Paraoxonase 1 activities and genetic variation in childhood obesity

A. I. Rupérez1, O. López-Guarnido2, F. Gil2, J. Olza1, M. Gil-Campos3, R. Leis4, R. Tojo4, R. Cañete3, A. Gil1 and C. M. Aguilera1*

1Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology, Centre of Biomedical Research, Laboratory 123, University of Granada, Avenida del Conocimiento s/n, 18100 Armilla, Granada, Spain
2Department of Legal Medicine, Toxicology and Anthropology, University of Granada, 18071 Granada, Spain
3Unit of Paediatric Endocrinology, Reina Sofia University Hospital, 14004 Córdoba, Spain
4Unit of Investigation in Nutrition, Growth and Human Development of Galicia, Paediatric Department, Clinic University Hospital of Santiago, University of Santiago de Compostela, 15706 Santiago de Compostela, Spain

(Submitted 19 December 2012 – Final revision received 16 April 2013 – Accepted 18 May 2013 – First published online 21 June 2013)

Abstract
Changes in paraoxonase 1 (PON1) activities have been observed in a variety of diseases involving oxidative stress, such as CVD. However, its role in obesity has not been fully established. In the present study, we aimed (1) to genotype sixteen PON1 SNP, (2) to measure serum PON1 activities and (3) to correlate these findings with the incidence of childhood obesity and related traits. We conducted a case–control study of 189 normal-weight and 179 obese prepubertal children, and we measured four different PON1 activities: lactonase; paraoxonase; arylesterase; diazoxonase. Although none of these activities was significantly different between the obese and normal-weight children, lactonase activity was found to be positively correlated with HDL-cholesterol and ApoA1 levels and negatively correlated with myeloperoxidase and fatty acid-binding protein 4 levels. Among the sixteen genotyped PON1 SNP, only the intronic SNP rs854566 exhibited a significant association with obesity (OR 0·61, 95 % CI 0·41, 0·91; P=0·016). This genetic variant was also associated with increased diazoxonase, lactonase and arylesterase activities and decreased paraoxonase activity. Other genetic variants exhibited different association patterns with serum activities based on their location within the PON1 gene, and SNP that were located within the promoter were strongly associated with lactonase, arylesterase and diazoxonase activities. The functional variant Q192R exhibited the greatest effect on paraoxonase activity (P=5·88 × 10⁻⁴²). In conclusion, SNP rs854566 was negatively associated with childhood obesity and with increased serum PON1 activities in prepubertal children. We determined that lactonase is a reliable indicator of PON1 activities and should be included in future studies of PON1 function.

Key words: Paraoxonase 1 gene; Genetic polymorphisms; Obesity; Children

In humans, paraoxonase 1 (PON1) is primarily expressed in the liver and can be detected circulating in the plasma in association with HDL(1). PON1 is involved in the metabolism of lipoprotein phospholipids and inhibits the lipid peroxidation of LDL and HDL(1,2). Changes in PON1 activities have been shown to lead to a variety of diseases that involve oxidative stress, including CVD, Alzheimer’s disease, chronic renal failure, the metabolic syndrome and chronic liver impairment(3).

With the prevalence of obesity and metabolic disorders, such as type 2 diabetes, being on the rise, these conditions will probably remain the major contributors to cardiovascular mortality and morbidity in the twenty-first century. A growing body of evidence that suggests that obesity-induced oxidative stress plays an important role in adults and children has emerged in the past few years. Recently, our group has demonstrated elevated levels of plasma myeloperoxidase (MPO) in prepubertal obese children, and we detected that the expression of this enzyme was correlated with biomarkers for inflammation and cardiovascular risk, such as high-sensitivity C-reactive protein, matrix metalloproteinase-9 and resistin(3). Taken together, these findings highlight the need to investigate the molecular mechanisms linking metabolic stress, obesity and, ultimately, CVD. Furthermore, the finding that PON1 is expressed in the interstitial space of adipose tissue(5) suggests a role for the PON1 enzyme in these disorders.

Relatively little work has been conducted to address the role of PON1 in obesity, and the small number of studies that have been carried out are not always in agreement. For example,

Abbreviations: HDL-C, HDL-cholesterol; LD, linkage disequilibrium; MPO, myeloperoxidase; ox-LDL, oxidised LDL; PON1, paraoxonase 1.

