Effect of Insulin Resistance on Serum Paraoxonase Activity in a Nondiabetic Population

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Paraoxonase is a high-density lipoprotein (HDL)-bound esterase that hydrolyzes various organophosphorus compounds and protects low-density lipoprotein (LDL) against accumulation of lipid peroxides. Paraoxonase activity is strongly affected by the polymorphism of the paraoxonase gene (*PON1*) at position 192. In addition, the enzyme activity shows a great variation within each genotype, although the underlying mechanism is unknown. Because paraoxonase activity is decreased in subjects with type 2 diabetes mellitus who have insulin resistance, we investigated the association between paraoxonase activity and insulin resistance in a nondiabetic population. The subjects were 237 healthy Japanese adults with fasting plasma glucose less than 7.0 mmol/L. Paraoxonase activity was measured using paraoxon as a routine substrate. Insulin resistance was assessed by homeostasis model assessment index (HOMA index). Paraoxonase activity was affected by HDL level. To reduce the effect of HDL on paraoxonase, paraoxonase activity/HDL ratio was used. When the subjects were divided into tertiles by HOMA index, the subjects with higher HOMA values had higher paraoxonase/HDL ratios, although the 3 groups were comparable in age, gender and the *PON1* genotype distribution. Paraoxonase/HDL ratio showed significant positive correlations not only with HOMA index, but also with body mass index, waist-to-hip ratio (WHR), whereas it correlated inversely with age at borderline significance. Multiple regression analysis indicated that the association between HOMA index and paraoxonase/HDL ratio was significant and independent of *PON1* genotype, age, and adipocity. The positive association between HOMA index and HDL-corrected enzyme activity was again significant when the enzyme activity was measured with diazoxon as an alternative substrate. These results suggest that insulin resistance or hyperinsulinemia is a factor contributing to the intragenotype variability of paraoxonase activity in a population without overt hyperglycemia.

PARAOXONASE IS AN esterase residing on high-density lipoprotein (HDL) that consists of 355 amino acids.[1] This enzyme has long been known to hydrolyze neurotoxic organophosphorus compounds such as paraoxon (metabolite of insecticide parathion), soman, and sarin,[2] although its endogenous substrates have not been established. Recent studies suggest that paraoxonase plays an important role in detoxification of modified phospholipids that accumulate in oxidized low-density lipoprotein (LDL)[3-5] and, therefore, in the protection against atherosclerosis.[6] Advanced atherosclerosis is induced in mice lacking the *PON1* gene that encodes paraoxonase.[6]

Human *PON1* gene has polymorphic sites at positions 55 (Leu-Met) and 192 (Gln-Arg)[7] of the amino acid sequence. A promoter polymorphism has also recently been reported.[8] Serum activity of paraoxonase measured with paraoxon as substrate varies depending on the 192 polymorphism, being the highest in Arg-Arg homozygotes (RR), the lowest in Gln-Gln homozygotes (QQ), and intermediate in Glu-Arg heterozygotes (QR).[7,9] The 55 polymorphism[7] and the promoter polymorphism also affect the enzyme activity to a lesser extent.[10] The 192 genotype is proposed to be a genetic predictor for coronary artery disease (CAD) based on the link between the R allele and an increased risk for CAD events in several,[11-16] but not all studies.[17-22] Paradoxically, in subjects with the R allele, coronary risk is elevated, whereas the enzyme activity toward paraoxon is higher. Davies et al[9] found that the relative activity among paraoxonase isoforms due to the 192 genotypes could be reversed when measured with diazoxon, sarin, and soman as alternative substrates. A recent study[23] reported that the protective effect of paraoxonase against LDL peroxidation was independent of its esterase activity toward paraoxon.

