Impaired Vitamin E Status in Patients With Parenchymal Liver Cirrhosis: Relationships With Lipoprotein Compositional Alterations, Nutritional Factors, and Oxidative Susceptibility of Plasma

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Vitamin E is a lipid-soluble vitamin and an important antioxidant that protects lipoproteins and cell membranes from lipid peroxidation. The aims of the present study were to investigate, in patients with parenchymal liver cirrhosis, the following: (1) nutritional and vitamin E status in relation to compositional changes in lipoproteins; and (2) the effects of these alterations on the patients' plasma susceptibility to copper-mediated oxidation. Patients (n = 55) with liver cirrhosis and 25 healthy volunteers had vitamin E in serum and in isolated lipoprotein fractions analyzed by high-performance liquid chromatography (HPLC). Plasma susceptibility to peroxidation was measured by incubation with Cu^{2+}. Nutritional status was assessed by anthropometry. Vitamin E concentration was significantly decreased ($P < .001$) in the serum and in very–low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) in cirrhotic patients. The decrease was related to the degree of liver impairment. There were significant correlations between cholesterol and vitamin E concentrations in serum and in all the lipoprotein fractions (r between 0.72 and 0.89; $P < .001$) in cirrhotic patients, but there were no significant relationships between vitamin E and any of the anthropometric indices of nutritional status. The plasma maximal oxidation rate was significantly increased in cirrhotic patients ($P < .01$) and was inversely related to the serum concentration of vitamin E ($P < .05$). We conclude that lipoprotein alterations and not nutritional factors should be regarded as major factors explaining serum vitamin E reduction in patients with parenchymal liver cirrhosis, and that vitamin E depletion is associated with an increased plasma susceptibility to oxidation.

THE TERM "vitamin E" encompasses a group of isomers of tocopherol and tocotrienol that are lipid-soluble vitamins and essential nutrients in humans.[1-3] The major food sources of vitamin E are fats, vegetables, cereals, seed oils, and olive oil.[1,4] Postprandially, vitamin E is absorbed from the gastrointestinal tract into chylomicrons that subsequently undergo lipolysis by the circulating enzyme lipoprotein lipase to form chylomicron remnants.[5] The remnants are taken-up by the liver, which then secretes vitamin E together with dietary lipids into nascent very–low-density lipoproteins (VLDL).[6,7] Tocopherol is transferred to other lipoproteins during the catabolism of chylomicrons and VLDL[8]. Although γ-tocopherol is the predominant form of vitamin E found in the human diet, α-tocopherol accounts for most of the vitamin present in lipoproteins and in the tissues, because this isomer is selectively incorporated into VLDL by hepatic α-tocopherol-transfer protein.[9] It is for this reason that α-tocopherol is the isomer that is most commonly measured for clinical purposes.

Vitamin E deficiency is rarely observed in healthy adults, but several studies have observed a significant decrease of α-tocopherol concentration in the plasma of patients with chronic liver diseases.[10-13] The causes of vitamin E deficiency in patients with parenchymal liver disease are far from clear. An impaired vitamin E intake secondary to malnutrition has been suggested to explain plasma α-tocopherol decrease in alcoholic patients with cirrhosis.[14,15] An alternative explanation would be an impaired α-tocopherol transport by lipoproteins since it is known that patients with liver disease present with alterations in lipoprotein structure, shape, and composition.[16,17]

Vitamin E is an important antioxidant that protects lipoproteins and cell membranes from lipid peroxidation. It inhibits unsaturated fatty acid oxidation and has free radical scavenging properties.[2,3]

The aims of the present study were to investigate, in patients with parenchymal liver cirrhosis, the relationships between: (1) the changes in α-tocopherol concentrations in serum and isolated lipoproteins composition in relation to nutritional status; and (2) α-tocopherol concentrations and the patients' plasma susceptibility to copper-mediated oxidation.

