Paraoxonase-1 and serum concentrations of HDL-cholesterol and apoA-I

Marie-Claude Blatter Garin,[1] Xenia Moren, and Richard W. James[2]

Clinical Diabetes Unit, Division of Endocrinology, Diabetes, and Nutrition, Medical Faculty, University Hospital, 1211 Geneva 14, Switzerland

Abstract **Paraoxonase-1 (PON1) and HDL are tightly associated in plasma, and this is generally assumed to reflect the need for the enzyme to associate with a hydrophobic complex. The association has been examined in coronary cases and age-matched controls. Highly significant ($P < 0.0001$), positive associations were observed between PON1 activities and concentrations and HDL-cholesterol and apolipoprotein A-I (apoA-I) concentrations in cases and controls. Corrected slopes were significantly different in cases (cases vs. controls: arylesterase, $r = 0.19$ vs. 0.38, $P < 0.02$ for apoA-I and $r = 0.15$ vs. 0.34, $P < 0.02$ for HDL-cholesterol) such that if PON1 should influence serum HDL, it would be less effective in coronary cases. When examined as a function of the *PON1* gene promoter polymorphism C−107T, highly significant differences ($P < 0.001$) in HDL-cholesterol and apoA-I were observed between genotypes for controls, with high expresser alleles having the highest HDL concentrations. This relationship was lost in cases with coronary disease. The coding region polymorphisms Q192R and L55M of the *PON1* gene showed no association with HDL. The promoter polymorphism was an independent determinant of HDL concentrations in multivariate analyses. These data are consistent with an impact of PON1 on plasma concentrations of HDL, with detrimental modifications to the relationship in coronary cases.**—Blatter Garin, M-C., X. Moren, and R. W. James. **Paraoxonase-1 and serum concentrations of HDL-cholesterol and apoA-I.** ***J. Lipid Res.*** **2006.** 47: **515–520.**

Supplementary key words atherosclerosis • oxidative stress • low density lipoprotein • high density lipoprotein • apolipoprotein A-I

A number of studies have demonstrated the close, physiological association between paraoxonase-1 (PON1) and HDL in plasma. HDL provides a vector that facilitates the secretion of the enzyme by liver (1), essentially by offering a hydrophobic harbor for the retained signal peptide of PON1 and coincidentally stabilizing the enzyme. The lipoprotein also furnishes a hydrophobic environment that could be important for PON1 function (2). In return, the enzyme prevents or limits the oxidation of HDL (3), although the extent to which this provides physiological benefit to HDL in vivo is less well established. The latter is of particular relevance given that HDLs appear to be the primary transporters of oxidized lipids in plasma (4) and that oxidation can compromise HDL function (5). Moreover, a recent study suggested that PON1 could influence HDL concentrations (6). That study, however, was performed under the particular conditions of patients with familial hypercholesterolemia accompanied by a high prevalence of atherosclerosis in the population. Thus, at present, PON1 is suggested to be a primary determinant of the antioxidant capacity of HDL, and the antioxidant function is evoked as the principal source of the beneficial impact of the enzyme on atherosclerotic disease (7).

Several independent studies have demonstrated that serum PON1 activity and concentration are decreased in patients with cardiovascular disease and other pathologies that increase the risk of vascular disorders (8–10). The cause has yet to be established, but reduced PON1 activity may contribute to the disease process. In this context, one prospective study has suggested that decreased serum PON1 status precedes the onset of vascular problems (11).

A potential extrapolation of these studies is that PON1 could have a beneficial impact on vascular disease by influencing serum HDL concentrations, the latter being a well-established, powerful cardiovascular disease risk determinant. To gain further insight into this possibility, the present study examined *i*) whether PON1 was an independent determinant of serum HDL concentrations in subjects without evidence of coronary disease (controls), and *ii*) whether the relationship between PON1 and HDL concentrations was modified in cases with confirmed cardiovascular disease.

METHODS

Study population

This study was based on a population with confirmed (angiography) coronary artery disease (CAD; n = 710, 79.5%

Manuscript received 5 July 2005 and in revised form 23 August 2005 and in re-revised form 22 November 2005.