Paraoxonase activity is decreased in some disease conditions including diabetes[24-27] and renal failure.[28,29] Serum paraoxonase activity correlates with HDL,[25] and the enzyme activity remained lower even when it was corrected for by HDL level.[28] Interestingly, these conditions are associated with insulin resistance[30,31] and advanced atherosclerosis.[32-34] No study is currently available in the literature that examines the relationship between insulin resistance and paraoxonase. Therefore, we examined in a nondiabetic population whether insulin resistance may affect paraoxonase activity.

SUBJECTS AND METHODS

Subjects

The subjects were 237 (159 females and 78 males) Japanese adults who participated in a health check program in Osaka City. Those whose fasting plasma glucose was greater than 7 mmol/L (126 mg/dL) were excluded as being diabetic according to the criteria of American Diabetes Association.[35] Also, the subjects were not regular users of multivitamin supplements (including vitamin E or C) that could affect lipoprotein oxidation and paraoxonase. No one received medications for hypertension, hyperlipidemia, or diabetes mellitus. The subjects gave informed consent to participate in the study. Table 1 gives characteristics of the subjects.

Homeostasis Model Assessment Index

The estimate of insulin resistance by the homeostasis model assessment (HOMA) was calculated by the following formula[36]: HOMA

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index = fasting insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5. Insulin concentration was measured by radioimmunoassay (Insulin RIABEAD II; Dinabot Co, Ltd, Tokyo, Japan) and plasma glucose by a glucose oxidase method. HOMA index was shown to correlate well with an insulin sensitivity index by the euglycemic clamp method by Matthews et al[36] and by us.[37]

Assay of Paraoxonase Activity

We measured serum paraoxonase enzyme activity with paraoxon as a routine substrate or diazoxon as an alternative substrate. Paraoxonase activity toward paraoxon was measured by adding serum to glycine buffer (0.05 mol/L, pH 10.5) containing 1.0 mmol/L $CaCl_2$ and 1.0 mmol/L paraoxon as described by Eckerson et al.[38] The final assay volume was 1 mL. The rate of hydrolysis of paraoxon was continuously monitored by measuring the liberation of p-nitrophenol at 412 nm at 25°C. The molecular extinction coefficient of p-nitrophenol was 16,900. Initial rates were linear for at least 5 minutes. One unit of the enzyme activity was defined as activity that generated 1 nmol of p-nitorophenol/minute and expressed as U/mL of serum.

Paraoxonase activity toward diazoxon was measured by adding serum to Tris-HCl (0.1 mmol/L, pH 8.5) containing 2.0 mol/L $NaCl_2$, 2 mmol/L $CaCl_2$, and 0.5 mmol/mL diazoxon at 24°C as described by Davies et al.[9] The final assay volume was 1 mL. Absorbance at 270 nm was continuously measured to monitor the appearance of 2-isopropyl-4-methyl-6-hydroxy pyrimidine (IMHP). One unit of the enzyme activity was defined as activity that produced 1 nmol IMHP/minute and expressed as U/mL of serum.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by standard methods.[39] PON-1 genotypes were determined following polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described by Humbert et al.[40] For the 192 polymorphism determination, we used the sense primer 5′TATTGTTGCTGTGGGACCTGAG3′ and antisense primer 5′CACGCTAAACCCAAATACATCTC3′, which encompass the 192 polymorphic region of the human *PON1* gene. PCR was performed for 40 cycles with each cycle consisting of 30 seconds of denaturation at 94°C, 30 seconds of anealing at 60°C, and 30 seconds of extension at 72°C. PCR products were digested with Alw 1 for 3 hours at 37°C. The digested products were then subjected to 3.0% agarose gel electrophoresis for 25 minutes at 100 V and visualized with 0.1% ethidium bromide staining.

Other Assays

Hemoglobin A_{1c} (HbA_{1c}) was measured by high-performance liquid chromatography. Total cholesterol and triglycerides were measured by enzymatic methods using commercially available kits (Cholesterol E-test by cholesterol oxidase-DAOS method; Triglycerides E-test by glycerol 3 phosphate oxidase-DAOS method, Wako Pure Chemicals, Osaka, Japan). HDL-cholesterol was measured by a dextran sulfate precipitation method.[41] Other measurements were performed by routine laboratory methods.

