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**Original Contribution****IMPAIRED RESISTANCE TO OXIDATION OF LOW DENSITY LIPOPROTEIN IN CYSTIC FIBROSIS: IMPROVEMENT DURING VITAMIN E SUPPLEMENTATION**BRIGITTE M. WINKLHOFFER-ROOB,* OULIANA ZIOUZENKOVA,[†] HERBERT PUHL,[†] HELMUT ELLEMUNTER,[‡] PETER GREINER,[§] GUIDO MÜLLER,^{||} MARTIN A. VAN'T HOF,[¶] HERMANN ESTERBAUER,[†] and DAVID H. SHMERLING*

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Abstract—Antioxidants such as vitamin E protect unsaturated fatty acids of LDL against oxidation. In the ex vivo model used, LDL was exposed to Cu²⁺ ions, a potent prooxidant capable of initiating the oxidation of LDL. The lag time, indicating the delay of conjugated diene formation in LDL due to antioxidant protection, was measured in 54 cystic fibrosis (CF) patients with plasma α -tocopherol levels below (Group A, $n = 30$) or above (Group B, $n = 24$) 15.9 $\mu\text{mol/L}$ (mean \pm 2 SD of Swiss population). Patients were reevaluated after 2 months on 400 IU/d of oral RRR- α -tocopherol. In group A, α -tocopherol concentrations in LDL increased significantly from 3.2 ± 1.6 mol/mol LDL to 8.2 ± 2.8 mol/mol ($P < 0.001$) and lag times increased from 79 ± 33 min to 126 ± 48 min ($P < 0.001$), whereas in the vitamin E sufficient group B no further increase neither in LDL α -tocopherol concentrations or in lag times was observed. LDL oleic acid concentrations were higher, and linoleic acid concentrations were lower in patients than in controls. After efficient vitamin E supplementation, lag times were positively related to LDL α -tocopherol ($P < 0.01$) and negatively to LDL linoleic and arachidonic acid content ($P < 0.001$). The maximum rate of oxidation correlated positively with linoleic and arachidonic acid concentrations, as did the maximum conjugated diene absorbance. These results indicate that LDL resistance to oxidation is impaired in vitamin E deficient CF patients but can be normalized within 2 months when α -tocopherol is given in sufficient amounts. Linoleic and arachidonic acid content exhibit a major influence on the LDL resistance to oxidation.

Keywords— α -Tocopherol, Low density lipoprotein, Oxidation resistance, Linoleic acid, Arachidonic acid, Cystic fibrosis, Free radicals

INTRODUCTION

As a consequence of fat malabsorption due to exocrine pancreatic insufficiency,¹ subclinical vitamin E deficiency is a frequent finding in patients with cystic fibrosis (CF). In contrast, symptomatic vitamin E deficiency is a rare event.² To prevent and treat vitamin E deficiency, oral vitamin E supplementation has become part of the therapeutic management of CF patients.³ However, vitamin E deficiency is not always reversed by routine vitamin E supplementation.^{1,4}

Thus far, data on the biological significance of sub-

clinical vitamin E deficiency in CF patients are limited. Chronic lung inflammation is suggested to cause an increased release of oxygen-derived free radicals in the presence of antioxidant deficiencies due to fat malabsorption, and the resulting oxidant-antioxidant imbalance could lead to an enhanced free radical attack of polyunsaturated fatty acids (PUFAs) of biomembranes and lipoproteins and, consequently, to tissue injury.⁵ Data on the occurrence of lipid peroxidation in CF patients is available from determinations of in vitro peroxide induced red cell hemolysis,⁶ thiobarbituric acid reactive substances,⁷ and hydrocarbon gas exhalation.^{8,9}

The aim of this study was to determine the significance of subclinical vitamin E deficiency on the LDL resistance to in vitro oxidative stress and the effects

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of correcting this deficiency in CF patients. In addition, relationships between the LDL resistance to oxidation and the fatty acid content of LDL were analyzed.

SUBJECTS

Patients

Fifty-four CF patients under long-term care in the CF outpatient clinics of 10 different hospitals in Switzerland, Austria, and Germany were enrolled. Their ages ranged from 3 months to 34 years (median 9 years). The diagnosis of CF had been established by typical clinical symptoms and elevated sweat electrolytes.¹⁰ All patients exhibited exocrine pancreatic insufficiency and were treated with pancreatic enzymes. Twenty-four patients were on long-term oral vitamin E supplementation in dosages of 50 to 800 IU/d prior to study entry. Shwachman scores,¹¹ indicating the clinical disease status, were 77 ± 19 . No changes were introduced in the therapeutic management during the study period, except for intermittent antibiotic treatment of acute pulmonary exacerbations. Patients were considered vitamin E deficient and assigned to group A if they had plasma α -tocopherol concentrations below mean $- 2$ SD of the Swiss population ($15.9 \mu\text{mol/l}$)¹²; accordingly, those with plasma α -tocopherol concentrations $> 15.9 \mu\text{mol/l}$ were assigned to group B.

Immediately after the baseline evaluation, patients without supplements ($n = 30$) or with dosages < 400 IU/d ($n = 12$) were switched to 400 IU/d of RRR- α -tocopherol, given in a single dose during breakfast. Patients who were already taking vitamin E supplements in dosages of 400 IU/d ($n = 9$), 600 IU/d ($n = 1$), and 800 IU/d ($n = 2$) continued to do so.

Forty-seven of 54 patients had a second evaluation after 2 months on vitamin E. Drop-outs were due to death ($n = 2$), movement to another country ($n = 2$), or vacation ($n = 3$). In two patients, either LDL α -tocopherol concentrations at baseline or lag time values at 2 months were missing. Thus, a complete set of data for statistical analysis was available from 53 patients at baseline and 46 patients at 2 months.

