Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis1–3

Didier Quilliot, Evelyne Walters, Jean-Paul Bonte, Jean-Charles Fruchart, Patrick Duriez, and Olivier Ziegler

ABSTRACT

Background: Patients with chronic pancreatitis (CP) are at high risk of antioxidant deficiencies. Furthermore, this disease can lead to diabetes mellitus (DM) that could exacerbate the severity of oxidative stress. Oxidative stress and the resulting LDL oxidation are a major cause of atherosclerosis.

Objective: The objective of the study was to ascertain whether diabetes significantly modifies oxidative status in patients with CP.

Design: CP patients with or without DM were compared with type 1 DM patients and healthy control subjects.

Results: Two-way factorial analyses showed that a decrease in the plasma concentrations of vitamin A, vitamin E, and carotenoids accompanied both CP and DM, and CP was also associated with lower plasma concentrations of selenium and zinc, lower catalase activity, and higher plasma concentrations of copper. The lag phase of LDL oxidation was lower in CP patients with or without DM than in the control subjects, whereas there was no significant difference between type 1 DM patients and control subjects. Multivariate analysis showed that LDL vitamin E (R² = 0.24, P < 0.0001) and fasting plasma glucose (R² = 0.32, P < 0.0001) concentrations were the main determinants of the lag phase of LDL oxidation.

Conclusions: Antioxidant status is altered in CP patients, particularly in those who also have DM. In these patients, a vitamin E deficiency and an elevated plasma glucose concentration were associated with significantly higher LDL oxidizability. Am J Clin Nutr 2005;81:1117–25.

KEY WORDS Chronic pancreatitis, diabetes mellitus, antioxidant, oxidized LDL, immune complexes, malondialdehyde

INTRODUCTION

Patients with exocrine pancreatic insufficiency are at a greater risk than are patients with exocrine pancreatic sufficiency of developing vitamin or trace-element deficiencies as a result of malabsorption (1–6). This finding is mostly in relation to the fat-soluble vitamins, although the role of water-soluble vitamins—particularly vitamin C—in this disease has been studied only sparingly (7). Some vitamins, such as vitamins E, C, and A and the carotenoids, have antioxidant properties, as do the trace elements selenium and zinc. The prevalence of lower plasma concentrations of these vitamins and trace elements in patients with chronic pancreatitis (CP) has been reported (8, 9). Moreover, lower concentrations of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, were implicated in cases of recurrent and acute pancreatitis (10, 11), which means that antioxidant deficiencies could have several serious consequences in that disease (12). Because of a heightened oxidative modification of LDL in CP (13), accelerated atherosclerosis could be one of several complications associated with this condition (14). It is surprising that, despite a low LDL-cholesterol concentration in patients with diabetes secondary to CP, those patients have the same prevalence and distribution of atherosclerosis as do patients with type 1 diabetes mellitus (DM) (15–17). With oxidative stress and the resulting LDL oxidation being the major cause of atherosclerosis, we hypothesize that antioxidant deficiencies in CP might be implicated in atherogenesis in this disease.

Early in vitro and in vivo trials of antioxidant supplements showed that antioxidants increase the resistance of LDL to oxidation (18). However, Estebauer et al (19) showed in vitamin E–deficient subjects that the resistance of LDL to oxidation, as ascertained by the duration of the lag phase in copper ion–induced oxidation, did not correlate with the LDL α-tocopherol content. In contrast, the nature of the relation between LDL oxidation resistance and LDL α-tocopherol content in patients with severe antioxidant deficiencies is not known. LDL fatty acid abnormalities, which we described previously in relation to CP (20), could also be responsible for modifying LDL oxidizability. Because of the antigenic properties of oxidized LDL, antibodies to oxidized LDL could serve as a useful index of in vivo LDL oxidation. Furthermore, LDL immune complexes (ICs) could play a role in atherogenesis (21). DM secondary to CP could exacerbate the severity of antioxidant deficiencies, given that DM accelerates Cu²⁺-induced ex vivo LDL oxidation (although these data are controversial; 22), whereas, in type 1 DM, the excess risk of atherosclerosis persists after control for lipidic factors (eg, LDL and HDL cholesterol) (23, 25). DM is also associated with deficiencies in vitamins and trace elements, particularly those of vitamin C (25–28), vitamin E (29, 30), zinc (8, 31, 32), and SOD (33).

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The main objectives of this study were to ascertain whether diabetes significantly modifies the oxidative status of patients with CP and to ascertain the extent of Cu\(^{2+}\)-induced ex vivo LDL oxidation and the concentrations of antibodies to oxidized LDL and LDL-ICs under low LDL-cholesterol conditions. We thus examined these variables in patients with DM secondary to CP and made a comparative analysis with findings in type 1 DM patients, patients with CP but without diabetes, and healthy control subjects.

**SUBJECTS AND METHODS**

**Subjects**

Ninety-two male subjects were classified into 4 groups: CP patients without diabetes (CP nondiabetic group; n = 12), patients with diabetes secondary to CP or insulin-treated diabetes (CP diabetic group; n = 35), patients with type 1 DM (control diabetic group; n = 10) and healthy control subjects (control nondiabetic group; n = 20). The patients with type 1 DM were individually matched with the CP diabetic patients for age and diabetes duration (±2 y). The control subjects were matched for social category and age with the CP nondiabetic patients (Table 1).

