0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(6):2957–2962

doi: 10.1210/jc.2003-031897

Protective Effect of Paraoxonase Activity in High-Density Lipoproteins against Erythrocyte Membranes Peroxidation: A Comparison between Healthy Subjects and Type 1 Diabetic Patients

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High-density lipoproteins (HDL) plays a key role in the protection against oxidative damage of lipoprotein and biological membranes. The aim of the present study was to investigate the relationship between the antioxidant role of HDL and the HDL-paraoxonase (PON) activity in healthy subjects and in type 1 diabetic patients. Moreover, the ability of HDL of controls and diabetic patients to protect and/or repair biological membranes from oxidative damage was studied.

HDL were isolated from 31 type 1 diabetic patients and 31 sex- and age-matched healthy subjects and immediately used to evaluate lipid hydroperoxides and HDL-PON activity. Erythrocyte membranes obtained from healthy subjects were oxidized with 2,2-azo-bis(2-aminidinopropane)dihydrochloride and then incubated in the presence of HDL isolated from healthy or type 1 diabetic subjects, with measurements of membrane lipid hydroperoxides before and after the incubation. HDL from type 1 diabetic patients showed higher levels of lipid hydroperoxides and a lower activity of HDL-PON than healthy subjects. Moreover, HDL of type 1 diabetic patients protected less efficiently erythrocyte membranes against oxidative damage compared with HDL from healthy subjects. A negative correlation was found between HDL-PON activity and the levels of hydroperoxides of HDL, confirming the relationship between PON and lipid peroxidation and suggesting that subjects with low PON activity are more exposed to oxidative damage than subjects with high PON activity.

The ability of HDL to protect erythrocyte membranes was positively correlated with HDL-PON activity and negatively correlated with the levels of lipid hydroperoxides of HDL of healthy subjects. These results confirm a linkage between PON activity and lipid peroxidation of lipoproteins and suggest that the ability of HDL to protect erythrocyte membranes might be related to the PON activity.

It might be hypothesized that the decrease of PON activity in diabetic patients and the lower HDL protective action against membrane peroxidation could contribute to acceleration of arteriosclerosis in type 1 diabetes mellitus. (*J Clin Endocrinol Metab* 89: 2957–2962, 2004)

HIGH-DENSITY LIPOPROTEINS (HDL) have been shown to be inversely correlated with the risk of atherosclerosis and coronary heart disease (1). The protective effect of HDL has been related to their role in the cholesterol reverse transport (from peripheral tissues to the liver) and to their ability to inhibit oxidation of low-density lipoproteins (LDL) and biological membranes (2, 3).

Using different models of Cu^{2+}-oxidized cells, HDL have been demonstrated to exert a protective role also against oxidative damage of cells (4, 5). It has been suggested that the antioxidant properties of HDL might be related to their ability to accept phospholipids containing hydroperoxides from oxidized membranes or lipoproteins (4). A transfer of hydroperoxides from oxidized LDL to HDL and an exchange of lipid peroxidation products between the lipoproteins were previously demonstrated (3).

Several lines of evidence suggest that the antioxidant effect of HDL is at least partially related to paraoxonase (PON), an enzyme associated with HDL surface (HDL-PON). In fact, previous studies have shown that PON is able to hydrolyze preformed lipid hydroperoxides and to delay or inhibit the initiation of oxidation induced by metal ions on lipoproteins (6, 7).

The enzymatic activity of HDL-PON varies widely among healthy humans, and it has been suggested that subjects with low PON activity may have a greater risk of developing diseases in which oxidative damage and lipid peroxidation are involved, compared with subjects with high PON activity (8). Moreover, previous studies have shown a relationship between PON activity and the antioxidant properties of HDL (9, 10) and the susceptibility of HDL to atherogenic modifications induced *in vitro*, such as glycation and homocysteinylation (11, 12).

Diabetes is associated with oxidative damage (13), and it has been suggested that the higher levels of lipid peroxidation products in plasma of diabetic patients (14) could be related to a higher susceptibility of plasma lipoproteins of diabetic patients to oxidation (15, 16) and/or to a decrease of plasma antioxidant defenses (17). A decrease of PON activity has been also observed in diabetic patients (18–20).