Published, JLR Papers in Press, December 3, 2005.
DOI 10.1194/jlr.M500281-JLR200

[1] Present address of M-C. Blatter Garin: Swiss Institute of Bioinformatics, University Medical Center, Medical Faculty, University of Geneva, Geneva, Switzerland.

[2] To whom correspondence should be addressed.
e-mail: richard.james@hcuge.ch

TABLE 1. Characteristics of control and case groups

Parameter	Cases	Controls	ANOVA (*P*)
Men/women	564/146	100/99	
Age (years)	60.5 ± 9.6	56.7 ± 10.7	< 0.0001
Diabetes (%)	18.0	9.3	< 0.01
Cholesterol (mmol/l)	5.77 ± 1.24	5.61 ± 1.04	0.09
Triglycerides (mmol/l)	1.65 ± 1.10	1.28 ± 0.63	< 0.0001
HDL-cholesterol (mmol/l)	1.13 ± 0.31	1.29 ± 0.34	< 0.0001
LDL-cholesterol (mmol/l)	3.90 ± 1.13	3.74 ± 0.94	0.1
ApoA-I (g/l)	0.94 ± 0.20	1.04 ± 0.24	< 0.0001
ApoB (g/l)	0.98 ± 0.23	0.91 ± 0.21	< 0.001
ARE activity (U/ml)	79.6 ± 21.8	83.9 ± 24.4	< 0.02
Ponb activity (U/ml)	176.3 (30–478)	264.8 (61–527)	< 0.01[a]
PON1 concentration (μg/ml)	92.8 ± 21.5	97.4 ± 21.6	< 0.03

apoA-I, apolipoprotein A-I; ARE, arylesterase activity (phenylacetate as substrate); Ponb, basal paraoxonase activity (paraoxon as substrate); PON1, paraoxonase-1. All values are means ± SD, except Ponb, which is median and (90% confidence intervals). Values for triglycerides, HDL-cholesterol, LDL-cholesterol, apoA-I, and apoB were log-transformed before analysis.

[a] Ponb values were analyzed by a nonparametric test (Wilcoxon ranked-sum test).

men) and controls (n = 199, 50.8% men) recruited within the 46–65 year range to minimize age differences with cases. Briefly, cases were recruited from patients referred consecutively to the Cardiology Division of the University Hospital, Geneva, for coronary angiography. All participants underwent angiography, and the presence of coronary disease was defined as stenosis estimated at >20% in at least one major epicardial artery. Participants with no detectable coronary artery anomaly were classified as coronary disease-negative (controls). The majority of controls were referred for examination of heart valve anomalies, with a minority being referred for investigation of chest pains. The latter were diagnosed as being of noncardiac origin. Each participant completed questionnaires on personal and family medical history with an interviewer and gave a fasting blood sample. All participants gave written, informed consent, and the ethics committee of the University Hospital approved the study.

The anthropomorphic and clinical characteristics of these populations have been described in detail in previous publications (9, 12) and are briefly recapitulated in **Table 1**.

Analyses

Plasma lipids, lipoproteins, and apolipoproteins were quantified as described (13, 14). PON1 enzyme activities were analyzed with phenylacetate [arylesterase activity (ARE)] and paraoxon [basal paraoxonase activity (Ponb)] as substrates (15, 16). PON1 protein concentrations and genotypes were analyzed as detailed previously (15, 16). Correlations between activities and PON mass for controls and cases were as follows: ARE, 0.83 and 0.79; Ponb, 0.72 and 0.69, respectively. Genotype frequencies did not depart from Harvey-Weinberg equilibrium (17).

Statistical analyses

Before analyses, variables that were not normally distributed [triglycerides, LDL-cholesterol, apolipoprotein A-I (apoA-I), and apoB] were subjected to natural log transformation. Quantitative parameters were compared between groups by ANOVA. HDL-cholesterol and apoA-I were adjusted for potential confounders [triglycerides, total cholesterol, age, gender, body mass index (BMI), diabetes category, and smoking status]. Correction for PON genotypes was by dummy variable regression (considering each genotype as two variables). Slopes of HDL/apoA-I versus PON1 variables were adjusted for covariates (age, gender, triglycerides, BMI, diabetes category, and smoking) before comparisons between case and control groups. Main effects were tested via *F* tests for slopes, and interactions were tested via *F* tests for differences between slopes. Analyses were performed using the SPSS statistical package version 11.0 (SPSS, Inc., Chicago, IL).