MATERIALS AND METHODS

Subjects

The study was performed in 55 patients (mean age, 58 years; range, 40 to 70) with liver cirrhosis (37 men, 18 women) who were receiving treatment in the outpatient clinic of Hospital Universitari de Sant Joan de Reus. The diagnosis of cirrhosis was based on liver biopsy or on clinical evidence that included echography to evaluate splenomegaly or portal vein dilation and fibrogastroscopy to detect the presence of gastroesophageal varices. The etiology of the disease was alcoholic in 30 patients (55%), viral in 17 (31%), combined in 4 (7%), and cryptogenic in 4 (7%). Patients were classified according to the Child-Pugh scale[18] into 3 groups: A (n = 32), B (n = 16), and C (n = 7). This classification estimates the severity of cirrhosis based on biochemical and clinical parameters. Grade of severity increases from A through to C. The control group of subjects was composed of 25 healthy volunteers (17 men, 8 women; mean age, 56 years; range, 38 to 64) chosen

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from the routine health-and-safety-at-work checks conducted in our area. None of the patients or the control subjects had been taking vitamin supplements at the time of the study. All of the procedures were in accordance with the ethical standards of Hospital Universitari de Sant Joan and with the anonymity of results being guaranteed.

All blood samples were drawn in the fasted state and collected into tubes containing EDTA as anticoagulant for lipoprotein fractionation or into tubes with lithium heparin for the measurement of plasma susceptibility to oxidation. Tubes with no anticoagulants were used for α-tocopherol measurement and all the other biochemical analysis that require serum.

Lipoprotein Fractionation

The separation of VLDL, intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) was by using standard techniques of sequential preparative centrifugation[19] in a Kontron ultracentrifuge (Kontron Instruments, Zurich, Switzerland).

Measurement of α-Tocopherol

The concentration of α-tocopherol in serum and in lipoprotein fractions was measured by high-performance liquid chromatography (HPLC).[20] Briefly, 100 μL of serum or HDL, 200 μL of VLDL or LDL, and 300 μL of IDL were mixed with an equal volume of ethanol containing 16 nmol of tocopherol acetate as an internal standard. Vitamin E was extracted with a volume of hexane equal to that of sum of the test sample plus the ethanol, centrifuged at 2,000 $\times$ *g* for 5 minutes, and the supernatant evaporated to dryness under a stream of N_2. The extract was stored at –20°C and, prior to analysis, reconstituted with 100 μL ethanol. The HPLC system (Kontron Instruments) consisted of an autosampler (model 360), a pump (model 422), and an ultraviolet detector (UV model 332). Absorbance was read at 292 nm. The column used was a Spherisorb ODS-2 (25 $\times$ 0.5 cm; Higgins Analytical Inc, Mountainview, CA) and the mobile phase was 12% dichloromethane in methanol. The flow rate was set at 1.5 mL/min. Retention times were 4.7 $\pm$ 0.3 minutes for α-tocopherol and 6.3 $\pm$ 0.5 minutes for tocopherol acetate.

Plasma Susceptibility to Oxidation

Plasma susceptibility to oxidation was measured as previously described.[21] Briefly, plasma (20 μL) was diluted with 2,950 μL of phosphate-buffered saline. Thirty microliters of 5 mmol/L $CuCl_2$ solution was added to initiate oxidation. Susceptibility of the plasma to oxidation was measured by monitoring the changes in absorbance at 234 nm every 6 minutes over a period of 10 hours. This measurement is an estimate of conjugated diene production.[21-23] Lag-phase duration and the maximal oxidation rate (estimates of initial and late stage plasma oxidation, respectively) were calculated as previously described.[24] Higher maximal oxidation rates and/or shorter lag phases indicate greater susceptibility to oxidation.

Other Biochemical Measurements

Standard laboratory methods (ITC Diagnostics, Barcelona, Spain) were applied in an ILab 900 automatic analyzer (Instrumentation Laboratories, Milan, Italy) to measure blood constituents. These included cholesterol and triglyceride concentrations in plasma and lipoprotein fractions, serum total protein, albumin, bilirubin and esterified bilirubin concentrations, and alanine aminotransferase, γ-glutamyl transferase, and alkaline phosphatase activities.

Assessment of Nutritional Status

The nutritional status of the cirrhotic patients was estimated by anthropometry. The dominant arm diameter, height, and weight were registered and skinfold measurements at the triceps, biceps, suprailiac and subscapular regions were measured using Holtain calipers (Holtain Ltd, Crosswell, UK). Measurements were by a single investigator using a standardized protocol. Skinfold determinations were performed in triplicate and the mean of the values used in the subsequent calculations.