Abnormal HDL composition and altered HDL subclasses

Abbreviations: AAPH, 2,2-Azo-bis(2-aminidinopropane)dihydrochloride; HbA_{1c}, glycated hemoglobin A1c; HDL, high-density lipoprotein(s); LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein(s); ns, not significant; PON, paraoxonase.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

distribution have also been observed in patients with type 1 diabetes (21). The compositional changes of HDL are reflected in modifications of functional activities, with a lower protection exerted by HDL from diabetic patients against LDL oxidation than HDL from healthy subjects (22) and a decreased capacity to induce cholesterol efflux from biological membranes (23).

The ability of HDL of diabetic patients to protect biological membranes from oxidative damage has not been investigated previously. The aim of this study was to further investigate the relationship between the antioxidant role of HDL and the HDL-PON activity in healthy subjects and in type 1 diabetic patients. Moreover, we compared the ability of HDL of controls and diabetic patients to protect and/or repair biological membranes from oxidative damage. The compositional changes of red blood cell membranes oxidized *in vitro* with 2,2′-azo-bis-(2-amidinopropane) have been well characterized (24). Therefore, oxidized erythrocytes represent a useful model to investigate the protective effect exerted by HDL against oxidative damage of biological membranes.

Subjects and Methods

Subjects

Thirty-one type 1 diabetic patients (16 women and 15 men, 42 ± 8 yr old; duration of disease, 12 ± 5 yr) and 31 healthy subjects (18 women and 13 men; 35 ± 8 yr old) were included in the study. Plasma lipids (total cholesterol, HDL-cholesterol, and triglycerides) were not significantly different in controls and patients (Table 1). Body mass index was similar in controls and diabetic patients (22 ± 3 kg/m^2 and 24 ± 4 kg/m^2, respectively). Patients did not show any chronic complication of the disease or microalbuminuria. Controls and patients were not taking lipid-lowering drugs, angiotensin-converting enzyme inhibitors, or antioxidants or other medication that can affect lipid metabolism. At the time of the study, glycated hemoglobin (HbA_{1c}) levels of type 1 diabetic patients were 9.6 ± 2% ($P < 0.0005$ *vs.* controls). None exhibited evidence of cardiac or renal failure, and all were euthyroid with normal liver function tests and had normal values for plasma urea, creatinine, and electrolytes. Smokers were excluded from the study because cigarette smoke has been shown to inhibit PON activity (25). Furthermore, subjects having a current or recent illness were excluded from the study because modifications of PON activity were described in human patients during the acute phase response (26). Blood samples were collected at 0800 h, after overnight fasting.

The study was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000, and informed consent was obtained from each participating subject.

TABLE 1. Plasma lipid concentrations, levels of lipid hydroperoxides and the activity of PON associated to HDL (HDL-PON activity) of healthy and type 1 diabetic subjects

	Healthy subjects (n = 31)	Diabetic patients (n = 31)
Age (yr)	35 ± 8	42 ± 8
BMI (kg/m^2)	22 ± 3	24 ± 4
HbA1c (%)		9.6 ± 2[a]
TC (mg/dl)	200 ± 30	200 ± 44
HDL-C (mg/dl)	61 ± 19	63 ± 18
TG (mg/dl)	85 ± 20	89 ± 50
HDL-PON activity (U/mg HDL proteins)	444.8 ± 285.8	71.2 ± 59.6[a]
HDL-lipid hydroperoxides (nmol/100 μg HDL proteins)	1.12 ± 0.48	2.15 ± 0.16[a]

BMI, Body mass index; TC, plasma total cholesterol; HDL-C, HDL cholesterol; TG, plasma triglycerides.

[a] $P < 0.001$ *vs.* healthy subjects.

Chemical substances

AAPH [2-azo-bis(2-aminidinopropane) dihydrochloride], butylated hydroxytoluene, dihydrogen sulfate (H_2SO_4), methanol, paraoxon (diethyl p-nitrophenyl phosphate), PBS, sodium chloride (NaCl), iron (II) ammonium sulfate, and xylenol orange were obtained from Sigma Chemical (St. Louis, MO).