RESULTS

There were significant correlations between PON1 mass and activities and serum concentrations of apoA-I and HDL-cholesterol for subjects free of CAD (**Table 2**). *PON1* gene expression is greatly influenced by the C−107T promoter polymorphism. It accounts for up to 25% of intraindividual variations in serum concentrations and activities of the enzyme (16). This was confirmed in the present study, where in controls the promoter genotype accounted for 23.6% and 7.7% of variations in ARE and Ponb (20.7% and 8.8%, respectively, for the cases). Corresponding variations

TABLE 2. Correlations between PON1 activity and HDL-cholesterol or apoA-I

Activity	*r*		*P* (Controls vs. Cases)
	Controls	Cases	
ARE vs.			
ApoA-I	0.38	0.19	<0.02
HDL	0.34	0.15	<0.02
Ponb vs.[a]			
ApoA-I	0.40	0.21	<0.01
HDL	0.42	0.19	<0.02
PON mass vs.			
ApoA-I	0.31	0.15	<0.04
HDL	0.35	0.18	<0.025
ARE vs.[b]			
ApoA-I	0.365	0.215	<0.05
HDL	0.32	0.17	<0.05
Ponb vs.[b]			
ApoA-I	0.34	0.19	<0.05
HDL	0.39	0.15	<0.002
PON mass vs.			
ApoA-I	0.29	0.13	<0.04
HDL	0.37	0.19	<0.02

All correlation coefficients are significant at $P < 0.01$. The *P* values are for comparison of slopes between cases and controls.

[a] Corrected for the Q192R genotype.

[b] Corrected for C−107T and Q192R genotypes, age, gender, body mass index (BMI), cholesterol, triglycerides, diabetes, and smoking categories.

TABLE 3. HDL-cholesterol and apoA-I as a function of *PON1* gene polymorphisms

Population	C−107T[a] CC	C−107T[a] CT	C−107T[a] TT	ANOVA
Control	n = 48	n = 90	n = 61	
ApoA-I	1.14 (0.03)	1.00 (0.02)	1.03 (0.03)	0.005
HDL-cholesterol	1.45 (0.04)	1.24 (0.03)	1.24 (0.04)	0.001
Cases	n = 164	n = 358	n = 188	
ApoA-I	0.91 (0.01)	0.93 (0.01)	0.97 (0.01)	0.02
HDL-cholesterol	1.12 (0.02)	1.10 (0.01)	1.18 (0.01)	0.006
		Q192R[b]		
	QQ	QR	RR	
Control	n = 88	n = 89	n = 22	
ApoA-I	1.08 (0.03)	1.03 (0.02)	0.96 (0.05)	0.14
HDL-cholesterol	1.28 (0.03)	1.30 (0.03)	1.31 (0.07)	0.94
Cases	n = 373	n = 292	n = 45	
ApoA-I	0.95 (0.01)	0.93 (0.01)	0.87 (0.03)	0.06
HDL-cholesterol	1.14 (0.01)	1.11 (0.01)	1.05 (0.04)	0.09
		L55M[c]		
	LL	LM	MM	
Control	n = 83	n = 95	n = 21	
ApoA-I	1.07 (0.03)	1.02 (0.02)	1.08 (0.06)	0.24
HDL-cholesterol	1.28 (0.04)	1.29 (0.03)	1.38 (0.06)	0.61
Cases	n = 249	n = 348	n = 113	
ApoA-I	0.93 (0.01)	0.93 (0.01)0	0.94 (0.02)	0.99
HDL-cholesterol	1.12 (0.02)	1.12 (0.01)	1.12 (0.03)	0.99

ApoA-I values are in g/l; HDL-cholesterol values are in mmol/l. Mean values and (SEM) are given. All values are corrected for age, gender, BMI, cholesterol, triglycerides, and smoking category.