Controls

Twenty-three clinically healthy normolipidemic nonsmoking staff members aged 26 ± 7 years from the Departments of Pediatrics, University of Zurich ($n = 21$), and University of Innsbruck ($n = 2$) served as controls because it was ethically not acceptable to draw the necessary amounts of blood from healthy children. None of the controls was taking vitamin supplements or medications other than oral contraceptives

($n = 6$). All were on a regular diet. Controls had a single evaluation including the same parameters as assessed in patients to allow, by comparison, validation of baseline values and evaluation of treatment efficacy.

The study protocol was approved by the Ethical Committee of the Department of Pediatrics, University of Zurich, and informed consent was obtained from the patients or their parents and from controls.

METHODS

Immediately after blood was drawn after an overnight fast, EDTA (0.1% final) plasma was prepared and protected from light with aluminum foil by the same investigator (B.M.W.R.) in all CF centers.

Determination of LDL antioxidants and LDL resistance to oxidation

The freshly prepared plasma samples (3 to 4 ml) were shipped on wet ice by overnight courier to the Institute of Biochemistry, University of Graz. LDL was isolated by ultracentrifugation in a discontinuous potassium bromide gradient containing 0.1% EDTA using a Beckman SW 41 Ti-rotor, as described previously.¹³ The LDL band was quantitatively collected and diluted with PBS to give the same volume as the centrifuged plasma sample (3 to 4 ml). The total cholesterol concentrations of the LDL stock solutions were determined with the CHOD-PAP kit from Boehringer Mannheim. Based on this analysis, the LDL concentration was calculated as mg LDL mass/ml and $\mu\text{mol LDL/l}$, respectively, assuming an LDL cholesterol content of 31.6 wt % and a molecular weight of 2500 kD both for CF patients and healthy subjects. An aliquot of the LDL stock solution was stored at 4°C for not longer than 6 h for the determination of antioxidants. Another aliquot was used immediately for the oxidation assay. For the latter, EDTA and salt were removed using prepacked Biogel P6 columns. Oxidation was then performed under standardized conditions¹³ with 0.1 μM LDL in PBS (equal to 0.25 ml LDL mass/ml) and 1.66 μM CuCl_2 . The oxidation of LDL was monitored spectrophotometrically by measuring the conjugated diene (CD) absorbance at 234 nm. The oxidation indexes derived from the CD versus time curves are lag time, maximum rate of oxidation (dA/min), and maximum increase in CD absorbance (A_{234}). This oxidation assay has proved to be reproducible with CV for multiple analyses of the same LDL sample of 1.85%¹³ and multiple analyses of LDL from a single donor of 8.8%.¹⁴

Antioxidants in LDL were determined as described

Table 1. Tocopherol and Carotenoid Concentrations in Plasma and in LDL at Baseline and After 2 Months of Vitamin E Supplementation in Group A (Plasma α -Tocopherol $< 15.9 \mu\text{mol/l}$) and Group B (Plasma α -Tocopherol $> 15.9 \mu\text{mol/l}$) as Well as in Controls

	Patients Baseline			2 Months				
	Group A (n = 30)	Group B (n = 24)	Group A vs. B ^a	Group A (n = 27)	Baseline vs. 2 Months ^b	Group B (n = 20)	Baseline vs. 2 Months ^b	Controls ^b (n = 23)
Plasma ($\mu\text{mol/l}$)								
α -tocopherol	9.0 \pm 4.2 ^c	28.3 \pm 9.3	< 0.0001	27.8 \pm 8.1	< 0.0001	35.3 \pm 9.0 ^d	< 0.05	26.9 \pm 5.1 ^{c,d}
γ -tocopherol	0.76 \pm 0.60	0.48 \pm 0.43	NS	0.34 \pm 0.33	< 0.001	0.25 \pm 0.17	< 0.01	1.59 \pm 0.61
α -carotene	0.02 \pm 0.05	0.06 \pm 1.11	< 0.05	0.03 \pm 0.07	< 0.05	0.05 \pm 0.10	NS	0.18 \pm 0.08
β -carotene	0.09 \pm 0.11	0.22 \pm 0.33	< 0.05	0.10 \pm 0.15	NS	0.21 \pm 0.40	NS	0.80 \pm 0.37
Lycopene	0.03 \pm 0.03	0.06 \pm 0.06	< 0.05	0.05 \pm 0.05	< 0.001	0.09 \pm 0.07	< 0.01	0.54 \pm 0.28
LDL (mol/mol)								
α -tocopherol	3.2 \pm 1.6 ^d	8.5 \pm 3.1 ^d	< 0.0001	8.2 \pm 2.8 ^d	< 0.0001	9.4 \pm 3.5 ^d	NS	5.0 \pm 0.8 ^d
γ -tocopherol	0.27 \pm 0.19	0.16 \pm 0.15	< 0.01	0.12 \pm 0.13	< 0.001	0.05 \pm 0.06	< 0.001	0.32 \pm 0.14
α -carotene	0.003 \pm 0.005	0.02 \pm 0.03	NS	0.008 \pm 0.02	NS	0.02 \pm 0.04	NS	0.04 \pm 0.02
β -carotene	0.04 \pm 0.06	0.09 \pm 0.18	< 0.05	0.04 \pm 0.04	NS	0.12 \pm 0.25	NS	0.25 \pm 0.12
Lycopene	0.02 \pm 0.04	0.04 \pm 0.05	< 0.05	0.04 \pm 0.03	< 0.05	0.05 \pm 0.05	NS	0.31 \pm 0.15
Lutein/Zeaxanthine	0.02 \pm 0.01	0.03 \pm 0.02	< 0.01	0.02 \pm 0.01	NS	0.03 \pm 0.02	NS	0.09 \pm 0.06
Cryptoxanthine	0.009 \pm 1.01	0.009 \pm 0.01	NS	0.01 \pm 0.01	NS	0.01 \pm 0.02	< 0.05	0.10 \pm 0.06

Values are mean \pm SD. Identical superscripts indicate significant differences for α -tocopherol between patients and controls.