Preparation of HDL

Blood samples of controls and type 1 diabetic patients were collected in heparin-containing vacutainer tubes. Plasma was removed by low-speed centrifugation (3000 rpm) at 4 C for 20 min and thereafter used for the preparation of HDL. HDL (d = 1.063–1.210 g/ml) were prepared by single vertical ultracentrifugation for 1.30 h as described by Chung *et al.* (27). After dialysis at 4 C for 24 h against 5 mM PBS (pH 7.4), protein concentration of HDL was determined by the method of Lowry *et al.* (28). HDL were stored at 4 C and immediately used to evaluate lipid hydroperoxides and HDL-PON activity.

Preparation of erythrocyte membranes and peroxidative treatment

Erythrocyte membranes were prepared as previously described by Burton *et al.* (29). Briefly, heparinized blood samples of healthy subjects were collected after overnight fasting and centrifuged (4,500 rpm) to remove plasma. Erythrocytes were washed twice with NaCl (0.9%) isotonic solution, lysed hypotonically in 5 mM ice-cold PBS (pH 8), and processed in a Centrikon H-401 (Kontron centrifuge) at 20,000 rpm. The resulting membranes were washed with PBS of decreasing molarity to completely remove the hemoglobin. Erythrocyte membrane proteins were evaluated by the method of Lowry *et al.* (28). A pool of erythrocyte membranes from the healthy subjects was used for the incubation experiments. An aliquot of the erythrocyte membrane pool (about 6 mg) was incubated with the hydrophilic free radical generator AAPH (5 mM) for 20 h at 37 C. After incubation, oxidation was stopped by refrigeration, and erythrocyte membranes were centrifuged at 15,000 rpm for 20 min. Oxidized membrane pellets were resuspended in 0.9% NaCl solution (ox-membranes) and used for incubation with HDL isolated from plasma of controls and type 1 diabetic patients.

Incubation of oxidized membranes with HDL

In a preliminary phase of the study, a pool of HDL isolated from plasma of different subjects was used to investigate the experimental conditions (different concentration of HDL, different times of incubation) to study the protective effect exerted by HDL on cell oxidative damage. A significant decrease in the levels of lipid peroxides of ox-membranes occurred after incubation of erythrocyte membranes for 3 h in the presence of 100 μg HDL. Therefore, these experimental conditions were used to study the effect of HDL on oxidized erythrocyte membranes. Briefly, an aliquot of ox-membranes (about 500 μg) was incubated in the presence of HDL (100 μg) isolated from healthy and type 1 diabetic subjects, at 37 C for 3 h. At the end of incubation, ox-membranes were separated from lipoproteins by centrifugation at 15,000 rpm and were resuspended in 0.9% NaCl solution and used for determination of lipid hydroperoxides.

Measurement of lipid hydroperoxides

The levels of lipid hydroperoxides in HDL and in ox-membranes incubated in the presence or in the absence of HDL from plasma of controls and diabetic patients were determined as previously described (30). Briefly, aliquots of HDL (about 100 μg) and erythrocyte membranes (about 500 μg) were incubated at 37 C for 20 min with ferrous oxidation of xylenol orange reagent (100 μM xylenol orange, 250 μM Fe^{2+}, 25 mM H_2SO_4, and 4 mM butylated hydroxytoluene in 90% methanol). After incubation, HDL and cell membranes were centrifuged at 4,500 rpm and 15,000 rpm, respectively, and the supernatants were used for the determination of the absorbance at 560 nm. Hydroperoxide content was

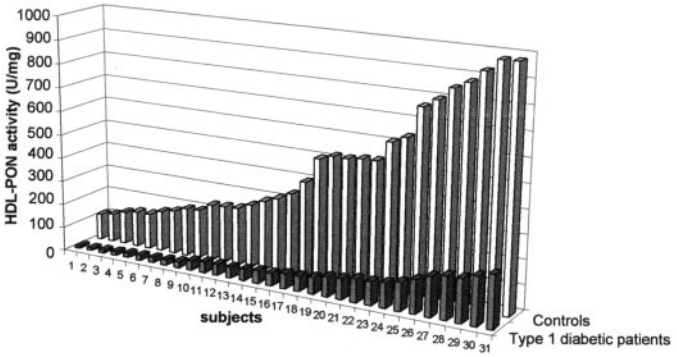

FIG. 1. Activity of PON associated with HDL (HDL-PON) isolated from control subjects (□) and type 1 diabetic patients (▨). Activity is expressed as units per milligram of HDL protein.

determined using a molar absorption coefficient of 4.3×10^4 M^{-1} cm^{-1}. Results are expressed as nanomoles of hydroperoxides in 100 μg proteins of HDL or milligrams of erythrocyte membranes.

