# Relationship of vitamin A and vitamin E intake to fasting plasma retinol, retinol-binding protein, retinyl esters, carotene, $\alpha$-tocopherol, and cholesterol among elderly people and young adults: increased plasma retinyl esters among vitamin A-supplement users[1-3]

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**ABSTRACT** We studied the relationships of supplemental and total vitamin A and supplemental vitamin E intake with fasting plasma biochemical indicators of vitamin A and vitamin E nutritional status among 562 healthy elderly people (aged 60–98 y) and 194 healthy young adult (aged 19–59 y) volunteers. All subjects were nonsmokers. For the young adults, plasma retinol was significantly greater in males than in females ($p < 0.01$); retinol was not related to supplemental vitamin A intake for either group. Fasting plasma retinyl esters demonstrated a significant increase with vitamin A supplement use. For supplemental vitamin A intakes of 5001–10 000 IU/d, a 2.5-fold increase over nonusers in fasting plasma retinyl esters was observed for elderly people ($p < 0.05$) and a 1.5-fold increase for young adults ($p > 0.20$). For elderly people, greater fasting plasma retinyl esters were associated with long-term vitamin A supplement use (> 5 y) and biochemical evidence of liver damage. Elderly people who take vitamin A supplements may be at increased risk for vitamin A overload. *Am J Clin Nutr* 1989;49:112–20.

**KEY WORDS** Vitamin A, retinol, retinol-binding protein, retinyl esters, carotene, vitamin E, $\alpha$-tocopherol, supplemental vitamin A, supplemental vitamin E, hypervitaminosis A

## Introduction

Vitamin A deficiency is uncommon among healthy, well-nourished elderly individuals. Reports from the 1940s and 1950s demonstrated that plasma vitamin A values are similar in persons aged 40–90 y (1–3). In the Ten-State Nutrition Survey (1968–1970) and the Health and Nutrition Examination Surveys of 1971–1974 and 1976–1980 (HANES I and II), plasma vitamin A values generally increased throughout adulthood reaching a plateau at approximately the sixth decade of life (4–6). In the HANES surveys plasma vitamin A values < 0.70 $\mu$mol/L were rare (< 0.3%) among individuals aged > 60 y (5, 6). Low plasma vitamin A values were also rare among healthy elderly men and women living in Utah (7), Missouri (8), and Oregon (9). Three studies showed that liver levels of vitamin A are maintained throughout life (10–12) indicating adequate hepatic vitamin A stores among elderly people.

In spite of normal plasma values and adequate liver stores of vitamin A among elderly people, HANES I also showed that ~50% of individuals aged > 60 y ate two-thirds or less the recommended allowance for vitamin A (5). The 1980 Recommended Dietary Allowance (RDA) for vitamin A from a mixed diet of plant and animal foods is 5000 IU for men and 4000 IU for women (13), but it has been argued that these intakes need not be so high to maintain adequate liver stores of the vitamin (14). The liver has a great capacity to store vitamin A (15) and it is possible that luxurious intakes of the vitamin throughout life may result in an excessive hepatic accumulation of vitamin A and a predisposition to vitamin A overload in later life. Vitamin A supplementation,

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which is common among elderly people (16, 17), may further challenge the capacity of the liver to store vitamin A and could eventually precipitate signs of vitamin A toxicity.

Megadose supplements of vitamin E (> 10 times the RDA) are also common among elderly people (16, 17). In animals large oral doses of vitamin E increased vitamin A uptake and storage (18, 19), which could contribute to an accumulation of hepatic vitamin A stores. Therefore, we examined the relationships of supplemental and total (dietary plus supplemental) vitamin A and supplemental vitamin E intake on fasting plasma retinyl esters, an indicator of vitamin A overload (20), as well as retinol, retinol-binding protein (RBP), carotene, $\alpha$-tocopherol, and cholesterol among healthy elderly people and young adult volunteers. We also determined the relationship of fasting plasma retinyl esters with hematologic indicators of liver damage.

## Subjects and methods

### *Elderly sample*

Seven hundred and forty-six noninstitutionalized volunteers (aged 60–98 y) participated in the Nutritional Status Survey (NSS) of elderly people living in the greater Boston, MA area. The study was conducted by the US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston. The survey methodology, including a description of recruitment guidelines, exclusion criteria, and dietary and supplement nutrient intakes, is described in detail elsewhere (17, 21). A review of dietary and supplement intake determinations, as it pertains to the present study, is presented below. Informed written consent was obtained from all volunteers under the guidelines established by the Human Investigation Review Committee of Tufts University and the New England Medical Center. Of the 746 subjects the following were excluded from the study: 55 who had incomplete food records, 23 who had no retinol and/or $\alpha$-tocopherol plasma values, and 106 who were current users of tobacco products. Data from the remaining 562 volunteers (181 males, 381 females) form the basis of this report.