Statistics

All data were presented as mean ± SE. Comparison among 3 groups was performed by 1-way analysis of variance (ANOVA) with Scheffe-type multiple comparison as a post hoc test. Effects of 2 factors on 1 variable were evaluated by 2-way ANOVA. Multiple regression analysis was performed to evaluate independent association of 1 dependent variable with more than 2 independent variables. Gender and genotype distributions between groups were assessed by χ^2 test. *P* values less than .05 were considered significant.

RESULTS

Effect of PON1 Genotype and HDL Level on PON1 Enzyme Activities

The distribution of the *PON1* 192 polymorphism in the total subjects was 28 (12%), 116 (49%), and 93 (39%) in the QQ, QR, and RR genotypes, respectively (Table 1). Allele fre-

Table 1. Comparison of the Subjects Among *PON1* Genotypes

	QQ	QR	RR	*P* Value
No.	28	116	93	—
Gender (M/F)	9/19	37/79	32/61	.950*
Current smoker (%)	25	30	26	.758*
Age (yr)	53.8 ± 1.6	55.3 ± 0.9	53.9 ± 0.9	.551
BMI (kg/m^2)	23.2 ± 0.4	22.4 ± 0.3	22.8 ± 0.3	.297
WHR	0.84 ± 0.01	0.84 ± 0.01	0.85 ± 0.01	.831
Glucose (mmol/L)	5.29 ± 0.09	5.26 ± 0.05	5.35 ± 0.05	.424
Insulin (pmol/L)	26.7 ± 3.0	26.6 ± 2.2	25.8 ± 1.4	.944
HOMA index	1.06 ± 0.13	1.06 ± 0.10	1.03 ± 0.06	.966
HbA_{1c} (%)	4.83 ± 0.06	4.89 ± 0.03	4.89 ± 0.04	.720
TC (mmol/L)	5.25 ± 0.18	5.34 ± 0.09	5.45 ± 0.09	.460
TG (mmol/L)	1.22 ± 0.16	1.26 ± 0.07	1.24 ± 0.08	.965
HDL-C (mmol/L)	1.61 ± 0.08	1.69 ± 0.04	1.76 ± 0.05	.256
PONp (U/mL)	166 ± 5	286 ± 6†	407 ± 8†‡	<.0001
PONp/HDL	110 ± 6	179 ± 5†	244 ± 7†‡	<.0001
PONd (U/mL)	11.33 ± 0.27	7.84 ± 0.16†	0.43 ± 0.10†‡	<.0001
PONd/HDL	7.51 ± 0.42	4.93 ± 0.14†	2.61 ± 0.08†‡	<.0001

NOTE. Mean ± SE. *P* values by ANOVA or by * χ^2 test.

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio; HOMA, insulin resistance by homeostasis model assessment; HbA_{1c}, hemoglobin A_{1c}; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; PONp, paraoxonase activity toward paraoxon; PONd, paraoxonase activity toward diazoxon.

† $P < .05$ *v* QQ genotype.

‡ $P < .05$ *v* QR genotype by Scheffe-type multiple comparison.

quency was 0.37 for the Q and 0.64 for the R allele, respectively. The distribution followed the predictions of the Hardy-Weinberg equation, and the allele frequency was in the range (0.60 to 0.74 for the R allele) that was previously reported for a Japanese population.[14,17,27] There was no difference in HOMA index or plasma lipid levels among the 3 genotypes. The enzyme activity toward paraoxon was higher in the order of the RR > QR > QQ genotypes.

Significant positive correlation was found between paraoxonase activity and HDL-cholesterol in the RR ($r = .376$, $P = .0002$), and QR ($r = .259$, $P = .005$) genotypes, whereas the correlation in the QQ genotype was not significant ($r = .086$, $P = .658$). To reduce the influence of HDL on paraoxonase activity, the enzyme activity was expressed as paraoxonase/HDL ratio.[27,28] The effect of the genotype on the enzyme activity remained significant after such correction (Table 1).