Measurement of HDL-PON activity

The activity of PON associated with HDL (HDL-PON) isolated from healthy and type 1 diabetic subjects was measured using a small volume of HDL (100 μg) resuspended in 5 mM Tris-HCl (pH 7.4), containing 0.14 M NaCl and 2 mM $CaCl_2$. The reaction was initiated by adding paraoxon (diethyl p-nitrophenyl phosphate) as a substrate, and the increase in the absorbance at 412 nm was monitored.

The enzyme activity was calculated from the rate of p-nitrophenol production (18 053 M^{-1} cm^{-1}) and was expressed as units per milligram of HDL proteins; a unit of PON activity (U) was defined as 1 nmol p-nitrophenol formed per minute under the above assay conditions (11, 12).

Statistics

Results are presented as mean ± SD. The differences in HDL-PON activity between type 1 diabetic patients and controls were evaluated by Mann-Whitney *U* test, whereas the Student's *t* test was used for the other parameters studied. Linear regression analysis was used to calculate correlation coefficients (r). Values were considered to be significant at *P* less than 0.05. (Microcal Origin 5.0, OriginLab, Northampton, MA).

Results

HDL-PON activity in controls and type 1 diabetic patients and correlation with lipid hydroperoxides

The individual values of PON activity associated with HDL isolated from healthy subjects showed a large variability in agreement with our previous studies (11, 12) (Fig. 1). The HDL-PON activity values ranged from 110–980 U/mg HDL proteins; the median value was 340 U/mg. Lower values of PON were observed in HDL isolated from type 1 diabetic patients (Fig. 1). The values ranged from 9.1–216 U/mg; the median value was 54 U/mg, about 6-fold lower than from controls. As shown in Table 1, the mean values of the enzyme activity in controls and patients were significantly different ($P < 0.001$).

The levels of hydroperoxides associated with HDL isolated from control subjects ranged from 0.43–2.1 nmol/100 μg. In HDL from type 1 diabetic patients, the levels of hydroperoxides were included between 0.94–5.13 nmol/100 μg. The mean values of the levels of hydroperoxides in healthy and diabetic subjects were significantly different (Table 1) ($P < 0.001$).

A significant negative correlation was found between the individual values of HDL-PON activity and the levels of lipid hydroperoxides associated with HDL from healthy subjects ($r = -0.83$, $n = 31$; $P < 0.001$), confirming the relationship between the PON activity and the biochemical indexes of HDL lipid peroxidation (11) (Fig. 2). On the contrary, no significant correlation was found between HDL-PON activity and levels of lipid hydroperoxides in the group of diabetic patients [$r = -0.49$, $n = 31$; not significant (ns)] (Table 2). Moreover, no significant correlation between the levels of HbA1c and HDL-PON activity was found in diabetic subjects ($r = 0.11$, $n = 31$; ns).

Effect of HDL on oxidized erythrocyte membranes

Incubation of erythrocyte membranes in the presence of 5 mM AAPH for 20 h at 37 C induced an increase of the levels of hydroperoxides compared with membranes incubated alone (0.010 ± 0.002 nmol/mg protein and 1.000 ± 0.005 nmol/mg protein in untreated and oxidized membranes, respectively) ($P < 0.001$).

As shown in Fig. 3, a significant decrease in the levels of lipid hydroperoxides was observed in ox-membranes incubated for 3 h at 37 C, in the presence of HDL obtained from healthy subjects compared with membranes incubated alone (0.46 ± 0.18 nmol/mg *vs.* ox-membranes; $P < 0.001$). These results confirm that HDL are able to protect erythrocyte membranes from oxidative damage.