The nutritional indices analyzed in the present evaluation were the mid-arm muscular area (MAMA; predictive of lean body mass), the percentage of body fat (BF%; predictive of adipose tissue), and the percentage of the ideal body weight (IBW%; an estimate of overall nutritional status). This last variable was calculated only in those patients without significant deposits of ascites fluid (n = 24) so as to preclude the influence of this parameter on the estimate of nutritional status.

MAMA was estimated using the following equation[17]: $MAMA = (DAD - \pi \cdot DST)^2/(4 \cdot \pi)$, where DAD = dominant arm diameter (cm) and DST = dominant arm skinfold measurement of the triceps (cm). BF% was estimated by the formula of Siri[25]: $BF\% = [(4.95/D) - 4.5] \cdot 100$, where D = body density calculated as D (males) = $1.1610 - 0.0632 \cdot \log \Sigma$ skinfolds, or D (females) = $1.1581 - 0.0720 \cdot \log \Sigma$ skinfolds.

The patients' MAMA% and BF% were compared to the theoretical values for a normal Spanish population.[26,27] Results were expressed as percentage of the ratio between the observed and the theoretical values at 50% percentile based on gender and age.

IBW% was calculated as the observed values relative to the theoretical vales with respect to height, gender, and age according to the standard Metropolitan Life Insurance Company tables.[28]

Patients were considered as malnourished when MAMA% or BF% values were less than the 5th percentile of the normal population or when IBW% was lower than 90th percentile.

Statistical Analysis

Differences between 2 means were assessed using Student's *t* test (parametric) or Mann-Whitney's *U* test (nonparametric). Differences between multiple means were analyzed by analysis of variance (ANOVA) and Scheffé post-hoc for multiple comparisons. Pearson or Spearman correlation coefficients were used to evaluate the degree of association between any 2 variables. Multiple linear regression analysis was employed to investigate the effect of several variables on the lag phase and the maximal oxidation rate. Calculations were performed with the SPSS software package (SPSS, Chicago, IL). Unless otherwise stated, all results are expressed as means $\pm$ SD.

RESULTS

Serum α-Tocopherol Concentration in Relation to Liver Function

Results of the biochemical measurements in the serum of the control subjects and in the patients with liver cirrhosis, segregated with respect to the Child-Pugh classification, are summarized in Table 1. Serum α-tocopherol concentration was decreased in the patients with liver cirrhosis. This decrease was related to the severity of the disease. There were no significant differences in the serum α-tocopherol concentrations in the patients groups irrespective of whether they were alcoholic or not (data not shown). Hence they were pooled for statistical analyses. There were significant decreases in the serum concentration of cholesterol, total proteins, and albumin in class B and class C patients. Also, there were significant increases in the serum alanine aminotransferase and γ-glutamyl transferase activities and bilirubin concentrations in the patient groups. Alkaline phosphatase activity was not significantly increased,

Table 1. Results (means ± SD) of Serum α-Tocopherol Concentrations and Standard Biochemical Tests in Control Subjects and Cirrhotic Patients According to Staging on the Child-Pugh Scale of Disease Severity

Parameter	Controls (n = 25)	Cirrhotic Patients		
		Child-Pugh A (n = 32)	Child-Pugh B (n = 16)	Child-Pugh C (n = 7)
α-Tocopherol (μmol/L)	32.2 ± 8.3	26.3 ± 8.7	21.4 ± 9.8*	15.5 ± 5.2*†
Cholesterol (mmol/L)	5.76 ± 0.78	4.77 ± 1.27	3.63 ± 1.46*	2.94 ± 1.10*†
Triglycerides (mmol/L)	1.99 ± 1.74	1.27 ± 0.67	0.95 ± 0.36	0.93 ± 0.42
Total proteins (g/L)	72 ± 4	73 ± 3	66 ± 8*†	65 ± 4*†
Albumin (g/L)	44 ± 3	42 ± 5	27 ± 4*†	25 ± 6*†
ALT (μkat/L)	0.45 ± 0.16	0.74 ± 0.66*	0.85 ± 0.40*	0.98 ± 0.57*
GGT (μkat/L)	0.45 ± 028	1.65 ± 2.80*	2.19 ± 3.67*	1.13 ± 1.20
ALP (μkat/L)	3.1 ± 1.1	4.2 ± 1.8	5.2 ± 2.3	5.1 ± 1.1
Bilirubin (μmol/L)	10 ± 3	19 ± 9*	91 ± 87*	212 ± 197*
Esterified bilirubin (μmol/L)	5 ± 1	7 ± 3	38 ± 45*	88 ± 77*

*$P < .05$ with respect to the control group.

