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Regulation of Serum Paraoxonase Activity by Genetic, Nutritional, and Lifestyle Factors in the General Population

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Background: **Paraoxonase may protect lipoproteins and cell membranes from peroxidation, and alterations in the activity of this enzyme have been associated with some chronic diseases. Serum paraoxonase appears to be mainly under genetic control, but some studies suggest that environmental factors may also modulate its activity. The aim of the present study was to investigate whether diet and lifestyle affect serum paraoxonase activity.**

Methods: **We studied a population-based sample of 388 individuals (194 women and 194 men; age range, 18–75 years) and assessed their daily dietary intake using a 3-day estimated food record. The variables studied included serum paraoxonase activity, paraoxonase polymorphisms at positions 55 and 192, age, gender, smoking status, physical exercise, body mass index, energy consumption, nutrient intake (total lipids, saturated fatty acids, β-carotenes, vitamins C and E), and serum lipid concentrations.**

Results: **Multiple linear regression analysis showed that only genetic polymorphisms, serum cholesterol, HDL-cholesterol concentrations, and cigarette smoking were significant predictors of serum paraoxonase activity. HDL-cholesterol concentrations were also related to body mass index, daily energy consumption, and saturated fatty acid intake.**

Conclusions: **The between-individual variability of serum paraoxonase activity is regulated mainly by genetic determinants. Although HDL-cholesterol and tobacco smoking may contribute to the modulation of this enzyme, the other nutritional and lifestyle factors do not seem to play a significant role.**

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Paraoxonase (PON1) is a calcium-dependent esterase that circulates in plasma associated with HDL *(1)* and contributes to the protective effect of this lipoprotein on LDL oxidation *(1–6)*. Some authors have extended this suggested antioxidant role of PON1 to a general prevention of peroxidative damage to cell membranes *(7, 8)*. However, the enormous variation in serum PON1 activity (~40-fold) observed among individuals *(9)* complicates its clinical interpretation. This variation can be explained by both genetic and environmental influences.

Polymorphisms in the promoter and coding regions of the *PON1* gene are the main determinants of its expression and the enzymatic activity *(10–14)*, but serum PON1 activity can be modulated by several environmental factors. Pathologic states such as renal disease, diabetes mellitus, cardiovascular disease, and liver cirrhosis are associated with decreased PON1 activity [reviewed in Ref. *(15)*], and various dietary and lifestyle factors have been reported to influence serum PON1 activity. Tobacco smoking has been associated with reduced PON1 activity and concentrations in patients with coronary artery disease *(16)*, and extracts of cigarette smoke inhibited PON1 activity in vitro *(17)*. Pharmacologic therapy with simvastatin *(18)* and hormone replacement therapy *(19)*, on the other hand, have been reported to increase serum PON1 activity.

The effect of dietary modulation on serum PON1 activity has been studied in laboratory animals. An atherogenic diet reduced PON1 activity in mice, and this decrease correlated with a decrease in plasma HDL-cholesterol *(5)*. To our knowledge, few data are available

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from human intervention studies. Pomegranate juice consumption *(20)* or the intake of vitamin C and E supplements *(21)* may increase serum PON1 activity. Ethanol has been shown to inhibit serum PON1 activity *(22)*, but moderate alcohol ingestion seems to increase it. This effect is probably secondary to the increase in HDL concentration *(23)*.

Research on interactions between genetic and nutritional components is particularly interesting, and attempts are being made to find modulators of serum PON1 activity for therapeutic purposes *(9)*. However, because most of the conclusions reported to date have been the result of experimental investigations or intervention studies with patients, it is still unclear how nutritional or other environmental agents affect PON1 activity in the general population. The aim of the present study was to investigate how several nutritional and lifestyle habits affect serum PON1 activity in a population-based study in a Mediterranean area.

Participants and Methods

study population

We randomly selected a representative sample of 388 individuals (194 women and 194 men) from the population registers of the town councils of two Catalonian villages (mean age, 40 years; range, 19–75 years). Participants were ostensibly healthy with no evidence of renal insufficiency, severe hepatic damage, neoplasia, oligophrenia, or dementia. Forty-three participants had hypertension (11.1%), 22 had dyslipemia (5.6%), and 14 had type II diabetes mellitus (3.6%). One patient had an ischemic cardiopathy treated with a coronary by-pass, and one had had an acute episode of myocardial infarction (5 years previously). Medication intake was not an exclusion criterion except in the case of drugs interfering with vitamin metabolism (methotrexate, tuberculostatics, theophylline, or vitamin B_6 antagonists). The population studied did not consume vitamin supplements or local food fortified with vitamins. Pregnant women were not included in the study. The protocol was approved by the Ethics Committees of the Hospital Universitari de Sant Joan and the Jordi Gol Foundation. All participants signed consent forms.