Variation of Enzyme Activities Within PON1 Genotype

Figure 1 shows the relationship between the enzyme activities toward paraoxon and diazoxon in each genotype. Plots were clearly separated by the genotype, and the 3 genotypes had different slopes for the fitting lines indicating different substrate preference. In addition to the among-genotype difference, a great variation of the enzyme activity was present within each genotype. This was also true when the activity was expressed as the ratio to HDL.

Relationship Between HOMA Index and Paraoxonase Activity

HOMA index was not different among the *PON1* genotypes (Table 1). Conversely, when the subjects were divided into 3 groups by HOMA index, the 3 groups were comparable in the PON1 genotype distribution, age, or gender (Table 2). When paraoxonase activity was not adjusted for HDL, it did not correlate significantly with HOMA index in the QQ, QR, or RR genotype (Fig 2). However, paraoxonase/HDL ratio was significantly higher in subjects with a higher HOMA value (Table 2). This positive relationship between HOMA index and the HDL-corrected paraoxonase activity toward paraoxon remained significant in 2-way ANOVA that took the effect of the genotype into account (Fig 3).

Correlation of Paraoxonase Activity With Variables Related to Insulin Resistance

In the total subjects, paraoxonase/HDL ratio showed significant positive correlations with HOMA index, fasting plasma glucose, fasting insulin, body mass index (BMI), and waist-to-hip ratio (WHR), whereas it showed a negative correlation with age by simple regression analysis (Table 3). These correlations were consistently found in the RR genotype, whereas some of the correlations were not significant in the QQ and QR genotypes.

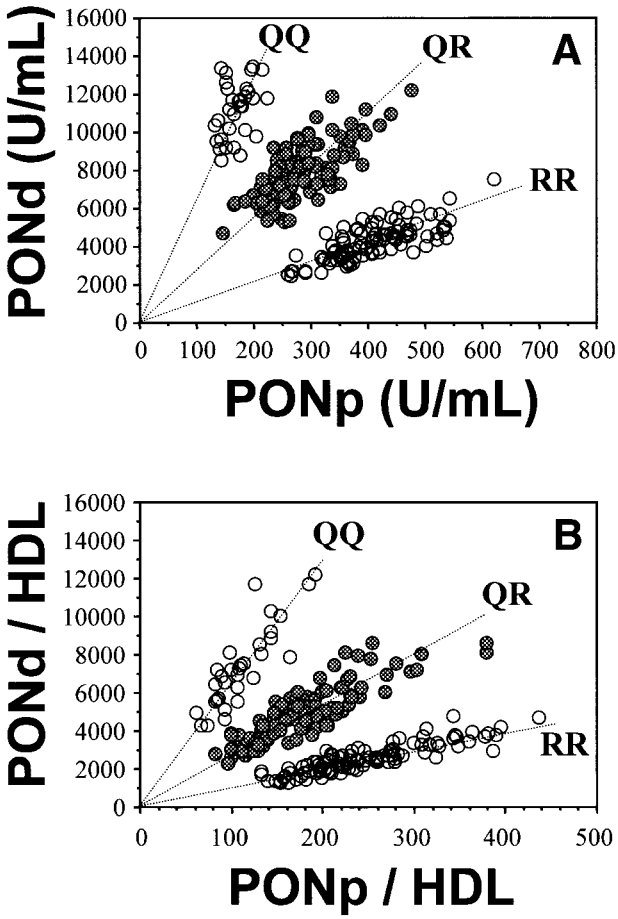

Fig 1. Variability of paraoxonase activities among the genotypes and within each genotype. Enzyme activity toward diazoxon (PONd) was plotted as a function of the enzyme activity toward paraoxon (PONp) without (A) and with adjustment for HDL (B). Note that there was a considerable variation within each genotype in addition to the among-genotype difference.

