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Antioxidant Paraoxonase 1 Activity in the Metabolic Syndrome

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Paraoxonase (PON1) is an antioxidant enzyme closely associated with high-density lipoproteins. Low PON1 has been shown in oxidative stress-associated processes such as dyslipidemia, diabetes mellitus, advancing age, and smoking. Indeed, oxidative stress is related to the degree of insulin resistance, a key component of the metabolic syndrome. Therefore, the possible relationship between PON1 activity and the metabolic syndrome was investigated.

From 1364 randomly recruited subjects, 285 were found to have the metabolic syndrome, according to the guidelines published by the National Cholesterol Education Program, Adult Treatment Panel III. PON1 activity, lipid peroxides, and *PON1* codon 192 genotypes, which strongly modulate PON1 activity, were determined.

Serum PON1 activity levels were found to be significantly lower, and lipid peroxide concentrations significantly higher, in subjects with the metabolic syndrome compared with unaffected subjects (*P* = 0.033 and < 0.001, respectively). Study subjects showed a significant decreasing trend in PON1 activity levels and a significant increasing trend in lipid peroxide concentrations, with the increase in the number of metabolic disturbances. No differences in the prevalence of *PON1* codon 192 genotypes were found among the categories of metabolic abnormalities.

In conclusion, a greater degree of severity of the metabolic syndrome is associated with a progressively worse antioxidant/oxidant balance, which is consistent with increased oxidative stress and lower antioxidant PON1 enzymatic capacity. (*J Clin Endocrinol Metab* 88: 5422–5426, 2003)

PARAOXONASE (PON1) IS a calcium-dependent esterase closely associated with the high-density lipoprotein (HDL) subfraction that contains apolipoprotein AI, but not apolipoprotein AII, in human serum (1). Several lines of evidence are emerging that suggest that HDL can prevent oxidation of low-density lipoprotein (LDL) and that some oxidized LDL phospholipids are physiological substrates for serum PON1 (2, 3).

It has been suggested that low PON1 activity is related to coronary heart disease (4, 5) and that this activity, usually measured using paraoxon as a substrate, is under genetic and environmental regulation and appears to vary widely among individuals and populations. PON1 enzyme activity for paraoxon as a substrate is modulated, among others at the *PON1* locus, by the *PON1* codon 192 polymorphism, which includes low paraoxon- and high paraoxon-activity alleles (Q192 and R192, respectively) (3).

The metabolic syndrome is made up of a constellation of metabolic abnormalities that represents major risk factors for increased mortality from cardiovascular diseases (6). The prevalence of the metabolic syndrome appears to be very high, and some evidence indicates that it could have increased in the last 15 yr (7). The *Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)* (ATP III report) (8) has recently provided valuable criteria for the diagnosis and management of the metabolic syndrome.

Whereas insulin resistance is thought to be the core (9), dyslipidemia, abdominal obesity, high blood pressure, and thrombotic and inflammatory states are considered as important components in the pathogenesis of the metabolic syndrome (8). Genetics, lifestyle, and environmental factors may also have a role (10, 11) in the expression of the cluster of metabolic abnormalities. There is also some evidence relating oxidative stress to the degree of insulin resistance and, hence, the metabolic syndrome. In this respect, it has been suggested that high oxidative stress promotes an impaired insulin action, which in turn may aggravate the degree of oxidation (11). In a similar way, the polymorphism Met-Leu 54 at the *PON1* gene locus, which is in strong linkage disequilibrium with the *PON1* 192 polymorphism, has recently been associated with the degree of insulin resistance in healthy subjects (12).

In view of the findings that establish a link between oxidative stress and antioxidant systems and the metabolic syndrome, we have analyzed PON1 activity and lipid peroxide levels in the setting of the metabolic syndrome in a large population-based study and investigated the possible influence of the *PON1* codon 192 polymorphism.

Subjects and Methods

Subject recruitment has previously been published in detail (13). Briefly, a cross-sectional study (the Registre Gironí del COR study), designed to establish the prevalence of main cardiovascular risk factors in the population of 25- to 74-yr-old people in the province of Girona,

Abbreviations: ATP III, Adult Treatment Panel III report; EEPA, daily energy expenditure in leisure-time physical activity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PON1, paraoxonase.

