Cardiovascular Aging Is Associated With Vitamin E Increase

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Background—Aging is an independent risk factor for the development of cardiovascular disease. Therefore, therapies to delay vascular aging may have enormous medical consequences. In this context, vitamin E is of particular interest, mainly because of its antioxidative properties.

Methods and Results—In 3-year-old rats, which are not susceptible to atherosclerosis, vitamin E levels, as measured by reversed-phase high-performance liquid chromatography, were markedly increased both in plasma and in major organs (P<0.01 to P<0.0001). The highest increase (at least 70-fold) was found in the aortic wall.

Conclusions—This unexpected accumulation of vitamin E appears to be a compensatory mechanism that attempts to counterbalance age-associated oxidative stress and that may represent a self-regulatory protective adaptation. (Circulation. 2002;105:1635-1638.)

Key Words: aging ■ cardiovascular diseases ■ vitamin E ■ antioxidative defense

Cardiovascular diseases become more frequent with increasing age.1 Therefore, the aging process itself becomes part of pathophysiological mechanisms.2 Recently, we have shown a markedly increased production of superoxide and subsequent formation of peroxynitrite with aging.3 Vitamin E is an important electron source for the reduction of peroxynitrite.4 Therefore, its therapeutical use in aging may seem particularly attractive.

Vitamin E is usually supplemented as α-tocopherol, which is its most biologically active form. The protective effect of additional vitamin E beyond normal dietary provision remains controversial. The Cambridge Heart Antioxidant Study (CHAOS)5 suggested that supplementation with α-tocopherol may reduce the risk of cardiovascular death, whereas the much larger Heart Outcomes Prevention Evaluation (HOPE) study6 indicated no significant reduction in death from cardiovascular causes in patients with vitamin E supplementation.

Although dietary addition of vitamin E is frequently marketed, little is known in regard to the requirement for vitamin E, especially for older people. Studies in other mammals have presented contradictory results on vitamin E–associated antioxidant capacity with increasing age.7,8 No studies have been performed, to date, that have estimated the concentration of vitamin E in the arterial vessel wall.

Our aim was to clarify whether tissue levels or plasma levels of vitamin E change with increasing age. To this end, we performed measurements of α-tocopherol in aorta, heart, liver, and plasma of α-tocopherol in plasma of young adult, middle-aged, and old healthy male rats. Rats are one of the most suitable models for studies of cardiovascular aging in mammals. Their life span is short, they do not develop atherosclerosis, and nutrition can easily be controlled.

We demonstrate an age-related vitamin E increase, in particular within the aortic vessel wall. This may be considered a counter-regulatory mechanism to prevent oxidative tissue damage.

Methods

Animals

F1 (F344xBN) healthy male rats, fed ad libitum, were obtained from the National Institute on Aging, Bethesda, Maryland. Animals belonged to three age groups: 4 to 6 months old (“young”), 19 months old (“middle-aged”), and 32 to 35 months old (“old”). Animals were fed a standardized, sterilized diet (NIH-31, Harlan Teklad Laboratory Diets, Indianapolis, Ind) that contained identical concentrations of vitamin E (45 IU/g) in all age groups.

Surgical Procedures

Rats were anesthetized, and, after medial sternotomy, the aorta was cannulated and blood was taken and centrifuged at 4000 rpm at 4°C for 10 minutes. The aorta, cleaned of adhering tissue, was used for vitamin E determination from the beginning of the renal arteries down to the bifurcation. The heart and the liver were excised in situ. Aortic tissue, heart, liver, and plasma were immediately snap-frozen in liquid nitrogen and stored at −70°C until further use for vitamin

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E determinations (n=11 for young, n=7 for middle-aged, and n=10 for old rats).

Reversed-Phase High-Performance Liquid Chromatography

Reversed-phase high-performance liquid chromatography was performed as described previously. Data were acquired via a chromatography server (Fission Instruments) and analyzed by a VAX-based multichannel data acquisition system (Multichrom, VG Data Systems Ltd). The separation was performed on a reversed-phase column (C18) at 30°C, and the tocopherols were detected fluorometrically. To guarantee reproducible results, the method has been monitored with internal control samples and by external quality assurance programs (National Institute of Standards and Technology, Gaithersburg, Md).

Electron Microscopy

From 2 additional young and 2 additional old animals, the aorta was isolated, fixed, and embedded for electron microscopy as described previously. Thin sections were viewed with a CM100 transmission electron microscope (Philips). Intimal thickness was defined as the distance from the luminal surface of the endothelium to the surface of the first elastic lamellae.

Measurement of Superoxide

- O2\(^{-}\) concentrations in aortic tissue were determined with a lucigenin-enhanced chemiluminescence method as described previously.

Calculations and Statistical Analysis

Results were analyzed by ANOVA followed by Bonferroni’s and Dunn’s correction. Data are presented as mean±SEM. Means were considered significantly different at *P*<0.05.

Results

In the plasma of old animals, α-tocopherol was increased 2-fold compared with young rats (Figure 1A). Plasma levels of γ-tocopherol were, given a detection limit of 0.02 mg/L, at least 10-fold higher in old rats (Figure 1B). Because γ-tocopherol was undetectable in the plasma of young animals, we did not investigate the concentration of tissue γ-tocopherol. We refrained from adjusting vitamin E plasma levels to cholesterol because vitamin E concentrations in plasma generally do not correlate with tissue levels.

