

CLINICAL STUDY

Q192R polymorphism of the paraoxonase-1 gene as a risk factor for obesity in Portuguese women

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Abstract

Introduction: Obesity became a major public health problem as a result of its increasing prevalence worldwide. Paraoxonase-1 (PON1) is an esterase able to protect membranes and lipoproteins from oxidative modifications. At the *PON1* gene, several polymorphisms in the promoter and coding regions have been identified. The aims of this study were i) to assess PON1 L55M and Q192R polymorphisms as a risk factor for obesity in women; ii) to compare PON1 activity according to the expression of each allele in L55M and Q192R polymorphisms; iii) to compare PON1 activity between obese and normal-weight women.

Materials and methods: We studied 75 healthy (35.9 ± 8.2 years) and 81 obese women (34.3 ± 8.2 years). Inclusion criteria for obese subjects were body mass index ≥ 30 kg/m² and absence of inflammatory/neoplastic conditions or kidney/hepatic dysfunction. The two PON1 polymorphisms were assessed by real-time PCR with TaqMan probes. PON1 enzymatic activity was assessed by spectrophotometric methods, using paraoxon as a substrate.

Results: No significant differences were found for PON1 activity between normal and obese women. Nevertheless, PON1 activity was greater ($P < 0.01$) for the RR genotype (in Q192R polymorphism) and for the LL genotype (in L55M polymorphism). The frequency of allele R of Q192R polymorphism was significantly higher in obese women ($P < 0.05$) and was associated with an increased risk of obesity (odds ratio = 2.0 – 95% confidence interval (1.04; 3.87)).

Conclusion: L55M and Q192R polymorphisms influence PON1 activity. The allele R of the Q192R polymorphism is associated with an increased risk for development of obesity among Portuguese Caucasian premenopausal women.

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Introduction

Serum paraoxonase-1 (PON1, E.C.3.1.8.1) is a glycosylated protein of 355 amino acids, with a molecular mass of 43–47 kDa. It is synthesized by the liver and secreted into the blood, where it circulates in association with high-density lipoprotein cholesterol (HDLc) (1, 2).

PON1 is a calcium-dependent esterase known for its ability to hydrolyze active metabolites of several organophosphates (3, 4). Its primary physiological role is the protection of low-density lipoprotein cholesterol (LDLc) and HDLc from oxidative modifications through enzymatic hydroxylation of oxidized phospholipids (1, 4–6).

The enzymatic activity of PON1 varies widely among healthy humans, and it has been suggested that subjects with low PON1 activity may have a greater risk of developing diseases in which oxidative damage and lipid peroxidation are involved (2, 3, 7). Several studies have demonstrated that the antioxidant activity of PON1

prevents oxidative stress mechanisms involved in pathophysiological processes associated with atherosclerosis and diabetes mellitus, among other common pathologies (8–11).

PON1 activity is modulated by environmental compounds and lifestyle, but also by genetic polymorphisms (7, 12). Human *PON1* gene is coded on chromosome 7q21.3–22.1, and several polymorphisms have been identified on its promoter and coding regions. Two polymorphisms in the coding region of *PON1* gene have been identified and widely studied: non-synonymous amino acid substitution Gln¹⁹² → Arg (Q192R) and Leu⁵⁵ → Met (L55M) (12, 13). The presence of each of these polymorphisms has shown to lead to different PON1 phenotype.

The presence of L55M (rs854560) has been associated with changes in the concentration of the enzyme but small effects on its activity. On the other hand, the polymorphism Q192R (rs662) leads to different PON1 enzymatic activity behavior dependent of the isoenzyme that is present (4). These polymorphism-dependent

changes in the PON1 phenotype have been related to several pathological states such as diabetes mellitus (14, 15), coronary disease (16, 17), and stroke (18, 19).

Obesity is a known risk factor for type 2 diabetes, dyslipidemia, hypertension, and atherosclerosis. Several studies have demonstrated an increase in oxidative stress in obese subjects, namely with a significantly higher susceptibility to lipid peroxidation (20). This pathophysiological mechanism has been implicated in the development of several comorbidities (21, 22).

Although PON1 expression has been associated with an obesity-related phenotype (23), less is known about the importance of this enzyme in the predisposition for the development of obesity. To clarify this relationship, we have studied the interaction between body mass index (BMI), PON1 activity, and Q192R and L55M polymorphisms.

Studying a population of obese and normal-weight Portuguese Caucasian premenopausal women, we intended to assess PON1 L55M and Q192R polymorphisms as a risk factor for obesity. Secondly, we compared PON1 activity according to the expression of each allele (in both polymorphisms) and between the two groups of women.