The decrease in the levels of hydroperoxides has been observed also in ox-membranes incubated with HDL isolated from type 1 diabetic patients; however, the decrease was lower than that of HDL of control subjects (0.71 ± 0.21 nmol/mg *vs.* ox-membrane; $P < 0.001$) (Fig. 3).

To investigate whether the protective effect exerted by HDL on lipid peroxidation of erythrocyte membranes was related to PON activity, we studied the correlations between HDL-PON activity and the percentage decrease in lipid peroxides in ox-membrane treated with HDL from healthy and type 1 diabetic subjects.

The percentage decrease in lipid hydroperoxides in ox-membranes incubated with HDL of controls showed a great variability and ranged from 18–95%. The percentage decrease in lipid hydroperoxides was lower in ox-membrane

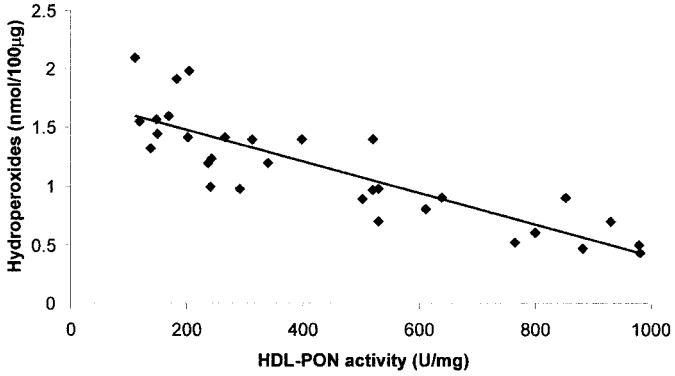

FIG. 2. Correlation between HDL-PON activity and levels of hydroperoxides in HDL isolated from plasma of healthy subjects (n = 31) ($r = -0.83$, $P < 0.001$).

TABLE 2. Correlations between HDL-PON activity, levels of hydroperoxides associated with HDL and HDL ability to protect oxidized erythrocyte membranes (% protection) in healthy subjects and type 1 diabetic patients

	Healthy subjects (n = 31)	Diabetic patients (n = 31)
HDL-PON activity *vs.* hydroperoxides associated with HDL	$r = -0.83$; $P < 0.001$	$r = -0.49$; ns
HDL-PON activity *vs.* % protection of ox-membranes	$r = 0.85$; $P < 0.001$	$r = 0.48$; ns
Hydroperoxides associated with HDL *vs.* % protection of ox-membranes	$r = -0.86$; $P < 0.001$	$r = -0.46$; ns

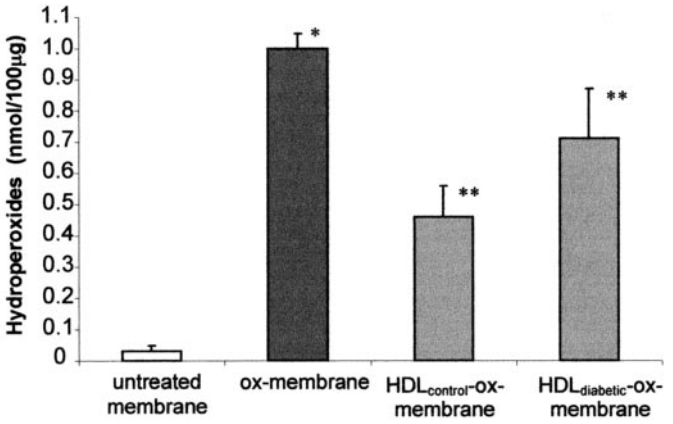

FIG. 3. Levels of hydroperoxides in untreated erythrocyte membranes (□) and in erythrocyte membranes incubated for 20 h at 37 C with 5 mM AAPH (▨) in the absence (ox-membrane) or in the presence of HDL isolated from healthy ($HDL_{control}$-ox-membrane) or type 1 diabetic subjects ($HDL_{diabetic}$-ox-membrane) (see *Subjects and Methods*). *, $P < 0.001$ *vs.* untreated membrane; **, $P < 0.001$ *vs.* ox-membrane.

