Vitamin E dietary supplementation significantly affects multiple risk factors for cardiovascular disease in baboons[1–3]

David L Rainwater, Michael C Mahaney, John L VandeBerg, and Xing Li Wang

ABSTRACT

Background: Oxidative stress is a widely accepted risk factor for cardiovascular disease (CVD), but the CVD benefit of dietary antioxidants, such as vitamin E, is controversial.

Objective: Therefore, we have investigated, in the baboon model, the effects of dietary vitamin E supplementation on risk factors for CVD.

Design: Pedigreed baboons ($n = 251$) were fed 2 atherogenic diets, high in fat and cholesterol, that differed in vitamin E concentrations. After 7 wk on each diet, blood samples were taken, and a panel of CVD risk factor traits (ie, indicators of lipoprotein metabolism and oxidative stress) were measured.

Results: Vitamin E supplementation caused significantly higher total antioxidant status (TAS) and lower oxidized LDL as expected. In addition, vitamin E caused 2 paradoxical effects on HDL metabolism: higher apolipoprotein A-I (apo A-I) concentrations and lower HDL sizes. We calculated a difference (Δ) variable for each trait as the value on the high-vitamin E diet minus that on the low-vitamin E diet and determined that several HDL concentration Δ variables were significantly correlated with Δ TAS, but only one, Δ apo A-I, was independently correlated. Genetic analyses showed that 2 Δ variables, Δ paraoxonase and Δ HDL_2, were significantly heritable, but that neither Δ TAS nor Δ apo A-I were heritable.

Conclusions: Thus, our data show that dietary vitamin E improves TAS and LDL quality. They also show 2 apparently paradoxical effects on HDL metabolism: lower HDL_2, which is mediated by genes, and higher apo A-I, which is not. These effects have contrasting associations with CVD risk and may help account for the mixed results from clinical trials of dietary vitamin E. *Am J Clin Nutr* 2007;86:597–603.

KEY WORDS Vitamin E, HDL, high-density lipoprotein, antioxidants, paraoxonase, apo A-I, apolipoprotein A-I, baboons

INTRODUCTION

Atherosclerosis is an inflammatory process (1) that is accelerated under circumstances of oxidative stress (2). Oxidative stress leads to damage of nearly all biological macromolecules and a cascade of events (including LDL oxidation, endothelial dysfunction, and enhanced inflammatory response) that contribute to atherosclerosis and ultimately lead to clinical manifestations, such as coronary heart disease. It has long been suggested that onset and progression of atherosclerotic lesions might be delayed by an effective supplementation of the antioxidant system.

Vitamin E is a fat-soluble vitamin that is associated with lipoproteins. It is an effective terminator of free radical chain reactions, but it is a relatively poor trapper of singlet oxygen (compared with β-carotene). Despite several good quality cohort studies reporting significant associations of vitamin E with lower rates of cardiovascular disease (CVD) events and mortality (3), reviews of several randomized clinical trials have concluded that vitamin E supplementation does not reduce cardiovascular mortality (3, 4). Although the sources for these discordant observations are unclear, we speculate that efficacy of vitamin E supplementation may depend on individual characteristics, such as genetic variants, sources of oxidative stress, and other coexisting biochemical environments, such as lipemia, that are pertinent to the redox balance. Understanding the bases of these discrepant findings may provide new insights into the interrelations between oxidative stress and CVD and potentially target therapies toward patients who are more likely to be responsive.

Animal models provide a practical means of assessing the effects of dietary supplements on biological indicators of CVD. In particular, baboons are an excellent model of complex human disease because of phylogenetic and physiologic similarities (5). In addition, our pedigreed baboon colony is structured to efficiently determine whether genes influence the traits we study or their responses to dietary environment. In this article, we address the issue of whether vitamin E dietary supplementation affects lipoprotein metabolism and associated indicators of oxidative stress and whether genes are involved.