methods of dietary assessment

The participants' dietary intake was assessed by use of a 3-day estimated food record *(24)*. Each participant was instructed to make a record of all food and beverages consumed (including snacks) over two nonconsecutive weekdays and one holiday. Quantities were estimated in household measures. The food record was checked with the study's dietitian, who verified and clarified the quantities and types of food. Portion sizes were confirmed by comparison with photographs various standard household measures. Each of the records was codified by the dietitian. The French and the Spanish Food Composition Tables were used to calculate daily nutrient intake *(25, 26)*.

Fifty-four percent of the participants reported that they consumed some alcoholic beverages. Of these, 100% reported consumption of red or white wine, 67% spirits, and 44% beer. Twenty-six participants (6.7%) reported to have stopped alcohol consumption for a period between 1 and 38 years (4 individuals had not consumed alcohol for 1 year, 12 had not consumed alcohol for 2–10 years, and 10 for >10 years:), and were considered as nondrinkers. Among these individuals, four reported to have drunk more than 80 g of alcohol/day.

Thirty-three percent of the participants reported current cigarette smoking. The mean (SD) cigarette consumption was 15.2 (15.9) pack-years (range, 0.3–95.0 pack-years). Eighty-three participants (21.4%) reported to have stopped smoking for a period between 1 and 40 years (3 individuals had stopped for 1 year; 34 had stopped for 2–10 years, and 46 had stopped for >10 years), and were considered as nonsmokers. Among these individuals, 22 reported to have smoked >20 cigarettes/day.

biochemical measurements

Serum PON1 activity was determined by measuring the rate of hydrolysis of paraoxon at 37 °C, based on the change in absorbance 410 nm *(27)*. The assay reagent contained 1 mmol/L paraoxon of >96% purity (Sigma), and 1 mmol/L $CaCl_2$ in 0.05 mol/L glycine buffer (pH 10.5). The assays were performed in an ILab 900® automated analyzer (Instrumentation Laboratories). The HDL-cholesterol concentration was determined by a homogeneous method *(28)*. Serum cholesterol and triglyceride concentrations were measured by standard techniques (ITC Diagnostics).

PON1 genotyping

$PON1_{55}$ and $PON1_{192}$ polymorphisms of the *PON1* gene were analyzed by PCR amplification and restriction isotyping as described previously *(29)*. PCR products of 171 bp for the $PON1_{55}$ and 99 bp for the $PON1_{192}$ polymorphisms were cleaved with the restriction enzymes *Nla*III and *Alw*I (New England BioLabs), respectively, and separated by electrophoresis in 12% polyacrylamide gels. The band patterns were developed by silver staining.

statistical analysis

We used the standard methods (Kolmogorov–Smirnov and Shapiro–Wilks) to check whether the variables were normally distributed when they were required for the application of a statistical test. To check differences between the PON1 tertiles on the variables studied, we used ANOVA or the Kruskal–Wallis method followed by the Mann–Whitney *U*-test corrected for multiple comparisons when appropriate. To verify the association between nutritional intake and PON1 activity when controlling for gender, age, tobacco and alcohol consumption, physical activity, genetics, and serum lipid concentrations, we

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fitted a multiple linear regression model that included all of these variables (ENTER method of selecting variables). The model took into account all of the factors that influence both PON1 activity and nutritional intake. To explore which of the nutritional factors significantly explained the variation in HDL-cholesterol values, we fitted a multiple linear regression model that selected only the most influential variables (STEPWISE method of selecting variables). Some control variables (gender, age, tobacco and alcohol consumption, and physical activity) had previously been forced (ENTER method). The χ^2 test was used to determine whether the genotype distributions of *PON1* polymorphisms were in Hardy–Weinberg equilibrium. Residuals were diagnosed to test whether the assumptions for the regression models were fulfilled. We rejected the null hypothesis when the P value was <0.05. Statistical analyses were performed with the SPSS 11.0 statistical package and the Epi-Info program (CDC).