[a] Corrected for Q192R and L55M genotypes.

[b] Corrected for C−107T and L55M genotypes.

[c] Corrected for C−107T and Q192R genotypes.

in these activities associated with other genotypes were as follows: for Q192R, 0.1% and 68.2% (controls) and 0.5% and 73.7% (cases); for L55M, 4.9% and 24.4% (controls) and 9.8% and 22.7% (cases), respectively.

Highly significant differences in serum HDL-cholesterol and apoA-I concentrations were observed as a function of the polymorphism, with carriers of the homozygous, high-expresser allele (−107CC) having significantly increased concentrations of both variables (**Table 3**). A similar analysis based on the coding region polymorphisms of PON1 (Q192R and L55M) showed no association of the genotypes with serum concentrations of apoA-I or HDL-cholesterol (Table 3).

When cases with angiographically proven coronary disease were analyzed, univariate analyses showed highly significant, positive correlations between PON1 arylesterase and paraoxonase activities (Table 2) and concentration (data not shown) and apoA-I and HDL-cholesterol ($P < 0.0001$ for all correlation coefficients). However, the slopes, corrected for covariates (Table 1), were significantly different between controls and cases for apoA-I ($P < 0.02$) and HDL-cholesterol ($P < 0.02$) (Table 2). This is illustrated in **Fig. 1**, which combines correlation plots for controls and cases. Similarly, slopes of HDL with paraoxonase activities (corrected for the position 192 polymorphism effects) were significantly different in cases (apoA-I, $P < 0.01$; HDL-cholesterol, $P < 0.02$) (Table 2). Analyses of HDL and apoA-I as a function of the promoter polymorphism C−107T revealed the −107CC homozygotes to be no longer associated with significantly higher concentrations of these parameters (Table 3). CAD cases showed no significant differences in HDL-cholesterol or apoA-I as a function of the coding region polymorphisms (Table 3).

To ascertain whether PON1 parameters were determinants of HDL, multivariate analysis was performed using the control populations, with apoA-I or HDL-cholesterol as the dependent phenotype. The results are illustrated in **Table 4** for HDL-cholesterol. Variables well known to influence serum HDL-cholesterol concentrations were significant, independent determinants, including gender, triglycerides, BMI, and smoking status. The promoter

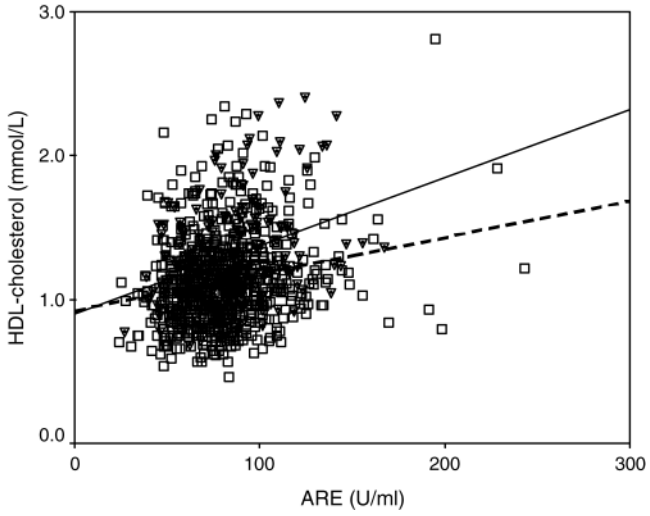

Fig. 1. Correlations between plasma HDL-cholesterol concentrations and paraoxonase-1 activity [arylesterase (ARE)]. Squares, controls; triangles, cases.