* Corresponding author: C. M. Aguilera, fax +34 958 248960, email caguiler@ugr.es
decreased levels of paraoxonase and arylesterase activities have been observed in obese adults\(^6\,\!^7\) and children\(^8\), although another study has observed no changes in paraoxonase activity in adults with the metabolic syndrome or obesity\(^9\). However, the lack of a strong link between PON1 activities and oxidative stress could be due to differences in measurement techniques and data analysis between these studies. For example, the actual physiological substrates of PON1 are unclear, and the available methods for testing PON1 activities use a variety of synthetic substrates, including paraoxon (paraoxonase activity), phenylacetate (arylesterase activity) and diazoxon (diazoxonase activity). Although less commonly reported in the literature, researchers have also tested PON1 lactonase activity using a variety of lactones, such as dihydrocoumarin. This activity has been proposed to be responsible for oxidised-lipid hydrolysis, HDL-mediated macrophage cholesterol efflux\(^10\) and homocysteine–thiolactone hydrolysis\(^11\). Due to these technical issues, measurements of PON1 activities will probably differ depending on the specific substrate used\(^12\,\!^13\). Another issue is that serum PON1 activities are strongly affected by enzymatic genotype as well as by exogenous factors, such as age, diet and drug, alcohol and tobacco use\(^14\). Of these factors, genetics are the main determinant of PON1 variability, and PON1 activities can differ by as much as a factor of 40 across a population of healthy individuals from the same ethnic group\(^15\). Although nearly 200 SNP have been described for PON1\(^16\), it is common for authors to state only two known coding polymorphisms in this gene: Q192R (rs662) and L55M (rs854560). To date, no strong links have been observed between the presence of these SNP and obesity, and although a number of association studies have been performed\(^17\–\!^19\), the findings of these studies are inconsistent. With this in mind, we carried out a comprehensive association study in obese prepubertal children that had additional PON1 SNP as well as the previously characterised missense variations. In addition, we measured serum PON1 activities using four different substrates (dihydrocoumarin, paraoxon, phenylacetate and diazoxon) and correlated these results with a wide variety of plasma biomarkers, including markers for oxidative stress, insulin resistance, obesity and cardiovascular risk, to determine their association with PON1 activities and genetic variability.

**Experimental methods**

**Study population**

In the present case–control study, we recruited 179 obese children (95 males and 84 females) and 189 normal-weight children (109 males and 80 females), aged 4–13 years, from two Spanish cities (Córdoba and Santiago de Compostela). Childhood obesity was defined according to Cole \(et\) \(al\).\(^20\). To be included in the study, it was required that the children were prepubertal and were not suffering from nutritional diseases or endogenous obesity. Children who were suffering from disease or malnutrition, currently in puberty or using medications to alter blood pressure, glucose levels or lipid metabolism were excluded from the study. Following an initial assessment at school or a primary-care centre, children who met the inclusion criteria were invited for a clinical examination at a participating hospital. The parents or guardians of the children were informed concerning the purpose and procedures of the study before written consent was obtained; all of the children agreed to participate in the study. Sex hormone levels were measured to confirm that the children were prepubertal (data not shown). The protocol was performed in accordance with the Declaration of Helsinki (Edinburgh 2000, revised), the recommendations of the Good Clinical Practice of the CEE (Document 111/3976/88, July, 1990) and the current Spanish regulations dictating clinical investigations in human subjects (RD 223/04 on Clinical Assays). The present study was approved by the Ethics Committee of the University of Granada, the Ethics Committee of the Reina Sofia University Hospital of Córdoba and the Bioethics Committee of the University of Santiago de Compostela.

**Anthropometric and biochemical measurements**

Anthropometric measurements were performed by a single examiner with the children in bare feet and dressed in their underwear. Body weight (kg), height (cm) and waist circumference (cm) were measured using standardised procedures and were used to calculate the BMI of the children. Obesity was defined according to BMI, using the age- and sex-specific cut-off points proposed by Cole \(et\) \(al\.)\(^20\) (linked to adult cut-offs of 25 and 30 kg/m\(^2\)). Blood pressure was measured three times for each individual by the same examiner, according to international recommendations. Blood samples were drawn from the antecubital vein after the children had fasted overnight. Biochemical analyses and measurements of specific biomarkers, including adiponectin, leptin, oxidised LDL (ox-LDL) and high-sensitivity C-reactive protein, were measured using ELISA (catalogue no. RD191036200R; BioVendor). Quantitative insulin sensitivity check index and homeostasis model assessment for insulin resistance scores were calculated using plasma glucose and insulin levels. Retinol, α-tocopherol and β-carotene levels were analysed using HPLC, as described previously\(^22\).

**DNA isolation and genotyping**

Genomic DNA was extracted using the QIAamp Blood kit (Qiagen). Based on their location with respect to the PON1 gene, a total of sixteen SNP were selected from the HapMap and NCBI databases. First, each missense variation was selected. Then, SNP were selected from the promoter 3′ untranslated region (UTR) and 5′UTR. All SNP had minor allele frequencies greater than 0.05 in the Caucasian
population. Table S1 (available online) describes the main characteristics of the SNP included in the present study.

