Serum Paraoxonase (PON1) Concentration in Patients Undergoing Hemodialysis

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Cardiac death from atherosclerosis is common in patients undergoing hemodialysis. Although the enzymic activity of human serum paraoxonase (PON1) has been reported to be decreased in such patients, serum PON1 concentrations have not been measured. We investigated serum PON1 concentrations in 81 patients undergoing hemodialysis and 103 age-matched healthy subjects using an enzyme immunoassay. The PON1 concentration was significantly lower in the patient group than the control group (mean $\pm$ SD: 6.78 ± 3.56 vs 18.01 ± 4.55 U/ml, respectively. $p < 0.0001$). There were no significant relationships between serum PON1 concentrations and the PON1 genetic polymorphisms, 55Leu/Met (L/M) and 192Gln/Arg (Q/R). The concentration adjusted for HDL-cholesterol or apolipoprotein A-I was also lower in the patient group. However, the specific activities (enzyme activity divided by the PON1 concentration) of paraoxonase and arylesterase were increased in the patient group compared with the control group. In the male patients, but not the female patients, PON1 concentrations were significantly lower in subjects with than without coronary heart disease (CHD) (mean $\pm$ SD: 4.48 ± 2.77 vs 7.34 ± 3.22 U/ml, respectively. $p < 0.01$). In conclusion, the serum PON1 concentration in hemodialyzed patients was significantly decreased, resulting in an attenuation of PON1 enzymic activity. This decrease may be in part involved in the development of cardiovascular disease. *J Atheroscler Thromb*, 2002; 9: 133–138.

Key words: Human serum paraoxonase, PON1, Hemodialysis

Introduction

Human serum paraoxonase (PON1) is an esterase associated with apolipoprotein (apo) A-I in high-density lipoprotein (HDL) (1, 2). PON1 inhibits the oxidation of low-density lipoprotein (LDL) *in vitro*, which therefore may contribute to the prevention of atherosclerosis (3, 4). The serum enzymic activity of PON1 decreases in patients with myocardial infarction (5), diabetes mellitus (6, 7) or other diseases. Decreases in the activity have also been reported in patients with uremia or in those undergoing he-

modialysis (8–10). This protein has two polymorphic sites; Leu-Met at position 55 of the amino acid sequence (L/M) and Gln-Arg at position 192 (Q/R) (11, 12), which are involved in the serum PON1 enzymic activity. We have shown that both the paraoxonase and arylesterase activities of PON1 were decreased independently of those genetic polymorphisms in patients undergoing hemodialysis (13).

Coronary heart disease (CHD) is the most frequent cause of death in hemodialyzed patients (14). Oxidized LDL is a major factor in the acceleration of atherosclerosis (15), and such abnormal lipoproteins may be implicated in the de-

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Abbreviations: PON1, human serum paraoxonase; CHD, coronary heart disease; ASO, arteriosclerosis obliterans; CVD, cerebral vascular disease; HDL-C, HDL-cholesterol; and ECG, electrocardiogram.

velopment of CHD in uremic patients (16). Therefore, the actions of PON1 against oxidized lipoprotein may be an important factor in the prevention of cardiovascular disease in patients undergoing hemodialysis.

The site of action of PON1 against the oxidation of LDL has not yet been established, but the cysteine at position 284 of the amino acid sequence is suspected to be an active site for anti-oxidation (17), which appears to be different from the enzyme-active site (18). Therefore, the serum PON1 concentration may be more important for the prevention of atherosclerosis than the enzymic activity. PON1 concentrations under hemodialysis have not previously been investigated. In this study, we examined serum PON1 concentrations in patients undergoing hemodialysis and in control subjects using an enzyme immunoassay, and studied the relationship with enzymic activity, or complications of cardiovascular disease.

Materials and Methods

Samples from 81 patients undergoing hemodialysis (50 males and 31 females) and 103 healthy subjects (54 males and 49 females) used in a previous study of PON1 enzymic activity (13) were employed for this study. All blood samples were obtained from patients immediately prior to hemodialysis. Serum and plasma samples were subdivided and stored at -80°C until examined. The subjects had no history of cardiovascular disease during the 6 months prior to the study. Control subjects were confirmed as being of normal status by physical and laboratory examinations including a normal resting electrocardiogram (ECG). Informed consent was obtained from each subject prior to this study.

