The effect of the paraoxonase 1 (PON1) T(−107)C polymorphism on serum PON1 activity in women is dependent on fatty acid intake

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ABSTRACT

Paraoxonase 1 (PON1) is an enzyme that prevents the peroxidation of lipoprotein and cell membranes. Our hypothesis is that the effect of the PON1 T(−107)C polymorphism on serum PON1 activity in healthy adult women is dependent on their fatty acid intake profile. This study included women (n = 39) who completed a food frequency questionnaire. Fatty acid intake was estimated based on the interview and a nutrient reference table. Blood samples were collected for genotyping and to measure serum PON1 activity. Serum PON1 activity was different among genotypes and was higher for women of the CC genotype (P < .001). Women in the study were categorized in 2 groups according to the median nutrient intake. Overall, there was a difference (P < .05) in serum PON1 activity between the CC and TT genotypes in women ingesting either above or below the median total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, omega 3 (n-3) and omega 6 (n-6; P < .05). However, genotype effects on serum PON1 activity were not observed in women ingesting below the median (15:1) ratio of n-6/n-3 (P > .05) but were observed in women ingesting above the ratio of n-6/n-3 (P < .05). This is partly because women of the CC genotype had decreased PON1 activity when ingesting a lower ratio of n-6/n-3 diet (P < .05), while women of the TT genotype had increased PON1 activity (P < .05). In conclusion, the overall presence of the C allele was associated with increased serum PON1 activity, although a diet with high saturated fatty acid or a low ratio of n-6/n-3 reduced PON1 activity in women with the CC genotype.
1. **Introduction**

Paraoxonase 1 (PON1) is an enzyme that is mainly associated with high-density lipoproteins (HDL), synthesized in the liver, and released in the blood stream [1,2]. PON1 has antioxidant activity and is able to hydrolyze lipid peroxides, catalyzing the breakdown of oxidized phospholipids from low-density lipoprotein (LDL) [3]. Therefore, PON1 plays an important role in lipid metabolism, preventing atherosclerotic plaque formation [4,5]. Lower serum PON1 activity is observed in patients with cardiovascular diseases [6]. In general, atherosclerotic plaque formation is slower in women than in men, but post-menopause plaque formation is faster and highly dependent on pre-menopausal risk factors [7]. Among the risk factors, polycystic ovarian syndrome is associated with decreased serum PON1 activity and an increased incidence of cardiovascular disease [8,9].

There are over 160 single nucleotide polymorphisms (SNPs) described in the PON1 gene [4]. Two important SNPs are located in the coding region of the gene, at codons 55 (L55M) [10] and 192 (Q192R) [11]. Both SNPs are associated with serum PON1 activity and the chance of developing cardiovascular disease [12,13]. Another important SNP is located at the promoter region, at the −107 position, and is referred as T(−107)C (rs705379), affecting PON1 gene expression and serum activity [6]. The CC genotype is associated with a 2-fold increased serum PON1 activity, and its frequency is increased in healthy octogenarians, indicating an association with the overall antioxidant capacity and aging diseases [14]. The TT genotype is associated with low serum PON1 activity and an increased risk of coronary artery disease [15]. The PON1 T(−107)C SNP is one of the most important factors affecting serum PON1 activity. It accounts for approximately 12% of the serum activity variation between individuals, contributing more than other SNPs, even in combination with other dietary factors [16]. Therefore, this was the SNP of choice for the present study.

In addition to genetic factors, numerous environmental factors, including diet, have been associated with PON1 activity [16–18]. A recent study demonstrated that the consumption of fatty acids and cholesterol accounts for approximately 6% of the variance in serum PON1 activity [16]. Other factors, such as age, gender, smoking and alcohol consumption, are responsible for approximately 11% of the variance in PON1 serum activity [16]. A positive association between the intake of cholesterol and fatty acids with serum PON1 activity has been found in previous studies [19,20]. In contrast, others have found no association between PON1 activity and the daily intake of lipids and saturated fatty acids (SFA) [21] or have even found a negative effect of SFA and cholesterol on PON1 activity [22]. These studies indicate that, although there are reports linking nutrient intake and PON1 activity, there is still some controversy, which may be elucidated by gene-nutrient interaction studies.