Multiple Regression Analysis of Factors Affecting Paraoxonase Activity

Because BMI, WHR, and age are known to affect insulin resistance, multiple regression analysis was performed to examine whether the observed association between insulin resistance and paraoxonase was independent of these confounding variables (Table 4). The result indicated that the positive association between HOMA and paraoxonase/HDL ratio was significant and independent of age, gender, the *PON1* genotype (R allele number), BMI, and WHR.

Enzyme Activity Toward Diazoxon and Its Relationship With Insulin Resistance

When diazoxon was used as an alternative substrate for paraoxonase assay, the effect of the *PON1* genotype on the enzyme activity was reversed, and the activity toward diazoxon was the highest in the QQ genotype and the lowest in the RR genotype (Table 1). When the enzyme activity was not adjusted for HDL, it did not correlate significantly with

Table 2. Comparison of the Three Groups Divided by HOMA Index

	Low	Middle	High	*P* Value
No.	77	81	79	—
HOMA index	0.483 ± 0.014	0.854 ± 0.015*	1.697 ± 0.065*†	<.0001
(HOMA range)	(0.128-0.666)	(0.674-1.086)	(1.098-4.078)	
Gender (M/F)	23/54	29/52	26/53	.730‡
Current smoker (%)	29	28	27	.954‡
Age (yr)	54.4 ± 0.9	53.6 ± 1.2	55.4 ± 1.1	.495
BMI (kg/m^2)	21.0 ± 0.2	22.9 ± 0.3*	24.0 ± 0.4*†	<.0001
WHR	0.83 ± 0.01	0.85 ± 0.01	0.86 ± 0.01*	.005
Glucose (mmol/L)	4.97 ± 0.06	5.39 ± 0.05*	5.52 ± 0.05*	<.0001
Insulin (pmol/L)	13.2 ± 0.4	21.6 ± 0.4*	41.7 ± 1.7*†	<.0001
HbA_{1c} (%)	4.83 ± 0.04	4.83 ± 0.04	4.99 ± 0.04*†	.008
TC (mmol/L)	5.30 ± 0.10	5.35 ± 0.10	5.50 ± 0.09	.313
TG (mmol/L)	0.99 ± 0.07	1.14 ± 0.08	1.61 ± 0.11*†	<.0001
HDL-C (mmol/L)	1.86 ± 0.05	1.78 ± 0.05	1.50 ± 0.05*†	<.0001
PONp (U/mL)	307 ± 12	338 ± 11	313 ± 11	.124
PONp/HDL	171 ± 7	197 ± 7	222 ± 10*	<.0001
PONd (U/mL)	6.92 ± 0.30	7.00 ± 0.29	6.77 ± 0.33	.866
PONd/HDL	4.08 ± 0.25	4.15 ± 0.21	4.74 ± 0.25	.105
PON1 genotypes (QQ/QR/RR)	10/41/26	7/41/33	11/34/34	.574‡

NOTE. Mean ± SE. *P* value by ANOVA or ‡ by χ^2 test.

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio; HOMA, insulin resistance by homeostasis model assessment; HbA_{1c}, hemoglobin A_{1c}; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; PONp, paraoxonase activity toward paraoxon; PONd, paraoxonase activity toward diazoxon.

* $P < .05$ *v* the low HOMA group.

† $P < .05$ *v* the middle HOMA group by Scheffe-type multiple comparison.