The NSS required two appointments. At the first appointment an overnight fasting blood sample was drawn. Instructions were then given to each volunteer, singly or in groups, for carefully collecting a 3-d record of all foods, beverages, medications, and nutritional supplements consumed. During the second appointment the food diary was reviewed by a dietitian for accuracy, completeness, and clarity. A nurse practitioner then conducted an interview and a physical examination and collected detailed information regarding frequency and duration of medication and nutrient supplement usage. Information was recorded directly from bottle labels.

Food records were coded and analyzed with the Nutrient Data Bank at the University of Massachusetts, Amherst. Although the present convention is to indicate vitamin A intake in retinol equivalents (13, 14), dietary vitamin A in the data bank was expressed as IU and includes preformed vitamin A as well as carotenoids with provitamin A activity. Because preformed vitamin A and provitamin A carotenoids were not indicated separately in the data bank, a direct conversion to retinol equivalents was not possible. For accuracy, therefore, all vitamin A intakes, including supplemental vitamin A, was expressed as IU/d. Total vitamin A intake was calculated as dietary plus supplemental vitamin A. Supplemental vitamin E was expressed as mg/d. Dietary vitamin E in the data bank is considered limited and was not used in this study. Users were those who used vitamin A and/or vitamin E supplements within the past year and nonusers were those who used neither vitamin A nor vitamin E supplements for at least 1 y. Nonusers may include users of nutrient supplements other than vitamin A or vitamin E.

### *Young adult reference sample*

To evaluate the biochemical data from the laboratory, data from healthy young adult subjects (aged 19–59 y) were obtained as a reference. The Young Adult Reference (YAR) study was not a survey as was the NSS and thus the recruitment guidelines varied slightly from the NSS. The YAR group was selected on the basis of age, sex, and nutrient-supplement usage. The YAR group was recruited by posting announcements at area schools and businesses and consisted primarily of personnel and students from Tufts University and the New England Medical Center, Boston. Efforts were directed toward recruiting 100 users of nutrient supplements and 100 nonusers, equally distributed by sex among age-decade groups.

An initial prescreening interview was conducted on interested young adult volunteers to obtain information on general health, medical history, and current usage of nutrient supplements. From the prescreening interview, subjects with any major disease, such as diabetes, chronic renal or liver failure, malabsorption, untreated hyperthyroidism or hypothyroidism, malignant neoplasm, chronic untreated inflammatory or infectious disease, untreated psychiatric illness or dementia, or any generalized metabolic disorder were excluded from the study. Subjects with a history of alcoholism or alcohol abuse (> 60 mL ethanol/d or > 2 beers/d) were excluded. Subjects who reported any of the following within the preceding 3 mo were excluded: surgery or use of any tobacco products (cigarettes, cigars, or pipe), prescriptive medications (including oral contraceptives), or over-the-counter medications regularly (at least three times per week). Women who were pregnant or lactating were also excluded.

Within 2 wk of the prescreening interview, an appointment with a nurse practitioner was scheduled. At this time an informed written consent was obtained from each young adult volunteer under the guidelines established by the Human Investigation Review Committee of Tufts University and the New England Medical Center. After a brief physical examination an overnight fasting blood sample was drawn for tests, which included hematologic, clinical, diagnostic, vitamin, mineral, lipid, and protein analyses. Before phlebotomy, subjects consumed no ethanol for 24 h and no over-the-counter medications for 3 d.

Detailed information regarding supplement usage of specific nutrients was also collected by the nurse practitioner during the scheduled visit. A questionnaire directed at current usage of nutrient supplements (including brand name, dosage, and frequency) was administered. Supplements were coded and analyzed for nutrient content with an expanded product content file based on the Drug Product Information File (22), which was also used in the NSS (17). From this information the mean daily intake for supplemental vitamin A and vitamin E was determined and expressed as IU/d and mg/d, respectively. Dietary data were not collected for the YAR subjects. Users were

those who used vitamin A and/or vitamin E supplements at least three times per week regularly for at least 3 mo and who used the last supplement within 1 wk of admission into the study. Nonusers were those who did not use any vitamin A or vitamin E supplements for at least 1 y. Nonusers may include users of nutrient supplements other than vitamin A or vitamin E.

Of 575 young adult subjects who underwent a prescreening interview, 200 subjects (100 nonusers and 100 users of nutrient supplements) were accepted into the study. Of the 200 subjects, 6 had no retinol and/or $\alpha$-tocopherol plasma values. The data from the remaining 194 subjects (96 males, 98 females) form the basis of the YAR group.