Results

The mean (SD) serum PON1 activity for all participants was 410.9 (153.9) U/L (range, 148.0–997.0 U/L; median, 376.0 U/L). These results show that the intersubject variability for serum PON1 was high. Table 1 summarizes the genotype and allele frequencies of the $PON1_{55}$ and $PON1_{192}$ polymorphisms. The distributions observed were in Hardy–Weinberg equilibrium and were similar to those reported previously *(13, 27, 30–32)* in Caucasian populations. A previously described linkage dysequilibrium *(33)*, showing an association between the $PON1_{192}$ *R* and the $PON1_{55}$ *L* alleles, was also observed in our sample.

The results of the nutritional assessment, serum lipid concentrations, and genotype frequencies classified according to tertiles of serum PON1 activity are shown in Table 2. There were highly significant differences in genotype frequencies ($P < 0.001$) among the three groups. As expected, individuals with a higher PON1 activity had a higher frequency of genotypes containing the *R* and *L* alleles. There were also significant differences in serum HDL concentrations ($P = 0.001$) and percentage of smokers ($P = 0.04$) among the three groups. We found no significant differences in the other variables when they were classified according to PON1 tertiles.

The combined effects of all of the nutritional and biochemical variables on PON1 activity are shown in Table 3. $PON1_{55}$ and $PON1_{192}$ genotypes were by far the main determinants of serum PON1 activity. Serum cholesterol and HDL concentrations also showed marked direct effects on this enzyme, and cigarette smoking was associated with significantly lower serum PON1 activities ($P = 0.007$).

We also investigated whether any of the recorded habits could indirectly be related to serum PON1 concentrations through their effect on HDL-cholesterol concentration. The results of the multiple linear regression analysis are shown in Table 4. Some of the selected variables were significantly associated with HDL-cholesterol. Male sex, saturated fatty acid intake, and cigarette smoking were associated with lower HDL-cholesterol concentrations. Although physical exercise, measured qualitatively, did not show any significant effect on HDL-cholesterol, lower body mass index and higher daily energy consumption were related to higher HDL-cholesterol concentrations. Vitamin C intake seemed to be directly associated with HDL-cholesterol concentrations, but this association did not reach statistical significance ($P = 0.07$).

Discussion

The present study shows that, in addition to genetic polymorphisms, HDL-cholesterol concentration and cigarette smoking are significant predictors of PON1 activity in our population, but that the other nutritional and lifestyle habits investigated do not play a significant role.

A 1 mmol/L increase in HDL-cholesterol was associated with a mean increase in serum PON1 activity of 53.5 U/L. This association was also reported by Jarvik et al. *(21)* and Nevin et al. *(34)* and seems logical because PON1 circulates in plasma linked to this lipoprotein *(1)*. We investigated whether any of the nutritional and lifestyle habits selected influenced HDL-cholesterol concentration and could therefore indirectly affect serum PON1 activity. Their effects on HDL-cholesterol were relatively small, and it is likely that their influence on PON1 is not very relevant. Physical exercise, although not independently associated with HDL-cholesterol, may play a positive role by decreasing body mass index and increasing energy intake. An unexpected finding was the inverse relationship between saturated fatty acid intake and HDL-cholesterol concentration. These results are contradictory to those reported in previous studies *(35–37)*, and we found no evident explanation.

Tobacco smoking was also shown to be an independent determinant of serum PON1 activity. This confirms

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Table 1. Genotype and allele frequencies and enzyme activity for *PON1* polymorphisms in the population studied.

$PON1_{192}$			$PON1_{55}$		
Genotype	Genotype frequency	Mean (SD) PON1 activity, U/L	Genotype	Genotype frequency	Mean (SD) PON1 activity, U/L
QQ	0.482	292 (59)	*LL*	0.394	487 (152)
QR	0.441	490 (101)	*LM*	0.466	394 (131)
RR	0.077	702 (130)	*MM*	0.139	247 (55)
Allele frequency (Q/R)	0.701/0.299		*Allele frequency (L/M)*	0.626/0.374	

Table 2. Nutritional variables, serum lipids, and genotype frequencies according to tertiles of serum PON1 activity.[a]

