Alterations in Plasma Vitamin E Distribution in Type 2 Diabetic Patients With Elevated Plasma Phospholipid Transfer Protein Activity

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Mouse studies indicated that plasma phospholipid transfer protein (PLTP) determines the plasma distribution of vitamin E, a potent lipophilic antioxidant. Vitamin E distribution, antioxidant status, and titer of anti–oxidized LDLs (oxLDL) autoantibodies were evaluated in plasma from control subjects (n = 31) and type 2 diabetic patients (n = 31) with elevated plasma PLTP concentration. Unlike diabetic and control HDLs, which displayed similar vitamin E contents, diabetic VLDLs and diabetic LDLs contained fewer vitamin E molecules than normal counterparts. Plasma PLTP concentration in diabetic plasmas correlated negatively with vitamin E in VLDL+LDL, but positively with vitamin E in HDL, with an even stronger correlation with the VLDL+LDL–to–HDL vitamin E ratio. Circulating levels of oxLDL were significantly higher in diabetic plasmas than in control plasmas. Whereas the titer of IgG autoantibodies to modified LDL did not differ significantly between diabetic patients and control subjects, diabetic plasmas showed significantly lower levels of potentially protective IgM autoantibodies. The present observations support a pathophysiological role of PLTP in decreasing the vitamin E content of apolipoprotein B–containing lipoproteins, but not of HDL in plasma of type 2 diabetic patients, contributing to a greater potential for LDL oxidation. *Diabetes* 53:2633–2639, 2004

The plasma phospholipid transfer protein (PLTP) mediates both net transfer and exchange of phospholipids between lipoproteins (1). Recently, the biological function of PLTP appeared more complex in nature, with its implication in the binding and transfer of other amphipathic compounds, including nonesterified cholesterol, diacylglycerides, lipopolysaccharides, and α-tocopherol, i.e., the main isomer of vitamin E (2–5). The atherogenic potential of PLTP in genetically engineered mice was initially explained in terms of its ability to raise apolipoprotein B (apoB)-containing lipoprotein production by the liver (6–8). Nevertheless, additional molecular mechanisms might mediate the antiatherogenic properties of PLTP deficiency. We recently demonstrated that PLTP deficiency in the mouse resulted in enrichment of vitamin E in atherogenic apoB-containing lipoproteins at the expense of HDL and cellular membranes, leading to a decreased susceptibility of LDL to oxidation (5). In contrast, in mice overexpressing PLTP, the PLTP-facilitated transfer of α-tocopherol out of the apoB-containing lipoprotein pool resulted in significant increases in the susceptibility to oxidation and in the autoantibody levels to oxidized LDL (oxLDL) (8). The physiological significance of the antioxidant properties of vitamin E and its role in preventing the oxidative processes of atherogenesis have been subjects of debate. Despite considerable epidemiological evidence in favor of an inverse relationship between vitamin E intake and cardiovascular disease (9), as well as convincing observations in vitro and in vitamin E–supplemented animal models (10,11), large prospective clinical trials in human populations have not supported a beneficial effect of vitamin E in the treatment of high-risk patients (12–15). In addition to a putative lack of sufficient antioxidant properties of vitamin E under specific plasma contexts (16), the negative outcomes were also explained in terms of inappropriate doses of vitamin E, heterogeneity in preexisting antioxidative status of studied population, or degree of severity of atherosclerotic lesions at the time of supplementation (12,13,15).

Beyond the confusing picture of most human studies, it must be pointed out that the evaluation of the vitamin E status of high-risk populations with elevated oxidative stress has been usually limited to the determination of total plasma levels of the antioxidant, with lack of detailed analyses of its lipoprotein distribution. The latter point is potentially of great importance because it can be predicted that the vitamin E content of apoB-containing lipoproteins, rather than total plasma vitamin E, would most accurately reflect the ability of vitamin E to block the oxidative process in apoB-containing lipoproteins, which occurs in the earliest step of atherogenesis (17). In this context, it is noteworthy that in vitro and in vivo studies (5) demonstrated that PLTP can promote the net mass redistribution of α-tocopherol between apoB-containing

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Received for publication 5 May 2004 and accepted in revised form 9 July 2004.

apo, apolipoprotein; ELISA, enzyme-linked immunosorbent assay; MDA-LDL, malondialdehyde-modified LDL; oxLDL, oxidized LDL; PLTP, phospholipid transfer protein.