CHD was defined as any one of the following: 1) acute myocardial infarction or confirmed non-acute myocardial infarction based on the serial reading of baseline and biennial electrocardiograms; 2) coronary artery disease requiring bypass surgery or angioplasty; or 3) angina confirmed by angiography or by ischemic changes during non-invasive testing. Cerebral vascular disease (CVD) was defined as neurological abnormalities with definite evidence as revealed by computed tomography or magnetic resonance imaging. Arteriosclerosis obliterans (ASO) was defined as evidence of claudication with objective findings including arteriography. ECG abnormalities as myocardial ischemic change were diagnosed according to criteria described previously (19). Briefly, signs of an ischemic ECG were defined as the presence of either a borderline wave (Minnesota codes I_3), significant or borderline ST segment depression (codes IV_{1-3}), or evidence of a complete left bundle branch block (code VII_1).

Serum PON1 enzymic activity and polymorphisms of the PON1 gene

Previous findings regarding the paraoxonase and arylesterase activities of PON1 for paraoxon and phenylacetate as substrates were utilized for this study (13). Briefly, serum was preincubated with $5\text{ }\mu\text{M}$ eserine for 10 minutes at room temperature to inhibit serum butyrylcholinesterase activity. Paraoxonase activity was measured by adding serum to 1 ml of a Tris/HCl buffer (100 mM, pH 8.0) containing 1 mM CaCl_2 and 1 mM paraoxon (Sigma). The rate of generation of *p*-nitrophenol was determined at 412 nM, 25°C using a continuously recording spectrophotometer. Arylesterase activity was measured by adding serum to 1 ml of Tris/HCl buffer (10 mM, pH 8.0) containing 1 mM CaCl_2 and 1 mM phenylacetate (Sigma). The rate of hydrolysis of phenylacetate was determined spectrophotometrically at 270 nm, 25°C . The 192Q/R (glutamine/arginine) and 55M/L (methionine/leucine) polymorphisms of the PON1 gene were also utilized from the previous study (13). Genomic DNA was extracted from whole blood using a commercial kit (SMI test; Sumitomo, Tokyo, Japan). The two polymorphisms of PON1 were analyzed using the polymerase chain reaction (PCR) and digestion of the amplified fragments with restriction enzymes. Primers for amplification of the polymorphic regions were 5'-GAAGAGTGATGTATAGCCCCAG3' and 5'-TTTAATCCAGAGCTAATGAAAGCC3' for 55L/M, and 5'-TATTGTTGCTGTGGGACCTGAG3' and 5'-CACGCTAAACCCAAATAC-ATCTC3' for 192Q/R. DNA was denatured and then amplified for 35 cycles at 94°C for 30 s, 61°C for 30 s and 72°C for 30 s. The PCR product was digested with *Hsp92II* for L/M and *A1* for Q/R. The digested products were separated on a 4% agarose gel and identified by ethidium bromide staining.

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Enzyme immunoassay (EIA) of serum PON1 concentrations

Serum PON1 concentrations were measured using an antigen-competitive EIA as described previously (20). Briefly, polyclonal antiserum was produced in a rabbit by immunization with a recombinant PON1 protein (PON284; including amino acids (a.a) 108-355 of PON1). Microplates were coated with coating buffer (10 mM Tris-HCl, pH 8.5, containing 100 mM NaCl) containing another recombinant PON1 protein (PON116; including a.a 223-338 of PON1) as a competitive antigen ($0.04\text{ }\mu\text{g/ml}$), overnight at 4°C . The plates were washed with buffer A (20 mM Tris-HCl, pH 7.4, containing 137 mM NaCl, 3 mM KCl and 0.05% Tween 20), and were blocked by a coating buffer containing 1% bovine serum albumin (BSA) for 1 h. at 37°C . After a wash with buffer A, test sera were diluted 15-fold with buffer A containing 0.1% BSA and urea (final concentration, 4 M), and 100 μl of diluted sera was mixed with 100 μl of rabbit anti-PON1 antibody diluted 250-fold. For the standard curve, 1 mg/ml of PON116 solution was diluted 10 - 8,000-fold with buffer A containing 0.1% BSA and urea (final concentration, 4 M), and mixed with the antibody. The mixed aliquots, in triplicate, were added to each well,