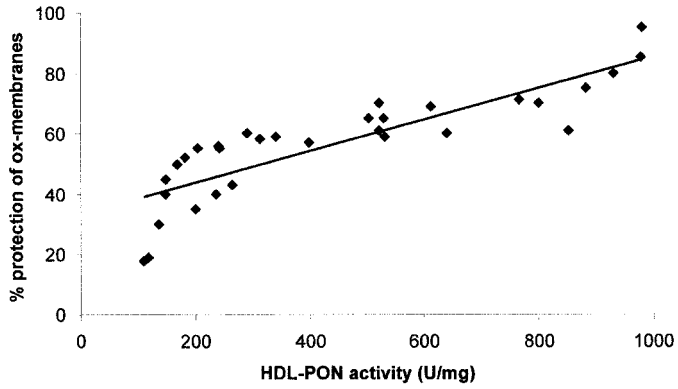

FIG. 4. Correlation between HDL-PON activity and ability to protect erythrocyte membrane from oxidative damage (percent protection) of HDL isolated from healthy subjects (n = 31) ($r = 0.85$, $P < 0.001$).

incubated with HDL isolated from plasma of type 1 diabetic patients (range, 13–60%).

A positive correlation was found between the percentage decrease in lipid hydroperoxides in ox-membranes and the individual values of HDL-PON activity of HDL in healthy subjects ($r = 0.85$, n = 31; $P < 0.001$) (Fig. 4), suggesting that HDL with low PON activity protect erythrocyte membranes less efficiently than HDL from subjects with high PON activity. The correlation was not statistically significant in type 1 diabetic patients ($r = 0.48$, n = 31; ns) (Table 2).

Moreover, a statistically significant negative correlation was found between the levels of HDL lipid hydroperoxide levels and the percentage decrease in membrane lipid peroxides. The correlation was significant in healthy subjects ($r = -0.86$, n = 31; $P < 0.001$) but not in diabetic patients ($r = -0.46$, n = 31; ns) (Table 2).

No significant correlation was established between the percentage decrease in membrane lipid peroxides caused by incubation with HDL obtained from diabetic subjects and HbA1c levels ($r = 0.22$, n = 31; ns).

Discussion

Previous studies have shown modifications of the levels of plasma lipoproteins isolated from type 1 diabetic patients (31) and alterations in the activity of enzymes involved in lipoprotein metabolism, such as cholesteryl ester transfer protein (CETP) (32), phospholipid transfer protein (PLTP) (33), and lecithin:cholesterol acyltransferase (LCAT) (34).

In the present study, we demonstrated that the activity of the enzyme PON associated with HDL of type 1 diabetic patients is significantly lower than that of healthy subjects. Moreover, our results showed higher levels of lipid hydroperoxides in HDL isolated from type 1 diabetic patients than from controls. These results confirm that diabetes is associated with oxidative damage, in agreement with previous studies. In fact, a decreased activity of serum PON (18–20) and a higher susceptibility to lipid peroxidation of lipoproteins of diabetic subjects have been observed (15, 16).

No differences in genotypic frequencies of PON between healthy subjects and type 1 diabetic patients have been demonstrated (20), and it has been suggested that the lower activity of PON in type 1 diabetic subjects might be related to alterations of the chemical composition of HDL. This hypothesis is supported by previous studies about the factors affecting the PON activity. In fact, the conformation of PON within the hydrophobic environment of HDL is crucial for its activity (35), and compositional modifications have been observed in HDL isolated from diabetic patients (21, 33). Therefore, it has been hypothesized that the compositional alterations of HDL of diabetic subjects could affect the binding of PON to HDL, leading to a conformational change of the enzyme and/or to a reduced availability of substrate in the hydrophobic region of HDL in which PON is active, resulting in a decreased enzyme activity (20).

The negative correlation found between HDL-PON activity and the levels of hydroperoxides of HDL isolated from control and type 1 diabetic subjects confirms the relationship between PON and lipid peroxidation and demonstrates that subjects with low PON activity are more exposed to oxidative damage than subjects with high PON activity (8, 11).

