Original Article

Defective Metabolism of Oxidized Phospholipid by HDL From People With Type 2 Diabetes

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HDL protects against atherosclerosis development. Defective functioning of HDL in type 2 diabetes may be one cause of increased cardiovascular disease associated with type 2 diabetes. HDL modulates LDL oxidation through the action of paraoxonase-1 (PON1), which is one of the major mechanisms by which HDL is antiatherogenic. We have compared the ability of HDL from people with type 2 diabetes (n = 36) with no coronary heart disease (CHD) to metabolize oxidized palmitoyl arachidonyl phosphatidylcholine (ox-PAPC), a major product of LDL oxidation and a PON1 substrate, with that of HDL isolated from healthy control subjects (n = 19) and people with CHD but no diabetes (n = 37). HDL from people with type 2 diabetes metabolized 11% less ox-PAPC, and HDL from people with CHD metabolized 6% less, compared with HDL from control subjects (both $P < 0.01$). The ability of HDL from control and type 2 diabetic subjects containing the PON1-192RR alloform to metabolize ox-PAPC was significantly reduced compared with PON1-192QQ or QR genotypes ($P < 0.05$). The defective ability of HDL to metabolize ox-PAPC was reflected in a significant increase in circulating plasma oxidized LDL concentration in the two patient groups (37 ± 5, 53 ± 7, and 65 ± 7 mmol/l for control, CHD, and type 2 diabetic subjects, respectively; $P < 0.001$), with PON1-192RR genotype carriers having the highest concentrations. In the control group, there was a significant negative correlation between serum PON1 activity and oxidized LDL concentration ($r = 0.856$, $P < 0.001$); however, this correlation was not evident in the patient groups. HDL from type 2 diabetic subjects without CHD had a decreased ability to metabolize oxidized phospholipids, which could lead to increased susceptibility to develop cardiovascular disease. *Diabetes* 55:3099–3103, 2006

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apo, apolipoprotein; CHD, coronary heart disease; MCP-1, monocyte-chemotactic protein-1; ox-PAPC, oxidized palmitoyl arachidonyl phosphatidylcholine; PON1, paraoxonase-1.

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The oxidation of LDL in the artery wall is believed to be the primary event leading to the initiation and progression of atherosclerosis (1,2). HDL, on the other hand, is protective against the development of atherosclerosis (3,4). The primary protective effect of HDL was believed to be its pivotal role in reverse-cholesterol transport; however, HDL also has antioxidative, anti-inflammatory, and antithrombotic properties (5).

HDL-associated paraoxonase-1 (PON1) is primarily responsible for the antioxidative properties of HDL in retarding the oxidation of LDL (6–10). By modulating the oxidation of LDL, PON1 abolishes the oxidized LDL–stimulated induction of monocyte-chemotactic protein-1 (MCP-1) production by endothelial cells, thereby preventing monocyte–endothelial cell interaction in one of the earliest processes of atherosclerosis (11,12). PON1 is low in patients with diabetes (13–15), leading to dysfunctional HDL with impaired antioxidant capacity (15). In type 2 diabetes, there is an inverse relationship between PON1 activity and circulating oxidized LDL levels (16,17), indicative of the major role of PON1 in retarding LDL oxidation. We have recently shown that adenovirus-mediated overexpression of human PON1 in a mouse model of metabolic syndrome significantly inhibited atherosclerosis development by reducing oxidized LDL in both plasma and the artery wall (18). Type 2 diabetes is associated with a three- to fourfold increased susceptibility for coronary heart disease (CHD), compared with people without diabetes, and HDL cholesterol is lower in type 2 diabetic than in nondiabetic individuals (19). Oxidized palmitoyl arachidonyl phosphatidylcholine (ox-PAPC) is a primary proinflammatory product of LDL oxidation and a substrate for PON1 (20).

To determine whether HDL from type 2 diabetic individuals is defective in its ability to metabolize oxidized phospholipids, we analyzed the ability of HDL isolated from healthy control subjects, people with type 2 diabetes, and those with nondiabetic CHD to metabolize ox-PAPC.