†$P < .05$ with respect to Child-Pugh A group of patients.

Abbreviations: ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase.

indicating that cholestasis was not a major feature of the liver disease of these patients.

In cirrhotic patients, there were significant ($P < .001$) direct relationships between serum α-tocopherol and albumin ($r = 0.48$) and between α-tocopherol and total protein ($r = 0.56$) concentrations, and a significant ($P < .05$) inverse relationship between α-tocopherol and esterified bilirubin ($r = -0.31$). These relationships were not observed in the control group.

Relationship Between α-Tocopherol and Lipoprotein Alterations

Figure 1 displays the concentrations of α-tocopherol in the lipoprotein fractions of the control subjects and of the patient groups. Cirrhotic patients presented with a progressive decline of VLDL α-tocopherol concentration from Child-Pugh A to Child-Pugh C patients. Also, a decrease of HDL α-tocopherol concentration, which became statistically significant in the Child C group, was observed. Thus, VLDL accounted for most of the loss of α-tocopherol in the lipoproteins of Child-Pugh stage A cirrhotic patients while this alteration was accompanied by a decrease of the vitamin content in HDL in the more severe classes of patients.

Cholesterol and triglyceride concentrations in lipoprotein fractions are summarized in Table 2. There were significant decreases in VLDL cholesterol and triglycerides, as well as of LDL cholesterol in the patient groups. Child-Pugh stage A cirrhotic patients presented with a significant increase of HDL cholesterol and triglyceride concentrations, probably associated with the alcoholic etiology of the condition in the majority of these patients.[17]

Table 3 summarizes the results of the ratios of α-tocopherol to cholesterol concentrations in serum and lipoprotein fractions. There were no significant differences between groups except for a small increase in LDL in Child-Pugh stage C patients.

The relationships between cholesterol and α-tocopherol concentrations in serum and in lipoprotein fractions are shown in

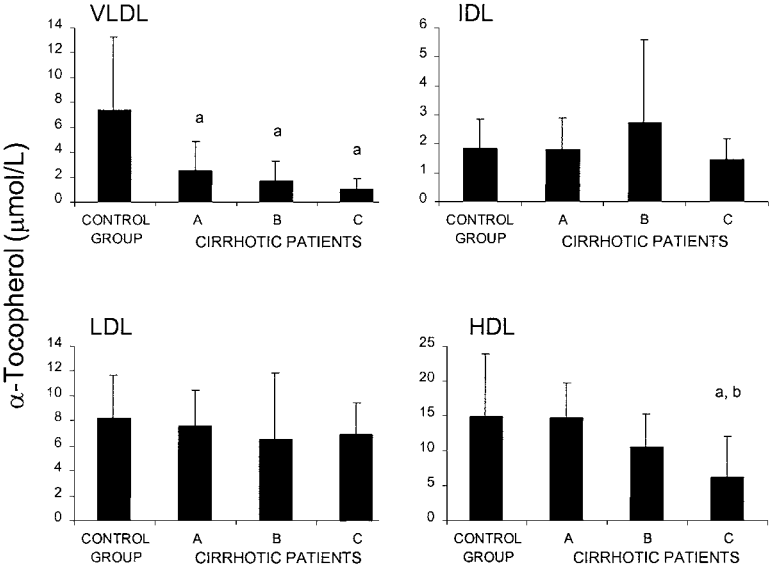

Fig 1. α-Tocopherol concentrations in lipoprotein fractions of the control subjects and cirrhotic patients classified according to Child-Pugh scale of the severity of the disease. [a]$P < .05$ with respect to the control group; [b]$P < .05$ with respect to the Child-Pugh A group of patients.