Spain, was performed in 1995–1996. A random sample of 3000 subjects from this province was recruited. Of the 3000 subjects selected, 596 were not eligible because of errors in census data or due to their being either institutionalized or temporarily absent. The eligible sample was composed of 2404 subjects of whom 1748 (72.7%) participated in the study: 839 men and 909 women. Finally, 713 men and 651 women from whom data were available were included in this study. Anthropometric data, as well as data on the daily energy expenditure in leisure-time physical activity (EEPA), tobacco smoking, and medications were obtained. Participants were requested to precisely describe their food and beverage intakes during the previous 3 d. Daily dietary fat intake was calculated from the 72-h recalls with the software Diet Analysis Nutritionist IV (N Squared Computing, San Bruno, CA). Dietary fat was adjusted for dietary energy intake by the residual method (14). Blood pressure determination was made using a periodically calibrated mercury sphygmomanometer. The operators followed a certification process in the standardized measurement technique at a central laboratory, and all determinations were always made by the same person. Measurements were performed after a 5-min rest. Two measurements were taken: the interval between the first and second was at least 20 min. The value used was the arithmetic mean of both determinations. All subjects gave their written informed consent to participate. The protocol was approved by an ethics committee.

According to the ATP III report (8), participants with three or more of the following metabolic abnormalities were considered to have the metabolic syndrome: 1) abdominal obesity: waist circumference greater than 102 cm in men and greater than 88 cm in women; 2) hypertriglyceridemia: at least 150 mg/dl (1.69 mmol/liter); 3) low HDL: less than 40 mg/dl (1.04 mmol/liter) in men and less than 50 mg/dl (1.29 mmol/liter) in women; 4) systolic blood pressure: at least 130 mm Hg, or diastolic blood pressure at least 85 mm Hg, or antihypertensive medication use; 5) high serum glucose: at least 110 mg/dl (≥6.1 mmol/liter). Participants using antidiabetic drugs (insulin or oral agents) were also included in this category.

Laboratory analyses

Blood samples were collected after an overnight fast. Serum was used for the determination of lipids and glucose. Serum cholesterol, triglycerides, and glucose concentrations were determined enzymatically (Hoffmann-La Roche Diagnostica, Basel, Switzerland). HDL cholesterol was measured as cholesterol after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic-Mg^{2+} (Roche Molecular Biochemicals, Mannheim, Germany). Analyses were performed in a Cobas Mira Plus (Hoffmann-La Roche Diagnostica). LDL cholesterol was calculated by Friedewald's formula (15).

Analysis of PON1 activity

For PON1 activity analysis, serum samples frozen at −70 C and thawed just before the beginning of each assay were used. PON1 activity toward paraoxon was measured after the reaction of paraoxon hydrolysis into *p*-nitrophenol and diethylphosphate, essentially as previously described (16). PON1 activity was determined from the rate of *p*-nitrophenol production (after subtracting the spontaneous paraoxon hydrolysis) at 37 C and recorded at 405 nm by a Cobas Mira Plus autoanalyzer (Hoffmann-La Roche Diagnostica). Forty microliters of serum were added to a basal assay mixture to reach final concentrations of 5 mM paraoxon, 1.9 mM $CaCl_2$, 90 mM Tris-HCl (at pH 8.5), and 3.6 mM NaCl. A PON1 activity of 1 U/liter was defined as 1 μmol *p*-nitrophenol formed per minute per serum liter. The molar extinction coefficient of *p*-nitrophenol is 18,053 $M^{-1} \cdot cm^{-1}$ at pH 8.5. The intra- and interassay coefficients of variation were under 1.7%.

Lipid peroxidation

Lipid peroxides were assessed as the generation of malondialdehyde equivalents and measured by the thiobarbituric acid reactive substances method. Plasma collected with EDTA was separated by centrifugation at 1000 × *g* at 4 C for 15 min, and samples were frozen at −80 C. The method involves heating the sample with thiobarbituric acid under acidic conditions and reading the absorbance of the malondialdehyde-thiobarbituric acid adduct formed at 532 nm (17). Values were expressed in micromoles per liter. Intrarun and between-run imprecisions were 4.24 and 6.87%, respectively.

PON1 codon 192 genotype determination

Genomic DNA was isolated from white cells by the salting-out method (18). PCRs and determination of *PON1* codon 192 genotypes were performed using primer sequences derived from published data (19).

Statistical analysis

Comparisons of biochemical variables between two groups were performed with Student's *t* test or the Mann-Whitney *U* test. The χ^2 test was used to analyze the association between categorical variables. The prevalence of the metabolic syndrome was age-adjusted using the sampling weights. ANOVA with a general linear model was used to determine the association between the variation in PON1 activity and lipid peroxide levels and the number of metabolic abnormalities. The model was adjusted for confounding variables, and the significance of trends of PON1 and lipid peroxide levels among categories of metabolic abnormalities was also calculated.

