Nutritional Status of Antioxidant Vitamins (A, E, and Beta-Carotene) in Elderly Japanese

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Summary  The nutritional status with respect to vitamins A and E, and beta-carotene was examined in elderly Japanese subjects in two institutions at Osaka and Kyoto. Only the plasma vitamin E level has been determined in the majority of previous investigations. In this study, vitamin E levels were determined in red blood cells (RBCs), platelets (PLT), mononuclear cells (MN), polymorphonuclear cells (PMN), and buccal mucosal cells (BMC), using HPLC with electrochemical detection. Alpha-tocopherol levels in plasma and RBCs did not differ between elderly and young adults, while those in PLT, MN, and BMC were lower in the elderly. Thus, the vitamin E status of elderly Japanese individuals appears to be inadequate of the cellular levels. The daily vitamin E intake of the elderly subjects was below the recommended dietary allowance for the Japanese population. Plasma levels of retinol and beta-carotene were also assessed. The vitamin A status did not differ between elderly and young adults on the basis of the levels of retinol and beta-carotene, which was more prominent in the young adults and became smaller in the elderly. This sex difference was partly attributable to a difference in plasma total lipids. No clear age-related trend was noted.

Key Words  elderly human, retinol, retinol binding protein, beta-carotene, alpha-tocopherol, plasma, red blood cells, platelets, white blood cells, buccal mucosal cells
In view of the rapid increase of elderly individuals in the Japanese population, maintaining health and function for as long as possible has become an increasingly important national concern. A major factor in health maintenance and prevention of diseases related to aging is appropriate and adequate nutrition. There has been great interest in the possibility that, together with ascorbate, fat-soluble antioxidant vitamins (retinol, beta-carotene, and alpha-tocopherol) may also play a role in scavenging free radicals. The production of free radicals is thought to be related to diseases common in the elderly, such as cancer, atherosclerosis, and ischemic disorders (1-5). In this study, we investigated the elderly individuals in two institutions located in Osaka and Kyoto prefectures in order to assess the nutritional status of elderly Japanese with respect to antioxidant vitamins.

MATERIALS AND METHODS

Subjects

Table 1 shows the sex and age profile of the subjects and of the young adults used as controls examined in the two institutions. None of the subjects were taking any vitamin preparations. The characteristics of the subjects differed between the two institutions. The subjects in the Osaka institution were reasonably healthy, and ate well, although they had mild dementia. By contrast, the Kyoto institution included patients with cerebral atherosclerosis or stroke as one third of the population and their dietary habits were variable. The young adult controls were volunteers from among our laboratory personnel. The study protocol was approved by the ethics committees of the hospitals and institutions concerned, and the study was only performed after informed consent was obtained.

Sample collection. Blood specimens and buccal mucosal cells (BMCs) were collected from the subjects in the early morning before breakfast. BMCs were collected by scraping the buccal mucosa with a spatula, as described previously (6).

Assays. The alpha-tocopherol level in plasma, red blood cells (RBCs),

Table 1. Mean age and number of the subjects of each sex.

|                | Male age (n) | Female age (n) |
|----------------|--------------|----------------|
| Young adults   |              |                |
| Elderly        | 22±1 yrs (30) | 24±3 yrs (17)  |
| Osaka          | 78±10 yrs (21)| 78±8 yrs (52)  |
| Kyoto          | 78±6 yrs (9)  | 80±8 yrs (10)  |
| M±SD           |              |                |

J. Nutr. Sci. Vitaminol.
Platelet levels (PLTs), polymorphonuclear white blood cells (PMNs) and mononuclear white cells (MNs) was measured using HPLC with electrochemical detection and HPLC analysis after the separation of each specimen by the procedure shown in Fig.1.

Plasma levels of vitamin A (retinol) and beta-carotene were separately assayed using HPLC (7, 8). Retinol binding protein was assayed by the routine quantitative immunodiffusion method (9).

RESULTS AND DISCUSSION

Vitamin E

The plasma level of vitamin E was found to increase gradually with age up to the seventh decade in several earlier studies (10, 11). However, other studies have failed to show any relationship of age to plasma vitamin E levels (17), and some studies have even demonstrated a decline in vitamin E levels after the age of 65 (12-15). Plasma tocopherol generally depends on the plasma lipid level, so these changes of vitamin E may be related to changes of plasma total lipids in the elderly. Horwitt (16) has suggested that it is more accurate to express the plasma vitamin E level relative to the plasma total lipids. In a study by Vatassery et al. (17), no relationship was found between the vitamin E/lipid ratio and age, although they reported an age-related decrease in platelet tocopherol levels.

