α-Tocopherol supplements and high-density-lipoprotein–cholesterol levels

BY JOHN H. KALBFLEISCH[1,3], JOSEPH J. BARBORIAK[1,4], BARBARA A. ELSE[2], C. VINCENT HUGHES[2,5] AND FELIX E. TRISTANI[2,5]

Research Service[1] and Cardiopulmonary Rehabilitation Center[2], Veterans Administration Medical Center, Milwaukee, Wisconsin 53193, USA, and Departments of Anesthesiology[3], Pharmacology[4] and Medicine[5], The Medical College of Wisconsin, Milwaukee, Wisconsin 53226 USA

(*Received 14 June 1985 – Accepted 9 August 1985*)

1. In a randomized, double-blind 6-month study, α-tocopherol (728 mg) or placebo were administered daily to seventy-eight volunteers (forty-nine men, twenty-nine women) to investigate the possible enhancing effect of vitamin E on plasma high-density-lipoprotein–cholesterol (HDL–C) levels. In addition, the available reported values from short-term (4–6 weeks) studies, as well as the 4-week results from the present study, were combined and analysed for factors which may modify the effect of α-tocopherol on HDL–C.

2. No consistent effect of α-tocopherol on plasma HDL–C levels was observed either in the combined 4-week values or in the 6-month study. Further analysis of the combined short-term values and 6-month values indicated that, in subjects with low initial HDL–C levels, treatment with α-tocopherol or placebo did not produce significantly different HDL–C changes.

Results of some preliminary short-term studies have suggested that administration of a large daily dose of α-tocopherol (545–728 mg) can increase the plasma levels of high-density-lipoprotein–cholesterol (HDL–C) (Herman *et al.* 1979; Herman, 1981*a*,*b*). In view of the reported attenuating effects of high HDL–C levels on the development of coronary heart disease and the relative difficulty in achieving a significant and reproducible rise in HDL–C by dietary or pharmacologic means, the reports on the effect of α-tocopherol on this lipid class attracted considerable attention. However, a series of other studies (Schwartz & Rutherford, 1981; Barboriak *et al.* 1982; Ehnholm *et al.* 1982; Howard *et al.* 1982; Kesaniemi & Grundy, 1982; Chapkin *et al.* 1983; Serfontein *et al.* 1983), employing relatively small groups of subjects, failed to confirm the general effectiveness of α-tocopherol on HDL–C levels. Few of the reports were based on controlled randomized trials that included a placebo group, and they used a short (4–6 weeks) treatment period.

Therefore, we decided to study the effect of α-tocopherol on plasma HDL–C levels in a long-term, double-blind study using patients most likely to benefit from such a treatment, i.e. patients entering a cardiac rehabilitation programme. In addition, we have pooled available reported short-term values on the effect of α-tocopherol on plasma HDL–C in order to investigate the possible variables modifying such an effect. The findings of both the long-term and pooled-values studies indicate little or no consistent effect of oral α-tocopherol on plasma HDL–C levels.

MATERIALS AND METHODS

Long-term study

Subject selection. A group of forty-nine male patients (24–73 years of age), who were enrolled in a cardiac rehabilitation programme, volunteered for the study. A group of twenty-nine female subjects (24–71 years of age), who were spouses of the male patients,

Table 1. *Summary of α-tocopherol effects on plasma high-density-lipoprotein–cholesterol (HDL–C) in short-term (4 week) studies*