^a Mann-Whitney tests, two tailed.

^b Wilcoxon signed ranks test.

^c $P < 0.0001$.

^d $P < 0.001$.

previously.^{15,16} In brief, a sample of the LDL stock solution containing 0.5 mg LDL mass was mixed with 0.2 ml methanol and extracted with 1 ml hexane, and the extract was dried with nitrogen and taken up in 0.1 ml HPLC solvent. α - and γ -Tocopherol were determined by HPLC (ODS-2), using methanol as solvent and fluorimetric detection at 335 nm with 292 nm excitation. Carotenoids were separated on an ODS-2 column with acetonitrile/dichloromethane/methanol 67/19/4 and UV/VIS detection at 436 nm. Calibration was based on standards separated under identical conditions after every 10 samples.

Determination of fatty acids in LDL

The freshly prepared EDTA plasma samples (2.5 to 3.0 ml) were immediately frozen at -80°C and kept at this temperature for not longer than 2 months until analysis. LDL was prepared from the freshly thawed samples by 2 h ultracentrifugation using a single-step discontinuous gradient in a Beckman NVT65 rotor¹⁷ (note that this rotor, which allows isolation of LDL within 2 h, was not yet available at the time when LDL oxidation experiments were performed). The plasma volume after centrifugation was in the range of 2.5 to

Table 2. Parameters of LDL Resistance to Oxidation at Baseline and After 2 Months of Vitamin E Supplementation in Group A and Group B as Well as in Controls

	Patients Baseline			2 Months				
	Group A (n = 30)	Group B (n = 24)	Group A vs. B ^a	Group A (n = 27)	Baseline vs. 2 Months ^b	Group B (n = 19)	Baseline vs. 2 Months ^b	Controls (n = 23)
Lag time (min)	79 \pm 33 ^c	111 \pm 30	< 0.001	126 \pm 48 ^c	< 0.001	127 \pm 29 ^d	NS	99 \pm 29 ^{c,d}
Oxidation rate								
dA/min	0.011 \pm .003	0.011 \pm 0.003	NS	0.010 \pm 0.004	NS	0.011 \pm 1.004	NS	0.012 \pm 0.006
Time (min)	119 \pm 40	151 \pm 40	< 0.01	173 \pm 61	< 0.001	169 \pm 35	NS	141 \pm 39
CD absorbance								
A 234 nm	0.66 \pm 0.14	0.69 \pm 0.23	NS	0.69 \pm 0.14	NS	0.77 \pm 0.13	< 0.05	0.70 \pm 0.17
Time (min)	188 \pm 55	212 \pm 52	NS	236 \pm 71	< 0.01	245 \pm 69	NS	200 \pm 48

Values are mean \pm SD.

^a Mann-Whitney tests, two tailed.

^b Wilcoxon signed ranks tests.

Identical superscripts indicate significant differences between patients and controls: ^c $p < 0.05$, ^d $p < 0.01$.