MATERIALS AND METHODS

Animals and diet protocol

Two hundred fifty-one pedigreed baboons (*Papio hamadryas*) on a basal diet low in fat and cholesterol were subjected to a dietary challenge protocol. First, they were fed a diet rich in fat

[1] From the Department of Genetics (DLR, MCM, JLV, and XLW) and the Southwest National Primate Research Center (MCM and JLV), Southwest Foundation for Biomedical Research, San Antonio, TX, and the Division of Cardiothoracic Surgery, Texas Heart Institute, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX (XLW).

[2] Supported by NIH grants P01 HL028972, P51 RR013986, C06 RR014578, and C06 RR015456.

[3] Reprints not available. Address correspondence to DL Rainwater, Southwest Foundation for Biomedical Research, PO Box 760549, San Antonio, TX 78245-0549. E-mail: david@sfbrgenetics.org.

Received November 29, 2006.

Accepted for publication April 20, 2007.

(40% of calories from lard) and cholesterol (6 mg/g) (6) for 7 wk before taking a blood sample (HF sample). The animals were then returned to the basal diet (washout period) for 7 wk before feeding them the same high-fat high-cholesterol diet supplemented with 1000 IU vitamin E acetate/kg (BioServe, Frenchtown, NJ) for 7 wk before taking a second blood sample (HF + E sample). Both HDL- and LDL-cholesterol concentrations were substantially higher on the HF diet than on the basal diet (7). Previous studies have shown maximal LDL-cholesterol and HDL-cholesterol responses to occur by 4 wk on diet (8), and a pilot study, involving 60 baboons that were sampled on the basal diet and again after 7 wk on the HF diet plus 7 wk on the basal diet (9), showed that LDL cholesterol and HDL cholesterol were similar in the 2 basal diet samples ($\bar{x} \pm$ SD: 48.4 $\pm$ 18.4 compared with 49.2 $\pm$ 19.8 mg/dL for LDL cholesterol and 78.9 $\pm$ 22.3 compared with 74.0 $\pm$ 20.6 mg/dL for HDL cholesterol).

Blood samples (serum and plasma) were taken from the femoral vein of overnight-fasted baboons immobilized with ketamine. Plasma was collected in heparin tubes and made to 100 μmol diethylenetriaminepentaacetic acid/L and 20 μmol 3,5-di-*tert*-butyl-4-hydroxytoluene/L (Sigma Chemical, St Louis, MO). Samples were prepared by low-speed centrifugation (800 $\times$ *g*, 15 min, 6°C) and stored at −80 °C in small, single-use aliquots as described previously (10). All animal procedures were done under supervision of a veterinarian and approved by the Institutional Animal Care and Use Committee. Southwest Foundation for Biomedical Research is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Biochemical procedures

Serum cholesterol concentrations were measured enzymatically with reagents supplied by Boehringer Mannheim Diagnostics (Indianapolis, IN) and with the use of a Ciba-Corning Express Plus clinical chemistry analyzer (Norwood, MA). Cholesterol in HDL was estimated after heparin-Mn^{2+} precipitation of apolipoprotein B (apo B)–containing lipoproteins (11), and LDL cholesterol was estimated as the difference between total and HDL cholesterol. Serum triacylglycerol concentrations were estimated enzymatically with the use of reagents from Stanbio (San Antonio, TX). Serum concentrations of apo A-I and apo B were estimated with the use of commercial immunoturbidometric assays (DiaSorin, Stillwater, MN). CVs for control products in these assays were 2.0% for total cholesterol, 6.3% for HDL cholesterol, 5.0% for triacylglycerols, 4.1% for apo A-I, and 6.3% for apo B. Distributions of cholesterol among size-resolved lipoprotein classes were estimated by nondenaturing gradient gel electrophoresis and staining with Sudan black B (12–14). Serum samples were run in duplicate on composite gels; gels were calibrated, a gel-specific baseline was subtracted, and size fractions were calculated as described previously (13). In addition, we estimated a median diameter for HDLs (8.2–24 nm) and for LDLs (24–30 nm), which was defined as the diameter in which half the HDL (or LDL) absorbance was on larger and half on smaller particles and may be considered to be a weighted average diameter for HDLs and LDLs (13). CV for a control run on each gel was 2.9% for HDL median diameter (n = 249 gels) and 0.9% for LDL median diameter (n = 243 gels). Plasma oxidized LDL concentrations were measured immunologically with the use of a sandwich-style enzyme-linked immunoabsorbent assay that uses 2 monoclonal antibodies directed against different antigenic determinants on oxidized LDL (Mercodia Oxidized LDL ELISA; ALPCO Diagnostics, Salem, NH). Between-plate CV for this assay was 5% for a control product supplied with the kit.