CP was diagnosed on the basis of clinical history and the presence of morphologic pancreatic abnormalities, in particular that of pancreatic calcifications detected by X-ray and confirmed by abdominal ultrasonography, computerized tomography, or endoscopy. All CP diabetic patients and 10 of the 12 CP nondiabetic patients had calcific pancreatitis. One patient was diagnosed by using an ultrasonography scanner and another by using endoscopy.

All patients in the CP diabetic group had ceased or greatly reduced alcohol intake by the time of the study. On average, they had consumed <30 g alcohol/d for the 5 y immediately before the study. Their previous alcohol consumption averaged 111 ± 66 g/d (range: 50–450 g/d) for a period of 25.5 ± 11.4 y. Three of the CP diabetic patients had undergone a cholecystectomy, and 4 had undergone cystic derivations. Patients who had undergone pancreatectomy or gastrectomy were excluded. All patients in the CP diabetic group had diabetes according to criteria of the American Diabetes Association (34, 35) and had been taking insulin for ≥3 mo. No patient had an episode of acute pancreatitis in the 3 mo before the study. All the patients in the CP nondiabetic group had a normal fasting plasma glucose concentration—ie, <110 mg/L—on the day they entered the study.

Eleven of the 12 CP nondiabetic patients and 20 of the 35 CP diabetic patients had had or were currently having recurrent attacks of pain (painful pancreatitis). Fifteen of the 35 CP diabetic patients had a family history of diabetes.

Patients being treated with pancreatic enzymes underwent a 5-d washout period. CP patients on a home-based diet provided a 72-h stool sample after the wash-out period. Blood samples were collected after the overnight fast, before the insulin injection, and on day 6 of the washout period. None of the patients or control subjects received additional vitamin or trace element supplementation or had been assigned to a special diet.

In addition to a physical examination and an antioxidant analysis, each subject with DM underwent the following investigation: evaluation of the major vascular risk factors [ie, blood pressure, smoking status, and body mass index (BMI; in kg/m\(^2\)], electrocardiogram, and continuous-wave Doppler velocimetry.

Diabetic patients underwent an ophthalmologic examination and retinal fluorescein angiography.

Written informed consent was obtained from all subjects. The study was approved by the Nancy University ethics committee for the Protection of Human Subjects.

**Analytic methods**

Plasma concentrations of glucose were measured by using the glucose oxidase method in an automated glucose analyzer. HPLC (Diamat; BioRad laboratories, Hercules, CA) was used to determine concentrations of glycated hemoglobin (HbA1c), which were expressed as a percentage of total hemoglobin (normal range: 4.5–6%).

The fecal fat content in patients in the CP patient groups was analyzed according to Van de Kamer et al (36). HDL-cholesterol concentrations were obtained after the precipitation of apolipoprotein (apo) B–containing lipoproteins with sodium phosphotungstate and magnesium chloride (Boehringer Mannheim, Mannheim, Germany). Total-cholesterol and triacylglycerol concentrations were ascertained by using commercial kits (Boehringer Mannheim) adapted to a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, IN). LDL-cholesterol concentrations were calculated according to the method of Friedewald et al (37).

**Preparation of LDL**

Plasma LDL was prepared by ultracentrifugation with a Beckman TL100 centriuge and a TLA-100.4 rotor (Beckman Instruments, Inc, Palo Alto, CA) within a density cutoff range of 1.019 to 1.063 g/mL. EDTA (1 mg/mL) was added to plasma before ultracentrifugation. First the LDL was dialyzed against sodium chloride (0.15 mol/L) in EDTA (1 mg/mL) with the pH adjusted to 7.4; next it was gassed with N\(_2\), and then it was sterilized by filtration (0.45 μm) and stored at 4 °C. Apo B concentrations were analyzed with an immunonephelometric assay on a laser reader and by using commercial polyclonal antibodies (both: Behring, Marburg, Germany).

**Fatty acid composition of LDL and lipoprotein chemical composition**

The fatty acid composition of LDL was ascertained after lipid extraction (38). Lipids were methanolized with methanolic H\(_2\)SO\(_4\) for 2 h at 70 °C. Fatty acid methyl esters were extracted with the use of heptane and analyzed by gas chromatography on a Varian 3400 chromatograph (Sunnyvale, CA) equipped with a flame ionization detector and a capillary wall-coated open tubular column containing cyanopropylpolysiloxane (Chrompack, Middelburg, Netherlands). Cholesterol, phospholipids, and triacylglycerol were analyzed in VLDL, LDL, and HDL after separation by sequential ultracentrifugation.