and the plates were incubated for 105 min. at room temperature. After a wash, peroxidase-conjugated goat anti-rabbit IgG diluted with buffer A containing 0.1% BSA was added and incubated for 90 min. at room temperature, and a substrate solution of *o*-phenylenediamine containing 0.003% H₂O₂ was added after an additional washing. Color development proceeded for 90 min. at room temperature, and the optical density (OD) at 490 nm was measured using a microplate reader. The concentrations were expressed as arbitrary units. One unit was 1 μ g of PON116.

Statistical analysis

All values were expressed as means $\pm$ SD. Statistical differences between the two groups were determined using an unpaired Student *t*-test. The stepwise regression analysis for the serum PON1 concentration was conducted using various independent variables: age (years), male or female (coded as 1 or 2, respectively), 55L/M polymorphism (M-allele number), 192Q/R polymorphism (R-allele number), and serum lipid and apolipoprotein levels (mg/dl) using a software program for use with a personal computer (JMP, ver 3.1, SAS Institute Inc. Cary, NC).

Results

Clinical characteristics of the study subjects

Clinical characteristics of hemodialyzed patients and normal subjects are shown in Table 1. There are no differences in the mean age and sex between the 2 groups, while the mean BMI, and concentrations of total cholesterol, HDL-cholesterol, apo A-I and apo A-II were significantly lower in the patient group than in the control group.

Serum PON1 concentrations

The mean serum PON1 concentration was much lower in the patient group than the control group (62% decrease). The levels of PON1 adjusted to HDL-cholesterol (HDL-C) or apo A-I (PON1 divided by HDL-C or apo A-I) were also significantly lower in patients than in control subjects (42% and 46% decrease, respectively) (Table 2). There was no difference in serum PON1 concentration between the hemodialyzed patients with and without diabetes mellitus (*n* = 18 vs 63).

Specific enzymic activity of PON1

Both paraoxonase and arylesterase activities were significantly lower in the patient group than the control group. In contrast, the specific paraoxonase activity (enzymic activity divided by the PON1 concentration) was higher in the patient group. The specific arylesterase activity was also higher in the patient group (Table 2).

Multiple regression analysis of the serum PON1 concentration

One model of multiple regression analysis is shown in Table 3. In the normal subjects, age, apo A-I, apo B, and apo A-II among independent factors affected the serum PON1 concentration. However, in the patients, only age affected the concentration, while apo A-I and apo A-II did not. The 55L/M and 192Q/R polymorphisms of the PON1 gene were not significant contributors for serum PON1 concentration either in the control group or in the patient group.

Table 1. Characteristics of patients with hemodialysis and normal subjects.

	Patients (<i>n</i> = 81)	Controls (<i>n</i> = 103)	<i>P</i> ^a
Gender (M/F)	50/31	54/49	n.s.
Age, Male (y)	64 $\pm$ 11	63 $\pm$ 11	n.s.
Age, Female (y)	64 $\pm$ 7	61 $\pm$ 5	n.s.
BMI (kg/m ²)	21.2 $\pm$ 3.0	22.5 $\pm$ 2.	< 0.005
Duration of HD (y)	4.0 $\pm$ 4.7	—	—
Total cholesterol (mg/dl)	149 $\pm$ 38	206 $\pm$ 31	< 0.001
Triglyceride (mg/dl)	106 $\pm$ 60	115 $\pm$ 67	n.s.
HDL-cholesterol (mg/dl)	40 $\pm$ 13	58 $\pm$ 13	< 0.001
Apo A-I (mg/dl)	97 $\pm$ 24	137 $\pm$ 23	< 0.001
Apo A-II (mg/dl)	22 $\pm$ 6	31 $\pm$ 5	< 0.001
Serum creatinine (mg/dl)	10.1 $\pm$ 2.8	—	—
Blood urea nitrogen (mg/dl)	76.1 $\pm$ 16.9	—	—
Uric acids (mg/dl)	8.3 $\pm$ 1.5	—	—
Diabetes mellitus (<i>n</i>)	18	—	—

^aGender was tested by the chi-square test and the others by an unpaired Student *t* test. n.s.: not significant.