Table 2. Cholesterol and Triglyceride Concentrations (means ± SD) in Isolated Lipoprotein Fractions in Control Subjects and Cirrhotic Patients According to Staging on the Child-Pugh Scale of Disease Severity

Parameter	Controls (n = 25)	Cirrhotic Patients		
		Child-Pugh A (n = 32)	Child-Pugh B (n = 16)	Child-Pugh C (n = 7)
VLDL				
Cholesterol	0.54 ± 0.45	0.33 ± 0.26	0.19 ± 0.13*	0.19 ± 0.11*
Triglycerides	1.34 ± 1.52	0.51 ± 0.37	0.23 ± 0.10*	0.18 ± 0.11*
IDL				
Cholesterol	0.20 ± 0.08	0.25 ± 0.14	0.41 ± 0.51	0.24 ± 0.13
Triglycerides	0.22 ± 0.12	0.18 ± 0.05	0.19 ± 0.09	0.16 ± 0.09
LDL				
Cholesterol	3.97 ± 0.74	2.37 ± 0.76*	2.12 ± 1.18*	1.70 ± 0.58*
Triglycerides	0.29 ± 0.08	0.27 ± 0.10	0.40 ± 0.23	0.50 ± 0.34*†
HDL				
Cholesterol	1.05 ± 0.31	1.82 ± 0.55*	0.91 ± 0.39†	0.81 ± 0.68†
Triglycerides	0.14 ± 0.12	0.31 ± 0.24*	0.13 ± 0.11†	0.09 ± 0.05†

*$P < .05$ with respect to the control group.
†$P < .05$ with respect to Child-Pugh A group of patients.

Table 4. In control subjects, there were significant direct relationships between both parameters in VLDL, IDL, and HDL, but not in serum or in LDL. In cirrhotic patients, the correlations became, in general, stronger and were statistically significant in serum and in all the lipoprotein fractions.

α-Tocopherol and Nutritional Status

Most of the patients with liver cirrhosis were well nourished, irrespective of whether the measurements of MAMA%, BF%, or IBW% had been employed in estimating the nutritional status (Table 5). There were no significant relationships between α-tocopherol concentrations and any of the nutritional indices (MAMA%: $r = 0.29$; BF%: $r = 0.25$; IBW%: $r = 0.28$; $P =$ not significant).

Relationship Between Serum α-Tocopherol and Plasma Susceptibility to Oxidation

The maximal oxidation rate was significantly increased in patients with liver cirrhosis ($P < .01$). The lag phase was not significantly different to that of the control subjects (Table 6). Multiple linear regression analysis was performed to evaluate the combined effect of α-tocopherol, bilirubin, and albumin on plasma oxidizability. Results indicated that, when the effects of albumin and bilirubin were taken into account, there was a significant direct relationship ($P < .05$) between serum α-tocopherol concentration and the maximal oxidation rate in the control group, and an inverse relationship ($P < .05$) in cirrhotic patients. Also, there was a significant inverse relationship ($P = .05$) between serum α-tocopherol concentration and the lag phase in the control group, but not in cirrhotic patients.

DISCUSSION

The specific processes involved in the depletion of serum α-tocopherol concentration in patients with cirrhosis have not been fully clarified. A decreased secretion of conjugated bile acids into bile causing malabsorption of dietary lipids and fat-soluble vitamins seems to explain the reduced concentration of serum α-tocopherol in patients with cholestasis.[10,14,29] Nevertheless, this need not be the explanation for patients with parenchymal cirrhosis since these patients do not present with significant alterations in bile acid metabolism. The received wisdom is that vitamin E intake is impaired in alcoholics with parenchymal cirrhosis because of malnutrition or a poor dietary habit associated with the alcohol-dependency lifestyle.[13,15] However, malnutrition cannot totally explain α-tocopherol depletion in these patients, since body stores of vitamin E can persist for several years and are difficult to deplete.[13] A previous investigation observed dissociation between nutritional status and α-tocopherol depletion in cirrhotic patients.[11] However, the study cannot be considered conclusive since some of the

Table 3. Ratios (means ± SD) of α-Tocopherol to Cholesterol Concentrations in Serum and Isolated Lipoprotein Fractions in Control Subjects and Cirrhotic Patients According to Staging on the Child-Pugh Scale of Disease Severity

Parameter	Controls (n = 25)	Cirrhotic Patients		
		Child-Pugh A (n = 32)	Child-Pugh B (n = 16)	Child-Pugh C (n = 7)
Serum	5.6 ± 1.3	5.4 ± 1.3	6.1 ± 1.7	6.0 ± 1.8
VLDL	13.6 ± 5.9	6.1 ± 5.1	16.2 ± 36.7	15.6 ± 29.6
IDL	10.8 ± 10.3	6.8 ± 4.8	7.4 ± 4.9	5.1 ± 3.9
LDL	2.2 ± 1.6	3.1 ± 1.0	3.0 ± 1.8	4.3 ± 1.5[a]
HDL	14.8 ± 9.0	8.3 ± 2.8	12.3 ± 4.2	9.4 ± 5.2

[a]$P < .05$ with respect to the control group.