TABLE 4. Multivariate analysis of factors associated with plasma concentrations of HDL-cholesterol in control subjects

	Model 1		Model 2	
Factor	β	*P*	β	*P*
Triglycerides	− 0.372	< 0.0001	− 0.384	< 0.0001
Cholesterol	0.335	< 0.0001	0.284	< 0.0001
C−107T	0.229	< 0.001	NT	
Gender	0.210	< 0.001	0.163	< 0.01
BMI	− 0.176	< 0.01	− 0.172	< 0.01
Smoking	− 0.175	< 0.01	− 0.171	< 0.01
PON1 activity	NT		0.261	< 0.0001
Adjusted r^2	0.36		0.37	

Forward, stepwise regression analysis was performed with either PON1 promoter genotype C−107T (model 1) or PON1 activity (arylesterase, model 2) as the independent function. Gender was categorized as 1 (male) or 2 (female). Smokers were categorized as 1 (never smoked), 2 (ex-smokers), or 3 (current smokers). Age, the Q192R and L55M genotypes, and the presence of diabetes were also tested but did not enter either model. NT, not tested.

polymorphism C−107T was also a significant, independent determinant of HDL-cholesterol concentrations (Table 4). This model accounted for 36% of intraindividual variations in serum concentrations of HDL-cholesterol. When PON1 activity (arylesterase) was introduced into the model in place of the promoter polymorphism (model 2), it was also an independent determinant of HDL-cholesterol. This model accounted for 37% of the variation of HDL-cholesterol. Similar results were obtained when apoA-I replaced HDL-cholesterol in the multivariate analysis. Both the promoter polymorphism, C−107T ($P < 0.001$), and PON1 activity ($P < 0.0001$) were determinants of apoA-I concentrations.

After submission of this study for review, a publication by Rozek et al. (18) prompted us to extend our analyses to LDL. We observed highly significant, positive correlations between LDL-cholesterol and ARE ($r = 0.17$, $P < 0.0001$) and PON1 concentration ($r = 0.25$, $P < 0.0001$) for cases. Correlation coefficients were lower and nonsignificant for the control group (LDL-cholesterol vs. arylesterase, $r = 0.12$, $P = 0.08$; LDL-cholesterol vs. PON1 concentration, $r = 0.12$, $P = 0.10$). Elimination of patients on lipid-lowering therapy or diabetic patients did not modify these conclusions.

Table 5 shows LDL-cholesterol concentrations as a function of *PON1* gene polymorphisms. The C−107T promoter polymorphism in cases showed no significant difference in LDL-cholesterol concentrations between genotypes, with CC homozygotes having the highest concentrations. There were no significant differences in LDL-cholesterol concentrations between promoter genotypes in the control group, in which CC homozygotes tended to be associated with lower LDL-cholesterol concentrations. Analysis of the Q192R genotypes revealed a significantly higher level of LDL-cholesterol in the RR subgroup of cases (Table 5). A similar, albeit nonsignificant, trend was observed in the control subjects.

In multivariate analyses, PON1 activity (arylesterase and paraoxonase) and concentration were independent determinants of serum LDL-cholesterol concentration in cases (data not shown). The Q192R genotypes were also independent determinants of LDL-cholesterol concentrations, whereas the PON1 promoter polymorphism C−107T was not.

DISCUSSION

The three salient observations of the present analysis are that PON1 promoter genotype is associated with plasma HDL concentrations, that promoter genotypes (and PON1 activity/concentration) are independently associated with serum HDL-cholesterol and apoA-I concentrations, and, finally, that the relationship between PON1

TABLE 5. LDL-cholesterol as a function of PON1 polymorphisms

	LDL-Cholesterol			
Population	CC	CT	TT	ANOVA
C−107T[a]				
Control	n = 48	n = 90	n = 61	
	3.61 (0.14)	3.69 (0.10)	3.88 (0.12)	0.38
Cases	n = 164	n = 358	n = 188	
	4.03 (0.08)	3.88 (0.06)	3.80 (0.08)	0.11
	QQ	QR	RR	
Q192R[b]				
Control	n = 88	n = 89	n = 22	
	3.60 (0.11)	3.75 (0.10)	4.22 (0.20)	0.045
Cases	n = 373	n = 292	n = 45	
	3.84 (0.06)	3.89 (0.06)	4.37 (0.17)	0.018
	LL	LM	MM	
L55M[c]				
Control	n = 88	n = 89	n = 22	
	3.71 (0.12)	3.72 (0.10)	3.86 (0.24)	0.85
Cases	n = 373	n = 292	n = 45	
	3.92 (0.08)	3.88 (0.06)	3.88 (0.12)	0.96

Values are in mmol/l and (SEM). All values are corrected for triglycerides, HDL-cholesterol, gender, BMI, and diabetes.