TABLE 1
Baseline clinical characteristics and lipid parameters

	Control subjects	Type 2 diabetic patients	*P*
n	31	31	—
Age (years)	50.2 ± 12.5	62.3 ± 16.6	<0.05
Sex (M/F)	16/15	12/19	NS
BMI (kg/m^2)	25.12 ± 5.18	28.43 ± 6.54	<0.05
Glycemia (mmol/l)	5.21 ± 0.48	10.73 ± 4.58	<0.001
HbA_{1c} (%)	Not determined	9.12 ± 1.60	—
Total cholesterol (mmol/l)	5.65 ± 0.88	5.28 ± 1.21	NS
VLDL total cholesterol (mmol/l)	0.47 ± 0.46	0.72 ± 0.54	<0.05
LDL total cholesterol (mmol/l)	3.31 ± 0.68	3.51 ± 1.06	NS
HDL total cholesterol (mmol/l)	1.78 ± 0.57	1.50 ± 0.42	<0.05
Triglycerides (mmol/l)	0.99 ± 0.50	1.72 ± 1.04	<0.001
VLDL triglycerides (mmol/l)	0.60 ± 0.36	1.35 ± 0.93	<0.001
LDL triglycerides (mmol/l)	0.32 ± 0.08	0.43 ± 0.18	<0.005
HDL triglycerides (mmol/l)	0.11 ± 0.03	0.15 ± 0.06	<0.005
Phospholipids (mmol/l)	2.69 ± 0.41	2.75 ± 0.46	NS
VLDL phospholipid (mmol/l)	0.27 ± 0.19	0.48 ± 0.34	<0.005
LDL phospholipid (mmol/l)	1.16 ± 0.24	1.04 ± 0.30	NS
HDL phospholipid (mmol/l)	1.32 ± 0.34	1.24 ± 0.33	NS

Data are means ± SE.

lipoproteins and HDL in the absence of significant changes in total plasma levels of vitamin E.

Interestingly, increased plasma PLTP levels are known to be associated with both diabetes and the insulin resistance syndrome (18–21), probably reflecting a significant increase in the promoter activity of the human PLTP gene (22). The abnormally elevated plasma PLTP levels and the concomitant high lipoprotein oxidizability in type 2 diabetic patients (23) prompted us to monitor the impact of PLTP on plasma vitamin E distribution, antioxidant status, and autoantibody titers in these patients.

RESEARCH DESIGN AND METHODS

Control subjects (n = 31) were healthy subjects with no history of hyperlipidemia, coronary artery disease, or diabetes. Patients (n = 31) had type 2 diabetes and were treated with hypoglycemic drugs and/or insulin. Both control and diabetic subjects had normal thyroid, renal, and hepatic functions, and they were not receiving hypolipidemic agents. The protocol was approved by the local medical ethics committee (Bocage Hospital, Dijon, France), and all participants gave written informed consent.

Blood samples. Fasting blood samples were collected into EDTA-containing glass tubes. Plasma was separated by a 15-min centrifugation at 3,000*g* (4°C). Vitamin E was assessed on fresh plasma samples, whereas aliquots were frozen at −80°C for additional lipid analyses within a few months.