Table 4. Correlations Between α-Tocopherol and Cholesterol Concentrations in Serum and in Lipoprotein Fractions

	Control Group		Cirrhotic Patients	
	r	*P*	*r*	*P*
Serum	0.37	NS	0.84	<.001
VLDL	0.93	<.001	0.84	<.001
IDL	0.46	<.05	0.89	<.001
LDL	−0.26	NS	0.75	<.001
HDL	0.64	<.001	0.72	<.001

Abbreviation: NS, not significant.

Table 5. Summary of Anthropometric Evaluation of Nutritional Status in Patients With Cirrhosis

Nutritional Index	Mean (range)	Frequency of Malnutrition*
MAMA%	99.9 (51.5-212.7)	4/55 (7.3%)
BF%	77.3 (25.0-128.2)	14/55 (25.4%)
IBW%	132.2 (98.4-181.6)	0/24 (0%)

*Ratio (and percentage) between the number of malnourished individuals and the total number of patients studied with respect to each of the nutritional indices measured (see text for details).

Abbreviations: MAMA%, percentage mid-arm muscular area; BF%, percentage body fat; IBW%, percentage of ideal body weight.

nutritional indices used, such as the creatinine/height index, serum albumin, and prothrombin time, are not reliable in patients with liver disease.[15] Our study, using more appropriate anthropometric indices, confirmed the clear lack of a relationship between serum α-tocopherol reduction and the nutritional status in cirrhosis. Serum α-tocopherol concentrations were strongly decreased despite most of our patients being well nourished, according to the measurements reflecting muscular mass as well as adipose tissue. Furthermore, no significant correlations were observed between the anthropometric indices and serum α-tocopherol concentrations.

An alternative hypothesis to explain serum α-tocopherol depletion in cirrhosis could be that of an impaired transport by the lipoproteins which could be associated with their altered structure, composition, and, hence, metabolism. A positive correlation had been observed previously between serum cholesterol and α-tocopherol in normal subjects,[1] as well as cirrhotic patients.[13] The present study extends these observations by investigating the relationships between α-tocopherol, cholesterol, and triglycerides in isolated lipoprotein fractions. The results suggest that lipoprotein alterations play a major role in determining serum α-tocopherol concentrations in patients with parenchymal liver cirrhosis. We observed a marked reduction in α-tocopherol concentrations in serum, VLDL and HDL in cirrhotic patients. These alterations were associated with significant decreases in cholesterol concentrations in serum, VLDL, and LDL. Strong correlations between cholesterol and α-tocopherol concentrations were observed in serum and in all of the isolated lipoprotein fractions in patients with cirrhosis.

A debatable issue is whether the measurement of serum α-tocopherol concentration is a good estimation of vitamin E deficiency in patients with cirrhosis. Studies in patients with cholestatic liver disease noted a malabsorption of vitamin E secondary to alterations of bile composition that was better reflected by a decrease in the α-tocopherol:cholesterol ratio than by the absolute concentration of the vitamin.[10] An explanation for this observation is that since these patients present with hyperlipoproteinemia, the decrease of α-tocopherol content in the lipoprotein particles is compensated for by a concomitant increase in the numbers of particles. However, as Look et al[13] have recently pointed out, it is not clear whether this correction is valid in patients with parenchymal liver cirrhosis in which both α-tocopherol and lipoprotein concentrations decline in parallel with decreasing liver function. They observed that in this type of patient, the absolute serum α-tocopherol concentration correlates better with the severity of the disease than does the vitamin E:cholesterol ratio. The present study supports this conclusion, and similar relationships are noted in the isolated lipoprotein fractions.