HOMA index in the QQ, QR, or RR genotype (Fig 2). However, the positive relationship between HOMA index and the HDL-corrected enzyme activity was again significant regardless of substrate; 2-way ANOVA indicated that both HOMA index and the genotype had significant effects on HDL-corrected paraoxonase activity toward diazoxon (Fig 3). Also, HOMA index again showed a positive association with the HDL-corrected enzyme activity toward dia-

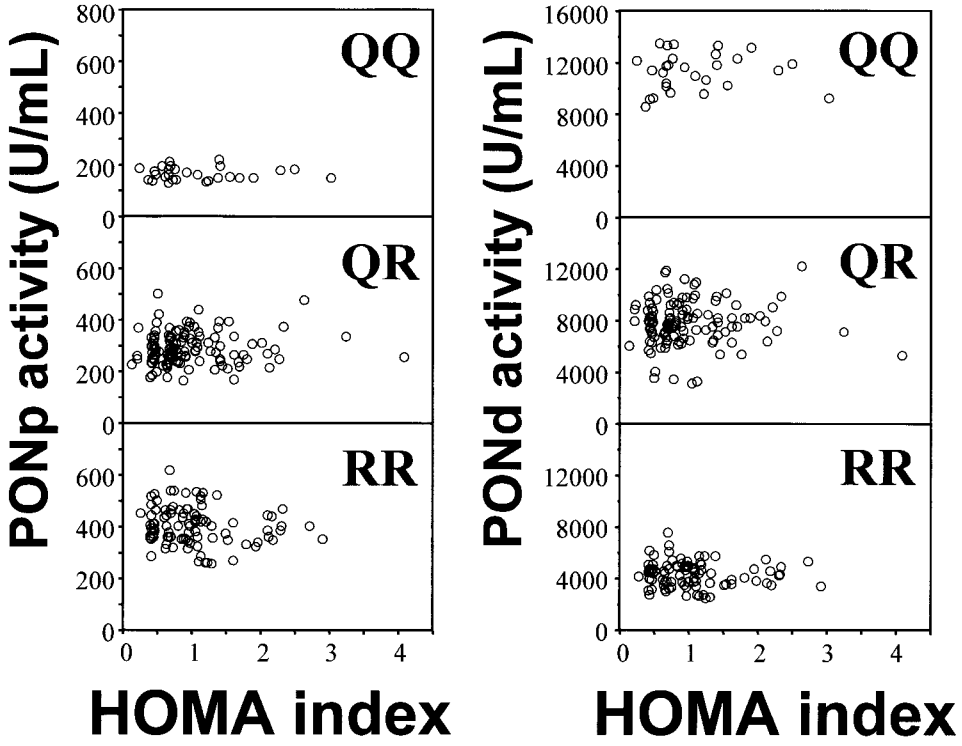

Fig 2. Relationship between HOMA index and serum paraoxonase activity in each *PON1* genotype. The subjects were divided into the QQ, QR, and RR genotypes and the correlation was then examined between HOMA index and serum paraoxonase activity toward paraoxon (PONp) or diazoxon (PONd). The enzyme activity was not adjusted for HDL levels. These correlations were all insignificant (correlation coefficients, −0.170 to 0.052; *P* values, .103 to .837).