No. of subjects	Sex	Age (years): mean or range	α-Tocopherol (mg/d)	Mean HDL–C (mg/l) Initial	Change	Reference
5	♂, ♀	34·8	545	168	+426	Herman *et al.* 1979
5	♂, ♀	34·2	545	454	+266	Herman *et al.* 1979
3	♂	26·7	728	317	+160	Herman, 1981*b*
26	♀	—	545	460	+70	Sundaram *et al.* 1981†
13	♀	42·4	728	659	+55	Barboriak *et al.* 1982
19	♂	45·8	728	411	+22	Barboriak *et al.* 1982
6	♂, ♀	38·2	728	668	+19	Hatam & Kayden, 1981*
15	♂	24·2	364	410	+15	Serfontein *et al.* 1983
15	♂	36·1	728	606	+11	Barboriak *et al.* 1982
39	♂, ♀	39·4	545	485	+3	Howard *et al.* 1982
5	♂, ♀	39·2	728	456	+2	Hatam & Kayden, 1981*
7	♂	41·0	545	376	0	Chapkin *et al.* 1983
15	♂, ♀	30–60	728	533	−4	Stampfer *et al.* 1983†
29	♂	58·4	728	473	−1	Present report‡
14	♂, ♀	49·2	545	382	−13	Herman, 1981*a*
6	♂, ♀	31–55	545	626	−15	Ehnholm *et al.* 1982*
11	♂	63·1	728	464	−34	Schwartz & Rutherford, 1981
5	♂, ♀	55·6	728	410	−36	Kesaniemi & Grundy, 1982
13	♀	44·5	728	668	−48	Present report‡
4	♂, ♀	25·0	728	595	−137	Herman, 1981*b*

* 6 weeks. † 8 weeks. ‡ 4-week values of 6-month study.

or hospital personnel, also volunteered for the project. Male subjects were survivors of a myocardial infarction and were participating in a rehabilitation programme consisting of supervised physical activity and dietary and psychological counselling.

Study procedures. Before treatment with either α-tocopherol or placebo, baseline levels of HDL–C, total cholesterol and triglycerides were obtained for each participant. Laboratory determinations of HDL–C were performed by the procedure suggested by the Lipid Research Clinical Manual (National Institutes of Health, 1979), while plasma total cholesterol and triglyceride levels were measured by the previously described automated procedure (Block *et al.* 1965; Kessler & Lederen, 1965). Our laboratory has been participating in the CDC-NHLBI High-Density Lipoprotein Standardization Program, which also includes the standardization of the total cholesterol procedure.

Subjects were randomized to a placebo or α-tocopherol (364 mg twice daily) schedule for the next 6 months. Lipid determinations were made at the 4-week and 6-month time-points. During the 6-month study, subjects were requested not to alter their usual life-style patterns nor make marked changes in diet or exercise and to report any unusual reactions or illnesses.

Combined short-term studies

To investigate the potential α-tocopherol effect on HDL–C with short-term treatment (4–6 weeks) more fully, we combined all the available reported values (Herman *et al.* 1979; Hatam & Kayden, 1981; Herman 1981*a*, *b*; Schwartz & Rutherford, 1981; Barboriak *et al.* 1982; Kesaniemi & Grundy, 1982) of HDL–C with the 1-month values of our 6-month study. Where necessary, all total cholesterol and HDL–C values were converted to mg/l (from mmol/l). These produced values for 196 subjects, of whom forty-nine received placebo and

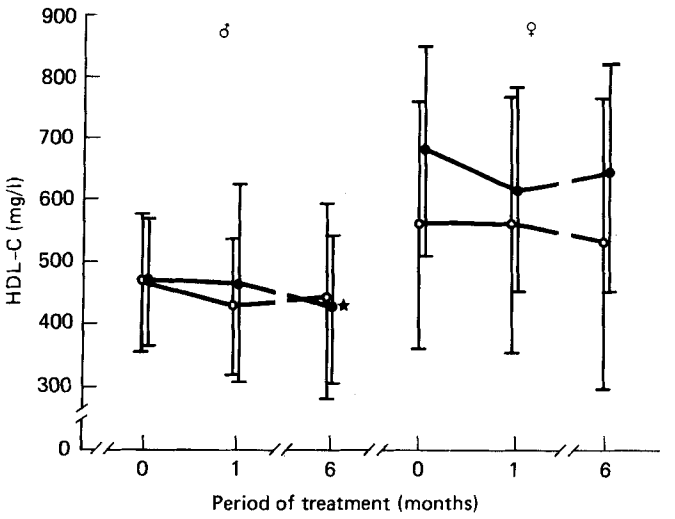

Fig. 1. The effect of α-tocopherol (728 mg/d, ●) and of placebo (○) on high-density-lipoprotein-cholesterol (HDL-C). Points are group means and standard deviations are represented by vertical bars. *Value was significantly different from the initial value ($P < 0.05$).