Total antioxidant status (TAS), reflecting the overall antioxidant capacity of serum, was defined as the ability to prevent oxidation of 2,2'-azino-di-(3-ethylbenzthiazoline sulfonate) by metmyoglobin with the use of a kit from Calbiochem (San Diego, CA). Between-plate CV for this assay was 5.0%. Serum lipoprotein-associated phospholipase A_2 (PLA2; also, platelet-activating factor acetylhydrolase) enzyme activity was measured at 30 °C with the use of a kit provided by Cayman Chemical Company (Ann Arbor, MI). Hydrolysis of the substrate, 2-thio PAF, produced a free thiol which was quantified with 5,5'-dithio-*bis*-(2-nitrobenzoic acid). The reaction was monitored at 405 nm with a Molecular Devices M4 microplate reader (Sunnyvale, CA) running in kinetic data acquisition mode. Rates were calculated from the linear phase during the first 6 min and converted to $\mu mol \cdot min^{-1} \cdot L^{-1}$ plasma (molar extinction coefficient = 13 600) Each sample was run in duplicate, and the average CV was 2.3% (n = 989); the between-plate CV, based on a pooled baboon serum control sample run on each plate, was 4.0% (n = 26). Serum paraoxonase enzyme activity was measured at 30 °C in a microplate format and followed a published protocol (15). Paraoxon (Chem Service, West Chester, PA) hydrolysis was monitored at 405 nm with the use of a Molecular Devices M4 microplate reader running in kinetic data acquisition mode. Rates were calculated from the linear phase during the first 10 min and converted to $\mu mol \cdot min^{-1} \cdot L^{-1}$ serum (molar extinction coefficient = 18 000). Each sample was run in duplicate, and the average CV was 1.3% (n = 1000); the between-plate CV, based on a pooled baboon serum control sample run on each plate, was 3.9% (n = 24).

Statistical analyses

Inferential statistical analyses were performed with the use of routines implemented in STATA 9.2 statistical software (StataCorp LP, College Station, TX). A paired t test was performed to assess the effects of dietary vitamin E supplementation on the means of the measured traits. To assess the association of change in TAS with change in each of the other traits, we performed a multiple regression analysis, with robust estimates of the variance, in a model that included the change variables and also their baseline values.

Because many of the 251 baboons for which we obtained data for this study belong to a larger, pedigreed breeding colony, we could exploit information on their biological relatedness (kinship) to detect the effects of genes on the measured traits. With the use of a maximum likelihood-based variance decomposition approach (16) implemented in SOLAR (version 4, copyright 1995–2007, Southwest Foundation for Biomedical Research, San Antonio, TX) (17), we conducted basic univariate quantitative genetic analyses to simultaneously assess the additive effects of genes on the traits while accounting for the mean effects of the covariates age, sex, and weight. With this approach we partitioned the residual phenotypic variance in each trait (σ^2_P) into components corresponding to additive genetic effects (σ^2_G) and environmental effects (σ^2_E). The heritability, or proportion of the residual phenotypic variation that can be attributed to additive genetic effects, is obtained as $h^2 = (\sigma^2_G)/(\sigma^2_P)$.