RESEARCH DESIGN AND METHODS

The study populations comprised 19 healthy control subjects attending a routine health check, who were matched for age (±5 years) and sex with the patient groups, and 36 subjects with type 2 diabetes attending the Manchester Royal Infirmary. Diabetes was diagnosed by World Health Organization criteria (21). All patients were receiving statin treatment, 4 were taking calcium channel blockers, 2 ACE inhibitors, 20 metformin, and 2 insulin; none had CHD. A total of 16 individuals were free of diabetes complications, and 20 had one or more complications (16 with peripheral neuropathy, 10 with nephropathy, and 11 with retinopathy). The third study group comprised 37 patients with angiographically assessed CHD, with >70% stenosis of at least

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TABLE 1
Characteristics of the study groups

	Control	Type 2 diabetes	CHD
n (male)	19 (10)	36 (21)	37 (22)
Age (years)	57.7 ± 4.8	57.7 ± 5.2	57.7 ± 5.4
BMI (kg/m^2)	21.2 ± 3.2	28.8 ± 5.0*	25.3 ± 4.0
Total cholesterol (mmol/l)	4.8 ± 1.6	5.1 ± 1.5	4.4 ± 1.0
Triglycerides (mmol/l)	1.05 (0.51–3.27)	2.24 (0.68–9.27)*	1.73 (0.64–3.21)*
HDL cholesterol (mmol/l)	1.55 ± 0.41	1.29 ± 0.59	1.05 ± 0.34*
apoB (mg/dl)	78 ± 59.5	88.0 ± 31.2	82.3 ± 21.0
apoA1 (mg/dl)	110.5 ± 38.3	128.7 ± 46.3	85.1 ± 20.0*
PON1 activity (nmol · min^{-1} · ml^{-1})	269.4 (68.2–487.2)	113.6 (40.2–409.4)*	150.9 (40.2–463.8)*
PON1 mass (μg/ml)	113.1 (35.2–239)	90.1 (17.6–175.9)†	83.5 (19.4–124.9)†

Data are means ± SD or median (range). Significantly different from control. *$P < 0.01$; †$P < 0.05$.

one coronary artery. All patients were receiving statins, seven calcium channel blockers, and five ACE inhibitors. The Central Manchester local research ethics committee approved the study, and all participants gave informed consent.

Venous blood was obtained after an overnight fast. Serum and EDTA-plasma were obtained by low-speed centrifugation. Plasma and serum were stored at −20°C until analysis. DNA was extracted from lymphocytes.

Biochemical analysis. Serum total cholesterol, triglycerides, HDL cholesterol, apolipoprotein (apo)A1, and apoB were determined using a Cobas Bios II autoanalyzer, using reagents and standards provided by the manufacturer (ABX Diagnostics, Shefford, U.K.). Serum PON1 activity toward paraoxon, PON1 concentration, and PON1 genotypes (Q192R, L55M, and C-108T) were determined by our previously published methods (13). Plasma oxidized LDL was determined by a commercial enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden).

ox-PAPC hydrolysis. The hydrolysis of ox-PAPC was investigated as described in detail previously (20). Briefly, PAPC (2 mg/ml in PBS) was oxidized by the addition of 5 μmol/l Cu^{2+} overnight at 37°C. Then, 1 mg of ox-PAPC was incubated with HDL in a final volume of 1 ml of PBS for 3 h at 37°C. The concentration of lipid-peroxides in the mixture was then measured using the cholesterol-iodide method (22).

Statistical analysis. All parameters were tested for normality using the one-sample Kolmogorov-Smirnov test. Statistically significant differences between variables with a Gaussian distribution were sought by Student's unpaired t test. Variables with a non-Gaussian distribution were compared using the Mann-Whitney U test. One-way ANOVA was used to show any effect of the PON1 polymorphisms on the variables investigated. Correlations between parameters were examined using Pearson's coefficient. The expected frequency of PON1 alleles were analyzed by the Hardy-Weinberg equilibrium test. The χ^2 test was used to determine the significance of differences in allele frequency. All statistical analyses were conducted using SPSS version 10.0.

RESULTS

The demographic details of the study groups are given in Table 1. Compared with the control subjects, both the type 2 diabetic and the CHD group had higher serum triglycerides and significantly lower PON1 activity and concentration. The group with type 2 diabetes had higher BMI, whereas the group with CHD had lower HDL cholesterol and apoA1. Serum total cholesterol was not significantly different between the groups, probably because all of the patients were receiving statin therapy. There were no differences in the distribution of the PON1-L55M, Q192R, or C-108T polymorphisms between the groups (Table 2).