There is also controversy as to whether a change in the vitamin E concentration takes place in other tissues with aging. Therefore, we studied the tocopherol levels in various circulating blood cells, including RBCs, leukocytes and platelets. In addition, buccal mucosal cells were also investigated, because the behavior of tocopherol in these cells differs from that in plasma, as described...
Fig. 2. Alpha-tocopherol levels in plasma, blood cells (RBC, PLT, MN and PMN), and BMCs in elderly and young adults. RBC, red blood cells; PLT, platelets; MN, mononuclear cells; PMN, polymorphonuclear cells; BMC, buccal mucosal cells.

in our previous report (18). Traber and Kayden (19) have previously investigated the significance of LDL tocopherol in the delivery of tocopherol to tissue cells via the LDL receptor. Among the cells examined this time, RBC and platelets have no LDL receptors, while the other cells express them. It may be more meaningful to examine cells both with and without LDL receptors when assessing the vitamin E status.

Figure 2 shows the differences in the vitamin E status between the young and elderly subjects, with stratification for the two institutions and for sex. No age-related difference in plasma alpha-tocopherol levels was noted. There was a slight reduction of the level in RBCs and buccal mucosal cells and a fall of the plasma tocopherol to lipid ratio in the elderly, but no significant differences were noted. However, the platelet and monocyte alpha-tocopherol levels were significantly lower in the elderly (p<0.05). The plasma total lipid level rose slightly with age, but this change was not significant. The vitamin E content of polymorphonuclear cells could not be determined, because these cells proved difficult to prepare adequately by our separation technique. Gamma-tocopherol levels in the majority of the specimens were also reduced in the elderly (data not
Table 2. Dietary intake of vitamins A and E in the two institutions.

| Institution | alpha-tocopherol (mg/day/person) | alpha-tocopherol equivalent (total tocopherol) | vitamin A: retinol equivalent (I.U./day/person) |
|-------------|---------------------------------|-----------------------------------------------|-----------------------------------------------|
| Osaka       | 6.25±1.54                       | 6.71±1.74                                     | 2,068±673                                     |
| Kyoto       | 5.72±1.42                       | 6.00±1.45                                     | 2,383±1,117                                   |

M±SD

These findings may be related to a poorer food intake in the elderly than in the young. Alpha-tocopherol levels in plasma, RBCs, and buccal mucosal cells in the elderly, and in the monocytes in the young adults, were higher in females than in males ($p < 0.05$). The higher plasma alpha-tocopherol level in elderly females was probably due to their higher plasma lipid levels, because there was no sex difference in the tocopherol to lipid ratio. An institutional difference in addition to a sex difference was also noted. In general, alpha-tocopherol concentrations were lower in Kyoto than in Osaka, especially in the RBCs, monocytes, and buccal mucosal cells. With respect to gamma-tocopherol levels, there was no clear difference between the sexes, while the difference between institutions was similar to that for alpha-tocopherol levels.

The daily dietary intake of vitamins E and A was calculated from the food menu of both institutions over 7 days (Table 2). The daily intake of vitamin E was lower in both institutions than that recommended in Japan, and the level in Kyoto was lower than that in Osaka. However, the daily vitamin A intake was almost adequate in both institutions, so the deficiency of vitamin E seemed to reflect the poor dietary intake by our elderly subjects. The poor vitamin E status was especially noted with respect to platelet and monocyte alpha-tocopherol levels. Females generally had richer vitamin E stores than males.

**Vitamin A**

The plasma retinol level was almost the same in the young and elderly adults (Fig. 3). It was higher in young males than in young females, but this difference was not seen in the elderly. The molar ratio of retinol to retinol-binding protein did not change with age or between the sexes. Previous reports have given some conflicting results regarding vitamin A. One study showed an upward trend with aging (20), while another showed a slight decrease (21). Our study found no change in vitamin A with aging, perhaps because the dietary intake remained sufficient even in the elderly.

**Beta-carotene**

Reports on the serum carotene level in relation to age have also varied. Some studies found no age-related trend (22), some found the highest carotene level in subjects aged 60–70 years (21), and some found the lowest level in the oldest age group investigated (23). In our study, the plasma beta-carotene level did not...
change with aging in males, but it decreased in females. However, the plasma beta-carotene level was highest in the young females; and, even after decreasing with aging, it remained higher than that in both young and elderly males. Plasma beta-carotene is known to depend on the plasma lipid level to some extent (24). The higher carotene level in elderly females may be attributable to their higher plasma lipid levels, because there was no sex-related difference in the carotene to lipid ratio among the elderly subjects. However, the finding of the highest plasma carotene level in the young females could not be explained by plasma lipid changes.

Assessment of vitamin nutrition status in the elderly is complex and difficult, because of the effect of certain variables such as dietary habit, smoking, undetectable diseases, and medications.

Although our study was performed on only a small population, it suggested that nutritional problems exist among elderly Japanese with respect to antioxidant vitamins, especially vitamin E. A larger study is now required in the elderly to obtain more precise information which may help to prevent diseases.
related to aging.

We are grateful to the New Products Planning & Development Department Health Care Division in Takeda Chemical Industries, Ltd., Japan, for kind support.

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