TABLE 1
Effect of vitamin E dietary supplementation on lipoprotein and oxidative stress measures[1]

Trait	*n*	HF diet	HF + E diet	*P*[2]
TAS (mmol/L)	250	1.096 ± 0.091[3]	1.139 ± 0.084	<0.0001
Apo A-I (mg/dL)	249	148.1 ± 28.1	151.3 ± 25.6	0.0086
HDL cholesterol (mg/dL)	251	90.1 ± 24.7	88.5 ± 24.9	0.082
HDL_{1A} (%)	251	1.3 ± 2.2	1.0 ± 1.4	0.0058
HDL_{1B} (%)	251	20.1 ± 11.6	17.3 ± 10.5	<0.0001
HDL_2 (%)	251	62.0 ± 9.6	61.2 ± 7.9	0.0075
HDL_3 (%)	251	16.3 ± 7.9	20.6 ± 9.6	<0.0001
HDL median diameter (nm)	251	11.37 ± 0.75	11.14 ± 0.70	<0.0001
Apo B (mg/dL)	250	56.2 ± 22.2	56.1 ± 22.1	0.46
LDL cholesterol (mg/dL)	251	89.4 ± 46.8	91.0 ± 49.9	0.20
$VLDL_1$ (%)	251	6.8 ± 4.3	6.9 ± 4.5	0.37
$VLDL_2$ (%)	251	12.7 ± 7.9	13.1 ± 7.8	0.13
LDL_1 (%)	251	28.1 ± 11.7	28.0 ± 11.7	0.44
LDL_2 (%)	251	32.2 ± 10.9	31.1 ± 10.3	0.024
LDL_3 (%)	251	16.2 ± 11.3	16.8 ± 12.0	0.16
LDL_4 (%)	251	4.1 ± 5.4	4.1 ± 5.8	0.45
LDL median diameter (nm)	251	27.68 ± 0.47	27.66 ± 0.49	0.21
Triacylglycerols (mg/dL)	251	64.4 ± 28.2	67.7 ± 29.7	0.016
PON1 ($\mu mol \cdot min^{-1} \cdot L^{-1}$)	251	67.4 ± 27.5	65.0 ± 29.2	0.011
PLA2 ($\mu mol \cdot min^{-1} \cdot L^{-1}$)	250	7.81 ± 2.16	7.62 ± 2.27	0.012
LDLox (U/L)	249	40.7 ± 16.2	36.7 ± 15.2	<0.0001

[1] HF, high fat; E, vitamin E; TAS, total antioxidant status; Apo, apolipoprotein; PON1, paraoxonase 1; PLA2, phospholipase A_2; LDLox; oxidized LDL.
[2] Determined from paired *t* tests.
[3] $\bar{x}$ ± SD (all such values).

RESULTS

Effect of vitamin E diet supplementation on TAS and selected indicators of lipoprotein metabolism and oxidative stress

The average values for a selection of lipoprotein and oxidative stress measures for the same animals fed 2 diets differing in concentrations of vitamin E are given in **Table 1**. A series of paired *t* tests indicated that a number of the traits were significantly different on the 2 diets. As expected, TAS was significantly higher in the HF + E diet. Among the lipid and lipoprotein phenotypes, apo A-I concentrations were significantly higher with vitamin E supplementation. However, HDL sizes were generally smaller, and this was detected by significantly more absorbance in HDL_3, less absorbance in HDL_1 and HDL_2, and smaller HDL median diameters (a global assessment of HDL size distribution). There were no significant effects of vitamin E on HDL-cholesterol, LDL-cholesterol, or apo B concentrations or on LDL size fractions (except a modest reduction of LDL_2).

For traits related to oxidative stress, we found that paraoxonase 1 (PON1; which is an HDL-associated enzyme) and PLA2 (which is an LDL-associated enzyme) were both reduced by dietary vitamin E. Finally, oxidized LDL is a specific indicator of lipoprotein oxidation, and this value was lower by ≈10% after the HF + E diet ($P < 0.0001$).