Genotyping was performed using the Illumina GoldenGate Assay (Illumina), as described previously\(^{21}\). The success rates for genotyping were >95% for nearly all of the SNP, with the exception of rs705379, which was excluded from further analysis. The Hardy–Weinberg equilibrium for each SNP was determined with the exact test using the PLINK software program (version 1.07; http://pngu.mgh.harvard.edu/~purcell/plink)\(^{23}\). The Hardy–Weinberg equilibrium P values were greater than 0.05 in both the obese and normal-weight groups for all the SNP, and the observed allele frequencies of the SNP in the present study were similar to those reported in the HapMap database for Caucasians (Table S1, available online). Linkage disequilibrium (LD) was analysed using the Haploview 4.2 software program (http://www.broad.mit.edu/mpg/haploview/)\(^{24}\).

**Determination of paraoxonase 1 activities**

Serum PON1 activities were determined using four different substrates (dihydrocoumarin, paraoxon, phenylacetate and diazoxon), as described previously\(^{15,25}\). Arylesterase, diazoxonase and lactonase enzyme assays were performed using a Lambda-2 spectrophotometer (Perkin Elmer Inc.). PON1 paraoxonase activity was determined using a multidetection microplate reader (BioTek Synergy HT; BioTek Instruments Inc.). The samples were assayed in duplicate, and the average value was used for further analysis. The non-enzymatic hydrolysis rate for each substrate was measured by substituting serum for buffer, and this value was subtracted from the total rate of hydrolysis.

**Statistical analyses**

All of the statistical analyses were performed using either the PLINK or SPSS software program (version 15.0.1; SPSS, Inc.). The normal distribution of the clinical parameter data was determined with the exact test using the PLINK software program (version 1.07; http://pngu.mgh.harvard.edu/~purcell/plink)\(^{23}\). The Hardy–Weinberg equilibrium P values were determined with the exact test using the PLINK software program (version 1.07; http://pngu.mgh.harvard.edu/~purcell/plink)\(^{23}\). The Hardy–Weinberg equilibrium P values were greater than 0.05 in both the obese and normal-weight groups for all the SNP, and the observed allele frequencies of the SNP in the present study were similar to those reported in the HapMap database for Caucasians (Table S1, available online). Linkage disequilibrium (LD) was analysed using the Haploview 4.2 software program (http://www.broad.mit.edu/mpg/haploview/)\(^{24}\).
Association of paraoxonase 1 SNP with the incidence of childhood obesity and related traits

The results of the logistic regression analysis and the SNP allele frequencies are given in Table 3. The only SNP that showed an association with obesity was rs854566, which showed a negative association (OR 0.61, 95% CI 0.41, 0.91; *P*=0.016) under the additive model of inheritance. This association was not significant after Bonferroni correction for multiple testing (data not shown). Other models of inheritance such as recessive model did not add strength to this association. Population stratification was discarded by conducting a meta-analysis using the individual association results from each of the examined populations (Córdoba and Santiago de Compostela) (Q = 0.906, I = 0.00). The missense SNP Q192R and L55M were distributed equally among the study group populations, and they did not show any association with obesity.

Phenotypic associations between the Q192R, L55M and rs854566 SNP and the anthropometric measurements as well as the biomarkers for metabolic, oxidative stress and cardiovascular risk are given in Table S3 (available online). The associations between SNP rs854566 and BMI, BMI z-score and weight were significant (*P*<0.001), although these associations lost their significance when the samples were divided into obese and normal-weight groups (data not shown). None of the oxidative stress biomarkers (ox-LDL, total antioxidant capacity, retinol, α-tocopherol and β-carotene) was weakly correlated with lactonase activity (*r*=0.172, *P*=0.001).
Effects of paraoxonase 1 SNP on paraoxonase 1 activities

All of the examined SNP showed a certain effect on PON1 activities, with the exception of rs757158, which is located in the 3'UTR (Table 4). The remaining SNP showed different association patterns that varied according to their position within the PON1 gene and according to their LD values. The LD analysis (Fig. 1) identified three blocks defined by high $r^2$ values between the SNP.

SNP located in the promoter region (rs854571, rs854572, rs854573, rs705382, rs757158 and rs13236941) were strongly associated with lactonase, arylesterase and diazoxonase activities, whereas their associations with paraoxonase activity were much weaker; only SNP rs854572 and rs757158 showed significant associations with paraoxonase activity. Block 3 corresponds to the SNP located in the promoter region.

