The Relationship between Serum Vitamin E Level and Risk Factors for Arteriosclerosis in Japanese Postmenopausal Women

Yuka NAKATSU1, Shumpei NIIDA2, Kiyoshi TANAKA3, Shigeo TAKENAKA1 and Akiko KUWARABA1*

1 Department of Clinical Nutrition, Graduate School of Comprehensive Rehabilitation, Osaka Prefecture University, 3–7–30 Habikino, Habikino, Osaka 583–8555, Japan
2 Medical Genome Center, National Center for Geriatrics and Gerontology, Otsu 474–8511, Japan
3 Faculty of Nutrition, Kobe Gakuin University, Kobe 651–2180, Japan

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Summary Since vitamin E is one of the most potent antioxidant and anti-inflammatory agents, vitamin E can play a role against arteriosclerosis through various actions. Then, we have studied the relationship between serum vitamin E status and risk factors for arteriosclerosis in Japanese postmenopausal women. One hundred and seven subjects (70.0±7.7 y) were evaluated for vitamin E status by measuring serum α- and γ-tocopherol (αT and γT) levels. The number of arteriosclerosis risk factors was defined by the existence of high blood pressure, hyperglycemia, and dyslipidemia. Median serum αT and γT concentrations were 24.32 and 2.79 μmol/L, respectively. In none of the subjects, serum αT level was below the cutoff value (<12 μmol/L) for vitamin E deficiency which causes fragile erythrocyte and hemolysis. While no significant differences were found in serum levels of αT and γT between the groups categorized by the number of arteriosclerosis risks, serum levels of αT adjusted by serum total cholesterol (TC) and triglyceride (TG) decreased with an increasing number of arteriosclerotic risk factors (p=0.074). Serum αT level adjusted by serum TC and TG was also a negative significant predictor for the number of arteriosclerosis risk factors controlled by covariates associated with arteriosclerosis. The present study described that serum vitamin E level was positively associated with a lower number of arteriosclerotic risks, and its role for preventing noncommunicable diseases was suggested.

Key Words antioxidant, α-tocopherol, insufficiency, metabolic syndrome, oxidative stress, serum lipids adjustment

Cardiovascular diseases (CVD) belong to the noncommunicable diseases (NCDs) and are serious health problems in developed countries including Japan. Primary prevention of arteriosclerosis is an urgent concern since it is a significant risk for CVD. Oxidative damage and free radical production in the endothelium play essential roles in the pathogenesis of atherosclerotic process (1).

Vitamin E is one of the most potent anti-oxidant and anti-inflammatory agents by non-enzymatic mechanisms. Of the eight derivative forms of vitamin E, α-tocopherol (αT) is known to be most effective in various aspects. It is the most active anti-oxidant (2), and also reduces oxidative stress-induced apoptosis (3), downregulates the genes involved in lipid peroxidation and inflammation (4). Additionally, αT prevents foam cell formation in human monocyte-derived macrophages (5, 6), lipotoxicity in macrophages (7), and the release of pro-inflammatory cytokines (8). Therefore, vitamin E is a quite promising candidate against arteriosclerosis through these actions. In a previous observational study, serum vitamin E levels were significantly lower in subjects with metabolic syndrome (MS) which is a condition closely associated with arteriosclerotic changes (9). In another intervention study, hexadecuterium-labeled RRR-αT (d6-αT) was administered to the study subjects, and measurement was done for its plasma level and urinary excretion of α-carboxyethyl hydroxychromanol (α-CEHC) which is known to positively correlate with dietary αT and plasma αT concentrations. Subjects with MS had significantly lower concentrations of plasma d6-αT and urinary α-CEHC. From the above results, together with the fact that subjects with MS had higher levels of biomarkers for oxidative stress and inflammation, the authors concluded that vitamin E requirement is higher in subjects with MS than in healthy individuals (10). Based on these considerations, we have studied the relationship between vitamin E status and risk factors for arteriosclerosis in Japanese postmenopausal women.