Correlated changes of TAS with selected indicators of lipoprotein metabolism and oxidative damage in response to dietary vitamin E supplementation

To investigate whether responses to vitamin E might be correlated, we calculated a difference (Δ) variable as a value on the HF + E diet minus that on the HF diet and tested for correlation of each Δ trait with Δ TAS in multiple regression models that also included the baseline (HF diet) values of each trait. Changes in HDL-cholesterol and apo A-I concentrations were strongly and positively correlated with Δ TAS, and PLA2 showed a marginal ($P = 0.044$) positive correlation (**Table 2**), but none of the LDL-related Δ traits, including oxidized LDL, were correlated with Δ TAS. However, in a multiple regression model that included all 3 significant Δ traits plus the corresponding baseline values, only Δ apo A-I and baseline TAS were significantly associated with Δ TAS. A scatterplot for the relation between Δ TAS and Δ apo A-I, which explains ≈24% of the variance, is shown in **Figure 1**.

Effect of genes on response to dietary vitamin E supplementation

We then subjected each Δ trait, representing the effect of vitamin E supplementation, to quantitative genetic analysis to determine whether genes controlled their individual responses to dietary vitamin E. Two of the Δ traits tested were significantly heritable (**Table 3**). Approximately 37% of the residual variance in Δ HDL_2 ($P = 0.007$) and ≈22% of the residual variance in Δ PON1 ($P = 0.030$) were explained by the additive effects of genes. A number of other Δ traits, including additional markers of HDL size, had moderate heritabilities (h^2: 0.2–0.3) that were not significant, probably because of the relatively small number of animals for this type of analysis. In contrast, neither Δ TAS nor the HDL-concentration variables, Δ HDL cholesterol and Δ apo A-I, were heritable.

DISCUSSION

In this study, we have fed baboons a high-fat diet enriched in vitamin E (1000 IU/kg) to identify its effects on CVD risk factor

TABLE 2
Difference (Δ) in traits and their relations with the difference in total antioxidant status (Δ TAS)[1]

Δ Trait	*n*	$\bar{x}$ (IQR)	β coefficient	*P*
Δ TAS (mmol/L)	250	0.043 (−0.01 to 0.10)	—	—
Δ Apo A-I (mg/dL)	249	3.4 (−10 to 17)	0.3220	<0.0001
Δ HDL cholesterol (mg/dL)	250	−1.6 (−13 to 10)	0.2756	<0.0001
Δ HDL_{1A} (%)	250	−0.3 (−0.4 to 0.25)	0.0456	0.50
Δ HDL_{1B} (%)	250	−2.8 (−6.4 to 1.3)	0.0091	0.88
Δ HDL_2 (%)	250	−1.2 (−5.5 to 2.8)	0.0208	0.77
Δ HDL_3 (%)	250	4.3 (0.5 to 7.8)	−0.0840	0.11
Δ HDL median diameter (nm)	250	−0.23 (−0.41 to 0.00)	−0.0143	0.82
Δ Apo B (mg/dL)	250	−0.1 (−11 to 10)	−0.0984	0.16
Δ LDL cholesterol (mg/dL)	250	1.6 (−16 to 17)	−0.0698	0.30
Δ $VLDL_1$ (%)	250	0.1 (−2.4 to 2.7)	0.0044	0.95
Δ $VLDL_2$ (%)	250	0.4 (−3.4 to 3.7)	0.0111	0.85
Δ LDL_1 (%)	250	−0.1 (−5.6 to 4.1)	0.0687	0.22
Δ LDL_2 (%)	250	−1.1 (−6.5 to 3.9)	−0.0262	0.68
Δ LDL_3 (%)	250	0.6 (−4.0 to 5.4)	−0.0580	0.22
Δ LDL_4 (%)	250	0.04 (−1.5 to 1.4)	−0.0066	0.89
Δ LDL median diameter (nm)	250	0.05 (−0.22 to 0.18)	0.0371	0.47
Δ Tricacylglycerols (mg/dL)	250	3.3 (−9 to 15)	−0.0806	0.37
Δ PON1 ($\mu mol \cdot min^{-1} \cdot L^{-1}$)	249	2.45 (−12.4 to 5.9)	0.0564	0.32
Δ PLA2 ($\mu mol \cdot min^{-1} \cdot L^{-1}$)	249	−0.19 (−1.03 to 0.75)	0.1134	0.044
Δ LDLox (U/L)	248	−4.1 (−9.6 to 1.6)	−0.1027	0.16

