MK group. The molecular weights of PK ($C_{31}H_{46}O_2$: MW=450.7) and MK-4 ($C_{31}H_{40}O_2$: MW=444.7) are very similar. PK and MK-4 were kindly supplied by Eisai Co., Ltd. (Tokyo, Japan).

Serum and tissue sampling. Four hours after administration, blood was collected from the abdominal aorta under ether anesthesia, and perfusion with saline was performed until the liver was blanched, in order to minimize the blood contamination of tissue samples. The small intestine was removed and divided into three regions. From the pylorus, we took the first 1 cm as the duodenum, and then separated the remaining part into the jejunum and ileum. The segments were slit longitudinally, rinsed with ice-cold saline, and scraped from the mice just after dissection. Each sample was homogenized using a Polytron homogenizer (Kinematica, Switzerland) with 10 mM Tris-buffered saline containing 1% Triton X-100 (pH 7.3) and 1 mM phenylmethylsulfonylfluoride (PMSF). The supernatant obtained after centrifugation at 7,000 ×*g* for 15 min was used as the enzyme extract.

Enzyme assay. ALP activity was determined with 10 mM *p*-nitro-phenylphosphate as a substrate in 100 mM 2-amino-2-methyl-1,3-propanediole HCl buffer containg 5 mM $MgCl_2$, pH 10.0, at 37°C, as previously reported (15). To analyze the biochemical properties of ALP, an inhibitory assay using levamisole (Lev) and L-phenylalanine (L-Phe) and a thermostability assay were performed, as previously described (15).

The enzyme activity was defined as the rate of hydrolysis of *p*-nitro-phenylphosphate and expressed in units (U=μmol *p*-nitro-phenol formed/min).

Protein concentrations were determined using BCA protein assay reagent (Pierce, Rockford, IL, USA).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Polyacrylamide gel (7.5%) electrophoresis in the presence of sodium dodecyl sulfate (SDS) was carried out according to the method of Weber et al. (16). After electrophoresis, ALP isozymes separated in the gel were stained by the coupling of β-naphthyl-phosphoric acid monosodium salt with Fast Violet B salt (17).

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR). Total RNA from the intestinal mucosa was extracted employing the acid guanidinium thiocyanate-phenol-chloroform method (18). As a template for PCR, single-strand cDNA was prepared from 1 μg of total RNA using Ready-to-go You-Prime First-Strand Beads (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, England). PCR primers were used for *Akp3* (19), *Akp2* (20), *Akp5* (21), and PXR (22). In order to detect *Akp6*, sense (*Akp6*-up) and anti-sense (*Akp6*-down) primers were designed on the basis of the *Akp6* nucleotide sequence (GenBank: NCBI sequence data: AK008000). *Akp6*-up spans nucleotide positions 882–902 and *Akp6*-down spans 1,333–1,354 (23). The PCR conditions were as follows: 5 cycles at 94°C (1 min), 50°C (1 min), and 72°C (1 min), and 25 cycles at 94°C (30 s), 55°C (30 s), and 72°C (30 s), followed by 10 min at 72°C. Negative controls were performed with each RT-PCR reaction, omitting the template. The efficiency of reverse transcription was verified by the detection of GAPDH (glyceraldehyde-3-phosphate dehydrogenase, forward: 5′-ACC ACA GTC CAT GCC ATC AC-3′, reverse:5′-TCC ACC ACC CTG TTG CTG TA-3′), as previously described (23).

The amplified samples were analyzed using 5.25% polyacrylamide gel electrophoresis (PAGE). The gels were stained with ethidium bromide and observed under UV light. The band intensity on PCR photographs was quantified by densitometry (AE6920M, ATTO, Tokyo, Japan). The PCR product was normalized to the intensity of the band for the house-keeping gene GAPDH, and is expressed as a ratio of the relative band intensity.