MATERIALS AND METHODS

Subjects. All of 176 serum samples and their clinical data were obtained from the National Center for Geriatrie, Osaka Prefecture University, and the Gerontological Biobank. We excluded subjects with smoking habit, prevalent CVD, cerebrovascular disease and liver disease. To exclude subjects with undeclared or unrecognized use of vitamin E sup-
Table 1. Subjects’ background profiles according to the number of arteriosclerosis risks.

|                           | All (n=107) | 0 (n=31) | 1 (n=51) | ≥2 (n=25) | p-value |
|---------------------------|-------------|----------|----------|----------|---------|
| Age (y)                   | 60.97±7.69  | 57.61±7.86| 60.94±7.98| 65.20±4.29| 0.001   |
| Alcohol consumption (n)   | 3           | 0        | 2        | 1        | 0.533*  |
| Height (cm)               | 154.60±5.62 | 156.11±5.34| 155.12±5.96| 151.65±4.14| 0.003   |
| Weight (kg)               | 52.79±6.07  | 51.37±4.75| 53.04±6.24| 54.04±7.00| 0.165   |
| Body mass index (kg/m²)   | 22.10±2.38  | 21.10±1.87| 22.04±2.25| 23.47±2.64| <0.001  |
| Waist circumference (cm)  | 81.23±7.42  | 78.44±7.08| 81.06±6.72| 85.05±7.82| 0.003   |
| Systolic blood pressure (mmHg) | 115.82±13.90 | 108.94±12.86| 115.49±11.53| 125.04±14.81| <0.001  |
| Diastolic blood pressure (mmHg) | 64.57±9.02   | 61.55±8.05 | 64.39±8.53 | 68.68±9.88 | 0.009   |
| Triglycerides (mg/dL)     | 82.00 (64.50, 107.00) | 68.00 (59.50, 98.00) | 84.00 (66.00, 103.50) | 94.00 (77.00, 131.00) | 0.005   |
| Total-cholesterol (mg/dL) | 223.65±30.88| 205.52±23.93| 234.84±28.93| 223.32±32.57| 0.012   |
| LDL-cholesterol (mg/dL)   | 136.69±28.25| 116.10±15.01| 147.25±28.57| 140.68±27.24| <0.001  |
| HDL-cholesterol (mg/dL)   | 78.51±17.55 | 83.29±18.90| 78.82±14.81| 71.96±19.44| 0.022   |
| Fasting blood glucose (mg/dL) | 94.82±11.47 | 91.71±5.69   | 91.71±7.05   | 105.04±17.17 | 0.006   |
| Serum α-tocopherol (μmol/L) | 24.32 (20.57, 28.24) | 24.32 (19.89, 26.82) | 24.47 (21.19, 29.01) | 23.72 (20.45, 28.11) | 0.655   |
| Serum γ-tocopherol (μmol/L) | 2.79 (2.20, 3.31)   | 2.68 (1.97, 3.34)  | 2.67 (2.30, 3.29)  | 2.92 (2.38, 3.30)  | 0.264   |
| Serum α-tocopherol/cholesterol+ triglycerides ratio (μmol/mmol) | 3.61 (3.12, 4.05)   | 3.85 (3.31, 4.31)  | 3.52 (3.09, 3.94)  | 3.44 (3.09, 3.94)  | 0.074   |
| Serum γ-tocopherol/cholesterol+ triglycerides ratio (μmol/mmol) | 0.40 (0.32, 0.47)   | 0.42 (0.31, 0.53)  | 0.40 (0.31, 0.45)  | 0.40 (0.35, 0.44)  | 0.755   |
| Serum α-tocopherol/γ-tocopherol ratio | 9.05 (6.93, 11.68) | 8.92 (6.60, 12.78) | 9.05 (7.19, 11.36) | 8.64 (7.74, 9.59) | 0.715   |

Average±SD, median (Q1, Q3). Jonckheere-Terpstra test.
*Chi-square test.
The Relationship between Serum Vitamin E Level and Arteriosclerosis Risk Factors

The background profiles and biochemical data are shown in Table 1. Compared with subjects without risk (0 risks), subjects with 1 risk and ≥2 risks were significantly older, had higher BMI, WC, BP (diastolic and systolic), and serum concentrations of TG, LDL-cholesterol (LDL-C), and total cholesterol (TC). Serum level of HDL-C was significantly lower in subjects with 1 risk and ≥2 risks. In total subjects, the median serum level of αT was 24.32 (Q1, Q3: 20.57, 28.24) μmol/L. Vitamin E is essential for maintaining membrane stability, and its deficiency causes fragile erythrocyte and hemolysis, which is considered to be a good indicator for vitamin E status, and the cutoff value has been considered as <12 μmol/L of serum αT (15). In none of the subjects, serum αT level was below this cutoff value. In 17 subjects (15.9% of total subjects), serum αT concentration was higher than 30 μmol/L, a level reported to be required for beneficial effects on human health by several prospective observational studies (16). Serum levels of γT and αT/γT ratio were 2.79 (Q1, Q3: 2.20, 3.31) μmol/L and 9.05 (Q1, Q3: 6.93, 11.68), respectively. Unfortunately, established criteria for γT level and αT/γT ratio is currently unavailable. Since vitamin E concentrations closely correlate with blood lipid concentrations, vitamin E adjustment was done as measured vitamin E values divided by serum levels of TC plus TG. There were no significant differences in these indices between groups. Serum levels of αT adjusted by serum TC and TG decreased with an increasing number...
of arteriosclerosis risk factors \( (p=0.074) \).