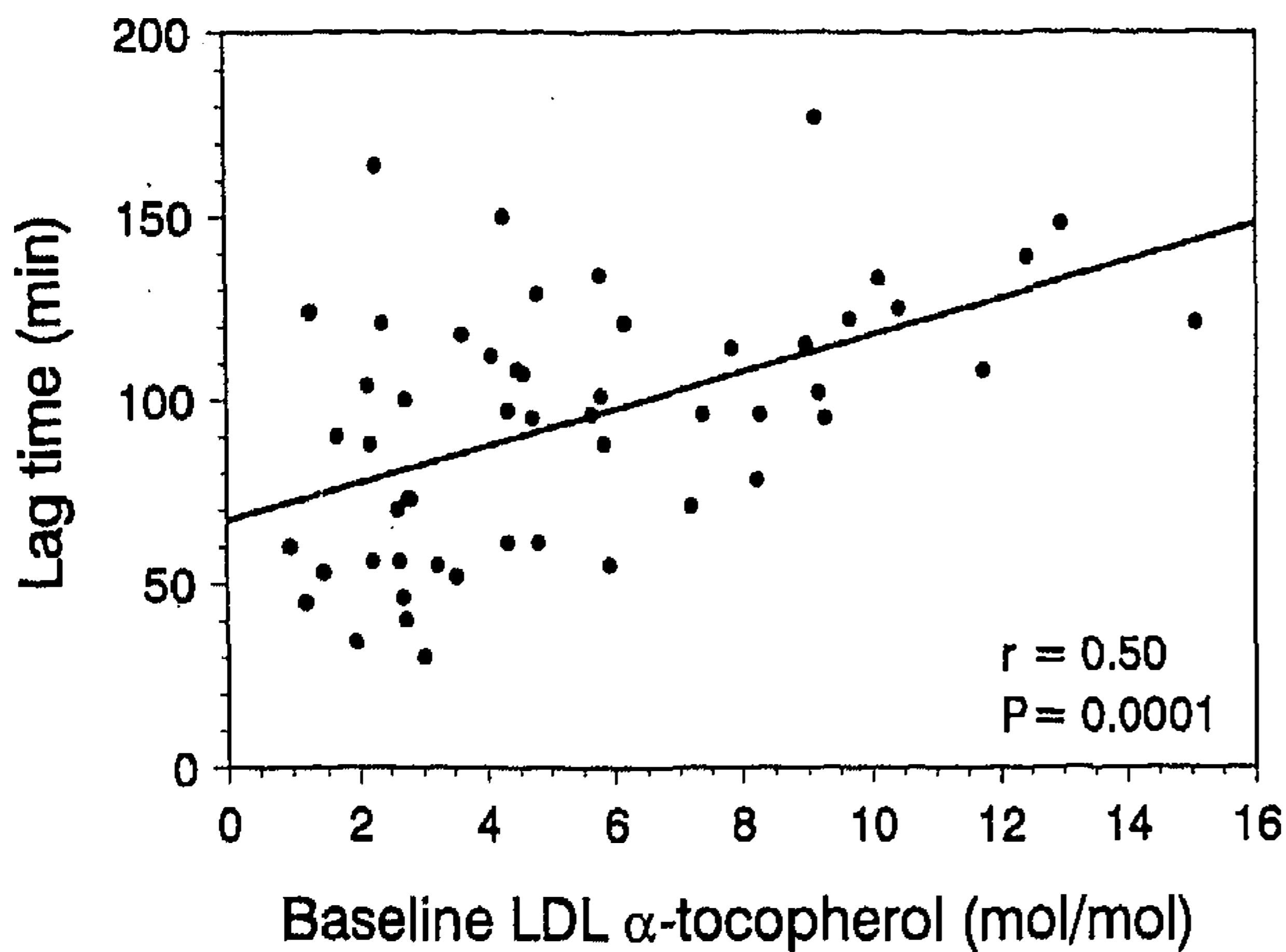


Fig. 1. Lag times of 53 CF patients are plotted against baseline LDL α -tocopherol concentrations. One patient is not included due to LDL α -tocopherol value missing.

3 ml. The LDL band was quantitatively collected and diluted with PBS to obtain exactly the same volume, as was the plasma volume originally present in the centrifuge tube. Cholesterol concentrations were determined as described earlier, and LDL cholesterol concentrations were calculated as mg LDL mass/ml and as $\mu\text{mol LDL/l}$, respectively. A sample of the LDL solution containing 1 mg LDL mass was spiked with 100 μg heptadecanoic acid and freeze dried in 10-ml Pyrex tubes. BHT (0.5 mg), BF_3 /methanol (1 ml), and benzene (0.5 ml) were added to the dry residues, and the samples were subjected to transesterification at 110°C for 90 min. The fatty acid methyl esters were extracted into hexane and separated by GC. The GC separation was performed with a HP5890 Series II instrument. More details of the whole procedure can be found in ref. 18.

Determination of plasma total cholesterol and LDL cholesterol concentrations

Plasma total cholesterol concentrations were measured enzymatically, using a commercially available kit from Beckman Ltd. (Brea, CA). Plasma LDL cholesterol concentrations were determined with a kit from BioMerieux Suisse SA (Geneva, Switzerland).

Determination of plasma antioxidants

Another aliquot of EDTA plasma was stored at -20°C for a maximum of 3 days before shipment on dry ice to the laboratories of the Vitamin Research Department of Hoffmann-La Roche Ltd. (Basel, Switzerland), where α - and γ -tocopherol and carotenoids

were determined in plasma by HPLC according to Hess *et al.*¹⁹

STATISTICAL ANALYSIS

Due to nonconformity of the data with the normality assumption, nonparametric tests were used for comparisons of antioxidant concentrations and the LDL resistance to oxidation at baseline and after 2 months of vitamin E supplementation. For within-group comparisons, Wilcoxon signed ranks tests were applied; for comparisons between groups, Mann-Whitney tests were used. Statgraphics (STSC Inc., Rockville, MD) Version 6 was used for all statistical procedures. Differences were considered significant at $P < 0.05$. All results are expressed as mean \pm SD.

RESULTS

Applying the cutoff value for plasma α -tocopherol concentrations of 15.9 $\mu\text{mol/l}$,¹² 30 patients were considered vitamin E deficient (group A), whereas 24 patients had a sufficient vitamin E status, in most cases due to long-term vitamin E supplementation prior to study entry (group B). Six patients of group A had been on supplements (median dosage, 100 IU/d) and 18 patients of group B (300 IU/d). Accordingly, baseline plasma and LDL α -tocopherol concentrations were significantly lower in group A compared with group B and with healthy controls (Table 1). Plasma α -tocopherol concentrations correlated positively with LDL α -tocopherol concentrations ($r = 0.85$, $P = 0.0001$). There were no differences between groups A