[1] Results of multiple regression analyses that also included the baseline (ie, high-fat diet) values for each pair of traits; the standardized β coefficient and *P* values are for the indicated trait. IQR, interquartile range; Apo, apolipoprotein; PON1, paraoxonase 1; PLA2, phospholipase A_2; LDLox; oxidized LDL.

variables. Our previous experiment showed a dietary dose-dependent elevation in plasma α-tocopherol (6); in that experiment, the HF + E diet resulted in ≈2.5-fold higher concentrations of plasma α-tocopherol than were the concentrations on the HF diet. This change in plasma α-tocopherol was associated in the present experiment with a complex pattern of significant changes in CVD risk factor variables that may help provide a biochemical basis for the contradictory findings in human clinical trials with the use of vitamin E as an antioxidant to lower oxidative stress and reduce risk of CVD.

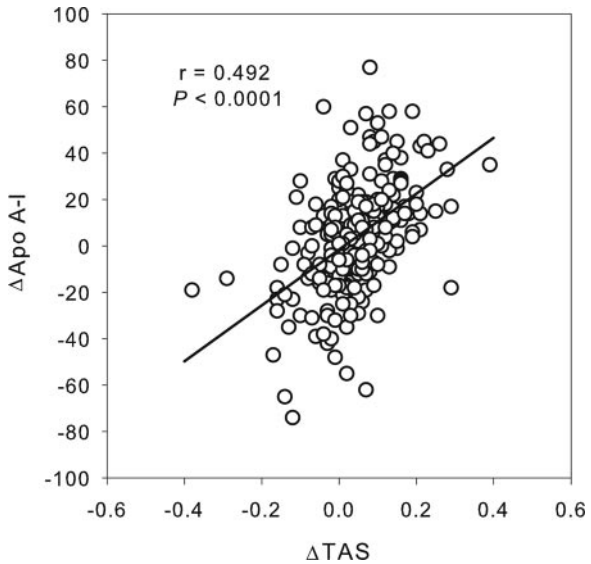

FIGURE 1. Scatter plot of the difference variable of total antioxidant status (Δ TAS) compared with the difference variable of apolipoprotein A-I (Δ apo A-I) with best-fit line from linear regression analysis ($n = 249$).

First, as might be expected, TAS was significantly higher in the HF + E diet than in the HF diet. TAS is a summary variable that measures the ability of serum to inhibit oxidation, so the present result suggests that serum is better able to suppress oxidative modification after vitamin E diet supplementation, confirming the general results of many previous studies (18–22).

Second, although we noted no significant change in concentrations or size properties of apo B-containing lipoproteins, oxidized LDLs were lowered substantially (by 10%). Oxidized LDLs are associated with a higher risk of CVD (23), and the present result confirms observations in humans of the reduction in plasma oxidized LDL with dietary vitamin E (24). Coupled with the lack of change in LDL concentration (ie, LDL cholesterol and apo B), reduction of oxidized LDL suggests that vitamin E diet supplementation improves the quality of LDL.

Third, there were modest, but significant, reductions in serum activities of 2 lipoprotein-associated enzymes, PON1 and PLA2, that are thought to help lower lipoprotein oxidation. Because the study used within-animal comparisons, the result suggests a reduction in circulating concentrations of active enzymes. In particular, the presence of PON1 on HDL particles is considered to be a major source of protection from lipoprotein oxidation (25). As such, therefore, reduction of PON1 activity may be considered to be prooxidant and proatherogenic. A number of studies have reported PON1 concentrations to be responsive to dietary environment (25), but the present results of a reduction in response to vitamin E diet supplementation contradict results in rabbits and humans (26, 27). This discrepancy may reflect differences in HDL size and composition properties among the species. Alternately, vitamin E-associated reduction in oxidative stress, and oxidized LDL, may have lowered the metabolic requirements for these enzymes to maintain the redox balance in