Table 2. Serum PON1 concentrations and enzymic activities.

	Patients (n = 81)	Controls (n = 103)	P
PON1 concentration (U/ml)	6.78 ± 3.56	18.01 ± 4.55	< 0.001
PON1 concentration/ HDL-C	0.19 ± 0.11	0.32 ± 0.10	< 0.001
PON1 concentration/ Apo A-I	0.07 ± 0.04	0.13 ± 0.04	< 0.001
Paraoxonase activity ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{l}^{-1}$)	94.7 ± 43.3	153.4 ± 54.7	< 0.001
Paraoxonase activity/ HDL-C	2.61 ± 1.42	2.70 ± 1.04	n.s.
Paraoxonase activity/ Apo A-I	1.03 ± 0.52	1.13 ± 0.39	n.s.
Paraoxonase-specific activity	17.33 ± 12.65	9.18 ± 4.39	< 0.001
Arylesterase activity ($\text{mmol}\cdot\text{min}^{-1}\cdot\text{l}^{-1}$)	71.8 ± 20.4	91.4 ± 22.0	< 0.001
Arylesterase activity/ HDL-C	2.00 ± 0.79	1.63 ± 0.50	< 0.001
Arylesterase activity/ Apo A-I	0.79 ± 0.27	0.68 ± 0.17	< 0.002
Arylesterase-specific activity	14.40 ± 15.92	5.45 ± 2.14	< 0.001

Paraoxonase-specific activity: paraoxonase activity ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{l}^{-1}$) divided by PON1 concentration (U/ml). Arylesterase-specific activity: arylesterase activity ($\text{mmol}\cdot\text{min}^{-1}\cdot\text{l}^{-1}$) divided by PON1 concentration (U/ml).
n.s.: not significant. HDL-C: HDL-cholesterol.

Table 3. Results of multiple regression analysis for serum PON1 concentration.

Normal controls				Adjusted R ² = 0.207
Parameter	Estimate	F ratio	P	
Age	-0.38	24.2	< 0.001	
Apo AI	+0.087	12.2	< 0.001	
Apo B	+0.054	8.2	< 0.01	
Apo AII	-0.377	7.6	< 0.01	
Gender	-1.21	2.0	0.16	
PON192		0.628	0.43	
PON55		0.00	0.93	

Patients				Adjusted R ² = 0.121
Parameter	Estimate	F ratio	P	
Age	-0.089	6.5	0.013	
Apo B	+0.024	3.0	0.087	
Apo AI	+0.021	1.6	0.21	
Apo AII		1.1	0.30	
PON192		0.7	0.41	
PON55		0.0	0.87	
Gender		0.0	0.98	

Gender (male and female) is coded as 1 and 2, respectively. PON 55L/M and 192Q/R genotypes are coded according to the numbers of M alleles and R alleles, respectively. The unit of apo A-I, A-II or B concentration is mg/dl.

Table 4. Comparison of serum PON concentrations between patients with and without complications of cardiovascular disease.

	Male		P
	Without n	With n	
CHD	37 7.34 ± 3.22	13 4.48 ± 2.77	< 0.01
CVA	38 6.51 ± 3.33	12 6.88 ± 3.45	n.s.
ASO	45 6.69 ± 3.27	5 5.77 ± 4.13	n.s.
ECG	24 7.72 ± 2.76	26 5.56 ± 3.52	0.02
	Female		P
	Without n	With n	
CHD	24 7.01 ± 4.24	7 7.29 ± 2.91	n.s.
CVA	22 7.55 ± 4.15	9 5.90 ± 3.28	n.s.
ASO	31 7.07 ± 3.94	0 -	n.s.
ECG	16 6.70 ± 3.51	15 7.47 ± 4.44	n.s.