Based on the evidence presented, our hypothesis is that the effect of the fatty acid intake on serum PON1 activity in healthy adult women is dependent on the genotype. Therefore, we aimed to observe the effect of the T(−107)C SNP, located at the PON1 gene promoter, on serum PON1 activity in healthy women according to the intake profile of fatty acids.

To accomplish this, a food frequency questionnaire was administered to a group of women to estimate their nutrient intake, and blood samples were collected for determining the PON1 genotype and serum PON1 enzyme activity. This gene-nutrient interaction study will help us to arrive at a better understanding of how the modulation of PON1 activity by nutrient intake is associated with this important PON1 promoter SNP.

2. **Methods and materials**

2.1. **Design and population**

A cross-sectional study of healthy women (n = 39) between 18 and 60 years old, regardless of nutritional status, was conducted at the gynecology section from the Federal University of Pelotas. Subject selection is depicted in Fig. 1. We excluded women younger than 18 years, those using any medicine that could compromise vascular function and the hypothalamic-pituitary-gonadal axis, and pregnant women [22]. The study was approved by the Federal University of Pelotas Ethics Committee. All subjects gave written, informed consent.

2.2. **Food consumption and anthropometric measures**

To estimate nutrient intake, a previously validated food frequency questionnaire was applied [23]. The survey asked about the average frequency of intake over the previous month of specified portions of 67 foods. From the questionnaires, the daily caloric intake (kcal/d), total fat intake (g/d),
and monounsaturated fatty acids (MUFA) intake as a percentage of the total daily intake of fat (%); and omega-3 (n-3; g/d) and -6 (n-6; g/d) and the ratio of n-6/n-3 were estimated according to the nutrient value of each food component [24].

Anthropometric measurements, including weight (kg), height (m), body mass index (BMI) and abdominal circumference (cm), were performed according to previously established criteria [25]. BMI was calculated according to the formula [BMI = weight/(height×height)]. Waist circumference was obtained as an estimation of central obesity and was measured approximately 2 inches above the navel; lesser curvature was located between the ribs and the iliac crest [25].

2.3. Blood collection and analyzes

Blood collection was performed after the interview in a clinical analysis laboratory. Approximately 12 mL of blood without anticoagulant and 5 mL of blood with EDTA were collected by venipuncture after 12 hours of fasting. The serum samples were processed immediately, and the sera were analyzed in an automated equipment for total cholesterol (Biosystems, RJ, Brazil), triglycerides (TG; Doles, GO, Brazil), glucose (Biosystems, RJ, Brazil) and HDL (Biosystems, RJ, Brazil), following the manufacturers’ instructions. The LDL concentration was estimated using the Friedewald equation: (total cholesterol – HDL – TG/5) [26].

PON1 activity was measured by its arylesterase activity as previously established [27]. Arylesterase activity was measured by the phenol formation rate by monitoring the increase in absorbance at 270 nm and 25°C. The working reagent consisted of 20 mM Tris/HCl, pH 8.0, containing 1 mM of CaCl₂ and 4 mM phenyl acetate as substrate. The samples were diluted 1:3 in a 20 mM Tris/HCl buffer and were added to the working reagent and the change in absorbance was recorded for 60 seconds. The activity was expressed in kU/L, based on the phenol extinction coefficient.