*Laboratory analysis*

Laboratory procedures were comparable for the NSS and YAR studies. Fasting (overnight) venous blood specimens were collected in tubes containing EDTA. They were immediately protected from light, placed on ice, and centrifuged at $1000 \times g$ for 15 min within 4 h to separate the plasma. A clinical blood-chemistry profile was conducted on all blood and included the following liver function tests: aspartate aminotransferase (AST); alanine aminotransferase (ALT); direct, indirect, and total bilirubin; alkaline phosphatase; and lactate dehydrogenase (LDH). The concentrations of retinol, RBP, retinyl esters, carotene, $\alpha$-tocopherol, and cholesterol in plasma were measured and the $\alpha$-tocopherol:cholesterol ratio (expressed as $\mu$mol:mmol) was determined and are henceforth referred to as the plasma nutritional indicators. Plasma retinol and $\alpha$-tocopherol were measured by reverse-phase high-performance liquid chromatography (HPLC) as described by Bieri et al (23). Plasma RBP was measured by a commercially available radial immunodiffusion kit (Behring Diagnostics, La Jolla, CA) (24). Total plasma carotene was measured by a standard spectrophotometric method (25). Plasma cholesterol was determined using a commercially available enzymatic procedure (Sigma Diagnostics, St Louis, MO) (26). In a subgroup of 158 elderly volunteers and in 174 young adult volunteers, total plasma retinyl esters were measured by normal-phase HPLC as described by Bankson et al (27). This method quantitates all the major plasma retinyl esters (ie, retinyl palmitate, stearate, and oleate) as a single peak in the chromatogram. Retinyl esters were expressed as nmol/L (1 nmol = 525 ng). The sample sizes for RBP, carotene, and retinyl esters were smaller than the overall sample because these assays were not standardized at the beginning of recruitment and plasma was not saved. There was no obvious selection bias except that the values represent plasma from the most recently recruited subjects.

*Statistical analysis*

Logarithmic transformations were applied to measures of vitamin A and vitamin E intake and to plasma levels of retinyl esters, $\alpha$-tocopherol, and cholesterol; square root transformations were applied to plasma levels of retinol, RBP, and carotene to normalize the distributions of these variables. Nutrient intakes were compared by sex using Student's $t$ test. The relationship of vitamin A and/or vitamin E intake, sex, and the plasma nutritional indicators were determined by partial correlation analysis and by one- and two-way analysis of variance. The relationship of abnormal hematologic indicators of liver function and elevated fasting retinyl esters were compared by chi-square analysis. The Scheffe test was used for all multiple comparison procedures (28). The probability level of $p < 0.05$ was set for statistical significance.

TABLE 1
Intake of vitamin A and vitamin E for elderly and young adult subjects

| | $n$ | $\bar{x} \pm$ SEM | Median |
|---|---|---|---|
| Elderly subjects | | | |
| Supplemental vitamin A (IU/d) | | | |
| Males | 56 | 8 454 ± 1 172 | 6 667 |
| Females | 145 | 7 310 ± 524 | 5 000 |
| Total vitamin A (IU/d) | | | |
| Males | 181 | 10 490 ± 674 | 8 317 |
| Females | 381 | 10 624 ± 402 | 8 380 |
| Supplemental vitamin E (mg/d) | | | |
| Males | 61 | 178 ± 28 | 27 |
| Females | 165 | 140 ± 17 | 27 |
| Young adults | | | |
| Supplemental vitamin A (IU/d) | | | |
| Males | 37 | 11 972 ± 2 100 | 8 571 |
| Females | 42 | 10 187 ± 2 396 | 5 000 |
| Supplemental vitamin E (mg/d) | | | |
| Males | 38 | 234 ± 90 | 27 |
| Females | 45 | 147 ± 27 | 27 |

## Results

Supplemental and total vitamin A and supplemental vitamin E intake for the entire NSS study was presented elsewhere (17, 21). However, because the NSS sample in the present study excludes current smokers, intakes for the present NSS group as well as supplemental vitamin A and vitamin E for the YAR group are shown in **Table 1.** Mean daily intakes did not differ significantly between males and females for either elderly or young adult subjects.

Sixty-five (36%) of the elderly male and 170 (45%) of the elderly female nonsmokers were users of supplemental vitamin A and/or vitamin E within the past year. Of these, 4 males and 5 females were users of only supplemental vitamin A and 9 males and 25 females were users of only supplemental vitamin E. Thirty-nine (41%) young adult males and 46 (47%) young adult females were users of supplemental vitamin A and/or vitamin E within the past year. Of these, one male and one female were users of only supplemental vitamin A and two males and four females were users of only supplemental vitamin E. Thus, of the supplement users 79% of the elderly subjects and 91% of young adults were using both supplemental vitamin A and vitamin E, largely as part of a multivitamin preparation.