CHD, coronary heart disease; CVA, cerebrovascular accident; ASO, arteriosclerosis obliterans; ECG, ischemic change (s) or old myocardial infarction in rest electrocardiogram.

PON1 concentrations of patients with and without cardiovascular diseases

We studied the relation between the PON1 concentration and cardiovascular complications (Table 4). Among the male patients, the PON1 concentration was significantly lower in the cases with than without CHD. The difference remained significant even when the PON1 concentration was

adjusted to the HDL-C or apo A-I concentration (PON1/ HDL-C: 0.12 ± 0.08 vs. 0.20 ± 0.11 , $p < 0.02$, PON1/Apo A-I: 0.05 ± 0.03 vs. 0.08 ± 0.04 , $p = 0.02$). A significant difference was also detected in the PON1 concentration between the cases with and without ischemic change or

myocardial infarction in rest ECG, while no differences were seen in the concentrations between the cases with and without CVA or ASO. Among the female patients, no differences were seen in the PON1 concentrations between the cases with and without those complications. There were no significant differences in the distributions of the 55L/M and 192Q/R polymorphisms between the patients with CHD and those without.

Discussion

Schiavon *et al.* first reported that serum paraoxonase activity was significantly reduced in hemodialyzed patients, and suggested that the altered HDL subfraction may be a principal cause of the reduction. They also showed a higher frequency of PON1 alloenzyme B in this population, which may be caused by the 192R allele of the PON1 gene, being another possibility (8). We previously reported that not only paraoxonase activity but also arylesterase activity was decreased in hemodialyzed patients independently of the 55L/M and 192Q/R polymorphisms of the PON1 gene (13). However, the cause of the decreases in these enzymic activities remains unclear.

In the present study, we showed that the serum PON1 concentration in hemodialyzed patients was markedly decreased, which could contribute to the reduction in PON1 activity. HDL-C and apo A-I concentrations were also low compared with the control subjects. Since PON1 is associated with apo A-I in HDL particles (2), the decrease in serum PON1 may be partly accompanied by a reduction in HDL particles or apo A-I. However, the PON1 concentration adjusted for HDL-C or apo A-I was still significantly lower in the patient group, suggesting that the reduction in the HDL components is not the only reason for the decreased PON1 concentration in the hemodialyzed patients. Multiple regression analysis showed that the apo A-I concentration was a significant contributor to the serum PON1 concentration in normal subjects, but not so in the hemodialyzed patients, which could indicate an altered association between PON1 and apo A-I in those patients. Although we have no direct evidence to support this, the catabolism of PON1 may be accelerated by alterations in the construction and composition of HDL particles or by other metabolic changes in the uremic milieu.

Recently, Kujiraoka *et al.* reported that subjects with CHD had significantly lower serum PON1 concentrations than healthy controls in a Japanese population (21). This appears to concur with the present observation that serum PON1 concentrations were significantly decreased in male hemodialyzed patients with CHD compared to those without CHD. Although the subject number in this study was too small to arrive at definitive conclusions, these findings suggest that the decrease in serum PON1 concentrations may be involved in the development of atherosclerosis in male patients undergoing hemodialysis. It was unclear

why the PON1 concentration in female patients with CHD was not decreased. Studies involving larger population numbers should be performed in order to clarify the relationship between the serum PON1 concentration and cardiovascular diseases.

In conclusion, the serum PON1 concentration in hemodialyzed patients was significantly decreased, resulting in an attenuation of PON1 activity. This decrease may be in part involved in the development of cardiovascular disease in such patients.