[a] Corrected for Q192R and L55M genotypes.
[b] Corrected for C−107T and L55M genotypes.
[c] Corrected for C−107T and Q192R genotypes.

and HDL is modified in coronary cases. An intriguing interpretation of these data is that PON1 is a determinant of serum levels of apoA-I and HDL-cholesterol. This effect would be compromised in patients with coronary disease, such that the enzyme would be less able to promote higher concentrations of this lipoprotein species.

The positive correlation between serum PON1 and HDL has been known for a number of years (19). The general assumption is that PON1 is the essential beneficiary of the association, with HDL determining serum levels of the enzyme. Our recent studies have provided a mechanistic justification for this dependency by showing the importance of HDL for PON1 release from cells and maintenance of enzyme activity (1). Moreover, the role of HDL in serum PON1 transport has been elegantly demonstrated by Sorenson et al. (20). This one-sided interpretation, however, is difficult to reconcile with the observation that PON1 promoter genotype is associated with differences in serum concentrations of HDL-cholesterol and apoA-I. There is no known linkage between the *PON1* gene and other genes influencing HDL, which could explain such an association, although linkage disequilibria exist within the *PON1* gene [between the promoter polymorphism, C−107T, and the L55M coding region polymorphism, and between the latter and the Q192R polymorphic site (16)]. There are reports, however, of genetic linkage between variations in serum HDL concentrations and the chromosomal region 7q21.3, which contains the PON gene cluster (21, 22). Moreover, the promoter has a strong influence on gene expression, and the high-expresser PON1 allele was associated with increased HDL and apoA-I concentrations. The genotype-HDL relationship may thus reflect an impact of PON1 activity on HDL and is consistent with the independent associations of genotype and activity with HDL in multivariate analyses. A recent study has also reported a significant association of the promoter genotype with serum HDL in patients with familial hypercholesterolemia (6), suggesting a beneficial influence on the lipoprotein. Our studies indicate that these observations are not limited to particular conditions that may prevail in familial hypercholesterolemia but can extend to an older population without coronary disease. A number of arguments can be marshaled to explain a beneficial influence of PON1 on HDL. Lipoprotein oxidation, which PON1 can limit, directly accelerates HDL catabolism (23). In addition, oxidation of apoA-I impairs its ability to remove cholesterol from cells (5, 24), which is part of the process of HDL maturation, leading to an increase in size. An inability to increase HDL size causes more rapid catabolism of the lipoprotein (25). Of relevance in this context is a recent study suggesting that PON1 can increase the capacity of HDL to efflux cholesterol from macrophages (26).

The association of the promoter genotype with apoA-I and HDL is notable for the higher concentrations of both in PON1 −107CC homozygotes. One explanation could be that it reflects the need for higher levels of activity for an impact of PON1 on serum HDL concentrations, presumably to counter the consequences of HDL oxidation. In this context, oxidative stress/lipoprotein oxidation is known to be more prevalent in patients with cardiovascular disease.

The second intriguing observation provided by these analyses is that the relationship between PON1 and HDL is altered in coronary cases. The association is significantly weaker in cases, such that PON1 would be associated with a less efficient HDL-increasing capacity. The lower PON1 activity reported in cardiovascular patients (8–10) would be a contributory factor to any reduced impact of the enzyme on HDL. However, the altered association between PON1 and HDL would appear to be of greater relevance. This suggests that factors other than absolute PON1 activity modulate the relationship between the enzyme and HDL. This may include an inability to fully exert the available PON1 antioxidant capacity (2). Our results are in broad agreement with the principal conclusion of a recent publication by Rozek et al. (18) that the association of PON1 with lipoprotein subfractions differs between controls and coronary cases. PON1 activity correlated more strongly with HDL in controls but correlated more strongly with LDL-cholesterol in coronary cases. However, the present study differs from the former study in that Rozek et al. (18) found no correlation between PON1 genotypes and serum lipid concentrations in either controls or carotid disease cases, in contrast with our observations. Patient selection and/or patient numbers could contribute to the differences between the two studies.