147 received α-tocopherol. The dose of α-tocopherol and length of treatment is indicated in Table 1.

Analysis of results

Information on age, sex and treatment group (placebo or α-tocopherol) of subjects in the 6-month study was coded and merged with the values for plasma HDL-C, total cholesterol and triglycerides for computer storage and analysis of results. The lipid levels for the placebo and α-tocopherol study groups at the initial, week 4 and 6-month time points were summarized by the mean and standard deviation. HDL-C values from the combined short-term studies are presented as a scatter plot of the end-of-treatment value (Y) *v.* the initial level (X).

Differences in lipid means within the study groups and differences between means (or changes in mean values) of placebo and α-tocopherol groups were assessed by analysis of variance and multiple comparison techniques (least significant difference (LSD) or Scheffe's procedure (Snedecor & Cochran, 1969)). Additionally, triglyceride values were analysed on the logarithmic scale because of the intrinsic positive skew in its probability distribution. To test for a change in lipid values across the range of observation, we regressed the end-of-treatment lipid determinations (Y) on the initial measurements (X) by the method of maximum likelihood, with a model $Y = a + bX$. The regression line for the α-tocopherol values was statistically compared with the regression line for placebo to detect a possible effect of α-tocopherol treatment and both regression lines were compared with the no-change reference line ($Y = X$). A probability level of 0·05 or smaller was used to indicate statistical significance.

RESULTS

The initial, 1- and 6-month values for HDL-C of the α-tocopherol and placebo groups in the present study are summarized in Fig. 1. Female subjects randomized to the α-tocopherol group had somewhat higher initial mean HDL-C levels than females assigned to the placebo

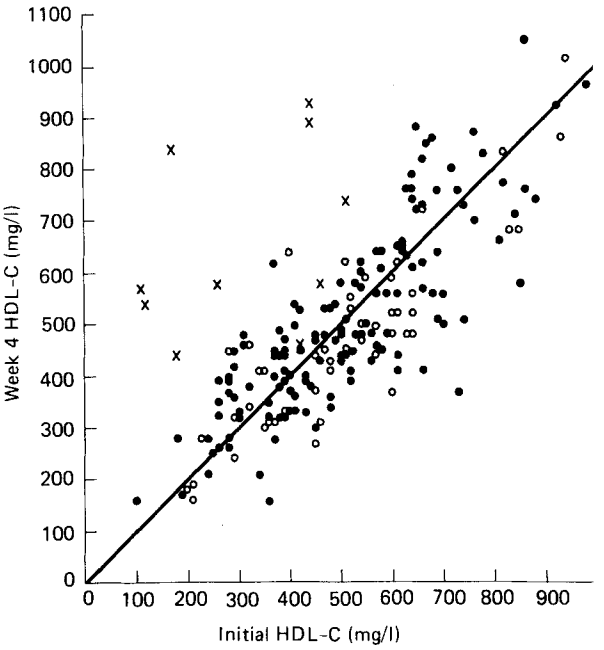

Fig. 2. Scatter diagram of the initial (X axis) *v.* 4-week (Y axis) high-density-lipoprotein–cholesterol (HDL–C) values derived from reported values and the present study. Values above the line indicate an increase, those below the line a decrease from the initial levels. ×, Values from Herman (1981*b*) were not included in the calculations. α-Tocopherol (●); placebo (○).

group, while males in the placebo and α-tocopherol groups had nearly identical initial mean HDL–C levels. Comparison of the 1- and 6-month mean HDL–C levels with the initial mean HDL–C values showed no significant change for either the female placebo or female α-tocopherol group. On the other hand, male subjects receiving α-tocopherol showed a significant reduction in mean HDL–C levels from the initial level (468 mg/l) to the 6-month level (423 mg/l, $P < 0{\cdot}01$ (LSD) and $P < 0{\cdot}05$ (Scheffe's)). Males on placebo showed no significant change in mean HDL–C. No significant differences were observed between the pattern of mean HDL–C responses across the study period for the α-tocopherol group *v.* the placebo group (the group × time-point interaction factor was not significant in the analysis of variance).