**Analysis of LDL oxidation, malondialdehyde-modified LDL antibodies, and LDL immune complexes**

LDL oxidation was studied in 65 subjects. LDL was dialyzed against phosphate-buffered saline that had been gassed with N\(_2\) containing no EDTA. Oxidation was induced by adding CuCl\(_2\) (final concentration: 1.66 μmol/L) to 100 μg LDL/mL (apo B). This mixture was incubated at 30 °C, and conjugated diene formation was followed by the measurement of absorption at 234 nm every 10 min for 8 h with a thermostat-controlled Kontron

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Uvikon 930 spectrophotometer equipped with a 10-position sample changer (Tegimenta AG, Rotkreuz, Switzerland). Analyses were performed in triplicate and expressed in nmol·min\(^{-1}\)·mg\(^{-1}\) of apo B. Malondialdehyde (MDA)-modified LDL antibodies and LDL-ICs were analyzed according to the method of Gunzler et al (44). Catalase activity was determined spectrophotometrically by using the 2,4-dinitrophenylhydrazine method with HPLC and by using a 600 E controller equipped with a refrigerated sample changer (Tegimenta AG, Rotkreuz, Switzerland). Analyses were performed in triplicate and expressed in nmol·min\(^{-1}\) at 25 °C; normal range: 60–100 U/mL.

Assessment of vitamins and trace elements

Serum vitamin A (normal range: 240–900 μg/L) and carotenoid concentrations were measured simultaneously by reversed-phase HPLC as described by Steghens et al (40); the normal range for β-carotene, α-tocopherol, and lycopene was 100–480, 27–200, and 50–380 μg/L, respectively. Measurement of vitamin E (α-tocopherol) in plasma and in LDL was performed by HPLC according to the method of Teissier et al (41) and by using a 600 E controller equipped with a refrigerated 717-plus autosampler and a Novapack C18 column and precolumn (all: Waters Associates, Milford, MA). Plasma concentrations of ascorbic acid (normal range: 4–14 mg/L) were measured by using the 2,4-dinitrophenylhydrazine method with HPLC and electrochemical detection. Atomic absorption spectrophotometry was used to measure the concentrations of zinc (normal range: 0.7–1.1 mg/L), copper (normal range: 0.8–1.6 mg/L), and selenium (normal range: 60–83 μg/L) by the method of Clavel et al (42).

Antioxidant enzymes

The concentration of the Cu-Zn-SOD enzyme in plasma (normal range: 2300–3800 UI/g hemoglobin) was ascertained by using an AbA-200 direct biochromatic analyzer (Abbott Laboratories, Irving, TX) and the technique of L’Abbe and Fisher (43). Plasma and erythrocyte concentrations of glutathione peroxidase were assayed by using the Ellman reaction as modified by Gunzler et al (44). Catalase activity was determined spectrophotometrically at 240 nm by using the method of Beers and Sizer (45) and was expressed in Bergmeyer units (1 U = decomposition of 1 g H\(_2\)O\(_2\)/min at 25 °C; normal range: 60–100 U/mL) (Beckman, Brea, CA).

Statistical analysis

Statistical analyses were performed with BMDP statistical software (version 7.0; BMDP, Los Angeles, CA). Results are expressed as mean (± SD), with data tested for normality by skewness and kurtosis tests. The 4 groups of patients were compared by using factorial analyses [2 × 2 analysis of variance (ANOVA)] and a Sheffé’s F test if the interaction was significant. Chi-square tests were used to compare the frequencies of vitamin and trace element deficiencies among the 4 groups.

Pearson’s correlation was used for testing 2-variable relations. Multivariate linear regressions were performed to ascertain the effects of certain factors on antioxidant concentrations. In the 2 subgroups of subjects with CP, multivariate linear regressions were performed to adjust for the effect of diabetes. In the CP diabetic subgroup, multivariate linear regressions were performed to adjust for the effect of CP. Multiple regression analysis was also used to assess the effects of several independent variables on lag phase. Age, tobacco consumption, and alcohol intake had no independent effect in any of the analyses. P values of < 0.05 were considered significant.

RESULTS

Subjects’ baseline characteristics

The anthropometric characteristics of the 4 groups of subjects showed that the CP groups (diabetic and nondiabetic) had significantly lower body weight and BMI values than did the control diabetic and control nondiabetic groups (Table 1). CP duration and the amount of steatorrhea were significantly less in CP nondiabetic than in CP diabetic patients. Pancreatitis duration was 8 ± 5.2 y in the CP nondiabetic group and 16.3 ± 7.7 y in the CP diabetic group (P < 0.001), and steatorrhea was 5.5 ± 5.2 g/d in the CP nondiabetic group and 12.0 ± 8.4 g/d in the CP diabetic group (P = 0.028).

The prevalence of macroangiopathy was 12% (3/25) in the control diabetic group and 28.5% (10/35) in the CP diabetic group (NS; chi-square test). The prevalence of retinopathy was 20% (5/25) in the control diabetic group and 28.5% (10/35) in the CP diabetic group (NS; chi-square test).
38% (8/25) in the control diabetic group and 40% (14/35) in the CP diabetic group (NS; chi-square test).

Among the CP patients, 11 of the 12 CP nondiabetic and 20 of the 35 CP diabetic patients had had bouts of pain. There was no significant difference between patients with or without pain in terms of age, diabetes and pancreatitis duration, steatorrhea, HbA1c, and BMI. The number of bouts of pancreatitis was significantly higher in the CP diabetic group than in the CP nondiabetic group (2.4 ± 2.5 and 4.4 ± 5.3, respectively; P = 0.006).