Variables/Tertiles	Tertile First (148–309 U/L)	Tertile Second (310–471 U/L)	Tertile Third (472–997 U/L)	*P*
Males, %	55.6	49.2	45.4	0.26
Age,[b] years	43 (15)	41 (16)	42 (15)	0.61
Age groups, %				
18–29 years	25.4	27.3	25.4	0.32
30–44 years	28.6	37.1	33.1	
45–54 years	21.4	12.9	24.6	
55–64 years	11.1	9.1	5.4	
≥65 years	13.5	13.6	11.5	
Current smokers, %	37.3	37.1	24.6	0.04
Cigarettes/day[b]	6.3 (10.1)	5.6 (9.0)	4.1 (8.8)	0.17
Current drinkers, %	42.1	37.9	36.2	0.61
Alcohol intake,[b] g/day	11.3 (18.7)	7.1 (12.6)	8.9 (16.9)	0.11
Physical exercise in leisure time, %				
None	54.0	56.0	52.3	0.07
1–5 h/week	20.6	20.5	32.3	
>5 h/week	25.4	23.5	15.4	
Body mass index,[b] kg/m^2	27.4 (5.1)	26.8 (5.4)	26.5 (4.8)	0.34
Total energy consumption,[b] kJ/day	9354 (2917)	9221 (2696)	9271 (2846)	0.93
Nutrient intake[b]				
Total lipids, g/day	103.9 (36.2)	103.2 (31.5)	102.6 (33.3)	0.95
Saturated fatty acids, g/day	29.9 (12.2)	29.2 (12.1)	28.1 (9.8)	0.43
β-Carotenes, μg/day	3008 (2659)	3307 (2796)	3242 (3014)	0.67
Vitamin E, mg/day	12.2 (6.6)	12.0 (4.7)	12.7 (6.2)	0.51
Vitamin C, mg/day	96.2 (65.8)	96.7 (71.9)	104.5 (67.5)	0.55
Serum cholesterol,[b] mmol/L	5.21 (0.98)	5.32 (1.16)	5.35 (0.94)	0.55
Serum triglycerides,[b] mmol/L	1.40 (1.15)	1.31 (0.80)	1.30 (0.89)	0.62
HDL,[b] mmol/L	1.46 (0.37)	1.52 (0.36)	1.64 (0.41)	0.001
$PON1_{192}$ genotype, %				
QQ	95.2	50.0	0.8	<0.001
QR	4.8	50.0	76.1	
RR	0.0	0.0	23.1	
$PON1_{55}$ genotype, %				
MM	38.1	4.5	0.0	<0.001
ML	51.6	47.0	41.5	
LL	10.3	48.5	58.5	

[a] Statistical analysis was performed by ANOVA or Mann–Whitney *U*-test corrected for multiple comparisons.
[b] Mean (SD).

previous studies in American veterans *(21)* and in patients with coronary artery disease *(16)*. The mean decrease reported in previous studies was 15%. We observed similar results: a single cigarette per day was associated with a mean decrease in serum PON1 activity of 1.18 U/L. This implies that smoking one pack of 20 cigarettes/day may decrease serum PON1 activity by ~25 U/L.

We found no significant association between PON1 and the daily intake of total lipids, saturated fatty acids, β-carotenes, vitamin C, or vitamin E. Other studies have recently investigated the relationship between dietary factors and serum PON1 activity in humans *(21, 38)*. Jarvik et al. *(21)* reported a direct relationship between vitamin C and E intake and serum PON1 activity. However, Kleemola et al. *(38)* did not confirm these results: they observed an inverse relationship between serum PON1 activity and the intake of β-carotenes. We found no such associations. Differences in the populations studied may help to explain these conflicting observations. The participants in the study by Jarvik et al. *(21)* were all males who attended several American veteran health centers. They were older (48–88 years), and a significant percentage of them took several types of medication. Kleemola et al. *(38)* studied basically young and healthy volunteers who were University students and employees; more than 70% of the group studied were women. In contrast, the participants in our study were randomly selected from the town council's registers, the age range was wide (18–75 years), and the proportion of sexes was

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Table 3. Association of genetic, nutritional, and lifestyle variables with serum PON1 activity.[a]

Variable	Mean (SE) effect on PON1, U/L	*P*
Male vs female	21.1 (11.2)	0.06
Age, by year	−0.47 (0.35)	0.18
Cigarettes/day	−1.18 (0.58)	0.045
Alcohol, g/day	−0.10 (0.36)	0.78
Interaction alcohol/tobacco	−0.009 (0.02)	0.70
Physical exercise, yes vs no	−6.76 (8.51)	0.43
Body mass index, kg/m^2	0.83 (0.97)	0.39
Energy consumption, kJ/day	-8.9×10^{-5} (0.02)	0.99
Nutrient intake		
Total lipids, g/day	0.71 (0.43)	0.09
Saturated fatty acids, g/day	−1.23 (0.98)	0.21
β-Carotenes, μg/day	-2.88×10^{-4} (0.002)	0.86
Vitamin E, mg/day	−0.15 (1.07)	0.89
Vitamin C, mg/day	−0.10 (0.07)	0.13
PON1$_{192}$ genotype		
RR vs *QQ*	373.2 (16.7)	<0.001
QR vs *QQ*	182.1 (9.1)	<0.001
PON1$_{55}$ genotype		
LL vs *MM*	84.7 (13.9)	<0.001
LM vs *MM*	49.6 (13.2)	<0.001
Cholesterol, mmol/L	12.4 (4.9)	0.01
Triglycerides, mmol/L	−5.72 (5.77)	0.32
HDL, mmol/L	53.5 (14.3)	<0.001