Regression analysis (Y = 6-month HDL–C, X = initial HDL–C) of the values for α-tocopherol-treated males produced the regression equation, $Y = -129{\cdot}4 + 1{\cdot}18X$, which was not significantly different from the regression equation for the placebo group, $Y = -62{\cdot}2 + 1{\cdot}11X$. The regression line for the placebo values did not differ significantly from the reference line ($Y = X$), but the intercept, −129·4, of the α-tocopherol group was significantly lower than the zero intercept of the reference line, thus indicating a slight decrease in mean HDL–C. The regression line for the α-tocopherol female group ($Y = -158{\cdot}9 + 1{\cdot}19X$) was not significantly different from the female placebo regression line ($Y = -155{\cdot}5 + 1.22X$) and neither line differed significantly from the reference line.

Since previous reports have indicated a more pronounced α-tocopherol effect on the HDL–C level in male subjects with initially low HDL–C (Hatam & Kayden, 1981; Barboriak *et al.* 1982), we retrospectively separated the HDL–C values of males into two sets according to the initial HDL–C level (< 400 or ⩾ 400 mg/l). Seven males with low initial HDL–C (mean 339 (SD 53) mg/l) in the α-tocopherol group did not show a significant

change after 6 months of treatment (mean 326 (SD 74) mg/l), and five placebo males showed no change for the 6-month period (mean 336 (SD 61) mg/l to 336 (SD 80) mg/l). The changes for the α-tocopherol and placebo groups were not significantly different.

The total cholesterol level was not significantly altered from the initial to 6-month values for any of the study groups (mean levels: α-tocopherol male 2424–2315 mg/l, α-tocopherol female 2396–2369 mg/l, placebo male 2388–2351 mg/l, placebo female 2441–2438 mg/l). Triglyceride levels showed no significant change for the male subgroups (α-tocopherol 1851–2059 mg/l, placebo 1506–1517 mg/l) and the female placebo group (1795–1746 mg/l). The female group receiving α-tocopherol showed a significant increase in mean triglyceride levels (1239–1529 mg/l, $P < 0{\cdot}05$ (LSD)).

The 1-month HDL–C values from the present study, combined with other published short-term α-tocopherol study values, are displayed as a scatter diagram in Fig. 2. The HDL–C values from the report of Herman (1981*b*) are identified separately in Fig. 2 and were not included in calculations using the combined values set because of the discrepancy with other reported HDL–C values. An equal number of α-tocopherol-treated subjects (*n* 64) fell above and below the no change reference line while eight values remained unchanged. By applying regression analysis to the combined short-term study values, the α-tocopherol regression line $Y = -6{\cdot}4 + 1{\cdot}01X$ was not significantly different from the placebo regression line $Y = -9{\cdot}5 + 0{\cdot}95X$, and neither regression line differed significantly from the $Y = X$ reference line. Similar analysis of HDL–C, expressed as a percentage of total cholesterol ($X\%$ and $Y\%$), gave the same result (α-tocopherol: $Y\% = 0{\cdot}01 + 0{\cdot}98X\%$; placebo: $Y\% = 0{\cdot}01 + 0{\cdot}94X\%$; not significantly different).

When the subjects' age and sex were used to divide the combined values set into subgroups, no significant changes in mean HDL–C were observed in α-tocopherol-treated or placebo-treated subjects. When low initial HDL–C levels were used to examine the effect of this variable (HDL–C < 350 mg/l, *n* 24), the combined group showed a statistically significant increase during the first 4 weeks of α-tocopherol treatment (from 270 (SD 50) to 320 (SD 90) mg/l, $P < 0{\cdot}01$). The HDL–C increase in the placebo group with similarly low initial values (*n* 10) was not statistically significant (from 270 (SD 52) to 300 (SD 113) mg/l, $P > 0{\cdot}05$). The differences between the HDL-C changes due to α-tocopherol or placebo were not statistically significant (*t* 0·78). A similar analysis of total cholesterol and triglyceride levels (not presented) also showed no significant changes in mean levels.