Antioxidant status

Plasma concentrations of vitamins and trace elements and enzyme activities are shown in Table 2 and Table 3. Plasma concentrations of vitamin E differed significantly among the 4 groups. Mean plasma vitamin E concentrations were significantly lower in both CP groups than in the 2 control groups (P < 0.0001) and significantly lower in the CP diabetic group than in the control diabetic group (P < 0.05). Vitamin E concentrations were significantly lower in the control diabetic group than in the control nondiabetic group (P < 0.01). The prevalence of a low plasma vitamin E concentration—i.e., below the lower limit of the normal range, <7 mg/L—was 71% in the CP diabetic group and 46% in the CP nondiabetic group. No control diabetic patient presented with a vitamin E concentration below this value (P < 0.0001; chi-square test). Compared with that in the control nondiabetic group, the LDL vitamin E concentration was significantly lower in both CP groups than in the control nondiabetic group; in addition, it tended to be lower in the control diabetic group than in the control nondiabetic group, but this latter difference was not significant.

Plasma concentrations of vitamin A and carotenoid (ie, \(\alpha\)-carotene, \(\beta\)-carotene, lycopene) were significantly lower in the CP diabetic group without diabetes than in the CP diabetic group with insulin-treated diabetes mellitus; DM, diabetes mellitus.

### Table 2

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Control nondiabetic (n = 20)</th>
<th>Control diabetic (type 1 DM) (n = 25)</th>
<th>CP nondiabetic (n = 12)</th>
<th>CP diabetic (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E (mg/L)</td>
<td>17.4 ± 3.7</td>
<td>12.9 ± 2.9</td>
<td>10.7 ± 2.9</td>
<td>5.8 ± 2.3</td>
</tr>
<tr>
<td>LDL vitamin E (mg/g)</td>
<td>0.398 ± 0.275</td>
<td>0.307 ± 0.192</td>
<td>0.108 ± 0.091</td>
<td>0.067 ± 0.069</td>
</tr>
<tr>
<td>α-Carotene (mg/L)</td>
<td>323.3 ± 114.1</td>
<td>126.6 ± 101.3</td>
<td>120.0 ± 133.5</td>
<td>68.0 ± 59.2</td>
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<tr>
<td>β-Carotene (mg/L)</td>
<td>165.8 ± 128.1</td>
<td>108.8 ± 82.6</td>
<td>70.8 ± 50.2</td>
<td>47.7 ± 40.4</td>
</tr>
<tr>
<td>Vitamin A (µg/L)</td>
<td>1028 ± 284</td>
<td>801 ± 316</td>
<td>682 ± 296</td>
<td>537 ± 215</td>
</tr>
<tr>
<td>Vitamin C (mg/L)</td>
<td>9.30 ± 4.46</td>
<td>8.80 ± 4.37</td>
<td>8.57 ± 6.71</td>
<td>7.33 ± 4.67</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Control nondiabetic (n = 20)</th>
<th>Control diabetic (type 1 DM) (n = 25)</th>
<th>CP nondiabetic (n = 12)</th>
<th>CP diabetic (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc (mg/L)</td>
<td>0.97 ± 0.01</td>
<td>0.94 ± 0.13</td>
<td>0.88 ± 0.18</td>
<td>0.85 ± 0.15</td>
</tr>
<tr>
<td>Copper (mg/L)</td>
<td>0.95 ± 0.20</td>
<td>1.12 ± 0.15</td>
<td>1.16 ± 0.12</td>
<td>1.13 ± 0.17</td>
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<tr>
<td>Zn:Cu</td>
<td>1.04 ± 0.30</td>
<td>0.85 ± 0.25</td>
<td>0.75 ± 0.13</td>
<td>0.78 ± 0.20</td>
</tr>
<tr>
<td>Selenium (µg/L)</td>
<td>84.6 ± 14.8</td>
<td>82.3 ± 14.2</td>
<td>62.2 ± 11.5</td>
<td>64.8 ± 14.2</td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td>2841 ± 332</td>
<td>2628 ± 344</td>
<td>2607 ± 382</td>
<td>2811 ± 589</td>
</tr>
<tr>
<td>Erythrocyte glutathione peroxidase (U/g Hb)</td>
<td>6.39 ± 1.25</td>
<td>5.86 ± 1.08</td>
<td>6.04 ± 1.25</td>
<td>5.97 ± 2.02</td>
</tr>
<tr>
<td>Plasma glutathione peroxidase (U/mL)</td>
<td>80.1 ± 24.2</td>
<td>82.8 ± 26.9</td>
<td>74.9 ± 17.8</td>
<td>81.5 ± 27.2</td>
</tr>
<tr>
<td>Catalase (U/mL)</td>
<td>81.7 ± 8.0</td>
<td>79.1 ± 9.3</td>
<td>73.4 ± 7.8</td>
<td>76.8 ± 9.9</td>
</tr>
</tbody>
</table>

1 CP nondiabetic, chronic pancreatitis without diabetes; CP diabetic, CP with insulin-treated diabetes mellitus; DM, diabetes mellitus.
2 ANOVA 2 × 2 with Scheffe’s test if the interaction was significant.
3 ± SD (all such values).
4 Significantly different from control nondiabetic group, P < 0.01.
CP subjects than in control subjects ($P < 0.01$). Vitamin A and lycopene concentrations were also significantly lower in diabetic subjects than in nondiabetics ($P < 0.01$). The prevalence of a low β-carotene concentration—ie, below the lower limit of the normal range, 0.100 mg/L—was 50% in the CP nondiabetic group, 74% in the CP diabetic group, and 16% in the control diabetic group. No control nondiabetic subjects had such a low concentration ($P < 0.0001$; chi-square test).