PLTP enzyme-linked immunosorbent assay. PLTP in human plasma samples was quantitated using a specific enzyme-linked immunosorbent assay (ELISA) as previously described (18,24). Briefly, plasma samples were mixed with anti-PLTP antibodies, and dilutions were made in albumin-phosphate buffer containing 1% Triton X-100 (Sigma, St. Louis, MO). Triton-containing mixtures were incubated for 16 h at 4°C, constituting a long-lasting treatment with detergent that is susceptible to improve the reactivity of human PLTP based on recent comparative studies (25). Aliquots of incubated mixtures were subsequently pipetted into immunoplates coated with a purified PLTP fraction. Bound anti-PLTP antibodies were detected with peroxidase-conjugated anti-IgG antibodies, and a standard curve was prepared using a plasma standard of known PLTP concentration.

PLTP activity measurements. PLTP activity was measured using a commercially available fluorescence activity assay (Cardiovascular Targets, New York, NY) according to the manufacturer's instructions. This fluorimetric assay measures the transfer (unquenching) of pyrene-labeled phosphatidylcholine from donor to acceptor synthetic liposomes. PLTP activity values were calculated as the initial slope of the phospholipid transfer curve, and they were expressed as initial phospholipid transfer rate (i.e., increase in fluorescence per minute) (24).

α-Tocopherol quantitation in plasma and isolated lipoproteins. The $d < 1.006$, $1.006 < d < 1.063$, and $1.063 < d < 1.210$ fractions were isolated from 400-μl fasting plasma samples by sequential ultracentrifugation in an Optima TLX Ultracentrifuge (Beckman, Fullerton, CA). The $d < 1.006$ fraction contained the triglyceride-rich lipoproteins (mainly VLDL), the $1.006 < d < 1.063$ fraction contained mainly LDL, and the $1.063 < d < 1.210$ fraction contained HDL. α-Tocopherol was quantitated by high-performance liquid chromatography analysis according to the general experimental procedure previously described (5). Tocol (Spiral, Dijon, France) was added to each sample as an internal standard before the extraction.

oxLDL measurements. The circulating levels of oxLDL in normolipidemic and diabetic plasmas were determined by the Mercodia Oxidized LDL ELISA kit (Mercodia, Uppsala, Sweden), which is a solid, two-site enzyme immunoassay based on the direct sandwich technique. Results, expressed in absorbance units, are proportional to the amount of oxLDL in the plasma sample.

Measurements of anti-oxLDL autoantibodies. Plasma autoantibody titers to epitopes of oxLDL were measured as previously described (26). Briefly, diluted plasma samples were added to microtiter wells coated with malondialdehyde-modified LDL (MDA-LDL). Bound autoantibodies were subsequently detected with either anti-human IgG or anti-human IgM antibodies coupled to alkaline phosphatase, and the amount bound was measured using a chemiluminescence technique. Results are expressed in relative light units per 100 ms.

Lipoprotein measurements. Phospholipids, total cholesterol, and triglycerides were assayed using commercially available enzymatic kits, i.e., PAP 150 (Biomérieux, Boulogne, France), Cholesterol 100, and Triglycerides 25 (ABX Diagnostics, Montpellier, France) kits, respectively. Total lipids were calculated as the sum of cholesterol plus phospholipids plus triglycerides. ApoB levels were determined in the VLDL and LDL fractions by turbidimetry using a commercially available kit (Sentinel Diagnostics, Milan, Italy).

Statistical analysis. The significance of the difference between data means was determined by using a t test for unpaired samples. Qualitative data between groups were compared by using the χ^2 test. Correlation coefficients were determined using linear regression analysis. Comparison of means after adjustment for a covariate (e.g., age) was performed by ANCOVA.