Table 2 shows the significant predictors for the number of arteriosclerotic risk factors analyzed by a multiple regression model with stepwise method. As shown in Table 2, serum \( \alpha T \) level adjusted by serum TC and TG was a negative significant predictor for the number of arteriosclerosis factors.

**DISCUSSION**

In this study, we have studied the association of serum vitamin E level with arteriosclerotic risks in Japanese postmenopausal women. Serum level of \( \alpha T \) adjusted by serum lipids significantly decreased with the increasing number of the risk factors for arteriosclerosis after adjustment by other additional covariates.

The association of serum vitamin E level with arteriosclerotic risk (e.g. MS) has been inconsistent between previous reports \((17–20)\). Li et al. observed no significant difference in serum vitamin E level between controls and MS subjects \((17)\). In another cross-sectional study, however, circulating vitamin E levels were significantly lower in patients with MS than healthy controls and vitamin E levels were correlated inversely with diastolic BP and positively with HDL-C in patients with MS \((18)\). The methodological problem in these studies in common is that the possible interference by the circulating lipid levels on vitamin E status was not taken into account.

The report by Ford et al. highlights the importance of adjustment by the serum lipid levels \((19)\). There was no difference in serum vitamin E level between subjects with MS and those without it in the initial analysis adjusted by many possible confounding variables, but not by serum lipid levels, whereas vitamin E concentrations were significantly lower in subjects with MS than those without it after additional adjustment for serum lipid concentrations. Their results are consistent with ours.

In almost all the previous studies, subjects with MS had higher serum lipid levels compared with control subjects. Since vitamin E concentration is positively affected by serum lipid concentrations, evaluation of vitamin E status by unadjusted vitamin E level is quite likely to overestimate the vitamin E status in subjects with MS. Therefore, when assessing the vitamin E status in subjects with dyslipidemia, adjustment by serum lipid levels is mandatory. In addition, an interesting result was reported regarding the impaired bioavailability of vitamin E in a randomized, crossover, double-blind study conducted in healthy and MS adults \((21)\). They were given encapsulated \( \text{d}_6-\alpha T \) \((15 \text{ mg}) \) with 240 mL nonfat \((0.2 \text{ g fat}) \), reduced-fat \((4.8 \text{ g fat}) \), or whole \((7.9 \text{ g fat}) \) milk. The randomized controlled trial \((RCT)\) revealed that \( \alpha T \) bioavailability was lower in MS adults potentially through greater inflammation and oxidative stress that limits small intestinal \( \alpha T \) absorption and/or impairs hepatic \( \alpha T \) trafficking. Since the study has employed the deuterium-labeled \( \alpha T \), their results would be free from interference by endogenous vitamin E status. Based on these findings, they suggested dietary requirement of \( \alpha T \) is high in MS adults. Moreover, a previous meta-analysis has described that omega-3 and vitamin E co-supplementation has beneficial effects on the lipids profile of patients with MS \((22)\). Unfortunately, however, the effect of vitamin E supplementation alone has not been clarified.