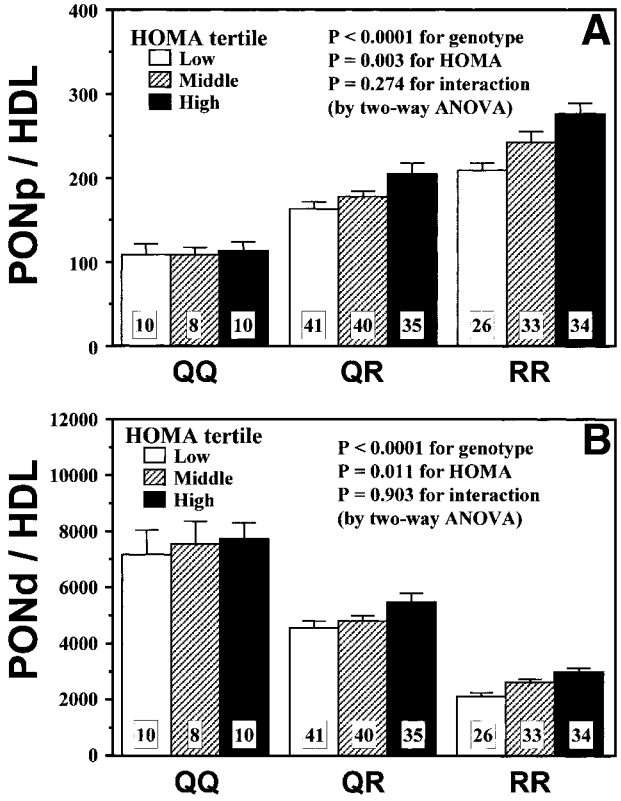

Fig 3. Effects of the genotype and insulin resistance on HDL-corrected paraoxonase activities. The subjects were divided into 9 groups by the *PON1* genotype and by tertile of HOMA index, and HDL-corrected enzyme activity toward paraoxon (PONd/HDL) was compared among the groups (A). The positive effect of HOMA index on the enzyme was significant when the effect of the genotype was taken into account by 2-way ANOVA. The effect of HOMA index on HDL-corrected enzyme activity toward diazoxon (PONd/HDL) was also positive and significant by 2-way ANOVA (B). The number in each column indicates the number of subjects; mean ± SE.

zoxon (β = .085, P = .05) that was independent of the *PON1* genotype, age, gender, BMI, and WHR (R^2 = .623, $P < .0001$) in multiple regression analysis.

DISCUSSION

The purpose of the present study was to examine the possible relationship between insulin resistance and paraoxonase activity. Because the *PON1* genotype and HDL level affect serum PON activity, we took these factors into account in this study. HDL-corrected paraoxonase activity was higher in those who had a greater HOMA index, and such association remained significant in multiple regression analysis that included the *PON1* genotype and other confounding variables as covariates. This is the first study suggesting that insulin resistance is an independent factor affecting intragenotype variability of paraoxonase activity in subjects without overt hyperglycemia.

We first expected that paraoxonase activity would correlate inversely with HOMA index, because the enzyme activity was reported to be decreased in type 2 diabetes mellitus,[25-27] which is known to show insulin resistance.[30] However, the present study has shown a positive association between HOMA index and the HDL-corrected paraoxonase activity. This is an unexpected result, and we have no clear explanation for it. However, this fact raises several possibilities. First, hyperglycemia, rather than insulin resistance, may be more important in the reduced paraoxonase activity in type 2 diabetes. This study included subjects without overt hyperglycemia. To date, however, no study is available that examines the direct effect of a high-glucose condition on the *PON1* gene expression, secretion, or serum paraoxonase activity.

Second, glycation of the enzyme protein in diabetes could result in functional changes of paraoxonase. Abbott et al[25] reported that the specific activity of paraoxonase was reduced in diabetes. Although it was not examined whether glycation was involved in the reduced specific activity of the enzyme, their study supports the possibility of posttranslational modification of the enzyme protein in diabetes.

Third, diabetic complications, rather than insulin resistance, may affect paraoxonase activity in type 2 diabetes patients. Serum paraoxonase activity is reduced in diabetic patients with neuropathy,[25] retinopathy,[27] and nephropathy.[27] Also, nondia-

Table 3. Univariate Correlation Between HDL-Corrected Paraoxonase Activity and Variables Related to Insulin Resistance

Variables	*PON1* Genotypes			
	Total	QQ	QR	RR
HOMA index	0.184*	0.133	0.146	0.446†
Glucose	0.239†	0.217	0.261*	0.296*
Insulin	0.172*	0.105	0.139	0.414†
WHR	0.290†	0.432‡	0.332†	0.351†
BMI	0.224†	0.114	0.195‡	0.399†
Age	−0.126‡	−0.190	−0.025	−0.292*

NOTE. The table gives simple correlation coefficients (r values). Paraoxonase activity was measured with paraoxon as substrate and corrected for by HDL-cholesterol.

Abbreviations: HOMA, insulin resistance by homeostasis model assessment; BMI, body mass index; WHR, waist-to-hip ratio.

* $P < .01$.

† $P < .001$.

‡ $P < .05$.