There was no significant difference in average ascorbate concentrations among the 4 groups. The prevalence of vitamin C deficiency—ie, vitamin C concentration below the lower limit of the normal range, 4 mg/L—was 33.3% in CP nondiabetic patients, 28.5% in CP diabetic patients, 25% in control diabetic patients, and 20% in control nondiabetic subjects (NS; chi-square test).

Plasma concentrations of zinc in the CP subjects were significantly ($P < 0.01$) lower than those in the control subjects: 17% of CP diabetic and 16% of CP nondiabetic subjects had concentrations below the lower limit of normal (0.7 mg/L). No subjects in either of the control groups had such low plasma concentrations of zinc ($P = 0.035$; chi-square test). All patients had plasma concentrations of copper in the normal range, but average values were significantly ($P < 0.01$) higher in both CP groups and the control diabetic group than in the control nondiabetic group.

Plasma concentrations of selenium were significantly lower in CP patients than in control nondiabetic patients ($P < 0.0001$): 33.3% of CP nondiabetic subjects, 42.8% of CP diabetic subjects, and 12% of control diabetic subjects were selenium deficient (<60 μg/L). No control subjects had such a deficiency ($P = 0.008$; chi-square test). Catalase activity was significantly ($P < 0.05$) lower in CP subjects than in the control groups, whereas SOD, erythrocyte glutathione peroxidase, and plasma glutathione peroxidase activities did not differ significantly between the groups.

**Analysis of the effect of CP on antioxidant status**

For the study population as a whole, multivariate analysis was performed to analyze the effects of CP and of diabetes after adjustment for the confounding variables age, alcohol intake (g/d), and number of cigarettes smoked/d. These variables had no significant effect on the analysis. CP was associated with significantly lower plasma concentrations of vitamin E ($β = -6.0 ± 0.7$ mg/L, $P < 0.0001$), vitamin A ($β = -289 ± 58$ μg/L, $P < 0.001$), carotenoids (α-carotene: $β = -73 ± 240$ mg/L, $P = 0.004$; lycopene: $β = -94 ± 33.9$ mg/L, $P = 0.007$; β-carotene: $β = -0.25 ± 0.04$ mg/L, $P < 0.001$), and trace elements (zinc: $β = -0.09 ± 0.03$ mg/L, $P = 0.003$; selenium: $β = -19.1 ± 3.0$ μg/L, $P < 0.001$).

In the subgroup of subjects with CP, the amount of steatorrhea was negatively correlated with vitamin E and β-carotene concentrations and positively correlated with SOD concentrations (Table 4). These relations were significant for vitamin E and β-carotene even after adjustment for the effect of diabetes.

**Analysis of diabetes-specific effects**

For the study population as a whole, multivariate analysis was performed to analyze the effects of CP and of diabetes after adjustment for the confounding variables age, alcohol intake (g/d), and number of cigarettes smoked/d. These variables had no significant effect on the analysis. DM was associated with significantly lower plasma concentrations of vitamin E ($β = -3.2 ± 0.8$ mg/L, $P < 0.0001$), vitamin A ($β = -190 ± 62$ μg/L, $P = 0.002$), and lycopene ($β = -111 ± 33$ mg/L, $P = 0.001$) and with nonsignificantly lower plasma concentrations of α-carotene ($β = -45 ± 24$ mg/L, $P = 0.06$) and β-carotene ($β = -0.07 ± 0.04$ mg/L, $P = 0.07$).

Univariate analyses of variables from the subgroups of diabetic patients (CP and control diabetic groups) showed that β-carotene and plasma glutathione peroxidase activities were negatively correlated with fasting plasma concentration of glucose ($P = 0.002$ and $P = 0.001$, respectively) and that plasma glutathione peroxidase activity was negatively correlated with HbA1c ($P = 0.014$) (Table 5).

**Antioxidant status of subjects with macroangiopathy or microangiopathy, or both, and effect of pain state**

There were no differences in antioxidant status between diabetic subjects with or without vascular complications, except for the plasma glutathione peroxidase activity, which tended to be lower in subjects with macroangiopathy than in those without macroangiopathy [86.4 ± 28.9 U/L ($n = 13$) and 69.9 ± 12.9 U/L ($n = 37$), respectively; $P = 0.051$]. The presence or absence of pain was not associated with an impaired or deficient antioxidant status in CP patients. The prevalence of such deficiencies was significantly lower in the CP nondiabetic group (1.27 ± 0.8; $n = 12$) than in the CP diabetic groups (painless CP diabetics ($n = 15$): 2.5 ± 0.8, $P < 0.001$; CP diabetics with pain ($n = 20$): 2.34 ± 1.0; $P < 0.05$).

**TABLE 4**

Correlation coefficients between steatorrhea and selected variables within chronic pancreatitis groups

<table>
<thead>
<tr>
<th></th>
<th>Simple linear regression</th>
<th>Adjusted on DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>Plasma vitamin E</td>
<td>-0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma β-carotene</td>
<td>-0.41</td>
<td>0.013</td>
</tr>
<tr>
<td>Plasma SOD</td>
<td>0.41</td>
<td>0.013</td>
</tr>
</tbody>
</table>

1 $DM$, diabetes mellitus; SOD, superoxide dismutase.