Results

The age-adjusted prevalence of the metabolic syndrome in the study population was 16.02%. There were no differences in the metabolic syndrome prevalence between sexes. As expected, subjects with the metabolic syndrome were overweight and had high blood pressure and glucose levels (Table 1). They were also older than the subjects without the metabolic syndrome and had less dietary fat intake, suggesting that they were adopting some measures to reduce fat intake. Unaffected individuals significantly expended more

TABLE 1. Clinical characteristics of the study participants with and without the metabolic syndrome

	Metabolic syndrome (−) (n = 1079)	Metabolic syndrome (+) (n = 285)	*P*
Sex (no. male/female)	560/519	153/132	0.319
Age (yr)	49.7 (13.3)	58.8 (11.1)	<0.001
BMI (kg/m^2)	25.7 (4.1)	29.7 (5.1)	<0.001
Waist-hip ratio	0.86 (0.09)	0.93 (0.08)	<0.001
EEPA (kcal/d)	502.7 (420.8)	412.8 (330.8)	0.001
Dietary fat (g/d)	88.1 (12.9)	83.2 (13.0)	<0.001
Smokers, n (%)	252 (23.3)	48 (16.8)	0.062
SBP (mm Hg)	127.8 (19.0)	145.5 (17.2)	<0.001
DBP (mm Hg)	75.7 (11.1)	83.7 (9.5)	<0.001
Glucose (mg/dl)	97.9 (27.9)	122.4 (38.9)	<0.001

Continuous variables are expressed as mean (SD). BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. The conversion factor to SI units for glucose is 0.055.

daily energy in leisure-time physical activity than affected subjects.

The lipid and lipoprotein profile of subjects with the metabolic syndrome was clearly atherogenic (Table 2). It is of interest that PON1 activity levels were found to be significantly lower, and lipid peroxide concentrations significantly higher, in subjects with the metabolic syndrome compared with unaffected subjects. However, the *PON1* codon 192 genotype distribution was similar in both groups.

In the next step, participants were stratified by the number of metabolic abnormalities present (from 0–5) in six categories, and PON1 activity and lipid peroxide levels were compared among groups in a general linear model adjusted for age, EEPA, and daily dietary fat intake (Table 3). Subjects showed a significant decreasing trend in PON1 activity levels, and a significant increasing trend in lipid peroxide concentrations, with the increase in the number of metabolic disturbances. Lipid peroxide concentrations were also significantly different among groups. No differences in the prevalence of *PON1* codon 192 genotypes were found among the categories of metabolic abnormalities.

Discussion

The prevalence of the age-adjusted metabolic syndrome according to the diagnostic criteria of the ATP III report (8) in our study population appears to be relatively high, taking into account that the age range was 25–74 yr. Its profound impact on mortality warrants the implementation of efforts directed to the advance in the knowledge of physiopathological mechanisms of the metabolic syndrome.

We found that lipid peroxide concentrations were significantly higher in subjects with the metabolic syndrome than in unaffected participants. Conversely, the former showed significantly lower PON1 activity levels. Remarkably, the magnitude and direction of the associations between low PON1 activity and high lipid peroxides and the increase of the number of metabolic abnormalities were consistent with a progressively worse antioxidant/oxidant balance. Therefore, a greater degree of severity of the metabolic syndrome resulted in an increased oxidative stress and lower antioxidant enzymatic capacity. Although a recent study showed a relationship between the degree of insulin resistance and the L allele of the *PON1* codon 54 polymorphism (12), which seems to be in linkage disequilibrium with the R allele, we could not demonstrate significant differences in the prevalence of the *PON1* codon 192 genotypes among the categories of metabolic abnormalities. However, PON1 activity was not determined in the study referred to above (12), and insulin resistance was not assessed in the present study. Furthermore, fat intake, which has been shown to diminish serum PON1 activity in human and animal studies (20, 21), was not a confounding factor in the relationship of the number of metabolic abnormalities with oxidative stress.

Within the cluster of metabolic abnormalities, insulin resistance constitutes the core of the metabolic syndrome. Increasing evidence in experimental and clinical studies suggests that oxidative stress and a simultaneous decline of antioxidant defense mechanisms can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance (22).

HDL-associated PON1 may be a major defense barrier against lipid peroxides from oxidized LDLs (23). In fact, the ability of HDL to attenuate the oxidation of LDL is largely attributable to PON1 (23). Besides lipid peroxides, PON1 has been found to hydrolyze hydrogen peroxides, which are a major reactive oxygen species produced by the arterial wall during atherogenesis (24). In addition to low PON1 activity in patients who suffered from myocardial infarction, a significant decrease in PON1 activity toward paraoxon hydrolysis has been shown in diseases with accelerated atherogenesis such as familial hypercholesterolemia (15, 25), and diabetes mellitus (15, 26), and also in oxidative stress-associated processes such as advancing age (27) and smoking (28).