[a] Statistical analysis was performed by multiple regression. Goodness of fit: $F_{(20,367)} = 57.123$; $P < 0.001$; $R^2_{corrected} = 0.74$.

equal. One important difference is that the use of vitamin supplements was a cause of exclusion in our study but not in the others, and this may contribute to the differences in the conclusions.

We also did not observe any significant association between serum PON1 activity and alcohol consumption. It has been suggested that consumption of red wine or flavonoid-containing drinks increases serum PON1 activity *(20, 39)*. However, the possible beneficial effect of these beverages may not be evident in our study because almost 50% of the participants reported that they did not drink alcohol and the remaining 50% tended to consume a variety of drinks, i.e., red or white wine, beer, or spirits, many of which do not contain flavonoids. Our results confirm those of another recent population study that reported a lack of association between serum PON1 activity and alcohol consumption in healthy men *(40)*. We cannot discount, however, the possibility that self-reported records underestimate the actual amounts of alcohol consumed by the participants because alcohol drinkers tend often to admit to a lower intake to that they actually consume. This possibility could explain the lack of association between alcohol intake and HDL-cholesterol in our participants, which is not consistent with the previous literature *(41, 42)*.

Table 4. Association of nutritional and lifestyle variables with HDL concentration.[a]

Variable	Mean (SE) effect on HDL, mmol/L	*P*
Male vs female	−0.33 (0.04)	<0.001
Age, by year	7.12×10^{-4} (0.001)	0.60
Cigarettes/day	-8.19×10^{-3} (0.002)	<0.001
Alcohol, g/day	8.39×10^{-4} (0.001)	0.48
Physical exercise, yes vs no	3.08×10^{-5} (0.03)	0.99
Body mass index, kg/m^2	−0.02 (0.004)	<0.001
Energy consumption, kJ/day	1.85×10^{-4} (0.001)	0.001
Saturated fatty acid intake, g/day	-7.96×10^{-3} (0.003)	0.007
Vitamin C, mg/day	4.60×10^{-4} (0.001)	0.07

[a] Statistical analysis was performed by multiple regression. Goodness of fit: $F_{(9,378)} = 18.169$; $P < 0.001$; $R^2_{corrected} = 0.29$.

The present study has other potential limitations. One limitation is that we did not analyze any of the *PON1* polymorphisms at the 5′ promoter region. Several *PON1* gene promoter polymorphisms have been described recently that influence the enzyme's serum concentration *(43–45)* and have been associated with cardiovascular disease *(46, 47)*. It is likely that the inclusion of these polymorphisms in the multiple regression model would modify the statistical association between *PON1* coding region polymorphisms and serum activity. However, this would not change the main conclusion of our study: that genetic background is the main determinant of serum PON1 activity in the general population, whereas most environmental factors play relatively minor roles. Another limitation is that we did not measure serum PON1 concentrations. However, in a recent prospective study, serum PON1 hydrolytic activity toward paraoxon was associated with cardiovascular disease events, whereas the PON1 concentration was not *(48)*. The authors of this study suggested that the differential substrate activity of PON1 is more critical than the enzyme concentration for its protective effect against atherosclerosis. They state that the reaction rate of PON1 against lipid peroxides is relatively low and that, therefore, the enzyme concentration may be not very important.

In conclusion, the between-individual variability in serum PON1 activity seems to be regulated mainly by genetic determinants, but increasing the HDL-cholesterol concentration by reducing the body mass index and stopping cigarette smoking seem to be beneficial behaviors that positively modulate the enzyme's activity and/or concentration. However, the evidence from our study does not suggest any specific recommendations for increasing serum PON1 activity by changing nutritional habits. We cannot discount the possibility that abrupt modifications to some dietary factors may seriously modulate serum PON1 activity, but we expect these effects to be limited in extent and duration. In our opinion, studies on genetically engineered human PON1, which has greater catalytic activity, such as those reviewed by Costa et al. *(15)* may

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provide more efficient tools for the therapeutic modulation of serum PON1 activity.

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