RESULTS

The baseline clinical characteristics of the study subjects are given in Table 1. Fasting glycemia was about twofold higher in type 2 diabetic patients than in normal subjects ($P < 0.001$), with HbA_{1c} 9.12% in diabetic plasmas. In diabetic patients, the fasting plasma triglyceride levels were twofold higher ($P < 0.001$), and all lipoprotein fractions (VLDL, LDL, and HDL) were enriched in triglycerides (Table 1). Although plasma total cholesterol levels did not differ between the two groups, in diabetic patients the cholesterol redistributed toward the VLDL fraction at the expense of the HDL fraction (Table 1). In accord with

TABLE 2
PLTP concentration and activity in control subjects and type 2 diabetic patients

	Control subjects	Type 2 diabetic patients	*P*
n	31	31	—
PLTP levels (mg/l)	4.59 ± 1.07	5.35 ± 1.38	<0.05
PLTP activity			
Initial transfer rate (AU/min)	762.71 ± 208.32	1,064.10 ± 291.02	<0.0001

Data are means ± SE in each group (statistical analysis by *t* test). PLTP concentrations were determined in frozen plasma from euglycemic and normolipidemic control subjects and from diabetic subjects using a competitive ELISA with a polyclonal PLTP antiserum. PLTP activity was measured using a commercially available fluorescence activity assay (Cardiovascular Targets), and activity values were expressed as initial phospholipid transfer rate. AU, arbitrary units.

previous studies (18) in similar subsets of control and diabetic subjects, plasma PLTP levels were significantly higher in diabetic patients than in control subjects, accounting for a significant rise in phospholipid transfer activity in diabetic plasmas (Table 2). As assessed by ANCOVA, similar differences between diabetic and control subjects were obtained after adjustment for age, and no correlations between age and PLTP activity or PLTP mass were found. Among diabetic patients, PLTP mass concentration correlated positively with total cholesterol and LDL cholesterol levels ($r = 0.50$, $P = 0.004$, and $r = 0.49$, $P = 0.006$, respectively). In contrast to previous studies (18) in type 2 diabetic patients with a mean 70% higher plasma PLTP mass concentration than in control subjects, no significant relationship of PLTP mass concentration with fasting glycemia was observed in the present study in diabetic patients, who displayed a moderate 17% rise in plasma PLTP mass concentration compared with control subjects ($r = 0.04$, NS).

In a first attempt to determine the impact of increased PLTP levels on plasma vitamin E, total α-tocopherol levels were measured in control and diabetic plasmas. As shown in Table 3, total plasma vitamin E levels were remarkably similar in the two groups, whether α-tocopherol levels were expressed as absolute concentrations or as α-tocopherol–to–total lipid ratios. Although absolute α-tocopherol contents of VLDL, LDL, or HDL did not differ significantly between diabetic patients and control subjects, detailed lipoprotein analysis revealed significant abnormalities in the concentration of α-tocopherol relative to lipids in diabetic lipoproteins (Table 3). Unlike diabetic and control HDL, which displayed similar α-tocopherol–to–total lipid ratios, diabetic VLDL and LDL both displayed significant decreases in α-tocopherol–to–total lipid ratios compared with normal VLDL and LDL. Diabetic VLDL contained fewer α-tocopherol molecules relative to triglycerides, total cholesterol, phospholipids, and apoB, indicating a lower α-tocopherol content per VLDL particle in diabetes (Table 3). α-Tocopherol content of diabetic LDL appeared lower than that of control LDL when expressed as α-to-

TABLE 3
α-Tocopherol levels and α-tocopherol–to–lipid ratio in total plasmas and lipoproteins from control subjects and diabetic patients