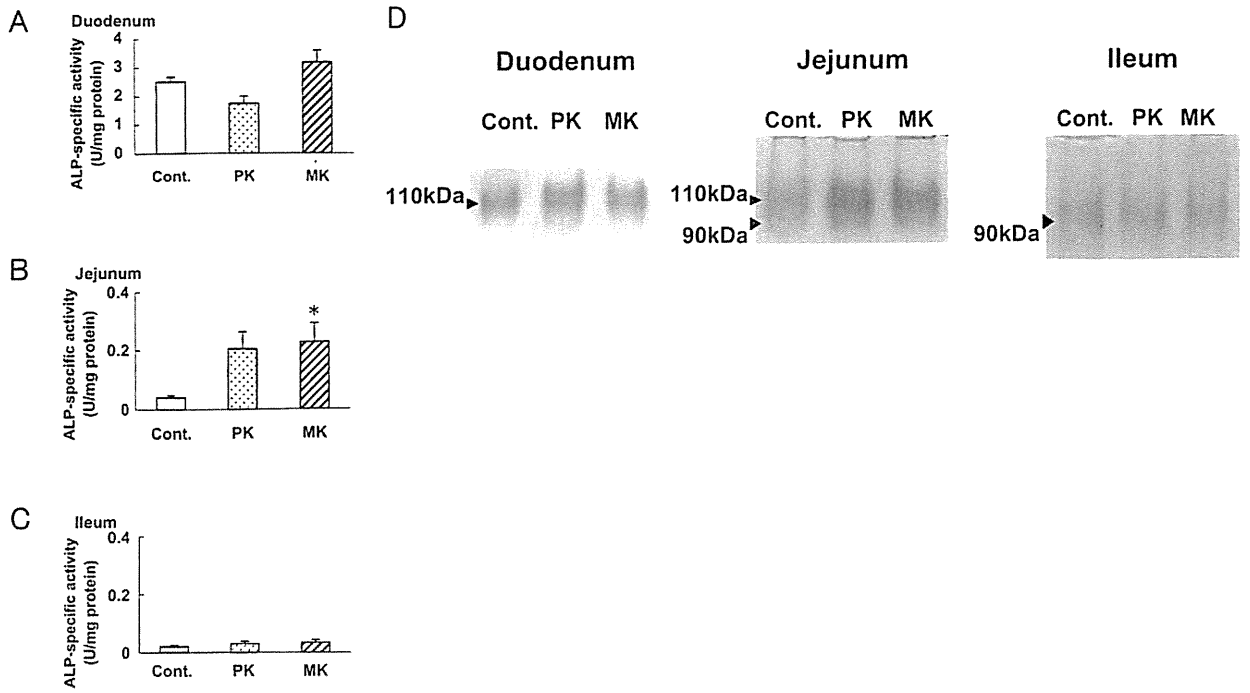

Fig. 1. ALP-specific activities of the duodenum (A), jejunum (B), and ileum (C). Results are the mean±SE of 7 animals. Significant difference between the MK and control groups (*$p<0.05$). D: Mouse intestinal ALP isozymes separated by polyacrylamide gel electrophoresis. The gels was stained for ALP activity with a β-naphtyl-phosphoric acid monosodium salt, Fast violet B salt. Cont.: control, PK: phylloquione, MK: menaquinone-4.

Statistical analyses. Values are shown as the mean± standard error (SE).

Dunnet's multiple comparison test was used after ANOVA to compare the significance of differences among the control and PK or MK. Differences were considered significant at $p<0.05$. Analysis was conducted using SPSS 18.0J (SPSS, Inc., Chicago, IL, USA).

RESULTS

ALP activity

To examine whether IAP was secreted from the intestinal mucosa on the oral administration of PK or MK, we measured the levels of serum ALP activity (mU/mL) in the control, PK, and MK groups, being 13.7±1.2, 10.3±2.0, and 14.1±1.8 (mean±SE), respectively, showing no significant differences among these groups.

ALP-specific activities in the intestine are shown in Fig. 1. There were no significant differences in ALP activities among these groups in the duodenum (Fig. 1A) and ileum (Fig. 1C). As presented in Fig. 1B, ALP activity of the MK group in the jejunum was significantly higher compared with the control group ($p<0.05$).

Molecular weight determination by SDS-PAGE

The molecular weights of ALPs of each intestinal segment were estimated employing SDS-PAGE analysis. As shown in Fig. 1D, the 110-kDa band of the major ALP isozyme was detected in the duodenum among these groups. In the jejunum, ALP enzymes were separated into two bands of 110 and 90 kDa, and the intensity of their enzymatic activity increased markedly in both PK and MK groups, similarly to the results regarding the specific ALP activity in the jejunum. In the distal part of the intestine (ileum), the ALP isozyme showed a main band of 90 kDa among these groups. No additional band was observed in any intestinal samples among the groups.

Table 1. Inhibitory effects of levamisole, L-phenylalanine and heat inactivation of ALP preparations of the jejunum.

Groups	Relative activity(%)		
	Levamisole (1 mM)	L-Phenylalanine (20 mM)	Heat inactivation (60°C 10 min)
Cont.	95.2±1.6	23.0±1.0	42.4±3.4
PK	94.2±2.1	23.5±1.4	46.1±2.3
MK	95.5±1.6	24.2±2.0	45.9±2.5

Each value represents mean±SE ($n=7$).
The ALP activity was assayed based on the rate of *p*-NPP hydrolysis. The effect of the inhibitor was determined in the presence of 5 mM $MgCl_2$ in the assay mixture. Remaining ALP activity with inhibitors or after heat treatment is expressed as a percent of non-treated controls. Results are the mean±SE of 7 animals.

Properties of ALP in the intestine

The enzymatic properties of ALP preparations of the jejunum which increased significantly on PK or MK administration were investigated employing an inhibition experiment with levamisole (Lev) and L-phenylalanine (L-Phe) and through a thermo-stability test. It is well known that IAP activity is not inhibited by Lev and is more stable to L-Phe and more heat-stable than TNSALP. As shown in Table 1, there was no significant