2.4. DNA extraction and analysis of the PON1 T(−107)C SNP

For genomic DNA extraction, a previously described solution-based salting out protocol for whole blood samples was used [28]. A 240-bp fragment from the promoter region of the PON1 gene was amplified by PCR, and digestion with the BsrBI restriction enzyme (New England Bio Labs, Cambridge, UK) was performed as previously described [14]. PCR was performed in 35 cycles (5 min at 94°C followed by 45 s at 94°C, 45 s at 67°C, and 45 s at 72°C) with a final extension of 5 minutes at 72°C. The following primers were used: forward AGCTAGCTGCG GACCCGGCGGGGAGGaG and reverse GGCTGCAGCCCTCACCA CAACC. The lowercase letter in the forward primer indicates a pairing error introducing at a restriction site for the BsrBI enzyme because there is no specific restriction site for cutting the original DNA sequence. After digestion for 2 hours at 37°C with 3 U of BsrBI, the DNA fragments were separated by electrophoresis on a 3% agarose gel stained with SYBR Safe (Applied Biosystems, Foster City, CA, USA). The presence of the C allele was identified by fragments of 28 and 212 bp, while the presence of the T allele resulted in an undigested 240-bp fragment, as previously established [14].

2.5. Statistical analyses

Statistical analyses were performed using SAS 9.2 software (SAS Institute Inc, Cary, NC, USA). The data are presented as the means ± SEM. The sample size was calculated using the power procedure for 1-way analysis of variance (ANOVA), considering P < .05 with a power of 0.80 and using the reference PON1 activity from Campo et al [14]. In addition, the actual sample size was doubled to allow enough power when dividing individuals in subgroups by the median nutrient intake. One-way ANOVA was performed to analyze the effect of genotype on PON1 activity and the HDL, LDL, cholesterol, TG, and glucose concentrations. Age, BMI, and total calorie intake were used as covariates, and as they had no effect on the serum PON1 activity, they were excluded from the model. In addition, the median nutrient intake was calculated, and individuals were categorized as ingesting above or below the median for each nutrient. One-way ANOVA was performed to test for the effect of the genotype in each category. In addition, the t-test was used to compare the 2 intake categories (below or above the median) for each genotype (CC, CT, and TT). The χ² test was used to test the population for Hardy-Weinberg equilibrium and also to compare the genotype distribution with other populations. P values below .05 were considered statistically significant.

3. Results

3.1. The PON1 T(−107)C polymorphism determines PON1 serum activity in women

The frequency of genotypes for the PON1 T(−107)C polymorphism for women in the studied population was 30.8% (12/39) for the CC genotype, 41.0% (16/39) for the CT genotype, and 28.2% (11/39) for the TT genotype. The genotype distribution was in Hardy-Weinberg equilibrium (P = .26). Serum PON1 activity was different among the three genotypes and was higher for the CC genotype and lower for the TT genotype (P < .001; Fig. 2). The genotype distribution was not different (P = .43) from that previously reported for European populations.

The serum concentrations of HDL, LDL, cholesterol, TG, and glucose and BMI and waist circumference are shown in

![Fig. 2](image-url) - Paraoxonase (PON1) activity (U/mL) among women (n = 39) from the CC, CT, and TT genotypes for the PON1 T(−107)C polymorphism. The data are presented as the means ± SEM. Different letters indicate significant differences at P < .05.
The genotype had no effect on serum HDL, LDL, cholesterol, triglycerides, glucose or BMI, and waist circumference ($P > .05$; data not shown).

### 3.2. Food components can influence PON1 activity depending on the PON1 T(−107)C genotype

The participants’ daily intake of energy, fat, SFA, MUFA, PUFA, cholesterol, n-3, and n-6 are presented in Table 1. For the analysis of the gene-nutrient interaction, women were divided as ingesting below or above the median for each component and analyzed for the genotype effect in each subgroup.

As stated before, the serum PON1 activity was overall affected by the genotype, increasing with the presence of the C allele. When categorizing women in the study by median nutrient intake, it was possible to observe that some food components interact with the genotype to affect serum PON1 activity. Overall, there was a difference ($P < .05$) in serum PON1 activity between the CC and TT genotypes in women ingesting above and below the median for total fat, SFA, MUFA, and PUFA (Fig. 3), indicating no gene-nutrient interaction. However, the serum PON1 activity was lower in women in the CC genotype ingesting more than 40% of fat from SFA ($P < .05$), although no difference was observed for women of the CT and TT genotypes (Fig. 3).