	Control subjects	Type 2 diabetic patients	P
n	31	31	—
Plasma α-tocopherol levels (μg/ml)	3.59 ± 0.77	4.02 ± 1.40	NS
Plasma α-tocopherol–to–lipid ratio (μg/mg)	0.69 ± 0.11	0.68 ± 0.13	NS
Lipoprotein α-tocopherol contents (μg/ml of plasma)			
VLDL	0.73 ± 0.46	1.02 ± 0.72	NS
LDL	1.85 ± 0.41	1.72 ± 0.43	NS
HDL	1.32 ± 0.37	1.27 ± 0.35	NS
Lipoprotein α-tocopherol–to–total lipid ratio (μg/mg of total lipids)			
VLDL	0.82 ± 0.15	0.57 ± 0.14	<0.0001
LDL	0.75 ± 0.09	0.67 ± 0.10	<0.001
HDL	0.73 ± 0.10	0.77 ± 0.13	NS
α-Tocopherol–to–triglyceride ratio (μg/mg of triglyceride)			
VLDL	1.41 ± 0.67	0.89 ± 0.22	<0.0001
LDL	6.92 ± 1.54	5.04 ± 1.65	<0.0001
HDL	15.17 ± 6.78	11.23 ± 4.91	<0.01
α-Tocopherol–to–total cholesterol ratio (μg/mg of total cholesterol)			
VLDL	4.98 ± 2.28	4.04 ± 1.41	<0.05
LDL	1.48 ± 0.29	1.60 ± 1.96	NS
HDL	1.96 ± 0.29	2.23 ± 0.39	<0.005
α-Tocopherol–to–phospholipid ratio (μg/mg of phospholipid)			
VLDL	3.99 ± 2.00	2.86 ± 0.73	<0.005
LDL	2.07 ± 0.27	2.17 ± 0.38	NS
HDL	1.29 ± 0.20	1.34 ± 0.23	NS
Lipoprotein α-tocopherol–to–apoB ratio (mol/mol)			
VLDL	8.64 ± 3.78	6.31 ± 3.70	<0.05
LDL	5.38 ± 5.72	2.78 ± 1.14	<0.05

Data are means ± SE in each group (statistical analysis by *t* test). α-Tocopherol was extracted from either total plasmas or ultracentrifugally isolated lipoproteins and assayed by high-performance liquid chromatography analysis as described under RESEARCH DESIGN AND METHODS.

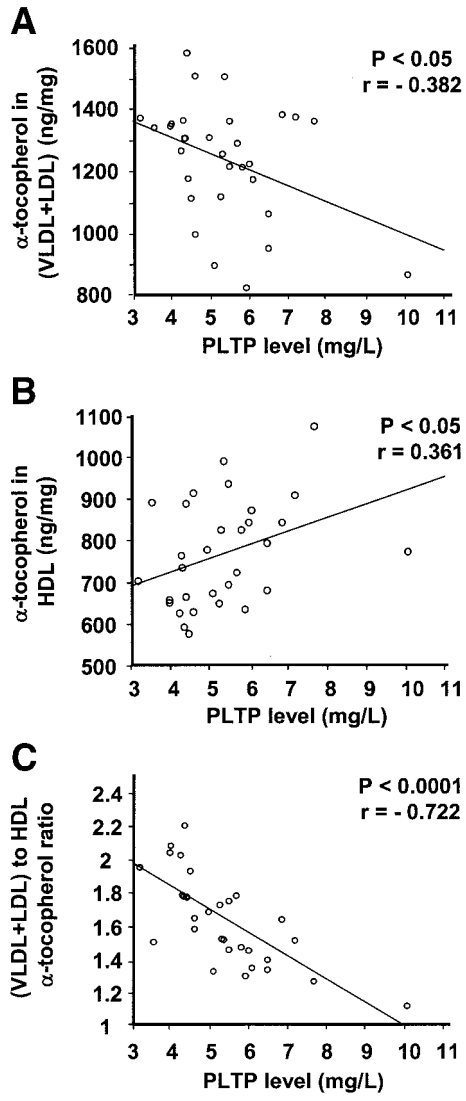

FIG. 1. Correlation between plasma PLTP levels and α-tocopherol content of lipoprotein fractions in diabetic plasmas. Plasma PLTP concentrations in diabetic plasmas were compared with the α-tocopherol–to–lipid ratio in either VLDL+LDL (*A*) or HDL (*B*). *C*: The correlation of plasma PLTP to the VLDL+LDL–to–HDL α-tocopherol ratio, which was calculated from values shown in *A* and *B*, is shown. Correlation coefficients were calculated by linear regression analysis.