DISCUSSION

The initial reports of Herman *et al.* (1979) and Herman (1981*b*) indicated that 4 weeks of α-tocopherol administration was associated with a considerable increase in HDL–C levels. Since the levels of total plasma cholesterol essentially did not change, the HDL–C elevation was believed to represent a redistribution of this lipid among the individual lipoprotein fractions. Following these encouraging initial reports, other investigators were unable to corroborate the HDL–C enhancing effects of α-tocopherol. In fact, most of the observed mean HDL–C changes from the initial level (Table 1) were quite small and statistically not significant.

The short-term studies summarized in Table 1 comprised a wide age range (21–76 years) of both males and females, normal healthy subjects (*n* 163) and selected groups of patients or individuals with lipid abnormalities (*n* 92). Apart from the 1-month values for the present study subgroups, there are thirteen reported studies of α-tocopherol-treated subjects but only four included a concurrently-randomized placebo group (*n* 213 α-tocopherol and *n* 33 placebo). Although the majority of reports did not corroborate the initial results of Herman *et al.* (1979) and Herman (1981*b*), only a few would have been able to make a parallel

placebo-group comparison had a more noticeable elevating effect on HDL–C been observed with α-tocopherol. The report of twenty-six female patients with mammary dysplasia (Sundaram *et al.* 1981) showed a statistically significant increase in mean HDL–C (from 460 to 530 mg/l). This initial mean HDL–C (460 mg/l) was rather low for females and there was no placebo-treated group for comparison.

Further examination of the HDL–C values of some of the short-term studies showed that a statistically significant increase in the mean HDL–C was primarily observed in α-tocopherol-treated subjects with low initial HDL–C (Herman *et al.* 1979; Herman, 1981*b*; Sundaram *et al.* 1981; Barboriak *et al.* 1982). Inspection of Fig. 2 also sugests that subjects with low initial HDL–C ($\leqslant$ 350 mg/l) tended to show an increase in HDL–C by the end of the treatment period. However, subjects with initially high HDL–C ($>$ 800 mg/l) frequently showed a reduction in HDL–C (Fig. 2). A large component of these changes may be statistical in nature (regression toward the mean) (Criqui *et al.* 1983; Ingelfinger *et al.* 1983), suggesting that the claims of an α-tocopherol effect for subjects with low HDL–C levels need re-examination. The small number of subjects in the extreme HDL–C regions makes a similar evaluation difficult for placebo values. Our regression analysis, which expresses the final HDL–C values in terms of the initial HDL–C level, indicates that there is no statistically detectable difference between regression lines for the α-tocopherol group, the placebo group, and the no-change reference line. The combined short-term values set consists of values from investigators not all using the same laboratory method for lipid determination, but we assumed that each laboratory always performed the same lipid analysis method. Consequently, we felt that differences between final and initial values and regression analyses of these values would not be overly biased by measurement techniques of different laboratories. Thus, examining the combined values, no short-term effect of α-tocopherol on plasma HDL–C levels could be demonstrated.

Young subjects ($\leqslant$ 35 years) were believed to be more responsive to α-tocopherol treatment than older ones (Herman *et al.* 1979; Herman, 1981*b*). However, our analysis of subjects in this age group (in the short-term study value set) did not show a significant effect of α-tocopherol on HDL–C levels. Therefore, with the exception of the first reported observations (Herman *et al.* 1979; Herman, 1981*b*), there is essentially no evidence for a marked HDL–C elevating effect with short-term administration of α-tocopherol.

The results of the current long-term randomized 6-month study tend to confirm the findings from the short-term studies. As shown in Fig. 1, there was little or no difference between α-tocopherol treatment or placebo in either male or female groups, and initially low HDL–C subgroups did not show a significant change in mean HDL–C after 6 months of treatment. Our male subgroups were primarily post-myocardial infarction patients and additional study would be needed to determine if our findings apply to a normal healthy male population of a similar age. Stampfer *et al.* (1983) reported no significant change in mean HDL–C levels following 8 and 16 weeks of treatment with α-tocopherol or placebo. The study by Stampfer *et al.* (1983) complements and extends our present findings, since their subjects were all healthy volunteers.