An extrapolation of these observations is that one aspect of the association of PON1 with vascular disease may involve an inability to promote higher concentrations of HDL. The data are consistent with the proposal that PON1 and HDL derive mutual benefit from their association in plasma. Our results, together with recent studies (6, 26), suggest that the relationship between HDL and PON1 status merits more detailed analysis.

The authors are particularly grateful to Professor A. Morabia (Clinical Epidemiological Division, University Hospital, Geneva) for advice on statistical analyses. This study was supported by grants from the Swiss National Research Foundation (Grants 32-043088.95 and 31-105310.01).

REFERENCES

1. Deakin, S., I. Leviev, M. Gomaraschi, L. Calabresi, G. Franceschini, and R. W. James. 2002. Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high affinity, saturable, desorption mechanism. *J. Biol. Chem.* **277:** 4301–4308.
2. James, R. W., and S. P. Deakin. 2004. The importance of high-density lipoproteins for paraoxonase-1 secretion, stability, and activity. *Free Radic. Biol. Med.* **37:** 1986–1994.
3. Mackness, M. I., B. Mackness, P. N. Durrington, A. M. Fogelman, J. Berliner, A. J. Lusis, M. Navab, D. Shih, and G. C. Fonarow. 1998. Paraoxonase and coronary heart disease. *Curr. Opin. Lipidol.* **9:** 319–324.
4. Bowry, V. W., K. K. Stanley, and R. Stocker. 1992. High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. *Proc. Natl. Acad. Sci. USA.* **89:** 10316–10320.