In the liver, which is considered to be a major site of storage for tocopherols, there was also a significant, 2-fold increase of α-tocopherol in parallel to the plasma levels in old rats as compared with young rats. The concentration of α-tocopherol in plasma and liver of middle-aged rats was in between that of young and old animals (Figures 1A and 2A).

In the heart, levels of α-tocopherol were nearly 3-fold increased in old animals as compared with young and middle-aged animals (Figure 2B).

The most astonishing finding was that α-tocopherol in the aorta was extremely high (at least 70 times greater) in old animals as compared with young animals in which it was below detection limits (Figure 2C). The aortic wall of old rats contained one-third of the concentration of α-tocopherol found in the liver, a fact that underlines the magnitude of α-tocopherol levels in the aorta of old animals. This marked increase in α-tocopherol was paralleled by a significant increase of -O2\(^{-}\)-generated chemiluminescence (Figure 2D).

Although aortic α-tocopherol levels in middle-aged animals were comparably high to those in old animals, oxidative stress was significantly lower (Figure 2, C and D).

Electron microscopy revealed no overt signs of atherosclerosis, such as accumulation of foam cells, smooth muscle cells, or extracellular lipid. Old aortas exhibited a marked increase in intimal thickness, which increased from 2.5±0.9 μm in young aorta (mean of 50 different measurements) to 8.7±3.4 μm in the old aorta (*P*<0.001; data not shown). This change is similar to that seen in human arteries in which an age-related increase in the intima-media thickness has been reported in the femoral arteries of old but healthy humans.

The steep increase of vitamin E content occurs independently of the development of atherosclerosis (although the increase in intimal thickness may possibly be an early precursor) and can be considered as primarily dependent on aging.

![Figure 1](http://circ.ahajournals.org/)[1636 Circulation April 9, 2002]

**Figure 1.** Levels of α-tocopherol (A) and of γ-tocopherol (B) in plasma of young, middle-aged, and old animals as measured by reversed-phase high-performance liquid chromatography. Values represent the mean±SEM. The limit of detection is defined as the x-fold signal of the baseline signal in which the standard deviation is still <7% of the mean signal. A, Young vs old, *P*<0.0001; middle-aged vs old, NS; and young vs middle-aged, *P*<0.001. B, Young vs old, *P*<0.0001; and young vs middle-aged and middle-aged vs old, NS.
Discussion

With the same model of aging, we have previously shown that vascular aging is closely associated with augmented \( \cdot \text{O}_2^\cdot \) generation and peroxynitrite formation that, in turn, exhibits new intracellular messenger functions. Vitamin E may reduce oxidative stress by directly scavenging reactive oxygen species. Therefore, our discovery of a specific tissue distribution and, in particular, of a dramatic increase in \( \alpha \)-tocopherol in the aortic vessel wall of old animals is likely to have distinct pathophysiological significance.

On the basis of these data, we postulate the existence of a compensatory antioxidant defense system to counteract oxidative stress–associated vascular aging. It is reasonable to assume that absorption of vitamin E can increase with age to reduce oxidative damage to vascular tissue. It would be expected to accumulate at the sites in which it is needed most to protect against the development of age-associated changes, such as endothelial dysfunction. Because mammals cannot synthesize vitamin E, intestinal absorption and subsequent transport determines how much of the ingested vitamin enters the circulation. An age-associated increase in the total amount of vitamin E absorbed in the intestine of old rats has already been described and would support the concept of a self-protective age-dependent adaptation. Under physiological conditions, the metabolism of vitamin E is incompletely understood to date. Therefore, we do not feel able to speculate on a possible decreased catabolism in aging.

Controlled upregulation, dependent on demand, may be determined by age-associated increases in oxidative tissue damage. Our concept is further strengthened by the facts that \( \alpha \)-tocopherol was particularly increased in the vasculature in which oxidant stress was high and is apparently still able to reduce oxidative radicals in the aorta of middle-aged animals, whereas in the extreme age group, it cannot.

In vitro experiments have shown that the \( \alpha \)-tocopheroxyl radical may exhibit pro-oxidant activities. However, the analytical method we used does not allow the quantification of \( \alpha \)-tocopherol radicals. It seems unlikely, albeit possible, that vitamin E itself may be involved in the cardiovascular aging process. The pathophysiological relevance of vitamin E as a pro-oxidant under in vivo conditions remains to be determined.

The interpretation of vitamin E plasma levels is possibly limited by the fact that they are not adjusted to plasma cholesterol. However, the main body of evidence (\( \alpha \)-tocopherol in major organs) we present remains unaffected by this.

In conclusion, it seems implicit that mammals are capable of regulating age-related, increased demand for vitamin E.
Our results suggest that sufficiently high levels of vitamin E may be built up from a normally balanced diet to counter-balance oxidative stress-associated vascular aging. This counter-regulation eventually remains unsuccessful because, in old age, oxidative damage occurs although the antioxidant defense is dramatically "switched on."

The relevance of our findings to aging in humans is not known. Corresponding studies in humans remain to be performed.

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