The next issue to be concerned would be the possible inverse effect of vitamin E on MS. Based on data from NHANES 2001–2006, Beydoun et al. reported that vitamin E level was directly related to the number of the MS count adjusted by covariates including serum TC, and TG \((20)\). This is in sharp contrast to the report by Czernichow et al. that antioxidant supplementation particularly vitamin E and \( \beta \)-carotene, for 7.5 y had no long-term benefits in the prevention of MS in a large-scale RCT \((23)\). Beydoun et al. ascribed the negative results mainly to the selection of study subjects \((20)\). In their study, subjects using vitamin E supplementation were included, resulting in the extremely high level of \( \alpha T \) \( \text{Q1}: 0.16–21.66 \mu mol/L; \text{Q2}: 21.67–27.35 \mu mol/L; \text{Q3}: 27.37–35.94 \mu mol/L; \text{Q4}: 35.95–303.81 \mu mol/L \). Although \( \alpha T \) exerts beneficial effects through its potent antioxidative action, the results from the intervention studies have been inconsistent. Huang and Appel hypothesized that large-dose \( \alpha T \) may impair the availability of other nutrients leading to the unfavorable outcomes at its extremely high dose \((24)\). After RRR-\( \alpha T \) supplementation \((400 \text{ IU daily}) \) in 184 adult nonsmokers, the serum concentration of \( \gamma T \) was reduced to 58\% of that before intervention. \( \gamma T \), despite its plasma level approximately one-tenth that of \( \alpha T \), has a higher antioxidant capacity and greater anti-inflammatory properties than \( \alpha T \) \((25)\). Besides, \( \gamma T \) is superior in trapping generated reactive nitrogen oxide species during inflammation \((26)\). Supplementation with \( \gamma T \) alone or in combination with \( \alpha T \) was more potent than \( \alpha T \) alone in reducing biomarkers of oxidative stress in patients with MS \((27)\) and attenuated exercise-increased coagulation as well as platelet aggregation \((28)\). Another apparently paradoxical explanation would be the “tocopherol-mediated peroxidation.”

An in vitro study demonstrated that \( \alpha T \), depending on oxidative conditions and presence of cooxidants, can act as a potent LDL prooxidant through \( \alpha \)-tocopheroxyl radical formed at early oxidation stages \((29)\). Further studies are required regarding the extrapolability of such in vitro finding to in vivo and optimal range of vitamin E status, especially its upper limit, for health promotion is needed.

Although most attention has been paid on \( \alpha T \) regarding the relationship between vitamin E and MS, there have been some studies on the relationship serum \( \gamma T \) and MS. In the present study, both unadjusted and adjusted \( \gamma T \) level were not associated with the number of arteriosclerosis risk. However, in the NHANES cohort, sedentary behavior was significantly associated with a higher risk for MS, and serum \( \gamma T \) levels, but not the level of other analogs, were inversely correlated with the number of daily steps which reflect physical activities. It is hypothesized that the lower \( \gamma T \) levels in
the active group are an adaptive response to oxidative stress induced by aerobic exercise, such as walking and running (30). The possible relationship of γT with MS awaits further studies.

The present study has several limitations. First, the participants were limited to postmenopausal women. However, since these subjects have a higher risk of arteriosclerosis caused by decreased estrogen secretion, investigation in such a study population would be of relevance. Second point is that we cannot argue the causality or the detailed underlying mechanism for the association between serum αT level and arteriosclerosis risk such as whether it is related to the anti-oxidant and anti-inflammatory potency of vitamin E since this is a cross-sectional study. The third one is that oxidative stress markers were not measured in the present study. However, previous reports described that patients with MS were characterized with elevated oxidative stress and decreased antioxidant protection in comparison with those without MS (17, 19, 31–33). Thus, elevated oxidative stress would be the basis for the increased number of arteriosclerosis risks in our study. Finally, the data on vitamin C status is unavailable. Antiatherogenic and anti-inflammatory functions of vitamin E are exerted by its reduced form. Vitamin C improves the reduction of vitamin E, and enhances its potency as a reductant (34).

Our paper is characterized by that the association of vitamin E status with arteriosclerosis risks in subjects without overt vitamin E deficiency. Recently, the importance of vitamin insufficiency has increasingly acknowledged. It is milder than deficiency and not accompanied by the phenotypic changes in the individual subjects, but associated with increased risk for various disease. In the case of vitamin E, its deficiency is rare in developed countries, but its insufficiency increases the risks of NCDs. The associations between vitamin E insufficiency and NCDs, however, have been scarce in Japan, and such epidemiological studies are required in the future for the health promotion.

In summary, serum vitamin E level was positively associated with a lower number of arteriosclerosis risks, and its role for preventing NCDs was suggested.

Authorship
Research conception and design: AK; investigation: YN, SN and AK; statistical analysis of the data: YN and AK; interpretation of the data: ST, KT and AK; writing of the manuscript: YN, ST and AK; supervision: KT.

Disclosure of state of COI
No conflicts of interest to be declared.

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