**Tables 2 and 3** show the mean, SEM, and median for the plasma nutritional indicators for elderly and young

TABLE 2
Plasma nutritional indicators for elderly subjects, by sex and vitamin supplement use

| Elderly subjects | Nonusers | | | Users | | |
|---|---|---|---|---|---|---|
| | *n* | $\bar{x} \pm$ SEM | Median | *n* | $\bar{x} \pm$ SEM | Median |
| Males | | | | | | |
| Retinol (μmol/L) | 116 | 2.52 ± 0.06 | 2.44 | 65 | 2.56 ± 0.08 | 2.51 |
| RBP (μmol/L) | 91 | 2.92 ± 0.07 | 2.81 | 45 | 2.80 ± 0.11 | 2.67 |
| Retinyl esters (nmol/L)* | 28 | 73 ± 13 | 57 | 21 | 108 ± 32 | 76 |
| Carotene (μmol/L) | 89 | 2.60 ± 0.12 | 2.37 | 44 | 2.43 ± 0.11 | 2.31 |
| α-Tocopherol (μmol/L)*† | 116 | 26.7 ± 0.8 | 23.9 | 65 | 37.2 ± 1.9 | 32.7 |
| Cholesterol (mmol/L)† | 115 | 5.5 ± 0.1 | 5.4 | 64 | 5.7 ± 0.1 | 5.5 |
| α-Tocopherol:cholesterol (μmol:mmol)* | 115 | 4.7 ± 0.1 | 4.5 | 64 | 6.7 ± 0.3 | 6.0 |
| Females | | | | | | |
| Retinol (μmol/L) | 211 | 2.45 ± 0.04 | 2.35 | 170 | 2.50 ± 0.05 | 2.44 |
| RBP (μmol/L) | 191 | 2.83 ± 0.05 | 2.76 | 152 | 2.77 ± 0.05 | 2.76 |
| Retinyl esters (nmol/L)* | 53 | 71 ± 8 | 57 | 56 | 157 ± 22 | 95 |
| Carotene (μmol/L) | 148 | 2.61 ± 0.08 | 2.49 | 121 | 2.68 ± 0.09 | 2.65 |
| α-Tocopherol (μmol/L)*† | 211 | 29.5 ± 0.7 | 27.6 | 170 | 38.5 ± 1.1 | 36.8 |
| Cholesterol (mmol/L)† | 210 | 6.1 ± 0.1 | 6.0 | 170 | 5.9 ± 0.1 | 5.9 |
| α-Tocopherol:cholesterol (μmol:mmol)* | 210 | 4.9 ± 0.1 | 4.6 | 170 | 6.6 ± 0.2 | 6.1 |

* Nonusers significantly different from users, $p < 0.001$.
† Males significantly different from females, $p < 0.001$.

adult subjects, respectively, by sex and vitamin supplement usage. For the elderly subjects (Table 2) plasma α-tocopherol and cholesterol were significantly greater in females than in males ($p < 0.001$ for each) but we failed to find a difference by sex for the α-tocopherol:cholesterol ratio. Plasma retinyl esters, α-tocopherol, and the α-tocopherol:cholesterol ratio ($p < 0.001$ for each) were all significantly greater in elderly users than in nonusers. Plasma retinol was not significantly related to sex or supplement use for the elderly subjects; none had plasma retinol values < 0.70 μmol/L. For the young adult subjects (Table 3) plasma retinol and RBP were significantly lower and plasma carotene was significantly higher in females than in males ($p < 0.01$ for each). Plasma retinyl esters ($p < 0.05$), carotene ($p < 0.01$), α-tocopherol ($p < 0.001$), and the α-tocopherol:cholesterol ratio ($p < 0.001$) were all significantly greater in young adult users than in nonusers. There were no statistically significant interactions between sex and supplement use for any of the plasma nutritional indicators in Tables 2 and 3.

A reference range for fasting plasma retinyl esters was determined. Fasting retinyl ester values below the limits of detection were considered normal; thus, a reference range was based on the 95th percentile rather than the 2.5th and 97.5th percentile. For nonusers of supplemental vitamin A and/or vitamin E, the 95th percentile for fasting plasma retinyl esters was 170 nmol/L for the elderly subjects and 130 nmol/L for young adults. The 95th percentile for the fraction of total vitamin A (retinol plus retinyl ester) circulating as retinyl ester was 13% for the elderly subjects and 11% for young adults.

In **Table 4** partial correlation analysis was conducted on supplement users to determine the relationships of supplemental vitamin A or vitamin E to the plasma nutritional indicators. When supplemental vitamin E and sex were controlled for, no statistically significant correlation between supplemental vitamin A and plasma retinol or RBP for either elderly subjects or young adults was found. There was a significant positive correlation between supplemental vitamin A and plasma retinyl esters in the elderly subjects ($p < 0.01$) but not in young adults ($p > 0.05$). When supplemental vitamin A and sex were controlled for, strong positive correlations ($p < 0.001$ for all cases) between supplemental vitamin E and both α-tocopherol and the α-tocopherol:cholesterol ratio was found for both elderly and young adult subjects. For the young adult group, supplemental vitamin E also demonstrated a significant positive correlation with plasma retinyl esters and cholesterol ($p < 0.05$ for each).