TABLE 3
Difference (Δ) in traits and heritability (h^2) values for effect of vitamin E dietary supplementation on lipoprotein and oxidative stress measures[1]

Δ Trait	*n*	$\bar{x}$	h^2	*P*
Δ TAS (mmol/L)	196	0.046	0	0.5
Δ Apo A-I (mg/dL)	196	2.5	0	0.5
Δ HDL cholesterol (mg/dL)	197	−2.8	0	0.5
Δ HDL_{1A} (%)	197	−0.3	0.298 ± 0.260[2]	0.099
Δ HDL_{1B} (%)	197	−2.6	0.090 ± 0.160	0.26
Δ HDL_2 (%)	197	−1.2	0.367 ± 0.194	0.007
Δ HDL_3 (%)	197	4.1	0.001 ± 0.130	0.5
Δ HDL median diameter (nm)	197	−0.23	0.235 ± 0.236	0.13
Δ Apo B (mg/dL)	196	−0.7	0.019 ± 0.168	0.45
Δ LDL cholesterol (mg/dL)	197	−1.3	0.100 ± 0.146	0.22
Δ $VLDL_1$ (%)	197	0.5	0	0.5
Δ $VLDL_2$ (%)	197	1.1	0.220 ± 0.252	0.16
Δ LDL_1 (%)	197	0.5	0.220 ± 0.185	0.072
Δ LDL_2 (%)	197	−1.3	0	0.5
Δ LDL_3 (%)	197	−0.3	0.268 ± 0.248	0.099
Δ LDL_4 (%)	197	−0.4	0	0.5
Δ LDL median diameter (nm)	197	0.01	0.179 ± 0.210	0.16
Δ Triacylglycerols (mg/dL)	197	3.7	0	0.5
Δ PON1 ($\mu mol \cdot min^{-1} \cdot L^{-1}$)	196	−3.1	0.223 ± 0.148	0.030
Δ PLA2 ($\mu mol \cdot min^{-1} \cdot L^{-1}$)	196	−0.37	0	0.5
Δ LDLox (U/L)	196	−4.3	0	0.5

[1] Quantitative genetic analyses performed in SOLAR (with polygenic routine); *P* values are for the heritability estimate. TAS, total antioxidant status; Apo, apolipoprotein; PON1, paraoxonase 1; PLA2, phospholipase A_2; LDLox, oxidized LDL.
[2] h^2 ± SEM (all such values).

the circulation. Then, through feedback mechanisms, the expression of PON1 and PLA2 may have been down-regulated.

Finally, vitamin E diet supplementation induced 2 seemingly contradictory changes in HDL phenotypes: smaller HDL size and higher apo A-I concentrations. The reduction in HDL size was detected as lower median diameters and corresponding changes in the various HDL size fractions, including a reduction in the proportion of cholesterol in HDL_2. This result confirms the findings in 39 persons of a significant reduction in HDL_2 concentration after taking antioxidant vitamins (28). Reduction of HDL size is associated with an increase in CVD risk in humans (29–34), whereas an elevation of apo A-I is associated with a decrease in CVD risk in humans (35–38). Therefore, on the basis of these population trends, the 2 effects of vitamin E on HDL might be expected to have opposite consequences for CVD risk. Because HDL cholesterol did not change in this experiment, the effect of vitamin E was to promote accumulation of small protein-rich HDLs, which may have a greater capability to support reverse cholesterol transport, a fundamental antiatherogenic process that lowers peripheral cholesterol stores.

The higher apo A-I concentrations we observed after vitamin E diet supplementation in baboons confirms findings in human populations (28, 39). Notably, there was a significant positive correlation of plasma α-tocopherol and apo A-I concentrations (39). On the basis of isoelectric point and size with the use of 2-dimensional gel electrophoresis, it appeared that primarily the proform of apo A-I was responsible for the higher apo A-I concentrations. Although higher concentrations of the pro-apo A-I might be responsible for some of the differences we observed in HDL phenotypes (ie, reduction of HDL size and PON1), in vitro and in vivo studies have found no qualitative differences between apo A-I and pro-apo A-I in terms of reverse cholesterol transport assessments (40, 41).