Methods

Subjects

The obese group consisted of 81 obese Caucasian premenopausal women, who attended the obesity outpatient clinic at the Curry Cabral Hospital (Lisbon, Portugal). Their age ranged from 18 to 50 years old, and their BMI was ≥ 30 kg/m². All of them exhibited a $<10\%$ variation in their body weight in the previous year.

The control group consisted of 75 healthy Caucasian premenopausal women, who either attended a routine health check or belonged to the health care staff of Curry Cabral Hospital. Their age ranged from 18 to 50 years old, BMI from 18.5 to 24.9 kg/m², and exhibiting $<10\%$ variation on their body weight in the previous year.

Although all women, obese and controls, presented in a fertile status, no one was pregnant or had been pregnant in the preceding 12 months. We considered only women without previous diagnosis of any acute/chronic conditions (except obesity for the obese group).

No woman was on any pharmacological regimen, except for oral contraceptives.

The study was conducted following the approval of the institutional scientific and ethical boards, and an informed consent was obtained from all participants.

Clinical evaluation

Each woman was anthropometrically characterized in terms of BMI, waist circumference, waist:hip ratio, and total body fat mass assessed by bioelectrical impedance (Tanita TBF-300A, Tanita Europe B.V., Hoofddorp, The Netherlands).

Sample collection

A fasting venous blood was collected from patients and controls. Serum samples were obtained by low-speed centrifugation and stored at -80 °C in an ultra-freezer, and thawed just before each assay. For genetic analysis, total blood was collected and stored at -20 °C.

Assessment of PON1 activity

PON1 activity assay used paraoxon (O,O-diethyl-p-nitrophenylphosphate; Sigma-Aldrich) as a substrate, following the rate of generation of p-nitrophenol (24). Spontaneous paraoxon hydrolysis was subtracted.

PON1 activity was initiated by adding serum samples to a reactive mixture containing 100 mM Tris-HCl buffer (pH 8.0), 2 mM CaCl₂, and 2 mM paraoxon. The reaction was followed at 412 nm at 25 °C, with the use of Helios β u.v.-Vis v2.04 spectrophotometer. Enzyme activity was calculated with a molar extinction coefficient of 18 290/M per cm.

Analysis of L55M and Q192R polymorphisms

Genomic DNA was isolated from white blood cells by phenol extraction. Genotyping was done through real-time PCR with TaqMan probes, using iCycler iQ Multicolor Real-Time PCR Detection System (Bio-Rad).

The design of the primers and probes (Table 1) was done using the software Beacon Designer (Primer

Table 1 Technical characterization of the primers and probes for real-time PCR genotyping for paraoxonase-1 polymorphisms.

Sequence		
Probes		Fluorophore
192-Q	5'-TCTCCCAGGATTGTAAGTAGGGGT-3'	FAM
192-R	5'-TCTCCCAGGATCGTAAGTAGGGGT-3'	TET
55-M	5'-CGCGATCGGCTCTGAAGACATGGAGATACTGGATCGCG-3'	TET
55-L	5'-CGCGATCGGCTCTGAAGACTTGGAGATACTGGATCGCG-3'	FAM
Primers		Polymorphism
Forward	5'-GAGCACTTTTATGGCACAAA-3'	Q192R
Reverse	5'-TAGTAGACAACATACGACCAC-3'	Q192R
Forward	5'-GTGAGGTGTGATAAAGAAATGG-3'	L55M
Reverse	5'-AAAGAAAACACTCACAGAGC-3'	L55M

Biosoft International, Palo Alto, CA, USA). Primers and probes were acquired to Thermo Company (Whatman BioScience, Newton, MA, USA).

For the study of both polymorphisms, we used TaqMan Universal PCR Master Mix (Applied Biosystems) with primers forward and reverse (300 nM) and probes FAM and TET (250 and 150 nM respectively). Amplification steps were 95 °C for 10 min, followed by 50 cycles at 92 °C for 15 s and 60 °C for 1 min.

Statistical analysis

Comparisons between the groups were made by Student's *t*-test. Odds ratio (OR) analysis was performed using binary logistic regression adjusted for age. Interaction between the polymorphisms and PON1 activity was evaluated by ANOVA test. PON1 allelic polymorphisms and genotypic distributions were analyzed by χ^2 test.

All determinations were performed using SPSS software, version 16.0 (Somers, NY, USA).

Results

The characteristics of the obese and control groups are depicted in Table 2. The groups were well matched for age but, as expected, were significantly different concerning all the anthropometric parameters.

No significant difference in PON1 activity was observed between control and study populations. A significant correlation between PON1 activity and BMI was presented in the obese group ($P=0.017$; $r=0.84$), but not in the control one. Nevertheless, no correlations of PON1 activity were observed with waist circumference, waist:hip ratio, and total body fat mass ($P>0.05$).