We also examined the relationships between total vitamin A intake and plasma nutritional indicators for the elderly subjects with partial correlations (data not shown). When supplemental vitamin E intake and sex were controlled for, plasma carotene demonstrated a significant relationship with total vitamin A intake (partial $r = 0.24$, $p < 0.001$). Total vitamin A includes dietary carotenoids with provitamin A activity. There was no statistically significant correlation between total vitamin A intake and plasma retinyl esters.

**Figure 1** demonstrates a dose response relationship between daily intakes of supplemental vitamin A and fasting plasma retinyl esters. For the elderly subjects the fasting plasma retinyl ester level in nonusers (group I) was 72 ± 14 nmol/L and was significantly increased ($p < 0.05$) to 182 ± 50 nmol/L in users of 5001–10 000 IU/

TABLE 3
Plasma nutritional indicators in young adults, by sex and vitamin supplement use

| Young adults | Nonusers | | | Users | | |
|---|---|---|---|---|---|---|
| | *n* | $\bar{x} \pm$ SEM | Median | *n* | $\bar{x} \pm$ SEM | Median |
| Males | | | | | | |
| Retinol (μmol/L)* | 57 | 2.50 ± 0.11 | 2.37 | 39 | 2.33 ± 0.13 | 2.14 |
| RBP (μmol/L)* | 55 | 2.57 ± 0.08 | 2.57 | 39 | 2.63 ± 0.11 | 2.52 |
| Retinyl esters (nmol/L)† | 49 | 62 ± 14 | 57 | 36 | 81 ± 10 | 76 |
| Carotene (μmol/L)*‡ | 54 | 2.42 ± 0.10 | 2.29 | 39 | 2.99 ± 0.12 | 3.00 |
| α-Tocopherol (μmol/L)§ | 57 | 21.1 ± 1.0 | 21.6 | 39 | 28.6 ± 1.8 | 27.2 |
| Cholesterol (mmol/L) | 57 | 4.9 ± 0.1 | 4.8 | 39 | 4.8 ± 0.1 | 4.8 |
| α-Tocopherol:cholesterol (μmol:mmol)§ | 57 | 4.3 ± 0.2 | 4.4 | 39 | 5.9 ± 0.3 | 5.7 |
| Females | | | | | | |
| Retinol (μmol/L)* | 52 | 2.08 ± 0.08 | 1.95 | 46 | 2.22 ± 0.09 | 2.01 |
| RBP (μmol/L)* | 52 | 2.35 ± 0.07 | 2.24 | 46 | 2.34 ± 0.08 | 2.31 |
| Retinyl esters (nmol/L)† | 48 | 57 ± 7 | 57 | 41 | 73 ± 12 | 57 |
| Carotene (μmol/L)*‡ | 50 | 3.03 ± 0.13 | 2.93 | 46 | 3.40 ± 0.18 | 3.40 |
| α-Tocopherol (μmol/L)§ | 52 | 21.6 ± 1.2 | 21.2 | 46 | 33.1 ± 2.1 | 32.9 |
| Cholesterol (mmol/L) | 52 | 5.1 ± 0.1 | 5.0 | 46 | 5.1 ± 0.1 | 5.0 |
| α-Tocopherol:cholesterol (μmol:mmol)§ | 52 | 4.3 ± 0.2 | 4.1 | 46 | 6.6 ± 0.4 | 6.2 |

* Males significantly different from females, $p < 0.01$.
† Nonusers significantly different from users, $p < 0.05$.
‡ Nonusers significantly different from users, $p < 0.01$.
§ Nonusers significantly different from users, $p < 0.001$.

d (group III) and to 221 ± 65 nmol/L in users of > 10 000 IU/d (group IV). For young adults the fasting plasma retinyl ester level was 58 ± 7 nmol/L in nonusers (group I) and 120 ± 24 nmol/L in users of > 10 000 IU/d (group IV) ($p < 0.05$). In contrast to the elderly subjects, mean plasma retinyl esters for young adult users of 5001–10 000 IU/d (group III) were similar to young adult nonusers (group I).

For the elderly subjects but not for young adults, information on duration of supplement use was available. **Figure 2** shows the mean ± SEM fasting plasma retinyl ester levels for the elderly subjects, by daily supplemental vitamin A intake and duration of use. Each group was separated by a duration of use of < 5 y or ≥ 5 y. For those elderly subjects using vitamin A for < 5 y, mean retinyl ester values were similar among the four groups of supplemental vitamin A intakes. However, note that for users of > 10 000 IU/d there were only three individuals who used vitamin A supplements for < 5 y. For those elderly subjects using vitamin A for ≥ 5 y the retinyl ester levels were 277 ± 102 nmol/L in users of 5001–10 000 IU/d (group III) and 283 ± 99 nmol/L in users of > 10 000 IU/d (group IV). These values were both significantly different ($p < 0.05$) from those in nonusers (group I).