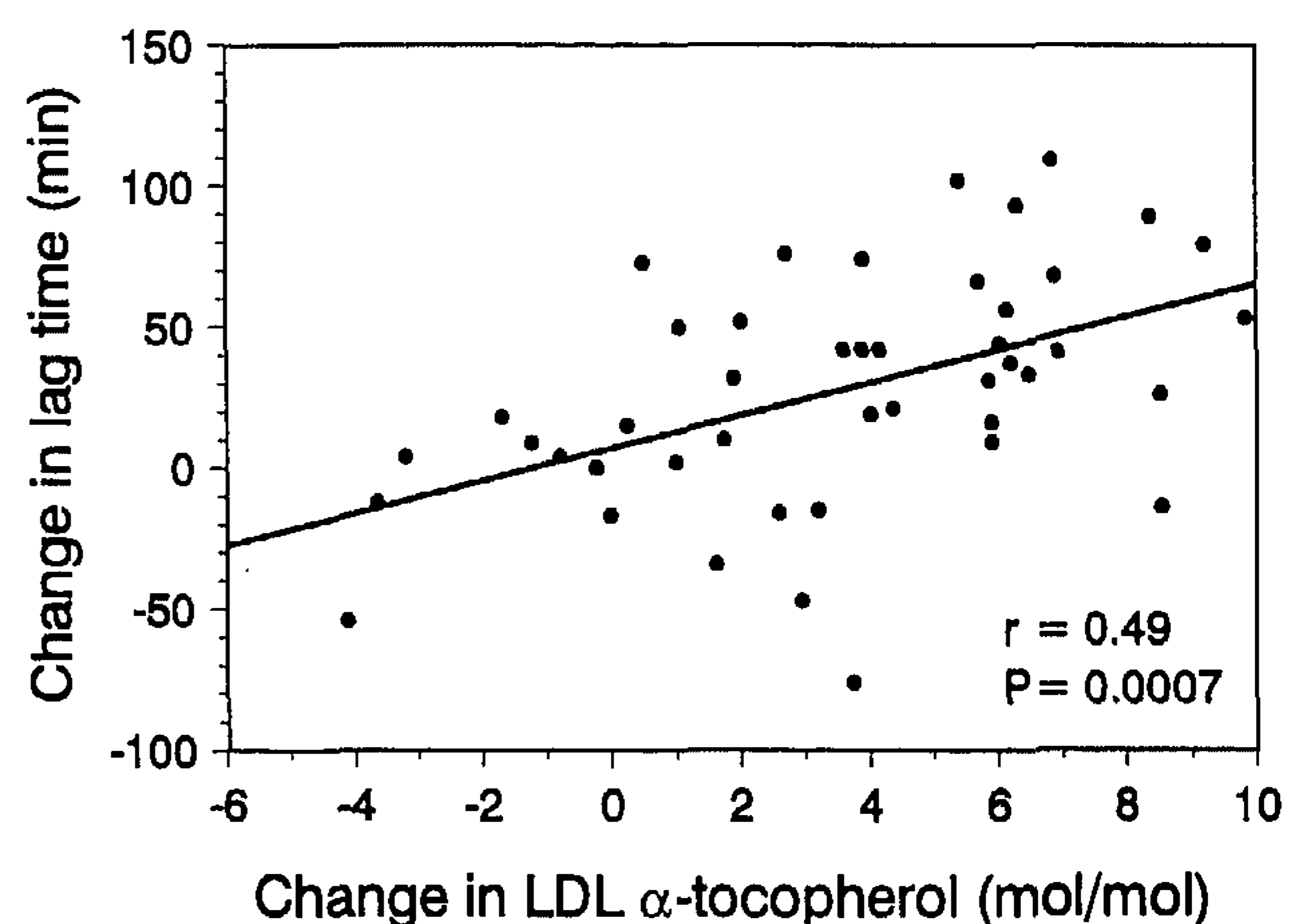


Fig. 2. Changes in lag times during vitamin E supplementation are plotted against changes in LDL α -tocopherol concentrations in 45 patients. One patient who completed the study is not included due to LDL α -tocopherol value missing at baseline.

Table 3. Fatty Acids in LDL at Baseline and After 2 Months of Vitamin E Supplementation in Group A and Group B

	Group A			Group B			Patients (n = 54)	Controls (n = 23)	P ^b
	Baseline (n = 30)	2 Months (n = 24)	P ^a	Baseline (n = 24)	2 Months (n = 20)	P ^a			
DB	3318.2 ± 906.7	3352.0 ± 881.8	NS	3532.0 ± 714.5	3380.4 ± 402.1	NS	3415.0 ± 824.6	3251.2 ± 452.4	NS
C16:0	744.0 ± 218.8	687.4 ± 142.6	NS	731.9 ± 190.6	637.1 ± 99.5	< 0.05	738.5 ± 204.7	533.6 ± 90.3	< 0.0001
C16:1n-7	130.1 ± 68.8	113.2 ± 57.7	NS	106.6 ± 46.5	87.7 ± 39.4	NS	119.5 ± 60.4	50.8 ± 18.8	< 0.0001
C18:0	201.1 ± 57.3	195.1 ± 47.0	NS	203.8 ± 47.4	182.5 ± 20.2	NS	202.3 ± 52.5	151.0 ± 20.5	< 0.0001
C18:1n-9	722.8 ± 224.1	696.1 ± 137.1	NS	745.8 ± 193.6	662.7 ± 119.8	NS	733.2 ± 209.2	458.5 ± 91.7	< 0.0001
C18:1n-7	60.6 ± 28.2	54.1 ± 19.4	NS	82.1 ± 150.5	44.3 ± 13.0	NS	56.8 ± 24.6	31.1 ± 6.3	< 0.0001
C18:2n-6	791.6 ± 32.98	820.8 ± 306.3	NS	858.4 ± 240.5	890.0 ± 165.4	NS	821.8 ± 291.5	949.0 ± 98.8	< 0.01
C18:3n-3	12.8 ± 4.4	12.8 ± 6.0	NS	13.5 ± 5.8	12.4 ± 6.7	NS	13.1 ± 5.0	13.5 ± 4.5	NS
C20:4n-6	145.5 ± 51.4	160.5 ± 57.1	< 0.05	151.0 ± 48.3	147.9 ± 35.2	NS	148.0 ± 49.6	134.2 ± 38.0	NS
C22:6n-3	33.4 ± 12.8 ^c	30.9 ± 12.6	< 0.05	39.3 ± 11.0 ^c	29.5 ± 10.7	< 0.05	36.1 ± 12.3	645.7 ± 14.8	< 0.01

As there were no differences between groups A and B except for C22:6n-3 ($P < 0.05$), groups A and B baseline were combined for comparison with controls. DB = Double bonds. Units are mol/mol; values are Mean ± SD.

^a Wilcoxon signed ranks tests.

^b Mann-Whitney tests, two tailed.

Identical superscripts indicate a significant difference between group A and B; ^c $P < 0.05$.

and B with regard to age (11.8 ± 8.3 years vs. 9.9 ± 8.2 years) and Shwachman scores (75.2 ± 17.9 years vs. 79.6 ± 19.2 years).

Oral vitamin E supplementation in a dosage of 400 IU/d was well tolerated in all patients, and none experienced any side effects, as detected by clinical examination and standard laboratory techniques. After 2 months of vitamin E supplementation, plasma α -tocopherol concentrations had significantly increased in both groups, whereas LDL α -tocopherol levels increased only in the vitamin E deficient group A. Plasma and LDL α -tocopherol concentrations were higher in the vitamin E sufficient group B than in controls (Table 1).