copherol–to–total lipid or α-tocopherol–to–triglyceride ratios, but not when expressed relative to total cholesterol or phospholipids. Nevertheless, and as observed with diabetic VLDL, diabetic LDL displayed significantly lower α-tocopherol–to–apoB ratios than control LDL, again indicating a lower α-tocopherol content per LDL particle in diabetes (Table 3). The α-tocopherol–to–lipid ratio of individual lipoproteins, rather than absolute α-tocopherol contents, seemed to constitute the most reliable marker of their antioxidant status. Indeed, in the present study, the significant decline in the relative α-tocopherol contents of diabetic VLDL and LDL was masked by concomitant increases of apoB-containing lipoproteins in diabetic plasmas (i.e., ~70% increases in both triglyceride and VLDL cholesterol levels) (Table 1).

As shown in Fig. 1, the plasma PLTP concentration in diabetic plasmas correlated negatively with the α-tocopherol–to–lipid ratio of apoB-containing lipoproteins (i.e., VLDL+LDL) (Fig. 1*A*), but positively with the α-tocopherol–to–lipid ratio of HDL (Fig. 1*B*). In support of a role of PLTP in decreasing the α-tocopherol content of apoB-containing lipoprotein, but not of HDL (5), an even stronger inverse relationship between PLTP concentration and the relative VLDL+LDL–to–HDL α-tocopherol ratio was observed (Fig. 1*C*). Plasma PLTP activity correlated strongly with PLTP mass concentration among both control subjects and diabetic patients ($r = 0.85$; $n = 62$; $P < 0.0001$). Accordingly, and as observed for PLTP mass concentration (Fig. 1), PLTP activity in diabetic plasmas correlated negatively with the α-tocopherol–to–lipid ratio of VLDL+LDL ($r = -0.38$; $n = 31$; $P < 0.05$) and with the VLDL+LDL–to–HDL α-tocopherol ratio ($r = -0.62$; $n = 31$; $P < 0.0005$) and positively with the α-tocopherol–to–lipid ratio of HDL ($r = 0.53$; $n = 31$; $P < 0.05$).

In accordance with a relative decrease in the antioxidative protection of apoB-containing lipoproteins, circulating levels of oxLDL, as determined using a commercially available ELISA (see RESEARCH DESIGN AND METHODS) were significantly higher in diabetic plasmas than in control plasmas (Fig. 2*A*), and there was a significantly negative correlation between the level of oxLDL and the relative α-tocopherol content of the plasma LDL fraction in diabetic patients (Fig. 2*B*).

Titers of IgG and IgM autoantibodies against MDA-LDL

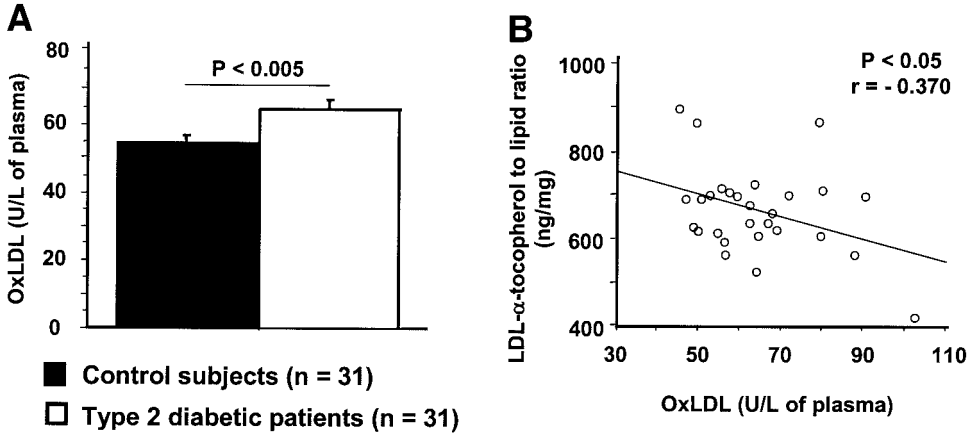

FIG. 2. oxLDL level (*A*) and its relationship to LDL–α-tocopherol content (*B*) in diabetic plasmas. The circulating levels of oxLDL in control and diabetic plasmas were determined by the Mercodia Oxidized LDL ELISA kit (Mercodia). Values are means ± SE in each group (statistical analysis by *t* test), and correlation coefficients were calculated by linear regression analysis.