HDL from all three populations was able to metabolize ox-PAPC in a concentration-dependent manner (Fig. 1). However, HDL from those with CHD and type 2 diabetes was significantly less able to metabolize ox-PAPC than HDL from control subjects ($P < 0.01$). The percent metabolism of ox-PAPC was 64% for control HDL, 58% for CHD HDL, and 53% for type 2 diabetes HDL ($P < 0.01$) (Fig. 2). Neither the PON1-L55M nor C-108T polymorphisms affected the ability of HDL to metabolize ox-PAPC. However, the ability of HDL from control subjects and type 2 diabetic subjects with the PON1-192RR genotype to metabolize ox-PAPC was significantly reduced compared with HDL from PON1-192QQ or QR genotypes ($P < 0.05$) (data not shown).

The defective ability of HDL from people with CHD or type 2 diabetes to modulate LDL lipid peroxidation was reflected in a significant increase in circulating plasma oxidized LDL in the two patient groups ($P < 0.01$) (Fig. 3). In the patient populations, individuals with the PON1-192RR had significantly higher plasma oxidized LDL concentrations than those with the QR and QQ genotypes ($P < 0.05$) (Fig. 4*A*). In general, oxidized LDL was higher in those with CHD and type 2 diabetes compared with control subjects, regardless of the PON1 genotype. In the type 2 diabetic population, individuals with the PON1–55LL genotype had significantly higher oxidized LDL concentrations than those with the LM and MM genotypes (Fig. 4*B*), and in the CHD population, those with the *L55M* genotypes had higher oxidized LDL concentrations than control subjects (Fig. 4*B*). The PON1-C-108T polymorphism had no effect on plasma oxidized LDL concentration. In the control group, there was a significant negative correlation between serum PON1 activity and oxidized LDL concentration ($r = 0.856$, $P < 0.001$) (Fig. 5); however, no such correlation was found in the groups with type 2 diabetes or CHD.

DISCUSSION

Several previous studies have shown that HDL from diabetic individuals are deficient in reverse-cholesterol transport or antioxidant activity (19,23). However, in this study, we have shown for the first time that HDL from people with type 2 diabetes but without CHD and from people

TABLE 2
Allele frequencies of the PON1 polymorphisms in the study groups

Position and allele	Gene frequency		
	Control	Type 2 diabetes	CHD
192			
Q	0.75	0.67	0.65
R	0.25	0.33	0.35
55			
L	0.63	0.68	0.65
M	0.37	0.32	0.35
−108			
C	0.55	0.52	0.56
T	0.45	0.48	0.44

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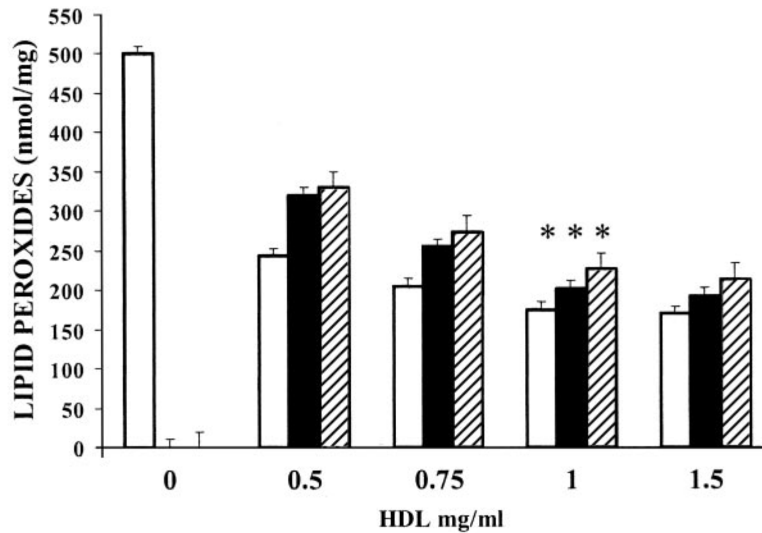

FIG. 1. Effect of HDL concentration on the metabolism of ox-PAPC in the control (□), CHD (■), and type 2 diabetic (▨) populations. *Significantly lower than 0.5 and 0.75 mg/ml HDL, $P < 0.01$.

with CHD but without diabetes were equally defective at metabolizing the model oxidized phospholipid, ox-PAPC, when compared with control HDL. Ox-PAPC is a known substrate for the HDL-associated antioxidant enzyme PON1 (20).