There were five elderly subjects and two young adults (all were users of supplemental vitamin A and vitamin E) with plasma retinyl esters ≥ 380 nmol/L. Chi-square analysis (with Yates correction) indicated that the prevalence of abnormally high AST activity (≥ 34 U/L) in plasma was significantly greater for the five elderly subjects (80%) with plasma retinyl esters ≥ 380 nmol/L than for the 147 elderly subjects (10%) with plasma retinyl esters < 380 nmol/L. Two of the five elderly subjects (40%) also had elevated ALT activity (≥ 39 U/L) ($p > 0.05$); the remaining liver function tests (direct, indirect, and total bilirubin, alkaline phosphatase, and LDH) in these individuals were all normal. For the two young adult subjects with plasma retinyl esters ≥ 380 nmol/L, all hematologic liver function tests were normal. Except for general antacid use, only one of the five elderly subjects with retinyl esters ≥ 380 nmol/L was taking any other medication.

## Discussion

Our findings confirm in elderly subjects what has been reported for young adults, with a few notable exceptions. For young adult subjects but not for the elderly, plasma retinol was significantly lower in women than in men. These data concur with previous findings for young adults (29–31). Failure to detect differences in plasma retinol by sex for the elderly subjects is supported by data from the Ten State Nutrition Survey and HANES I and II, which show that the difference in plasma vitamin A values between males and females are reduced with aging (4–6). For example, in HANES I the difference in mean plasma vitamin A values between males and females is 0.38 μmol/L for those aged 18–44 y and 0.23 μmol/L for those aged 45–74 y (6). Yearick et al (9) also demonstrated no difference in plasma vitamin A values between

TABLE 4
Partial correlations between supplement intake and plasma nutritional indicators

| Plasma nutritional indicator | Supplemental vitamin A intake, adjusted for supplemental vitamin E intake and sex* | | Supplemental vitamin E intake, adjusted for supplemental vitamin A intake and sex† | |
|---|---|---|---|---|
| | Partial *r* | *n* | Partial *r* | *n* |
| Elderly subjects | | | | |
| Retinol | −0.02 | 201 | 0.05 | 226 |
| RBP | 0.04 | 169 | −0.05 | 189 |
| Retinyl esters | 0.32‡ | 65 | 0.16 | 75 |
| Carotene | −0.13 | 135 | −0.01 | 159 |
| α-Tocopherol | −0.06 | 199 | 0.51§ | 226 |
| Cholesterol | −0.11 | 198 | 0.07 | 225 |
| α-Tocopherol:cholesterol | 0.05 | 198 | 0.50§ | 225 |
| Young adults | | | | |
| Retinol | −0.06 | 79 | −0.07 | 83 |
| RBP | −0.12 | 79 | −0.01 | 83 |
| Retinyl esters | 0.19 | 72 | 0.28‖ | 75 |
| Carotene | 0.10 | 79 | 0.13 | 83 |
| α-Tocopherol | 0.04 | 79 | 0.60§ | 83 |
| Cholesterol | 0.15 | 79 | 0.26‖ | 83 |
| α-Tocopherol:cholesterol | 0.04 | 79 | 0.49§ | 83 |

* Nonusers of supplemental vitamin A excluded.
† Nonusers of supplemental vitamin E excluded.
‡ $p < 0.01$.
§ $p < 0.001$.
‖ $p < 0.05$.

elderly men and women aged 63–96 y. Our data also support the previous finding that plasma retinol is unrelated to vitamin A intake (29, 31–33) although blinded intervention trials demonstrated a slight but significant rise in plasma retinol with oral vitamin A (34, 35).

For elderly subjects plasma carotene increased significantly with increasing total vitamin A intake, which includes provitamin A carotenoids. Our data for the elderly subjects confirm the previous findings both in cross-sectional studies (32, 36) and in intervention studies (33, 37) that plasma carotene levels are related to carotene intake.

For our young adult subjects, plasma carotene was significantly greater in women than in men. Although some reports failed to detect a difference in mean carotene values between young adult men and women (30, 32), in 362 young adults living in France, Herboth et al (31) observed significantly greater plasma carotene levels in women than in men. The differences for plasma carotene by sex may be true physiologic differences between males and females resulting from hormonal differences or may reflect greater consumption of foods rich in carotene content by young adult females than by males.