Our conclusions are: (a) The first reported marked elevations of HDL–C (Herman *et al.* 1979; Herman, 1981*b*) could not be reproduced by other investigators in short-term studies with similar or dissimilar subjects; (b) the results of short-term studies suggest a very small effect or no effect of α-tocopherol on HDL–C levels (even when expressed as a percentage of total cholesterol levels); (c) long-term administration of α-tocopherol (4–6 months) does not lead to a consistent increase in HDL–C levels. Our previous report (Barboriak *et al.* 1982) on a preliminary (non-randomized) study indicated an HDL–C level elevating effect of α-tocopherol in subjects with low initial HDL–C values. The findings of the present long-term randomized study indicate the importance and need for the use of placebo and

randomization for proper interpretation of such preliminary observations. Additionally, repeated determinations of study variates (particularly extreme values) at pertinent time intervals will help to reduce complicating design biases (regression toward the mean) (Criqui *et al.* 1983) when examining for possible treatment effects.

The authors wish to acknowledge the assistance of Mr Gerald Post and Ms Mary Joe Palessi, Pharmacy Service; the technical assistance of Mr Erwin Gehrke; and the administrative assistance of Mrs Norma Bachay, Veterans Administration Medical Center (Milwaukee, WI). They also wish to thank Dr Hemmige N. Bhagavan, Hoffmann-LaRoche Inc., Nutley, NJ, for supplying α-tocopherol and the corresponding placebo preparations.

REFERENCES

Barboriak, J. J., El Ghatit, A. Z., Shetty, K. R. & Kalbfleisch, J. H. (1982). *American Journal of Clinical Pathology* **77**, 371–372.

Block, W. D., Jarret, J. K. & Levine, J. B. (1965). In *Automation in Analytical Chemistry*, pp. 345–347 [L. T. Skeggs Jr, editor]. New York: Mediad, Inc.

Chapkin, R. S., Haberstrah, B., Liu, T. & Holub, B. J. (1983). *American Journal of Clinical Nutrition* **38**, 253–256.

Criqui, M. H., Frankville, D. D., Barrett-Connor, E., Klauber, M. R., Holdbrook, M. J. & Turner, J. D. (1983). *American Journal of Epidemiology* **118**, 52–59.

Ehnholm, C., Huttunen, J. K., Kostiainen, E., Lukka, M. & Aho, K. (1982). *Clinical Chemistry Acta* **121**, 321–325.

Hatam, L. J. & Kayden, H. J. (1981). *American Journal of Clinical Pathology* **76**, 122–124.

Herman, W. J. (1981*a*). *American Journal of Clinical Pathology* **76**, 124–126.

Herman, W. J. (1981*b*). *American Journal of Clinical Pathology* **76**, 844–845.

Herman, W. J., Ward, K. & Faucett, J. (1979). *American Journal of Clinical Pathology* **72**, 848–852.

Howard, D. R., Rundell, C. A. & Batsakis, J. G. (1982). *American Journal of Clinical Pathology* **77**, 86–89.

Ingelfinger, J. A., Mosteller, F., Thibodeau, L. A. & Ware, J. H. (1983). *Biostatistics in Clinical Medicine*. New York: Macmillan Publishing Company Inc.

Kesaniemi, Y. A. & Grundy, S. M. (1982). *American Journal of Clinical Nutrition* **36**, 224–228.

Kessler, G. & Lederen, H. (1965). In *Automation in Analytical Chemistry*, pp. 341–344 [L. T. Skeggs Jr, editor]. New York: Mediad, Inc.

National Institutes of Health (1979). *DHEW Publication* no. 75–628. **78**, 1661.

Schwartz, P. L. & Rutherford, I. M. (1981). *American Journal of Clinical Pathology* **76**, 843–844.

Serfontein, W. J., Ubbink, J. B. & de Villiers, L. S. (1983). *American Journal of Clinical Pathology* **79**, 604–606.

Snedecor, W. G. & Cochran, G.W. (1969). *Statistical Methods*, 6th ed. Ames, Iowa: Iowa State University Press.

Stampfer, M. J., Willett, W., Castelli, W. P., Taylor, J. O., Fine, J. & Hennekens, C. H. (1983). *American Journal of Clinical Pathology* **79**, 714–716.

Sundaram, G. S., London, R., Manimekala, S., Nair, P. P. & Goldstein, P. (1981). *Lipids* **16**, 223–227.