The precise mechanisms that explain the association between α-tocopherol depletion and lipoprotein alterations in cirrhotic patients cannot be ascertained from the present investigation, but may be easily hypothesized. VLDL α-tocopherol decrease may be secondary to a decrease in the number of lipoprotein particles synthesized by the liver. An inhibition of hepatic α-tocopherol-transfer protein, producing an impaired coupling of α-tocopherol to VLDL[9] could also play a role. Additionally, a decrease in HDL α-tocopherol could be related to the severe structural and compositional alterations that this lipoprotein undergoes in patients with advanced cirrhosis. These alterations include an increase in the cholesterol to apolipoprotein A1 ratio[30] and the lipid-depleted discoidal HDL together with lipoprotein particles that are intermediate in composition between nascent and mature HDL.[16,31]

Since vitamin E is considered an important antioxidant, the consequence of its depletion in patients with cirrhosis would be an increase in the plasma susceptibility to oxidation. This possibility was tested in our study by exposing the plasma to Cu^{2+} ions in vitro. As expected, the plasma from cirrhotic patients showed a significant increase in the maximal oxidation rate compared to control subjects, which indicates a greater oxidizability. Also observed was a significant inverse correlation between serum α-tocopherol concentration and the maximal oxidation rate. These results confirm those of Yamamoto et al[32] who observed an increase in plasma oxidative stress in cirrhotic patients, as estimated by the ubiquinone-10:ubiquinol-10 ratio.

In contrast, we observed a direct correlation between serum α-tocopherol concentration and plasma oxidizability within the control group, which suggests a pro-oxidant effect of vitamin E on the plasma of these subjects. This effect may be explained by the ability of α-tocopherol to produce α-tocopheroxyl radical when reacted with Cu^{2+}.[33] This toxic radical may be eliminated from plasma by water-soluble coantioxidants such as bilirubin, which can recycle it back to α-tocopherol.[34,35] Therefore, serum bilirubin would modulate the dual pro/antioxidant effect of α-tocopherol observed in vitro. Thus, α-tocopheroxyl production would be favored in control subjects with high α-tocopherol and low bilirubin levels while being inhibited in cirrhotic patients at the other end of the disease spectrum.

The increase of the plasma susceptibility to peroxidation in cirrhotic patients could have several pathophysiological consequences. It has been suggested that cirrhotic patients who have a reduced plasma α-tocopherol concentration,[36] or with an

Table 6. Indices of Plasma Oxidizability by Copper (means ± SD)

Group	Lag Phase (min)	Maximal Oxidation Rate*
Control subjects	114 ± 11	2.0 ± 0.6
Cirrhotic patients	127 ± 7	2.5 ± 0.3†

*Maximal oxidation rate was expressed as nmol conjugated dienes/(mg cholesterol × min).

†$P < .01$ with respect to control subjects.

increased plasma oxidative stress,[32] have an increased risk of developing hepatocellular carcinoma. The role played by oxygen radicals in the etiology of carcinogenesis is not very well established, but it seems reasonable to assume that increased oxidative damage of DNA could induce errors in the genetic information and lead to carcinogenesis. Other possible consequences are the development of increased red blood cell fragility and hemolytic anemia,[15] as well as neurological alterations including peripheral neuropathy and cerebellar degeneration.[8,9]

An interesting collateral contribution of the present study was to provide an insight into the concentrations of α-tocopherol in serum and the distribution among the different lipoproteins in a healthy Spanish population. As noted previously by Esterbauer et al,[37] data on vitamin E concentration in human plasma in ostensibly healthy populations from different countries are difficult to obtain. Our study confirmed the serum α-tocopherol concentration results obtained by Olmedilla et al[38] in a Spanish cohort, which were similar to those observed in other continental European countries but higher than those observed in the United Kingdom and the United States.[37] A novel finding of our study is that these higher values of α-tocopherol appear be mainly due to an increased content of the vitamin in the HDL fraction compared to that reported by other investigations.[39-41] These differences result, most probably, from regional variations and the dietary habits of the populations studied.

In summary, the present study shows that lipoprotein alterations, and not nutritional factors, need to be regarded as the major factors in explaining serum α-tocopherol reduction in patients with parenchymal liver cirrhosis. Depletion of α-tocopherol is associated with a moderate increase in the susceptibility of the patients' plasma to oxidative modification. The clinical consequences of these alterations warrant further investigation.

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