Both type 1 and type 2 diabetes are typified by low levels of serum PON1 (13,15), particularly in type 2 diabetes with complications (14). Low PON1 has been shown prospectively to be an independent risk factor for CHD (24). HDL-associated PON1 is antiatherogenic because of its ability to metabolize LDL (and perhaps cell membrane)-associated oxidized lipids (6–10). In this study, we found a negative correlation between PON1 activity and circulating oxidized LDL concentration in the control population, but we were unable to find such an association in type 2 diabetic or CHD populations. This is contrary to previous findings in diabetes (16,17). The reasons for these discrepancies are not obvious but could be caused by differences in study populations, number of subjects studied, or diabetes control. The latter may be particularly important because glycation of HDL appears to inhibit PON1 activity (25), which may explain the distorted PON1 activity-to-mass ratio found in the patient groups, and therefore the worse the glycemic control, the more glycation and the less PON1 activity (26), leading to a decreased ability to metabolize oxidized LDL. However, the PON1 polymorphisms also appear to play a role in the metabolism of plasma oxidized LDL in our study. We and others have previously shown that the PON1 alloforms from individuals with the PON1-192R and 55L polymorphisms are least able to metabolize lipid peroxides. These are in linkage disequilibrium, which may account for the poor metabolism by the 55L polymorphism (20,27). In the current study, this was only the case for the 55L polymorphism in those with type 2 diabetes. These findings would indicate an additional factor(s) acting to cause a derangement in PON1 activity. This could be attributable to a number of factors; however, the most likely are *1*) increased glycation, which is known to inhibit PON1 and which is also increased in subjects with CHD (28), and *2*) increased oxidative stress, which is prevalent in both diabetes and CHD (29,30) and which is also known to inhibit PON1 (31). On a cautionary note, the number of subjects studied was relatively small, and the possibility of a type II error cannot be excluded but seems unlikely given the clear-cut nature of the results.

In the artery wall, oxidized LDL stimulates the recruitment and retention of monocyte/macrophages via an in-

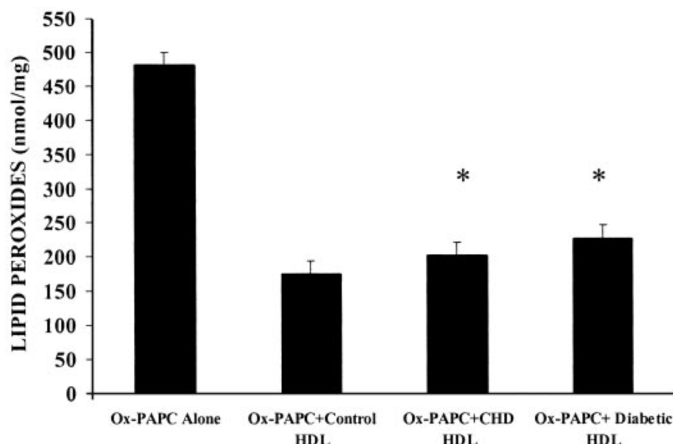

FIG. 2. Effect of HDL from control, CHD, and type 2 diabetic populations on ox-PAPC. *Significantly different from control subjects, $P < 0.01$.

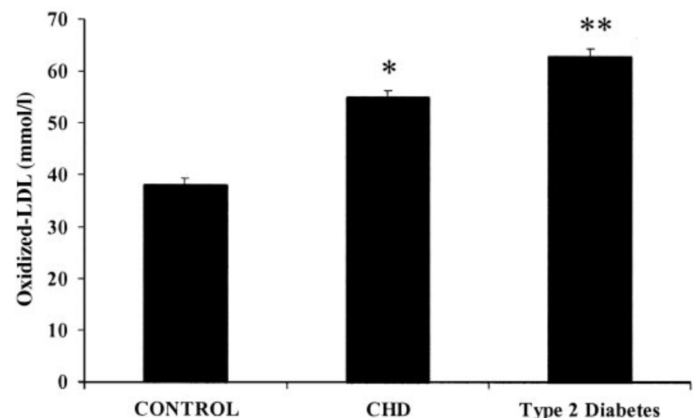

FIG. 3. Plasma oxidized LDL concentration in the three study populations. CHD and type 2 diabetes groups were significantly different from control subjects. *$P < 0.05$; **$P < 0.01$.