We attempted to identify the CVD risk factor variables whose responses to vitamin E were associated with change in TAS. Of all the traits measured, only Δ apo A-I was independently correlated (r^2: ≈24%). Apo A-I is readily oxidized and, as part of native HDL, is specifically oxidized at 2 methionine residues (42). Apo A-I oxidation can increase its immunoreactivity (43), but this would predict a negative correlation of Δ apo A-I with Δ TAS instead of the positive correlation we have observed. Given recent interest in apo A-I and its mimetic peptides as antioxidants (44, 45), it is tempting to speculate that apo A-I is one of the antioxidant molecules whose effects are detected in the TAS assay. However, on average, the vitamin E supplementation was associated with apo A-I concentrations that were ≈1.3 μmol/L higher (ie, 3.2 mg/dL), whereas TAS increased by ≈43 μmol/L and our previous study would predict that raw plasma α-tocopherol concentrations were ≈24 μmol/L higher in this experiment (6). Therefore, at most, the change in apo A-I can account only for a modest proportion of the change in TAS.

We also performed analyses to determine whether genes influence responses of the CVD risk factor variables to vitamin E diet supplementation. There were <200 baboons in these analyses, so standard errors for heritability were large (ranging from 0.13 to 0.26); a planned expansion of this study will reduce those standard errors and, therefore, may detect additional traits with significant genetic effects. Nevertheless, we observed that 2 of the Δ traits were significantly heritable in this study: proportion of cholesterol in HDL_2 and PON1. We have previously reported modest, albeit significant, genetic control of HDL response to dietary challenges (ie, increasing amounts of dietary fat and cholesterol) (46), but we are unaware of any report of genes controlling HDL response to dietary vitamin E concentrations in any species. In the case of HDL_2, we observed that ≈37% of the

residual phenotypic variance in Δ HDL_2 was explained by the additive effects of genes.

PON1 activity is highly heritable in both humans and baboons (47, 48), but the present result indicates that the response of PON1 to vitamin E dietary supplementation is also under genetic control, with a heritability of ≈22%. Vitamin E was reported to regulate transcription or activity of a number of genes involved in oxidative stress, proliferation, inflammation, and apoptosis (39, 49, 50). In several instances, the effect on transcription appears to be independent of antioxidant capacity and is specific to the α-tocopherol form of vitamin E (51, 52). The present findings suggest that genes influence the effect of vitamin E on HDL and PON1 metabolism by unknown mechanisms. These genetic mechanisms may involve direct effects on gene transcription or indirect effects on the redox environment. Ongoing experiments are under way to increase the number of animals available for analysis so that we may identify individual genes mediating the effects of vitamin E on CVD risk factors. Such exploration may show novel regulatory pathways linking lipoprotein and redox metabolisms.

The major finding of this study is a significant effect of vitamin E dietary supplementation on the metabolism of HDLs. Moreover, the effect appears to be exerted at 2 points in HDL metabolism. First, we observe that an effect of vitamin E is to cause smaller HDL size, including a lesser amount of cholesterol in the HDL_2 subfraction. This effect of vitamin E is under significant genetic regulation. Second, we observe higher concentrations of apo A-I. This is not under genetic control but is strongly correlated with change in antioxidant status and may, in part, help account for it. These 2 effects are oppositely associated with CVD susceptibility whether vitamin E supplementation might be proatherogenic or antiatherogenic for an individual may depend on which of these 2 effects on HDL metabolism predominates. This observation is consistent with the mixed results of clinical trials for vitamin E and may help explain the controversial findings to date.

We thank Perry H Moore Jr, Elizabeth D Rainwater, Jane S VandeBerg, and Joel S Whitehead for excellent technical assistance.

The authors responsibilities were as follows—DLR, MCM, and XLW: design; DLR and JLV: biochemical assays; DLR and MCM: statistical analyses; DLR: first draft; DLR, MCM, JLV, and XLW: final approval. None of the authors had any conflicts of interest.

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