The reduction of PON1 activity with the severity of metabolic abnormalities introduces the inactivation of PON1 as a likely consequence of oxidative stress in the metabolic syndrome, exceeding the antioxidant capacity of the enzyme. Free radicals are disproportionately formed in metabolic abnormalities, such as chronic hyperglycemia (22) and dyslipidemia (29), by oxidation, and the subsequent oxidative degradation of proteins. Free radicals oxidize DNA, proteins, and lipids and indirectly induce damage to tissues by activating a number of cellular stress-sensitive pathways (30). In addition, pancreatic β-cells exposed to hyperglycemia produce free radicals that may cause dysfunction of β-cell and suppress glucose-induced insulin secretion (31). Moreover, there is evidence that prolonged oxidative stress impairs

TABLE 2. Lipids, lipoproteins, PON1 activity, *PON1* 192 genotypes, and lipid peroxides in subjects with and without the metabolic syndrome

	Metabolic syndrome (−) (n = 1079)	Metabolic syndrome (+) (n = 285)	*P*
Cholesterol (mg/dl)	219.8 (43.1)	237.5 (46.3)	<0.001
Triglycerides (mg/dl)	93.8 (45.2)	167.3 (133.6)	<0.001
LDL cholesterol (mg/dl)	146.6 (39.5)	162.8 (39.2)	<0.001
HDL cholesterol (mg/dl)	55.1 (14.5)	42.9 (13.3)	<0.001
PON1 activity (U/liter)	232 (162–374)	212 (151–352)	0.033
Lipid peroxides (μmol/liter)	5.04 (3.32–8.14)	6.89 (4.14–14.21)	<0.001
PON1 192 genotypes			
QQ, n (%)	513 (47.5)	144 (50.5)	
QR, n (%)	455 (42.2)	116 (40.7)	0.591
RR, n (%)	111 (10.3)	25 (8.8)	

Continuous variables are expressed as mean (SD) or median (interquartile range). Conversion factors to SI units are 0.02586 for cholesterol, LDL cholesterol, and HDL cholesterol, and 0.01129 for triglycerides.

TABLE 3. PON1 activity and lipid peroxides adjusted for age, EEPA and daily dietary fat intake, and *PON1* 192 genotypes in subjects stratified by the number of metabolic abnormalities

	No. metabolic abnormalities						ANOVA *P*	*P* for trend
	0 (n = 348)	1 (n = 422)	2 (n = 309)	3 (n = 199)	4 (n = 67)	5 (n = 19)		
PON1 (U/liter)	233 (164–357)	236 (162–379)	226 (156–375)	226 (151–346)	218 (158–350)	153 (117–209)	0.400	0.043
Lipid peroxides (μmol/liter)	4.66 (3.05–7.45)	5.06 (3.28–8.18)	5.33 (3.60–4.18)	6.89 (3.81–14.18)	6.42 (4.60–11.76)	16.56 (3.81–31.32)	<0.001	<0.001
PON1 192 genotype								
QQ, n (%)	173 (49.7)	198 (46.9)	142 (46.0)	100 (50.3)	31 (46.3)	13 (68.4)		
QR, n (%)	138 (39.7)	179 (42.4)	138 (44.7)	82 (41.2)	29 (43.3)	5 (26.3)		
RR, n (%)	37 (10.6)	45 (10.7)	29 (9.4)	17 (9.4)	7 (10.4)	1 (5.3)		

Continuous variables are expressed as median (interquartile range).

insulin-induced glucose transporter-4 translocation in adipocytes, resulting in a grade of insulin resistance (32). Finally, insulin resistance or hyperinsulinemia *per se* seems to stimulate endothelial superoxide anion production via nicotinamide adenine dinucleotide phosphate hydrolase (33), and, hence, can worsen the degree of oxidative stress. Taken together, these observations strongly suggest that the activation of oxidative-stress pathways is a key component of the metabolic syndrome, which contributes to ascertaining the complex metabolic interrelationship among the different components of the syndrome.

PON1 activity has been shown to be reduced in the course of oxidative incubation with Cu^{2+}-induced peroxidation of LDL (34). Oxidized LDL appears to inactivate PON1 through interactions between the enzyme-free sulfhydryl group and oxidized lipids, which are formed during LDL oxidation (35). Therefore, low PON1 activity may reflect an increased oxidative stress present in the metabolic syndrome, which probably compromises and inactivates PON1 function. Whereas this seems a plausible explanation, at present, however, the possibility that low PON1 activity fails to effectively protect against continuous oxidative damage present in the metabolic syndrome cannot be ruled out.

In summary, our study firmly suggests for the first time that PON1 activity is adversely associated with a higher degree of the metabolic alterations characteristic of the metabolic syndrome and that this association appears to be independent of the *PON1* codon 192 polymorphism. PON1 activity is likely to be one of the antioxidant mechanisms involved in the beneficial effects of physical exercise (28), proper nutrition (36), and drug therapies (25), which are consistently recommended for the management of the metabolic syndrome (8).

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