References

- (1) La Du BN: Human serum paraoxonase/arylesterase. In: Pharmacogenetics of Drug Metabolism. ed by Kalow W, pp 51-91, Pergamon, New York, 1992
- (2) Blatter MC, James RW, Messmer S, Barja F, and Pometta D: Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. *Eur J Biochem*, 211: 871-879, 1993
- (3) Mackness MI, Arrol S, and Durrington PN: Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Lett*, 286: 152-154, 1991
- (4) Mackness MI, Arrol S, Abbott C, and Durrington PN: Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*, 104: 129-135, 1993
- (5) McElveen J, Mackness MI, Colley CM, Peard T, Warner S, and Walker CH: Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. *Clin Chem*, 32: 671-673, 1986
- (6) Abbott CA, Mackness MI, Kumar S, Boulton AJ, and Durrington PN: Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arterioscl Thromb Vasc Biol*, 15: 1812-1818, 1995
- (7) Ikeda Y, Suehiro T, Inoue M, Nakauchi Y, Morita T, Arai K, Ito H, Kumon Y, and Hashimoto K: Serum paraoxonase activity and its relationship to diabetic complications in patients with non-insulin-dependent diabetes mellitus. *Metabolism*, 47: 598-602, 1998
- (8) Schiavon R, De Fanti E, Giavarina D, Biasioli S, Cavalcanti G, and Guidi G: Serum paraoxonase activity is decreased in uremic patients. *Clin Chim Acta*, 247: 71-80, 1996
- (9) Hasselwander O, McMaster D, Fogarty DG, Maxwell AP, Nicholls DP, and Young IS: Serum paraoxonase and platelet-activating factor acetylhydrolase in chronic renal failure. *Clin Chem*, 44: 179-181, 1998
- (10) Dantoine TF, Debord J, Charmes JP, Merle L, Marquet P, Lachatre G, and Leroux-Robert C: Decrease of

- serum paraoxonase activity in chronic renal failure. *J Am Soc Nephrol*, 9: 2082-2088, 1998
- (11) Adkins S, Gan KN, Mody M, and La Du BN: Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *Am J Hum Genet*, 52: 598-608, 1993
- (12) Humbert R, Adler DA, Disteché CM, Hassett C, Omiecinski CJ, and Furlong CE: The molecular basis of the human serum paraoxonase activity polymorphism. *Nat Genet*, 3: 73-76, 1993
- (13) Itahara T, Suehiro T, Ikeda Y, Inoue M, Nakamura T, Kumon Y, Kawada M, and Hashimoto K: Serum paraoxonase and arylesterase activities in hemodialysis patients. *J Atheroscler Thromb*, 7: 152-158, 2000
- (14) Lowrie EG, Lazarus JM, Mocelin AJ, Bailey GL, Hampers CL, Wilson RE, and Merrill JP: Survival of patients undergoing chronic hemodialysis and renal transplantation. *N Engl J Med*, 288: 863-867, 1973
- (15) Witztum JL: The oxidation hypothesis of atherosclerosis. *Lancet*, 344: 793-795, 1994
- (16) Maggi E, Bellazzi R, Falaschi F, Frattoni A, Perani G, Finardi G, Gazo A, Nai M, Romanini D, and Bellomo G: Enhanced LDL oxidation in uremic patients: an additional mechanism for accelerated atherosclerosis? *Kidney Int*, 45: 876-883, 1994
- (17) Aviram M, Billecke S, Sorenson R, Bisgaier C, Newton R, Rosenblat M, Eroglu J, Hsu C, Dunlop C, and La Du B: Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. *Arterioscl Thromb Vasc Biol*, 18: 1617-1624, 1998
- (18) Cao H, Girard-Globa A, Berthezene F, and Moulin P: Paraoxonase protection of LDL against peroxidation is independent of its esterase activity towards paraoxon and is unaffected by the Q → R genetic polymorphism. *J Lipid Res*, 40: 133-139, 1999
- (19) De Bacquer D, De Backer G, Kornitzer M, and Blackburn H: Prognostic value of ECG findings for total, cardiovascular disease, and coronary heart disease death in men and women. *Heart*, 80: 570-577, 1998
- (20) Inoue M, Suehiro T, Nakamura T, Ikeda Y, Kumon Y, and Hashimoto K: Serum arylesterase/diazoxonase activity and genetic polymorphisms in patients with type 2 diabetes. *Metabolism*, 49: 1400-1405, 2000
- (21) Kujiraoka T, Oka T, Ishihara M, Egashira T, Fujioka T, Saito E, Saito S, Miller NE, and Hattori H: A sandwich enzyme-linked immunosorbent assay for human serum paraoxonase concentration. *J Lipid Res*, 41: 1358-1363, 2000