The LDL cholesterol content of plasma was significantly lower in patients (1.56 ± 0.38 mmol/l) compared with controls (2.94 ± 0.59 mmol/l) ($P < 0.0001$). It did not differ between group A (1.56 ± 0.38 mmol/l) and group B (1.80 ± 0.54 mmol/l) at baseline and did not change during the study period (2 months: group A, 1.53 ± 0.46 mmol/l; group B, 1.75 ± 0.62 mmol/l). The same was found for plasma total cholesterol concentrations (at baseline, group A,

2.85 ± 0.59 mmol/l; group B, 3.17 ± 0.77 mmol/l, compared with controls, 4.83 ± 0.68 mmol/l).

Both in plasma and in LDL, γ -tocopherol and carotenoid concentrations were significantly lower in patients than in controls (Table 1). β -carotene concentrations were higher in group B compared with group A; they did not change during the study period. In contrast, γ -tocopherol concentrations decreased in both groups, and some of the carotenoids increased either in plasma or in LDL or in both, but still remained lower than in controls (Table 1).

At baseline, lag times were significantly shorter in group A compared with group B ($P = 0.001$) and with controls ($P = 0.03$) (Table 2). Lag times correlated positively with LDL α -tocopherol concentrations (Fig. 1). After 2 months of vitamin E supplementation, a significant increase in lag times was observed only in group A ($P = 0.0001$). Changes in lag time correlated with changes in LDL α -tocopherol concentrations (Fig. 2). After supplementation, lag times of group A and group B were similar and even significantly longer than those of controls (Table 2).

Table 4. Results of Regression Analyses for Indexes of the LDL Oxidizability and LDL α -Tocopherol and Fatty Acid Content

	α -Tocopherol	C18:2n-6	C20:4n-6
Baseline			
Lag time	$P = 0.0001$	NS	NS
Oxidation rate	NS	$P = 0.0003$	NS
Maximum CD absorbance	NS	$P = 0.00001$	$P = 0.006$
2 Months			
Lag time	$P = 0.002$	$P = 0.0002$	$P = 0.0006$
Oxidation rate	NS	$P = 0.0003$	$P = 0.0001$
Maximum CD absorbance	$P = 0.00005$	$P = 0.00001$	$P = 0.0001$

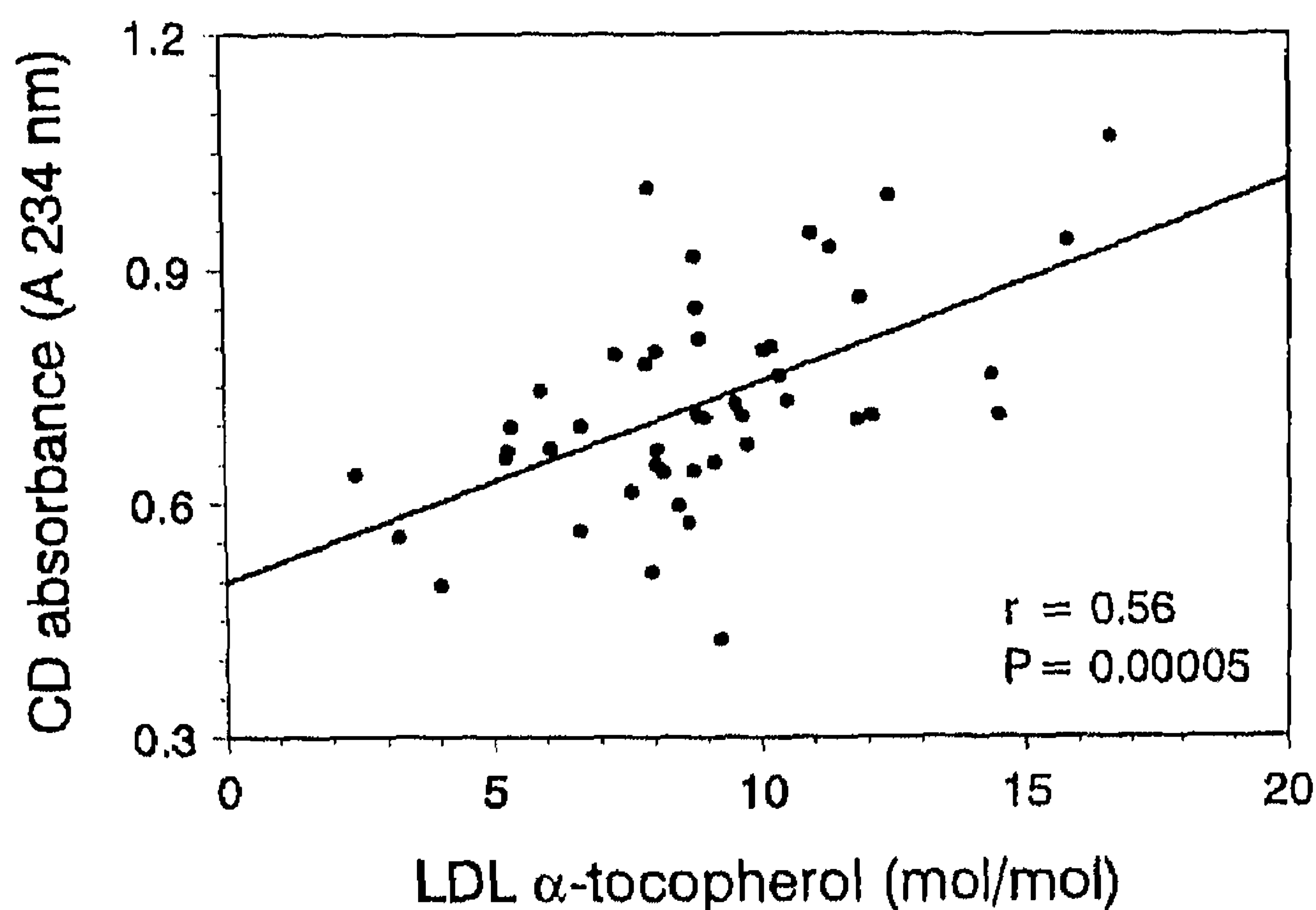


Fig. 3. The maximum CD absorbance after 2 months of vitamin E supplementation is plotted against the LDL α -tocopherol content for 46 patients who completed the study.