TABLE 4
MDA-LDL autoantibody titers in plasmas from control subjects and diabetic patients

	Control subjects	Type 2 diabetic patients	*P*
n	31	31	—
MDA-LDL IgG (RLU)	4,757.16 ± 2,579.75	5,432.75 ± 2,634.10	NS
MDA-LDL IgG–to–oxLDL ratio	92.47 ± 58.33	86.85 ± 44.37	NS
MDA-LDL IgM (RLU)	16,262.03 ± 14,695.57	9,841.25 ± 6,368.53	<0.05
MDA-LDL IgM–to–oxLDL ratio	324.30 ± 348.02	150.74 ± 79.87	<0.05

Data are means ± SE in each group (statistical analysis by *t* test). Plasma IgG and IgM autoantibody titers to epitopes of oxLDL were measured as described previously (26). Values are expressed either in relative light units (RLU), reflecting the absolute levels of each autoantibody subclass, or as a ratio of MDA-LDL autoantibody titer to circulating oxLDL level.

were compared in diabetic patients and control subjects. As shown in Table 4, the titer of IgG to MDA-LDL did not differ significantly between diabetic and control plasma whether anti–MDA-LDL IgG were expressed in absolute values or as relative values, e.g., IgG-to-oxLDL ratios. In contrast, compared with normal controls, diabetic plasmas showed significant decreases in absolute (38%) and relative (54%) anti–MDA-LDL IgM titers, respectively.

DISCUSSION

PLTP has been shown (5,8) to play a key role in the intravascular transport and metabolism of vitamin E in genetically engineered animal models, with drastic alterations in plasma PLTP levels. The results of the present study provide the first evidence in support of a physiological role of PLTP in determining the distribution of α-tocopherol in plasma of diabetic patients, who are known to display significant increases in plasma PLTP concentration. Because the diabetic state is associated with both high oxidative stress and greater LDL oxidizability (23,27–30), increased PLTP-mediated flux of α-tocopherol out of apoB-containing lipoproteins may be of pathophysiological relevance in the etiology of associated vascular disorders. The role of plasma PLTP activity in determining the vitamin E content of lipoproteins might offer a rationale for the lack of a significant relationship between the dietary intake of vitamin E and its plasma concentration in diabetic patients (31). In fact, several studies have previously reported that the vitamin E content of diabetic LDL is reduced (27,28). Even more importantly, PLTP activity might well constitute a previously unrecognized factor influencing vitamin E availability in apoB-containing lipoproteins, providing in part an explanation for the disappointing outcome of most of the human secondary prevention studies in which vitamin E supplementation in high-risk patients, including diabetic patients, did not result in a significant reduction in cardiovascular events (32–36).

In previous studies, the depletion of vitamin E in apoB-containing lipoproteins was proven to constitute one of the most dramatic effects of plasma PLTP expression in vivo. The main reservoir of vitamin E in PLTP-deficient mice was shown to be either VLDL or LDL, depending on the relative abundance of these two lipoprotein classes in mice of different genetic backgrounds, i.e., LDLR0/PLTP0 and apoBTgCETPTg/PLTP0 (high LDL levels) or apoE0/PLTP0 (high VLDL levels) (5). We now report that the role of PLTP in determining the α-tocopherol content of circulating lipoproteins also applies to the human situation. Unlike HDL, apoB-containing lipoprotein particles from diabetic plasmas with abnormally elevated PLTP mass and activity contain fewer α-tocopherol molecules compared with control plasmas. In addition, plasma PLTP concentrations correlated negatively with the relative α-tocopherol content of apoB-containing lipoproteins (VLDL+LDL), while it in turn correlated positively with the α-tocopherol content of HDL. In addition to earlier animal studies (5), the present observations in diabetic patients support a role for PLTP to mediate a shift of α-tocopherol from apoB-containing lipoproteins to other lipid-containing structures (i.e., HDL and cellular membranes).