Serum PON1 activity was also different between the CC and TT genotypes, independent of the n-3 and n-6 ingestion level ($P < .05$, Fig. 4). However, when comparing women ingesting below (<15:1) and above (>15:1) the median ratio of n-6/n-3, we observed an effect of the genotype on serum PON1 activity for women ingesting above the median ($P < .05$), but no genotype effect for women ingesting below the median ($P > .05$; Fig. 4). In this sense, women of the CC genotype had increased PON1 activity when ingesting a diet with a higher ratio of n-6/n-3 ($P < .05$), while women of the TT genotype had a reduction in serum PON1 activity ($P < .05$; Fig. 4).

### 4. Discussion

The PON1 gene promoter polymorphism T(−107)C has a strong influence on serum PON1 activity, with the CC genotype associated with the highest levels of serum PON1 activity [6,14,29]. In the present study, this polymorphism was studied in women of the Southern Brazil region, with further indication that the CC genotype is associated with increased serum PON1 activity. In addition, the genotype distribution found in the current study was not different from that found in European populations [14,15,29]. Therefore, we hypothesized that because PON1 activity is also affected by the profile of fatty acid intake, a gene-nutrient interaction could exist for this specific SNP in the promoter of the gene. Our hypothesis was confirmed, and we were able to demonstrate that the level of fatty acid intake can affect serum PON1 activity depending on the PON1 T(−107)C genotype.

As stated before, the presence of the C allele had an overall positive effect on serum PON1 activity. However, for women ingesting below the median ratio of n-6/n-3 (<15:1), no effect of the genotype on serum PON1 activity was observed at all, while the genotype effect was preserved for women ingesting above the median ratio of n-6/n-3. This is because of the ingestion of a ratio of n-6/n-3 below 15:1 reduced serum PON1 activity in women with the CC genotype and increased PON1 activity in carriers of the TT genotype. PUFAs, including n-6 and n-3 fatty acids, are known for inhibiting PON1 activity [30], and others have shown that the ingestion of arachidonic and eicosapentaenoic acids is negatively correlated to serum PON1 activity [19]. Increased production of free radicals after n-3 supplementation has been identified as the cause of decreased serum PON1 activity [31], while some reports indicate an increased serum PON1 activity after n-3 supplementation [5,32,33]. Nevertheless, our study points to an important divergent response between women of the CC and TT genotypes to the ratio of n-6/n-3 intake, and further work is needed to elucidate this interaction and adjust recommendations for this group. It should be taken into account that the n-3 intake of the studied population averaged 0.4 g/d and is well below the recommended 2 to 4 g/d [34]. The marked change in the ratio of n-6/n-3 may also be the reason for the contradictory results compared to populations with a higher n-3 intake or when n-3 is exogenously supplemented. Previous gene-nutrient interaction studies of n-3:n-6 fatty acid intake have shown an interaction between diet and 5-lipoxygenase for intimamedia thickness [35] and between the APOA4, APOA4, and APOC3 genotypes and the serum lipid profile [36]. It is important to consider carefully the interactions observed in the present study due to the small sample size, the fact that only women were analyzed and, especially, that nutrient intake was estimated based only on a food frequency questionnaire and that there were no blood markers for the level of fatty acid intake. Nonetheless, the present study suggests an interesting interaction between the gene and nutrient intake and new studies should be performed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Means ± SEM</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>41.2 ± 2.4</td>
</tr>
<tr>
<td>PON1 (U/mL)</td>
<td>98.4 ± 3.9</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>57.7 ± 2.3</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>129.4 ± 5.5</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>212.8 ± 6.4</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>143.3 ± 11.2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>88.9 ± 2.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8 ± 0.7</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>93.4 ± 1.8</td>
</tr>
<tr>
<td>Daily nutrient intake</td>
<td></td>
</tr>
<tr>
<td>Calories (kcal/d)</td>
<td>2,104.6 ± 90.6</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>55.5 ± 3.0</td>
</tr>
<tr>
<td>SFA (g/d)*</td>
<td>21.1 ± 1.1 (38.5%)</td>
</tr>
<tr>
<td>MUFA (g/d)*</td>
<td>14.0 ± 0.8 (25.5%)</td>
</tr>
<tr>
<td>PUFA (g/d)*</td>
<td>11.6 ± 0.9 (20.4%)</td>
</tr>
<tr>
<td>n-3 (g/d)</td>
<td>0.7 ± 0.0</td>
</tr>
<tr>
<td>n-6 (g/d)</td>
<td>10.6 ± 0.9</td>
</tr>
<tr>
<td>Ratio of n-6/n-3</td>
<td>16.1 ± 0.6</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>268.6 ± 14.8</td>
</tr>
</tbody>
</table>