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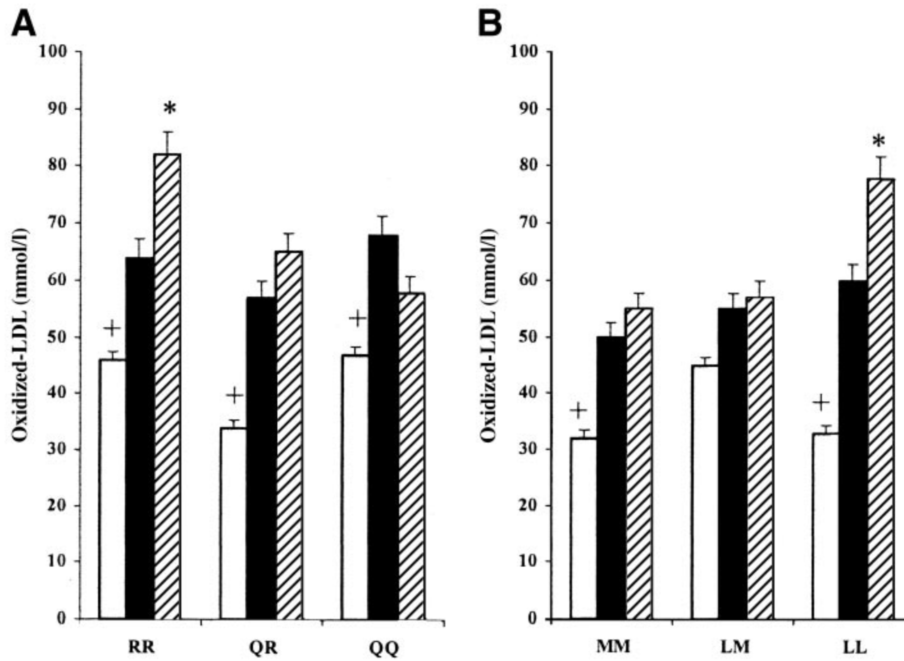

FIG. 4. Effect of the PON1 −192 (*A*) and −55 (*B*) polymorphisms on plasma oxidized LDL concentration. *Significantly higher than other genotypes, $P < 0.05$; +significantly lower than patient populations, $P < 0.05$. □, control; ■, CHD; ▨, type 2 diabetes.

crease in endothelial cell adhesion molecules and chemoattractants, such as MCP-1 (32), and the formation of macrophage foam cells (the progenitor of atheroma). HDL has been shown to inhibit the expression of endothelial cell adhesion molecules (32,33), whereas HDL and isolated human PON1 have been shown to inhibit monocyte transmigration in a coculture system (8,11). This is caused by the ability of PON1 to inhibit the oxidized LDL upregulated MCP-1 secretion by endothelial cells in the process of endothelial cell activation (12). Therefore, the derangement of the ability of HDL in type 2 diabetes to metabolize oxidized LDL would increase endothelial cell activation, leading to increased cardiovascular disease and diabetes complications.

In conclusion, we have shown that HDL from type 2 diabetic subjects shows a decreased ability to metabolize oxidized phospholipids, the consequences of which could lead to increased susceptibility to develop cardiovascular disease.

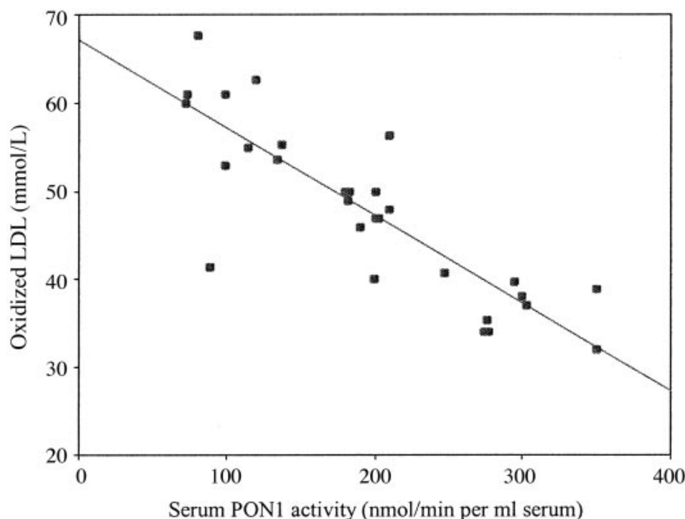

FIG. 5. Correlation between PON1 activity and oxidized LDL concentration in the control population.

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