At baseline, no differences were observed in the maximum rate of oxidation and the maximum CD absorbance, neither between the two patient groups nor between patients and controls. During vitamin E supplementation, the maximum rate of oxidation and the maximum CD absorbance did not change in group A, but the maximum CD absorbance increased in group B ($P = 0.02$) (Table 2).

Concentrations of saturated and monounsaturated fatty acids in LDL, such as palmitic (C16:0), palmitoleic (C16:1n-7), stearic (C18:0), and oleic acid (C18:1n-9), were higher and those of linoleic acid (C18:2n-6) and docosahexaenic acid (C22:6n-3) were lower in patients compared with controls; no differences were observed in α -linolenic (C18:3n-3) and arachidonic acid content (C20:4n-6) (Table 3). During the study period, arachidonic acid concentrations increased in group A and those of docosahexaenoic acid decreased in both groups, whereas palmitic acid concentrations decreased in group B (Table 3). There was a significant correlation between LDL linoleic (but not arachidonic) acid and LDL α -tocopherol concentrations ($r = 0.38$, $P = 0.009$).

In Table 4, the results of regression analyses for indexes of the LDL oxidizability and α -tocopherol and fatty acid content of LDL are summarized. Lag times (Fig. 1) and the maximum CD absorbance (Fig. 3) correlated positively with LDL α -tocopherol concentrations, whereas the maximum rate of oxidation did not. Lag times were negatively, and both the maximum rate of oxidation and the maximum CD absorbance were positively related to LDL linoleic and arachidonic acid concentrations (Fig. 4).

DISCUSSION

This study documents that LDL resistance to oxidation is impaired in vitamin E deficient CF patients but can be normalized within 2 months of oral α -tocopherol supplementation. Thus far, data on LDL resistance to oxidation comprised, with one exception,²⁰ healthy subjects with a sufficient vitamin E status before and after vitamin E supplementation^{15,21-23} or with variations in dietary fatty acid intake,^{24,25} but not a larger cohort of vitamin E deficient subjects and, in particular, not of CF patients. Whereas lag times correlated poorly with LDL α -tocopherol concentrations^{16,26-29} in healthy subjects, the study patients, exhibiting a wide range of LDL α -tocopherol values, showed a significant positive correlation. Interestingly, a few patients had unexpectedly long lag times despite a low LDL α -tocopherol content (Fig. 1), an observation in agreement with that of Kleinveld *et al.*²⁰ but lacking a final explanation.

During supplementation with 400 IU/d of RRR- α -tocopherol, plasma and LDL α -tocopherol concentrations increased about threefold in the vitamin E deficient, but not in the vitamin E sufficient group, with plasma and LDL α -tocopherol concentrations higher than in healthy controls. This is in contrast to data suggesting that LDL α -tocopherol concentrations can be increased during vitamin E supplementation without saturation in vitamin E sufficient healthy subjects^{15,21-23} and perhaps indicates a plateau effect due to low LDL content of plasma in CF patients. However, because a high proportion of patients in the vitamin E sufficient group had already been on vitamin E supplements prior to study entry and the increase in the dosage was small compared to that in the vitamin E deficient group, final conclusions cannot be drawn for a ceiling effect from this study.

Lag times increased about twofold over baseline values in the vitamin E deficient, but not in the vitamin E sufficient group, reflecting the α -tocopherol increase in LDL and resulting in lag times that were even longer in vitamin E sufficient patients compared with healthy controls.

The maximum rate of oxidation and CD absorbance did not differ between vitamin E deficient and sufficient patients and healthy controls, and vitamin E supplementation had no effect on these indexes. In contrast, healthy subjects showed significantly lower propagation rates after vitamin E supplementation compared with baseline.²¹ CD absorbance was positively related to the LDL α -tocopherol content only after vitamin E supplementation in the present study, suggesting that α -tocopherol does exert this particular

protective action only at relatively high levels, as achieved after 2 months of supplementation. Recently, LDL of vitamin E deficient patients with fat malabsorption has been found less susceptible to oxidation

compared with controls.²⁰ However, these patients also showed a low LDL content of linoleic acid, a major substrate for CD formation.