We found strong positive correlations between supplemental vitamin E intake and both the plasma α-tocopherol and the α-tocopherol:cholesterol ratio for both elderly and young adult subjects. These data confirm the previous finding that vitamin E supplements increase plasma vitamin E levels (37–40).

A new finding with possible clinical relevance is that supplemental vitamin A is associated with greater levels of circulating retinyl esters in fasting blood. The data suggest that fasting plasma retinyl esters are more sensitive to supplemental vitamin A for the elderly subjects than for young adults. For example, moderate daily vitamin A supplementation of 5001–10 000 IU/d for males is associated with an approximate 2.5-fold increase from nonusers in fasting plasma retinyl ester levels for the elderly subjects, whereas, there is only a modest increase (1.5-fold) in plasma retinyl esters (Fig 1) for young adults. Long-term use of vitamin A supplements (≥ 5 y) appears to be an important determinant for greater fasting plasma retinyl ester values among the elderly subjects (Fig 2). Finally, evidence for liver damage is demonstrated by a greater prevalence of elevated AST activity in the plasma of elderly subjects with fasting plasma retinyl esters ≥ 380 nmol/L than in subjects with retinyl esters < 380 nmol/L.

The physiological determinant for greater fasting plasma retinyl esters among vitamin A supplement users is unknown. In the few hours after vitamin A ingestion and in hypervitaminosis A, plasma retinyl esters are increased. In the postabsorptive state after vitamin A ingestion, retinyl esters in triglyceride-rich lipoproteins represent the transport of vitamin A from intestine to liver (41). In hypervitaminosis A, fasting plasma retinyl esters

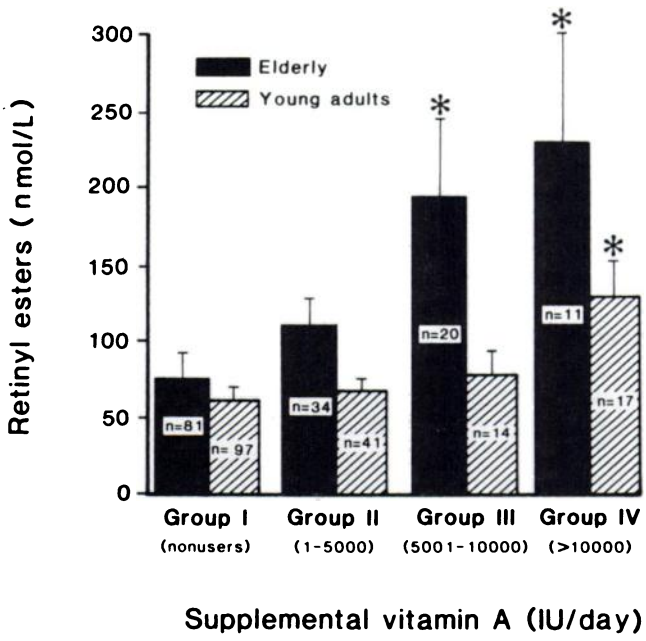


FIG 1. Mean ± SEM fasting plasma retinyl ester values by daily supplemental vitamin A intake for elderly subjects and young adults. *Significantly different from group I by the Scheffe test, $p < 0.05$.

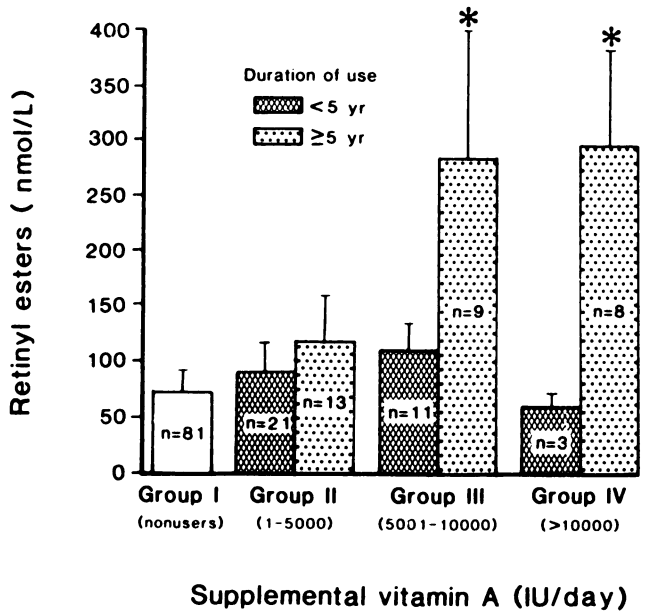


FIG 2. Mean ± SEM fasting plasma retinyl ester values by daily supplemental vitamin A intake and duration of use for the elderly subjects. *Significantly different from group I by the Scheffe test, $p < 0.05$.

are elevated representing > 30% of circulating vitamin A (20) and may result when intake of the vitamin exceeds the capacity of the liver to remove it from the circulation and store it at a sufficient rate. Alternatively, retinyl esters may be released directly from the liver in hypervitaminosis A but no data to support either hypothesis have been reported.