Table 2 Characterization of the obese and control groups included in the study. Values are represented as mean \pm s.d.

	Control group	Study group	P value
<i>n</i>	75	81	
Age (years)	35.9 \pm 8.2	34.3 \pm 8.2	0.23
BMI (kg/m ²)	21.5 \pm 1.8	43.1 \pm 8.5	<0.001
Waist circumference (cm)	71.6 \pm 5.7	117.8 \pm 15.7	<0.001
Waist/hip ratio	0.74 \pm 0.05	0.88 \pm 0.07	<0.001
Fat mass (%)	25.1 \pm 4.5	47.4 \pm 5.3	<0.001
Systolic blood pressure (mmHg)	108.7 \pm 13.6	124.3 \pm 17.5	<0.001
Diastolic blood pressure (mmHg)	69.4 \pm 9.8	79.9 \pm 11.2	<0.001
Glycemia (mg/dl)	83.0 \pm 6.8	90.7 \pm 21.9	<0.001
HbA1c (%)	5.3 \pm 0.3	5.7 \pm 0.9	0.002
Triglycerides (mg/dl)	77.8 \pm 27.8	123.0 \pm 80.9	<0.001
Total cholesterol (mg/dl)	173.0 \pm 28.8	184.9 \pm 33.3	0.017
HDL-cholesterol (mg/dl)	67.6 \pm 14.6	51.2 \pm 11.7	<0.001
LDL-cholesterol (mg/dl)	108.0 \pm 26.8	118.7 \pm 27.8	0.014
High-sensitivity C-reactive protein (mg/dl)	0.23 \pm 0.3	0.95 \pm 0.82	<0.001
Women on oral contraceptives (%)	42.7%	45.7%	0.751

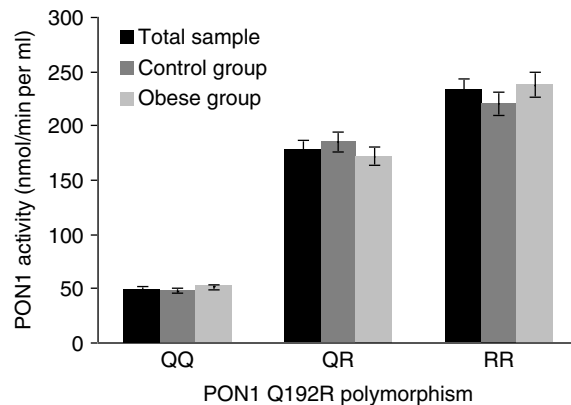


Figure 1 Serum PON1 activity in control and study populations according to the presence of different genotypes of the Q192R polymorphism ($P<0.01$ with respect to the other two genotypes, for the same population).

The two polymorphisms are in linkage disequilibrium ($P<0.001$). There is statistical linkage and physical linkage, as they are in the same chromosome.

When we analyzed the activity of PON1, according to the polymorphisms L55M and Q192R of *PON1* gene, there were significant differences in enzymatic activity between genotypes. For the Q192R polymorphism (Fig. 1), we found that the PON1 activity was significantly higher in the presence of the genotype RR and minor for QQ, with QR heterozygotes showing an intermediate activity ($P<0.01$). For polymorphism L55M (Fig. 2), a similar profile was verified: higher PON1 activity for genotype LL, lower PON1 activity for MM genotype, and intermediate PON1 activity for the heterozygotes ML ($P<0.01$).

The polymorphism Q192R was in accordance with the Hardy–Weinberg equilibrium in both groups. The L55M polymorphism was in accordance with the Hardy–Weinberg equilibrium in the obese group, but not in the control one, where it presented a higher prevalence of LL homozygotes ($P<0.05$; Table 3). Nevertheless, when we analyzed the differences between the allelic and genotypic distribution of the L55M polymorphism, no significant dissimilarity was observed (Table 3). Conversely, the polymorphism Q192R showed significant differences in allelic and genotypic distributions between the control and obese populations ($P<0.05$), with an increased frequency of the R allele in the obese group (Table 3).

OR analysis was performed for both Q192R and L55M PON1 polymorphisms (Table 4). For the L55M polymorphism, no significant influence was shown in obesity ($P>0.05$), neither in genotypic nor in the allelic form. The genotypic expression of the Q192R polymorphism did not represent a significant risk for the development of obesity (Table 4). Nevertheless, the presence of the R allele (QR + RR) represented a twofold

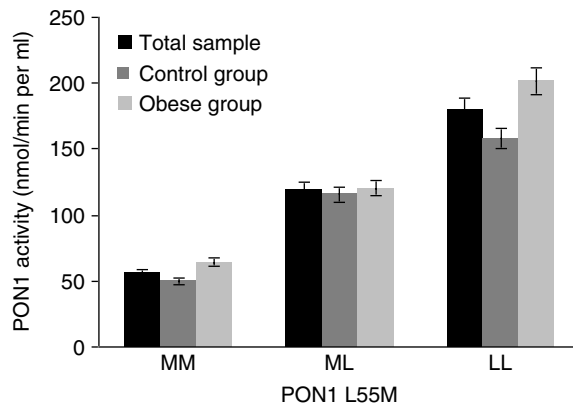


Figure 2 Serum PON1 activity in control and study populations according to the presence of different genotypes of the L55M polymorphism ($P < 0.01$ for MM genotype with respect to the other two genotypes, for the same population).