Consistent with the significantly higher oxidizability of α-tocopherol–depleted apoB-containing lipoproteins observed in mice overexpressing plasma PLTP (8), the relative decrease in the α-tocopherol content of diabetic apoB-containing lipoproteins was accompanied by a significant rise in the circulating level of oxLDL compared with controls. These observations suggest that the PLTP-mediated acceleration of α-tocopherol depletion in diabetic apoB-containing lipoproteins may have led, at least in part, to the greater oxidation susceptibility of these lipoproteins in vivo. Beyond the causal high PLTP/low α-tocopherol relationship, it is worthy to note that hyperglycemia per se is also likely to have contributed significantly to the concomitant rise in oxidized apoB-containing lipoproteins. Indeed, glucose has been shown (37) to accelerate the rate of LDL oxidation once vitamin E has been consumed. Hyperglycemia, as observed in the type 2 diabetic patients of the present study, might exert a dual deleterious effect. First, hyperglycemia contributes directly to the rise in plasma levels of PLTP, the α-tocopherol carrier, probably through upregulation of the PLTP gene expression (18,21,22). Second, hyperglycemia is known (37) to accelerate the oxidation process once vitamin E depletion of apoB-containing lipoproteins has occurred.

Beyond the assessment of circulating levels of oxLDL in vivo, there has also been considerable interest in autoantibodies to epitopes of oxLDL. In addition to being possible markers of the occurrence of lipoprotein oxidation in vivo, they reflect the extent of atherosclerotic lesions in animal models (38), and the baseline levels of anti-oxLDL IgG predict progression of atherosclerosis in some human studies (39,40). In contrast, recent experimental data have suggested that certain IgM antibodies to oxLDL may be atheroprotective (41–43), and several recent studies in humans have reported that plasma IgM autoantibodies to oxLDL display an independent inverse relationship to

measures of atherosclerosis (44,45). Our observation that IgM titers were lower in the diabetic population would be consistent with a depletion of such potentially atheroprotective anti-oxLDL autoantibodies. In another report (23), the levels of anti-oxLDL antibodies tended to be lower in patients with coronary artery disease than in control subjects. We now report that type 2 diabetes is associated with a significant and selective twofold decrease in circulating levels of anti-oxLDL IgM, a decrease not observed with the IgG isotypes. The decreased IgM could be due to decreased production or to increased consumption as a result of immune complex formation. In either case, we speculate that an insufficient titer of "protective" IgM might contribute to the higher atherosclerosis susceptibility of diabetic patients, a hypothesis that will deserve further attention in future studies.

In conclusion, the significant rise in plasma PLTP expression in diabetic patients is associated with the redistribution of α-tocopherol between plasma lipoproteins, contributing at least in part to weaker antioxidant defenses of diabetic apoB-containing lipoproteins despite unchanged total plasma vitamin E levels. In conclusion, the PLTP-mediated α-tocopherol transfer process is proposed to contribute to the greater oxidizability of circulating apoB-containing lipoproteins, including LDL. Together with concomitant decrease in the titer of protective IgM autoantibodies, the occurrence of elevated levels of LDL with a lower antioxidative potential in diabetic plasma might contribute to the acceleration of atherosclerosis.

ACKNOWLEDGMENTS

This work was supported by the Centre Hospitalier Universitaire de Dijon, the Institut National de la Santé et de la Recherche Médicale (INSERM), the Université de Bourgogne, the Conseil Régional de Bourgogne, and the Fondation de France.

We thank Jean-Paul Pais de Barros and Naig Le Guern for excellent technical assistance.

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