**TABLE 5**

Correlation coefficients between dependent variables and fasting plasma glucose and HbA1c in diabetic patients with or without chronic pancreatitis

<table>
<thead>
<tr>
<th></th>
<th>Fasting plasma glucose</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>-0.32</td>
<td>0.002</td>
</tr>
<tr>
<td>Plasma glutathione peroxidase</td>
<td>-0.42</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma glucose peroxidase</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Erythrocyte glutathione peroxidase</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 HbA1c, glycated hemoglobin, expressed as a percentage of total hemoglobin.

2 Adjusted for effect of chronic pancreatitis.
Lipids and LDL composition

The mean values for fasting plasma concentrations of lipid, lipoprotein, and apo B are reported in Table 6. Average total cholesterol, LDL-cholesterol, and apo B concentrations were significantly lower in the CP subjects than in the control nondiabetic and diabetic groups. These 3 variables were also significantly lower in diabetic than in nondiabetic subjects (P < 0.01). No significant differences in HDL-cholesterol or triacylglycerol concentrations were observed between groups. An analysis of the plasma LDL composition found significantly (P < 0.05) higher concentrations of triacylglycerol in CP patients than in the control diabetic and nondiabetic subjects.

BMI was correlated with LDL cholesterol in the whole population of CP patients, even after adjustment for the effect of diabetes (r = 0.35, P = 0.014; n = 47), and in the CP diabetic group (r = 0.42, P = 0.021; n = 35). Biliary acid content of the stool was significantly higher in CP patients than in control subjects (1446 ± 1054 and 386 ± 208 µmol/24 h, respectively; P = 0.041) and was significantly correlated with the amount of steatorrhea (r = 0.61, P = 0.007) in the entire population of CP patients.

LDL composition of fatty acids

Table 6 lists the mean (±SD) percentag of the LDL fatty acid content. Patients with CP had mean percentages of monounsaturated fatty acids (MUFA) that were significantly higher than those in the control diabetic or control nondiabetic subjects (P < 0.0001). However, no relation with steatorrhea, BMI, or LDL cholesterol was found. These high MUFA percentages were associated with a slightly but significantly lower polyunsaturated fatty acid content in CP patients (P < 0.01). There was an inverse and significant correlation between MUFA percentages and α-tocopherol concentrations (r = 0.46, P < 0.001) in LDL for the entire population of subjects.

Susceptibility of LDL to oxidation

Two-way ANOVA showed that the lag phase and LDL oxidation were lower in CP subjects with or without diabetes (Table 7), whereas there was no significant effect of DM. However, the lag phase and LDL oxidation were significantly lower in CP nondiabetics than in CP diabetics (DM × CP interaction and Scheffe’s F test: P < 0.05).

For the population as a whole (n = 65), univariate regression analyses showed that the lag phase in LDL oxidation was positively correlated with the LDL vitamin E concentrations (r = 0.49, P < 0.001; Figure 1) and plasma concentrations of β-carotene (r = 0.49, P < 0.001) and negatively correlated with the fasting plasma concentration of glucose (r = −0.34, P = 0.006). The lag phase was also positively correlated with LDL vitamin E concentrations in each subgroup of diabetic patients (control diabetic group: r = 0.48; P = 0.043; CP diabetic group: r = 0.40; P = 0.053), whereas the correlation with HbA1c was not significant. Lag phase was not correlated with the concentration of any fatty acid or that of any component of LDL, such as cholesterol, triacylglycerol, phospholipids, or apo B. The LDL oxidation rate was not significantly correlated with any antioxidant factor or HbA1c, but it was weakly and negatively correlated with the fasting plasma concentration of glucose (r = −0.23, P = 0.059) and negatively correlated with the MUFA concentration (r = −0.32, P = 0.014).

In a multivariate analysis, lag phase was negatively correlated with fasting plasma concentrations of glucose after adjustment for LDL vitamin E concentration (glucose: r = −0.27, P = 0.015; LDL vitamin E: r = 0.44, P < 0.0001). This analysis

---

**Table 6**

Lipids; LDL composition in cholesterol, triacylglycerol phospholipid, and apolipoprotein (apo) B; and fatty acid composition