WC, waist circumference; n-3, omega 3; n-6, omega 6; * indicates the percentage of SFA, MUFA, and PUFA from total fat intake.
Fat consumption did not have an interaction with the genotype, although it was previously reported that fat intake is a modulator of serum PON1 activity [19]. In this regard, although the daily fat intake was within the recommended range [37], the quality of the ingested fat was compromised. The intake of SFA was 7.6% above current recommendations [38], while the intake of MUFA was 6.5% below the recommendations [37]. The PUFA intake was 8.5% and was within the recommended range [38]. We observed that women of the CC genotype who ingested more than 40% of SFA had reduced serum PON1 activity compared to women of the CC genotype ingesting less than 40% of SFA. Previous studies in animal models suggested that a high-fat diet, rich in cholesterol and SFA, was linked to reduced serum PON1 activity [30,39,40]. Studies in humans also reported that a Mediterranean diet increases PON1 activity, while a Western-type diet decreases PON1 activity [21]. However, other authors have found no direct association between PON1 activity and cholesterol and SFA intake [41] or even increased PON1 activity in cholesterol- and SFA-rich diets [16]. Therefore, it is possible that gene-nutrient interactions, such as the one observed in our study of the relationship between PON1 and SFA intake, can be the source of conflicting results observed elsewhere and should be further explored in larger populations. In this regard, another gene-nutrient study has shown a reduction of PON1 activity induced by the intake of a high vegetable diet, which was greatest among women with the PON1_192Arg allele and PON1_55Leu/Leu genotype, although interaction with the PON1T(-107)C was not tested [42]. In the above-mentioned study, the high vegetable diet had a higher amount of PUFA and vitamin C than the low vegetable diet.

Although numerous studies indicate a positive correlation between serum PON1 activity and HDL [20,43] and a negative correlation with LDL, TG, and glucose [44,45], we did not find any associations between these variables and PON1 serum activity. We also did not find an effect of the genotype on the serum lipid profile, which is consistent with a previous report indicating that none of the known PON1 polymorphisms had a significant effect on the lipid profile [46]. The calorie intake and BMI of women in this study were 2104.6 kcal/day and 26.8 kg/m², respectively. These values indicate a slightly overweight population [25], although the average reported daily caloric intake is considered sufficient for the majority of the population [37]. The BMI and caloric intake results are in accordance to previous reports of the Brazilian population [47], indicating that the studied population is representative of the population weight and consumption behaviors.

In conclusion, the presence of the C allele was associated with increased serum PON1 activity in adult women in the southern region of Brazil. A gene-nutrient interaction was identified and indicated that a diet high in SFA, or with a low ratio of n-6/n-3, had a negative impact on serum PON1 activity, specifically for carriers of the CC genotype.

**Conflict of Interest**

The authors declare that they have no competing interests.
Fig. 4 – Mean paraoxonase (PON1) activity (U/mL) of women with the CC, CT, and TT genotypes of the PON1 T(−107)C polymorphism. The intake group was divided based on the median intake from the studied population of omega 3 (n-3, g/d) and omega 6 (n-6, g/d) and the ratio of n-6/n-3. The data are presented as the means ± SEM. Asterisks indicate differences between intake categories within the same genotype (*P < .05, **P < .01). Different letters indicate differences among genotypes in each intake category at the significance level of P < .05.

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REFERENCES