The fatty acid content of LDL had a major impact

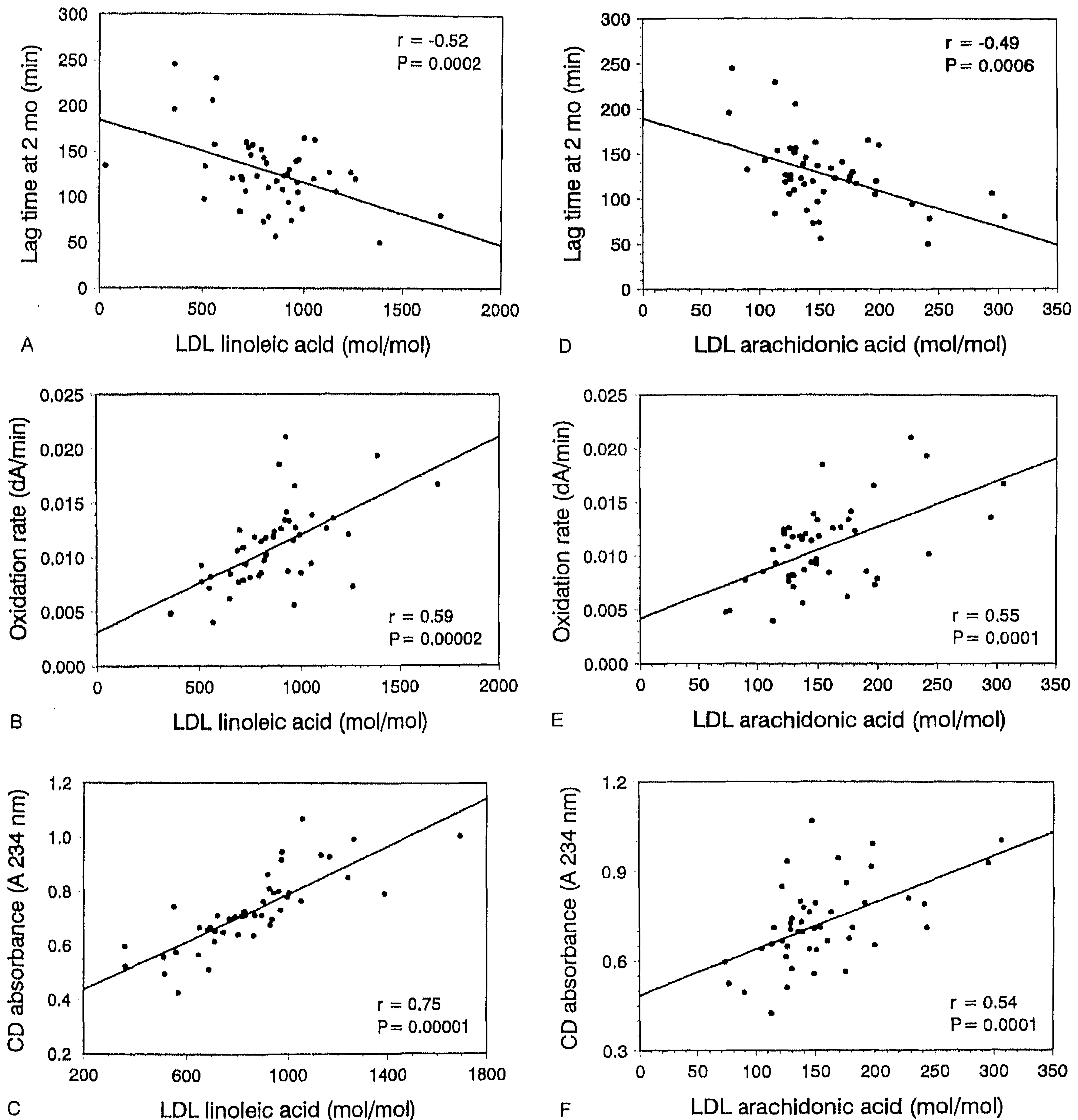


Fig. 4. Correlations between indexes of LDL oxidizability and LDL linoleic and arachidonic acid concentrations after 2 months of supplementation. Lag times (A, D), maximum rate of oxidation (B, E), and maximum conjugated diene (CD) absorbance (C, F) are plotted against LDL linoleic acid concentrations (left) and arachidonic acid concentrations (right) for 46 patients who completed the study.

on the resistance to oxidation: Lag times were negatively and rate of oxidation and CD absorbance were positively related to linoleic and arachidonic acid concentrations. This is in line with data from healthy subjects focusing on LDL linoleic acid.^{20,24,25} It is not surprising that linoleic acid plays a key role in LDL peroxidation because it represents the major fatty acid in LDL, accounting for 35 to 50% of fatty acids in healthy subjects²⁴ and $29 \pm 7\%$ in our patient population (compared to $5 \pm 1\%$ for arachidonic acid), and easily undergoes oxidation.³⁰ However, this study shows for the first time a significant effect of arachidonic acid content on the LDL resistance to oxidation.

CF patients have long been known to exhibit essential fatty acid deficiency³¹; however, the mechanisms involved are not precisely known. In the present study, linoleic and docosahexaenoic acid concentrations in LDL were lower only in the vitamin E deficient but not in the vitamin E sufficient group, compared with controls, and LDL linoleic (but not arachidonic acid) and α -tocopherol content were related to each other, indicating either a similarity in the underlying pathophysiological mechanism of fat malabsorption with subsequent alterations in the vitamin E and fatty acid status or a direct protective effect of α -tocopherol on the loss of LDL linoleic acid. The latter mechanism is further supported by an increase in arachidonic acid concentrations in the vitamin E deficient group during supplementation. However, there was a decrease in docosahexaenoic acid during supplementation in both groups that does not support this mechanism.

The effect of LDL α -tocopherol concentrations on lag times, but not on the rate of oxidation and CD absorbance, and the influence of linoleic acid and arachidonic acid content on the latter two indexes can at least in part be explained by the concept that, in the model of copper-induced LDL oxidation, α -tocopherol and other endogenous antioxidants are consumed during the lag phase and will therefore no longer be available for affecting the rate of oxidation or CD absorbance. The PUFA content of LDL, particularly of linoleic and arachidonic acid, as the substrate for oxidation will then become the major determinant of the LDL oxidizability.

In summary, this study documents that subclinical vitamin E deficiency in CF patients exerts a significant effect on the LDL resistance to oxidation in vitro. Impaired LDL resistance to oxidation of vitamin E deficient patients can be normalized within 2 months of 400 IU/d of RRR- α -tocopherol supplementation. We hypothesize that impaired resistance to oxidation of PUFAs is not confined to lipoproteins but might affect several tissues and, particularly, surfactant lipids in

the lung of CF patients. Hence, efficient vitamin E supplementation of CF patients is recommended as part of the therapeutic regime. However, dietary supplementation with unsaturated fatty acids may increase the oxidizability of lipids and, consequently, increase the vitamin E demands of CF patients for an efficient protection of PUFAs against oxidation.

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ABBREVIATIONS

- CD—conjugated dienes
 CF—cystic fibrosis
 EDTA—ethylenediamine tetraacetic acid
 GC—gas chromatography
 HPLC—high-performance liquid chromatography
 LDL—low density lipoprotein
 MDA—malondialdehyde
 PBS—phosphate-buffered saline
 PUFAs—polyunsaturated fatty acids