<table>
<thead>
<tr>
<th>Lipids (mg/dL)</th>
<th>Control nondiabetic (n = 20)</th>
<th>Control diabetic (type 1 DM) (n = 25)</th>
<th>CP nondiabetic (n = 12)</th>
<th>CP diabetic (n = 35)</th>
<th>P&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Diabetes effect</th>
<th>Chronic pancreatitis effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>214 ± 23&lt;sup&gt;3&lt;/sup&gt;</td>
<td>194 ± 28</td>
<td>192 ± 35</td>
<td>157 ± 30&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>115 ± 43</td>
<td>101 ± 39</td>
<td>136 ± 43</td>
<td>107 ± 51</td>
<td>0.042</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>144 ± 23</td>
<td>131 ± 24</td>
<td>115 ± 25</td>
<td>94 ± 23</td>
<td>0.002</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>41 ± 17</td>
<td>46 ± 11</td>
<td>51 ± 16</td>
<td>40 ± 13</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Apo B</td>
<td>120 ± 19</td>
<td>107 ± 26</td>
<td>103 ± 19</td>
<td>85 ± 25</td>
<td>0.004</td>
<td>0.0003</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td><strong>LDL composition (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>39.8 ± 1.6</td>
<td>39.9 ± 1.1</td>
<td>40.0 ± 1.9</td>
<td>38.9 ± 1.65</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>5.8 ± 1.7</td>
<td>6.3 ± 1.4</td>
<td>6.5 ± 1.5</td>
<td>7.7 ± 2.0</td>
<td>0.039</td>
<td>0.011</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Phospholipids</td>
<td>27.2 ± 1.1</td>
<td>27.3 ± 1.2</td>
<td>26.7 ± 3.7</td>
<td>27.0 ± 0.9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Apo B</td>
<td>27.2 ± 0.9</td>
<td>26.4 ± 1.8</td>
<td>26.8 ± 2.03</td>
<td>26.4 ± 1.1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td><strong>LDL fatty acid (% by wt of total fatty acids)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated</td>
<td>42.3 ± 4.5</td>
<td>42.0 ± 3.9</td>
<td>43.0 ± 3.6</td>
<td>40.1 ± 4.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>16.9 ± 2.5</td>
<td>17.3 ± 2.9</td>
<td>20.1 ± 1.8</td>
<td>20.8 ± 3.2</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>40.8 ± 4.1</td>
<td>40.6 ± 3.3</td>
<td>36.9 ± 3.7</td>
<td>39.1 ± 4.9</td>
<td>NS</td>
<td>0.007</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>n = 3</td>
<td>1.7 ± 1.1</td>
<td>1.3 ± 0.7</td>
<td>1.4 ± 0.9</td>
<td>1.0 ± 0.7</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>n = 6</td>
<td>39.1 ± 4.5</td>
<td>39.4 ± 3.5</td>
<td>35.3 ± 4.0</td>
<td>38.1 ± 5.2</td>
<td>NS</td>
<td>0.017</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> CP nondiabetic, chronic pancreatitis without diabetes; CP diabetic, CP with insulin-treated diabetes; DM, diabetes mellitus.

<sup>2</sup> ANOVA 2 × 2 with Scheffe’s test if the interaction was significant.

<sup>3</sup> ± SD (all such values).

<sup>4</sup> Significantly different from the other 3 groups, P < 0.01.
showed that concentrations of LDL vitamin E and fasting glucose (in plasma) were the main determinants of the lag phase \( (R^2 = 0.32) \). After adjustment for LDL vitamin E concentrations, \( \beta \)-carotene was not significantly correlated with the lag phase, but concentrations of those 2 antioxidants were highly correlated \( (r = 0.61, P < 0.001) \).

### TABLE 7

**LDL oxidation**

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Control nondiabetic ((n = 13))</th>
<th>Control diabetic ((n = 19))</th>
<th>CP nondiabetic ((n = 9))</th>
<th>CP diabetic ((n = 24))</th>
<th>Diabetes effect</th>
<th>Chronic pancreatitis effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag phase (min)</td>
<td>104.9 ± 14.6</td>
<td>90.4 ± 27.1</td>
<td>75.7 ± 19.3</td>
<td>87.3 ± 20.8</td>
<td>NS</td>
<td>0.007</td>
<td>0.030</td>
</tr>
<tr>
<td>Propagation (nmol diene · min(^{-1}· \text{mg}^{-1}))</td>
<td>5.6 ± 1.18</td>
<td>5.02 ± 1.1</td>
<td>3.8 ± 0.81</td>
<td>4.2 ± 0.98</td>
<td>NS</td>
<td>0.0002</td>
<td>0.025</td>
</tr>
</tbody>
</table>

\(^1\) CP nondiabetic, chronic pancreatitis without diabetes; CP diabetic, CP with insulin-treated diabetes; DM, diabetes mellitus.

\(^2\) ANOVA 2 × 2 with Scheffe’s test if the interaction was significant.

\(^3\) \( \bar{x} \) ± SD (all such values).

\(^4\) Significantly different from control nondiabetic group, \( P < 0.05 \).

\(^5\) Significantly different from CP nondiabetic group, \( P < 0.05 \).

DISCUSSION

This study shows that antioxidant status is highly altered and that LDL resistance to oxidation is significantly impaired in patients with CP. A low LDL vitamin E concentration was the main variable that could explain the increase in LDL oxidizability. A high fasting glucose concentration also influences LDL oxidizability, whereas the MUFA content in LDL was associated with a lower oxidation rate. The concentration of anti-MDA-ICs was higher in diabetic patients with retinopathy or macroangiopathy or both.

The lag phase is usually poorly correlated with the vitamin E concentration in healthy subjects (18, 46–49), probably because of the small range of LDL \( \alpha \)-tocopherol values. The wide range of LDL \( \alpha \)-tocopherol values in CP patients with fat malabsorption, however, gives rise to a highly significant correlation between these 2 variables. The effect of vitamin E deficiency on LDL oxidation was previously analyzed in young \((\bar{x} \text{ age: } 9 \text{ y})\) cystic fibrosis patients (50). We confirm those findings here in older \((\bar{x} \text{ age: } 55 \text{ y})\) CP patients with DM. These older patients showed signs of advanced atherosclerosis, whereas the younger patients studied by Winklhofer et al (50) did not show extensive atherosclerotic development.