Table 4. Multiple Regression Analysis of Factors Affecting HDL-Corrected Paraoxonase Activity

Independent Variables	β Value	P Value
Age	−0.130	0.0088
Male gender	0.125	0.0366
PON1 genotype (R allele number)	0.583	<0.0001
BMI	0.122	0.0266
WHR	0.147	0.0219
HOMA index	0.118	0.0213
R^2 = .480 ($P < .0001$)		

NOTE. The table gives standard correlation coefficients (β values) by multiple regression analysis with level of significance. Paraoxonase activity toward paraoxon was measured and corrected for by HDL-cholesterol.

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio; HOMA, insulin resistance by homeostasis model assessment; R^2, coefficient of determination.

betic subjects with chronic renal failure have decreased paraoxonase activity.[28,29]

Fourth, assay methods for paraoxonase activity may have affected the results. Ortigoza-Ferado et al[42] pointed out that serum albumin has some paraoxon-hydrolyzing activity, and it may have some artifact on paraoxonase activity assay. Also, Davies et al[9] showed that the effect of the polymorphism at position 192 of *PON1 gene* on the enzyme activity was reversed when diazoxon was used as an alternative substrate; the RR genotype showed the highest enzyme activity toward paraoxon and the lowest activity toward diazoxon. To avoid the possible artifact by serum albumin and to rule out the possibility that insulin resistance may be associated positively with the enzyme activity toward paraoxon, but negatively with the enzyme activity toward diazoxon, we measured the diazoxon-hydrolyzing activity. We confirmed the observation by Davies et al,[9] but found that HOMA index was again positively associated with the enzyme activity toward diazoxon in 2-way ANOVA and in multiple regression analysis. These results indicate that the observed increase in paraoxonase activity in the insulin-resistant state was not a substrate-specific artifact and suggests that insulin resistance contributed to the within-genotype variation of the enzyme activities. Paraoxonase protein concentration may be increased in the insulin-resistant state.

Fifth, hyperinsulinemia may positively affect paraoxonase activity. In subjects with peripheral insulin resistance without hyperglycemia, circulating insulin concentration is elevated. Therefore, it is possible that hyperinsulinemia due to peripheral insulin resistance affects paraoxonase production by the liver. So far, the direct effect of insulin on hepatic *PON1* gene expression has not been reported. There is another lipoprotein-bound antioxidative enzyme, platelet-activating factor acetylhydrolase (PAF-AH) in plasma.[5] Plasma PAF-AH degrades an inflammatory phospholipid PAF into lyso PAF, and the enzyme may also play an important role in the protection against oxidative modification of LDL[43] and atherosclerosis.[5] Patients with acute myocardial infarction show reduced activity of PAF-AH,[44] as well as paraoxonase.[45] There is a study[46] reporting that plasma PAF-AH activity was increased in hyperinsulinemia. Taken together, some of the enzyme systems related to catabolism of oxidized LDL may be activated in response to hyperinsulinemia.

Finally, the observed association between HOMA index and HDL-corrected paraoxonase activity may be due to the effect of insulin resistance on HDL, but not on paraoxonase per se, because HDL-cholesterol was decreased, whereas serum paraoxonase activity was unchanged in insulin resistance. However, in other disease conditions showing insulin resistance, such as diabetes mellitus[27] and chronic renal failure,[28] paraoxonase activity was reduced regardless of adjustment for HDL. Although it is unknown which is better, paraoxonase activity on the basis of unit volume of serum or unit HDL, in expressing the antiatherogenic property of HDL, the increase in paraoxonase/HDL ratio in our study does suggest a functional change of HDL.

In conclusion, paraoxonase enzyme activity showed a positive association with HOMA index independent of the *PON1* genotype, HDL level, and other confounding variables. It is unknown whether the observed change in the enzyme activity is pro- or antiatherogenic. Further studies are needed to clarify the mechanisms and clinical implications of the altered paraoxonase status in insulin resistance.

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