Moreover, in the present study, we confirmed that HDL are able to protect and/or repair oxidative damage of cell membranes, as shown by the significant decrease in the levels of hydroperoxides in ox-erythrocyte membranes incubated with HDL. The protective effect of HDL against oxidized cell membranes has been previously reported (4, 5), and it has

been hypothesized that the antioxidant effect of HDL could be related to their ability to accept lipid hydroperoxides from oxidized lipoproteins or biological membranes (3, 4). We suggest that the heterogeneity in the ability of HDL of healthy subjects to protect oxidized membranes could be related to compositional factors and/or to the activity of PON associated with the HDL surface. This hypothesis is supported by the statistically significant negative correlation established between the individual levels of HDL lipid hydroperoxides and the percentage decrease of hydroperoxides in ox-erythrocyte membranes. Several studies demonstrated that lipid peroxidation induces significant changes of physico-chemical properties and functional activities of lipoproteins, such as exchange processes between lipoproteins and membranes (36–38). Therefore, we suggest that the compositional changes in HDL with higher levels of hydroperoxides could induce a reduced ability to stimulate the efflux of hydroperoxides from ox-membranes and/or to accept lipid hydroperoxides from oxidized erythrocyte membranes. Concerning the involvement of PON in the protective effect exerted by HDL, Watson *et al.* (6) demonstrated that PON purified from human plasma could hydrolyze oxidized fatty acids from phospholipids and repair oxidative damage of mildly oxidized LDL. The relationship between PON activity and the protective effect exerted by HDL on oxidized membranes, observed in the present study, suggests a higher ability of HDL with high PON activity to protect erythrocyte membranes against oxidative damage. Therefore, we suggest that HDL-PON could play an important role in the protective effect exerted by HDL against oxidative damage of cell membranes.

In the present study, HDL of type 1 diabetic subjects have been shown to possess a lower ability to protect erythrocyte membranes, compared with HDL of controls. These results are in agreement with previous studies demonstrating a decreased ability of HDL of type 1 diabetic patients to protect from LDL lipid peroxidation (22). In our experimental conditions, no significant correlation has been found between HDL-PON of diabetic patients and the ability of HDL to protect erythrocyte membranes. Some hypotheses can be advanced to explain these results. We hypothesize that the lower antioxidant role exerted by HDL from type 1 diabetic patients could be due to a decreased ability of HDL to stimulate the efflux of hydroperoxides from ox-membranes. Previous studies have shown that the transfer of lipids between cells and HDL depends on lipid composition of HDL, and Cavallero *et al.* (39) have shown that lipoprotein containing apoA-I isolated from diabetic patients exhibit a decreased capacity to induce cholesterol efflux.

Further studies are necessary to investigate whether other enzymes associated with the HDL surface, such as plasma platelet-activating factor acetylhydrolase, LCAT, and cholesteryl ester transfer protein (31), might be involved in the alterations of the protective effect exerted by diabetic HDL against oxidative damage of biological membranes. In fact, previous studies have shown, in diabetic patients, modifications of the activities of enzymes involved in the antioxidant role of HDL, such as LCAT (34). Moreover, it has to be stressed that a PON-independent inhibition of LDL oxidation by HDL has been observed by Graham *et al.* (40).

In our experimental conditions, no statistically significant correlations have been established between HbA1c levels and HDL-PON activity or the protective capacity of HDL against peroxidation in diabetic patients, suggesting that glycemic control is not directly involved in these HDL properties.

In conclusion, our study confirms the existence of modification of lipid composition and functional activities of HDL of type 1 diabetic patients with a lower activity of PON associated with HDL. This reduction in PON activity might play a central role in the atherosclerotic process, because HDL-PON activity modulates the susceptibility of HDL to atherogenic modifications such as glycation and homocysteinylation (11, 12). Moreover, the present study demonstrates a lower ability of diabetic HDL to protect erythrocyte membranes, compared with control subjects, in agreement with previous studies by us (16) and other authors (15) that demonstrated a higher susceptibility to lipid peroxidation of lipoproteins isolated from plasma of diabetic patients than of healthy subjects.

Oxidative stress and oxidation of lipoproteins and membranes have been implicated in the development of coronary heath disease and atherosclerosis. On the basis of the present study, it might be hypothesized that the decrease of PON activity and the compositional changes of HDL in diabetic patients could contribute to acceleration of the cellular oxidative damage and arteriosclerosis in type 1 diabetes mellitus.

Acknowledgments

Received October 31, 2003. Accepted March 7, 2004.

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