The low LDL-cholesterol and apo B concentrations in CP patients with diabetes had no influence on LDL oxidizability. This finding is in agreement with 2 studies in which the effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on this variable were analyzed, except for the 3-hydroxy-2-methylglutaryl coenzyme A reductase inhibitor fluvastatine, which could have an antioxidant effect of its own (51, 52). Conjugated dienes are formed when polyunsaturated fatty acids react with hydroxyl radicals. Consequently, as we show here, the increase in the MUFA content is associated with a slowing of the oxidation process. This protective effect of MUFAs on LDL...
oxidizability and on atherosclerosis is well known (53). However, in the current study, the effect was not sufficient to enhance the lag phase and to protect the vitamin E–deficient LDL from oxidation. An inverse and significant correlation between LDL MUFA and LDL α-tocopherol concentrations was identified here, which could lead to an underestimation of the effect of MUFA on LDL oxidation.

We also found a significant and independent correlation between the fasting plasma concentration of glucose and the lag phase duration. Glucose can generate the oxygen radical $O_2^-$ in the presence of transition metal cations, and glycation products may also generate radicals that cause lipid peroxidation. In addition, some antioxidant enzymes may be altered by hyperglycemia. We found a significant negative correlation between HbA1c and glutathione peroxidase activity, which suggested that this enzyme could be glycated (54, 55). However, the antioxidant enzyme activities measured here did not differ significantly between the diabetic and nondiabetic patients, which is in agreement with the findings reported by Akkus et al (56). In contrast, some reports have described a decrease in the activities of erythrocyte SOD (57), catalase (58), and glutathione peroxidase (59). We can explain this result by the relatively good glycemic control (HbA1c) in diabetic patients in our study.

In view of the findings presented here, it seems clear that DM alters the antioxidant status and that this condition increases the risk of antioxidant deficiencies in CP. However, it is also possible that CP patients with weak antioxidant defenses are prone to diabetes.

Oxidized LDL can trigger an autoimmune response that leads to the formation of antibodies (60). Autoantibodies to epitopes of oxidized LDL were shown in the sera of normal and Watanabe hyperlipidemic rabbits and in humans (60, 61) and were reported as an independent predictor of the progression of carotid atherosclerosis in Finnish men (61). In the same way, ICs were shown in patients with coronary disease and hyperlipidemia and in normal subjects. However, there are conflicting reports in the literature concerning the titration of antibodies to oxidized LDL and LDL-ICs (59, 62, 63). Contrary to the findings of a previous study (64), the ratio of oxidized LDL autoantibodies measured here was not significantly higher in the control diabetic group than in the other groups. In fact, Festa et al (64) reported the highest ratio in control diabetic patients, whereas patients with a high microangiopathy score had a lower antibody ratio than did patients without complications. The ratio of oxidized LDL autoantibodies in CP patients has never been reported. We found a higher concentration of oxidized LDL-ICs, which has been proposed to mask free autoantibodies (64), in control diabetic and CP diabetic subjects with microangiopathy or macroangiopathy than in those without. It has been reported that the induction of foam cell formation in vitro is more efficient when the incubation is carried out in the presence of LDL-ICs.

In agreement with other studies (65, 66), we observed a positive correlation between LDL cholesterol and BMI. The low LDL-cholesterol concentration can be mainly explained by the malabsorption of biliary acids, although the malabsorption of cholesterol is an additional factor that could contribute to the low LDL-cholesterol concentration (67). The ratio of polyunsaturated to saturated fats in the diet did not differ significantly among the 4 groups (data not shown). Consequently, LDL cholesterol appears to be a good marker of pancreatic insufficiency and a strong indicator of the nutritional status of CP patients.

A limitation of this study is that the association between vitamin E deficiency and an increase in LDL oxidizability does not indicate a causal relation. The possibility that both vitamin E deficiency and LDL oxidizability are under the influence of a common factor should be considered.

In conclusion, this study shows that antioxidant status is altered in CP patients. The perturbations are particularly pronounced in subjects with CP plus DM. Lower LDL vitamin E concentrations are associated with a significantly higher LDL oxidizability. We have yet to ascertain whether the differences in antioxidant status found in this study are simply the consequence of CP, DM, or both, or whether these differences in turn, contribute to the clinical expression of the diseases. Moreover, the efficacy of an antioxidant supplement, particularly that of a vitamin antioxidant, has yet to be studied.

We thank JM Virion (Centre d’Investigation Clinique, CHU-INSERM, Hôpital Jeanne d’Arc, Toul, France) for help with the statistical analysis.

DQ had responsibility for the conception and organization of the study, analysis of the results, subject recruitment, and writing. EW had responsibility for laboratory analysis and analysis of the results. JPB had responsibility for fatty acids analysis. JCF had responsibility for laboratory management. PD had responsibility for the conceptual basis of the study, laboratory management, analysis of the results, and writing the manuscript. OZ had responsibility for clinical department management, the conceptual basis of the study, and analysis of the results. None of the authors had any personal or financial conflict of interest.

REFERENCES