(95% confidence interval (CI): 1.04–3.87) increased risk for obesity.

No synergetic effect was observed in PON1 enzymatic activity or in obesity risk when we combined the two polymorphisms of *PON1* gene.

Discussion

In Portugal, the prevalence of overweight and obesity has increased throughout the last decade, increasing to more than half of the adult population (25), in accordance with obesity epidemics worldwide. Many of the metabolic pathways and interactions related to the genesis of obesity are still unknown. That makes this disease one of the major challenges of the 21st century both in the clinical and in the investigational fields.

PON1 is an antioxidant enzyme that has been related to several pathophysiological conditions, mainly diseases associated with oxidative stress unbalanced status. Two main common polymorphisms in the

Table 3 Allelic and genotypic distributions of Q192R and L55M polymorphisms for the participant populations^a.

	Obese population	Control population	<i>P</i> value
<i>Q192R</i>			
QQ	27 (33.3%)	37 (50%)	0.038
QR	40 (49.4%)	30 (40.5%)	
RR	14 (17.3%)	7 (9.5%)	
Q	94 (0.58)	104 (0.703)	0.033
R	68 (0.42)	44 (0.297)	
<i>L55M</i>			
MM	11 (13.6%)	16 (21.6%)	0.828
ML	39 (48.1%)	26 (35.2%)	
LL	31 (38.3%)	32 (43.2%)	
M	61 (0.38)	58 (0.39)	0.809
L	101 (0.62)	90 (0.61)	

^aValues represent the number of cases (frequency).

Table 4 Odds ratio analysis for the studied PON1 polymorphisms.

Polymorphisms	Odds ratio ^a (95% CI)	P value
Q192R		
QQ	1	–
QR	1.9 (0.95–1.80)	0.069
RR	2.5 (0.86–7.06)	0.091
QQ	1	–
QR + RR	2.0 (1.04–3.87)	0.036
L55M		
MM	1	–
ML	2.2 (0.89–5.64)	0.085
LL	1.4 (0.55–3.49)	0.483
MM	1	–
ML + LL	1.8 (0.76–4.14)	0.186

^aOdds ratio was calculated using binary logistic regression adjusted for age.

coding region of the *PON1* gene have been identified, Q192R and L55M. They have been recognized by some authors as genetic susceptibility factors for the development of some diseases (13, 26).

In this study, no significant difference in PON1 activity was observed between obese and normal-weight women. So, PON1 activity seems to have no direct influence on the development of obesity. These results could be due to the fact that PON1 activity is modulated by several different factors (7), leading to a wide variability in PON1 activity among individuals. However, it was possible to determine a correlation between PON1 activity and BMI, as already reported by other authors (25).

When we analyzed the PON1 activity according to the L55M and Q192R polymorphisms, it was possible to verify that the LL and RR genotypes respectively lead to a significant increase in the enzymatic activity of PON1 in control and obese populations. Previous studies have reported similar results in type 2 diabetes mellitus (14) and cardiovascular diseases (27). This behavior may be due to structural change promoted by the polymorphism or changes in affinities to the substrate of each isoenzyme (28).

It was possible to observe that the L55M polymorphism showed a deviation on the genotypic distribution, which led to an increased number of homozygote LL in the control population. However, these findings were inconclusive because L55M polymorphism was not found as a predictive risk factor for obesity. Further studies should be carried out to determine the importance of the LL genotype in obesity.

For the Q192R polymorphism, there was a prevalence of the R allele in the obese population. Through OR analysis, we could demonstrate that this allele was actually a risk factor for obesity *per se* which lead to a twofold increase risk for the development of obesity in women.

It seems contradictory that higher PON1 activity is associated with a higher risk of obesity. Nevertheless, recent studies have shown that obesity is characterized by a reduced redox state in adipose tissue, which would

promote a deleterious pro-adipogenic process (29). This might indicate that if an elevated PON1 activity is present, it means that it is actually a higher redox state in the body. In this setting, adipogenic process would be activated and obesity would be promoted.

The major contribution of this study is that we demonstrate a direct influence of Q192R PON1 polymorphism as a risk factor for obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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