5. Nagano, Y., H. Arai, and T. Kita. 1991. High density lipoprotein loses its effect to stimulate efflux of cholesterol from foam cells after oxidative modification. *Proc. Natl. Acad. Sci. USA.* **88:** 6457–6461.
6. van Himbergen, T. M., M. Roest, J. de Graaf, E. H. Jansen, H. Hattori, J. J. Kastelein, H. A. Voorbij, A. F. Stalenhoef, and L. J. van Tits. 2005. Indications that paraoxonase-1 contributes to plasma high density lipoprotein levels in familial hypercholesterolemia. *J. Lipid Res.* **46:** 445–451.
7. Navab, M., S. Y. Hama, G. P. Hough, C. C. Hedrick, R. Sorenson, B. N. La Du, J. A. Kobashigawa, G. C. Fonarow, J. A. Berliner, H. Laks, et al. 1998. High density associated enzymes: their role in vascular biology. *Curr. Opin. Lipidol.* **9:** 449–456.
8. Jarvik, G. P., L. S. Rozek, V. H. Brophy, T. S. Hatsukami, R. J. Richter, G. D. Schellenberg, and C. E. Furlong. 2000. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1(192) or PON1(55) genotype. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2441–2447.
9. Leviev, I., A. Righetti, and R. W. James. 2001. Paraoxonase promoter polymorphism T(−107)C and relative paraoxonase deficiency as determinants of risk of coronary artery disease. *J. Mol. Med.* **79:** 457–463.
10. Mackness, B., G. K. Davies, W. Turkie, E. Lee, D. H. Roberts, E. Hill, C. Roberts, P. N. Durrington, and M. I. Mackness. 2001. Paraoxonase status in coronary heart disease: are activity and concentration more important than genotype? *Arterioscler. Thromb. Vasc. Biol.* **21:** 1451–1457.
11. Mackness, B., P. Durrington, P. McElduff, J. Yarnell, N. Azam, M. Watt, and M. Mackness. 2003. Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. *Circulation.* **107:** 2775–2779.
12. James, R. W., I. Leviev, and A. Righetti. 2000. Smoking is associated with reduced serum paraoxonase activity and concentration in coronary artery disease patients. *Circulation.* **101:** 2252–2257.
13. James, R. W., and D. Pometta. 1990. Differences in lipoprotein subfraction composition and distribution between type 1 diabetic men and control subjects. *Diabetes.* **39:** 1158–1164.
14. James, R. W., and D. Pometta. 1991. The distribution profiles of very low density and low density lipoproteins in poorly-controlled male, type 2 (non-insulin-dependent) diabetic patients. *Diabetologia.* **34:** 246–252.
15. Blatter Garin, M-C., C. Abbot, S. Messmer, M. I. Mackness, P. Durrington, D. Pometta, and R. W. James. 1994. Quantification of human serum paraoxonase by enzyme-linked immunoassay: population differences in protein concentrations. *Biochem. J.* **304:** 549–554.
16. Leviev, I., and R. W. James. 2000. Promoter polymorphisms of the human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arterioscler. Thromb. Vasc. Biol.* **20:** 516–521.
17. Leviev, I., O. Poirier, V. Nicaud, A. Evans, F. Kee, D. Arveiler, C. Morrisson, F. Cambien, and R. W. James. 2002. High expressor paraoxonase PON1 gene promoter polymorphisms are associated with reduced risk of vascular disease in younger coronary patients. *Atherosclerosis.* **161:** 463–467.
18. Rozek, L. S., T. S. Hatsukami, R. J. Richter, J. Ranchalis, K. Nakayama, L. A. McKinstry, D. A. Gortner, E. Boyko, G. D. Schellenberg, C. E. Furlong, et al. 2005. The correlation of paraoxonase (PON1) activity with lipid and lipoprotein levels differs with vascular disease status. *J. Lipid Res.* **46:** 1888–1895.
19. La Du, B. N. 1992. Human serum paraoxonase/arylesterase. *In* Pharmacogenetics of Drug Metabolism. W. Kalow, editor. Pergamon Press, New York. 51–91.
20. Sorenson, R. C., C. L. Bisgaier, M. Aviram, C. Hsu, S. Billecke, and B. N. La Du. 1999. Human serum paraoxonase/arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids: apolipoprotein A-I stabilizes activity. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2214–2225.
21. Arya, R., J. Blangero, K. Williams, Y. Almasy, T. D. Dyer, R. J. Leach, P. O'Connell, M. P. Stern, and R. Duggirala. 2002. Factors of insulin resistance syndrome-related phenotypes are linked to genetic locations on chromosomes 6 and 7 in non-diabetic Mexican-Americans. *Diabetes.* **51:** 841–847.
22. Gagnon, F., G. P. Jarvik, M. D. Badzioch, A. G. Motulsky, J. D. Brunzell, and E. M. Wijsman. 2005. Genome scan for quantitative trait loci influencing HDL levels: evidence for multilocus inheritance in familial combined hyperlipidemia. *Hum. Genet.* **197:** 494–505.
23. Senault, C., F. H. Mahlberg, G. Renaud, A. Girard-Globa, and G. K. Chacko. 1990. Effect of apoprotein cross-linking on the metabolism of human HDL3 in rat. *Biochim. Biophys. Acta.* **1046:** 81–88.
24. Bergt, C., S. Pennathur, X. Fu, J. Byun, K. O'Brien, T. O. McDonald, P. Singh, G. M. Anantharamaiah, A. Chait, J. Brunzell, et al. 2004. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc. Natl. Acad. Sci. USA.* **101:** 13032–13037.
25. Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1994. Human HDL cholesterol levels are determined by apoA-I fractional catabolic rate, which correlates inversely with estimates of HDL particle size. Effects of gender, hepatic and lipoprotein lipases, triglyceride and insulin levels, and body fat distribution. *Arterioscler. Thromb.* **14:** 707–720.
26. Rosenblat, M., J. Vaya, D. Shih, and M. Aviram. 2005. Paraoxonase 1 (PON1) enhances HDL-mediated macrophage cholesterol efflux via the ABCA1 transporter in association with increased HDL binding to the cells: a possible role for lysophosphatidylcholine. *Atherosclerosis.* **179:** 69–77.