The retinyl ester values observed in our samples of vitamin A supplement users are not consistent with the elevated levels observed in clinical hypervitaminosis A. For example, Smith and Goodman (20) reported two cases of hypervitaminosis A with plasma retinyl esters of 3715 nmol/L and 4763 nmol/L, and Ellis et al (42) reported a case of malnutrition, hyperlipoproteinemia, and hypervitaminosis A with a plasma retinyl ester level of 15 773 nmol/L. It is known that hepatic inflammation and fibrosis are common and that the usual hematologic indicators of liver function, particularly AST and ALT activity, may be abnormal in hypervitaminosis A (20, 42–45). We demonstrate that elderly subjects with fasting plasma retinyl esters ≥ 380 nmol/L have biochemical evidence for liver damage (ie, elevated AST). It is possible that the elevated plasma AST activity reflects enzyme induction by drugs; however, four of the five elderly individuals with retinyl esters ≥ 380 nmol/L were taking no medications other than antacids. It is also possible that vitamin A itself induces liver enzymes; however, large oral doses of vitamin A had no effect on hepatic enzyme activity in rats (46). Therefore, our data suggest that fasting plasma retinyl esters ≥ 380 nmol/L may be an early indicator of liver damage. Whether the liver damage is due to vitamin A overload is not known as yet.

The lack of correlation between total vitamin A intake and plasma retinyl esters may represent differences in consumption and bioavailability of vitamin A from diet vs supplements. One difference between dietary and supplemental vitamin A is that dietary vitamin A is ingested throughout the day at meals whereas supplemental vitamin A is usually taken as a single dose of preformed vitamin A in a multivitamin. Further, the vitamin supplement generally contains more vitamin A than any single meal. A second difference is that the determination of total vitamin A includes dietary provitamin A carotenoids, which are less bioavailable than the preformed vitamin A commonly found in supplements. These differences may account for the different relationships between supplemental and total vitamin A and fasting plasma retinyl esters in the elderly subjects.

There are no known previous studies that examined plasma retinyl esters in a relatively large sample of healthy individuals. In a smaller study involving only 14 young fasting control subjects (aged 3–28 y), plasma retinyl esters were separated from retinol by alumina column chromatography and measured fluorometrically (20). In these subjects plasma retinyl esters represented < 5% of total circulating vitamin A. Using a new HPLC technique that quantitates all plasma retinyl esters together (27), we found that in elderly and young adult nonusers of vitamin A-containing supplements, retinyl esters represent up to 13 and 11% of total circulating vitamin A, respectively, which corresponds to a reference range for plasma retinyl esters of up to 170 nmol/L for the elderly subjects and 130 nmol/L for young adults. Our data confirm the previous finding (20) that retinyl esters represent a minor fraction of total circulating vitamin A in fasting blood. The slightly greater plasma retinyl ester fraction in our group of elderly subjects and young adults may reflect the different age range for the study samples and/or the different methodologies for measuring retinyl esters.

Supplemental vitamin E showed a positive correlation with plasma retinyl esters among young adult users of supplemental vitamin E (Table 4). Because most young adult users of supplemental vitamin E were also users of supplemental vitamin A (91%), it is possible that the observed relationship between supplemental vitamin E and plasma retinyl esters is confounded by the concomitant intake of supplemental vitamin A. Alternatively, vitamin E may increase vitamin A absorption and storage. In animals, liver stores of vitamin A are diminished during vitamin E deficiency and are increased during α-tocopherol supplementation (18, 19, 47, 48). Kusin et al (49) demonstrated that after administration of 200 000 IU of retinyl acetate (with radioactive tracer) in children, 500 mg of α-tocopherol reduced the total amount of radioactivity recovered in the stools over a 4-d collection (from control subjects receiving no vitamin E), suggesting that vitamin E increases vitamin A absorption. Napoli et al (50) demonstrated that vitamin E decreases the activity of hepatic retinyl ester hydrolase, the enzyme re-

sponsible for mobilization of stored vitamin A, suggesting a possible mechanism for increased vitamin A storage by vitamin E. Further interpretation of these data must await confirmation from intervention and/or biochemical studies.

Our study suggests that elderly people should limit their intake of supplemental vitamin A particularly over the long term. Our data may also indirectly support a lowering of the RDA for vitamin A. It was suggested that hepatic stores of vitamin A are maintained with lower intakes of the vitamin (14). Daily intakes throughout life of vitamin A at RDA levels may result in an accumulation of hepatic vitamin A stores over time so that the capacity to store vitamin A in later life is reduced. Consequently, the margin of safety for vitamin A intake may be decreased in elderly people predisposing them to hypervitaminosis A.

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