The intronic SNP rs854566, which was negatively associated with obesity, showed significant effects on all of the tested PON1 activities. The presence of the protective allele (A) was highly associated with increased diazoxonase, lactonase and arylesterase activities and more weakly associated with decreased paraoxonase activity. This SNP was not included in any block due to its weak LD values with neighbouring SNP.

With respect to the missense mutations, rs662 (Q192R) showed the greatest effect on paraoxonase activity, which was highly significant ($\beta = 131.5$, 95% CI 115.0, 147.9; $P = 5.88 \times 10^{-12}$). This variant was also excluded from the LD blocks due to its weak LD values ($r^2 < 0.28$) with the other SNP. With respect to rs854560 (L55M), individuals with the 55LL genotype showed greater diazoxonase, lactonase, arylesterase and paraoxonase activities. The intronic SNP rs705378 shared the same associations as rs854560 due to

Table 3. Logistic regression analysis of the PON1 SNP and obesity under the additive model of inheritance

(Odds ratios and 95% confidence intervals)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele 1/allele 2</th>
<th>Obese 11</th>
<th>Normal weight 12</th>
<th>MAF 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs13236941</td>
<td>G/A</td>
<td>135</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>rs757158</td>
<td>G/A</td>
<td>75</td>
<td>79</td>
<td>0</td>
</tr>
<tr>
<td>rs705382</td>
<td>G/C</td>
<td>86</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td>rs854573</td>
<td>A/G</td>
<td>109</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>rs854572</td>
<td>G/C</td>
<td>70</td>
<td>81</td>
<td>0</td>
</tr>
<tr>
<td>rs854571</td>
<td>G/A</td>
<td>97</td>
<td>74</td>
<td>0</td>
</tr>
<tr>
<td>rs854566</td>
<td>G/A</td>
<td>126</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>rs13236941</td>
<td>C/A</td>
<td>69</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>rs854560</td>
<td>A(A)/T(Met)</td>
<td>60</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>rs3917527</td>
<td>A/G</td>
<td>165</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>rs662</td>
<td>G(C)/G(Arg)</td>
<td>90</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>rs854552</td>
<td>G/C</td>
<td>117</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>rs854551</td>
<td>G/A</td>
<td>132</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>rs7375590</td>
<td>G/A</td>
<td>161</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>rs854550</td>
<td>G/A</td>
<td>133</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

MAF, minor allele frequency; allele 1/2, major/minor allele.

*Adjusted for sex and age.

†From the logistic regression analysis.

Table 2. Correlations between serum paraoxonase 1 lactonase activity and metabolic, cardiovascular risk and oxidative stress biomarkers

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>-0.057</td>
<td>0.274</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.062</td>
<td>0.240</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>-0.073</td>
<td>0.162</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>0.005</td>
<td>0.923</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>-0.075</td>
<td>0.167</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>-0.077</td>
<td>0.154</td>
</tr>
<tr>
<td>Glucose (mg/l)</td>
<td>0.034</td>
<td>0.525</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>-0.075</td>
<td>0.160</td>
</tr>
<tr>
<td>QUICKI</td>
<td>-0.056</td>
<td>0.300</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>-0.073</td>
<td>0.174</td>
</tr>
<tr>
<td>Total cholesterol (mg/l)</td>
<td>0.248</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAG (mg/l)</td>
<td>-0.037</td>
<td>0.480</td>
</tr>
<tr>
<td>HDL-C (mg/l)</td>
<td>0.235</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/l)</td>
<td>0.131</td>
<td>0.013</td>
</tr>
<tr>
<td>ApoA1 (mg/l)</td>
<td>0.254</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB (mg/l)</td>
<td>0.078</td>
<td>0.138</td>
</tr>
<tr>
<td>FABP-4</td>
<td>-0.227</td>
<td>0.033</td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>0.079</td>
<td>0.137</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>-0.024</td>
<td>0.650</td>
</tr>
<tr>
<td>ox-LDL (mg/l)</td>
<td>-0.043</td>
<td>0.410</td>
</tr>
<tr>
<td>Retinol (µg/ml)</td>
<td>0.259</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>α-Tocopherol (µg/ml)</td>
<td>0.167</td>
<td>0.001</td>
</tr>
<tr>
<td>β-Carotene (µg/ml)</td>
<td>0.075</td>
<td>0.158</td>
</tr>
<tr>
<td>TAC (mmol/l)</td>
<td>-0.042</td>
<td>0.426</td>
</tr>
<tr>
<td>hsCRP (mg/l)</td>
<td>-0.006</td>
<td>0.915</td>
</tr>
<tr>
<td>MMP-9 (µg/l)</td>
<td>-0.063</td>
<td>0.230</td>
</tr>
<tr>
<td>MPO (µg/l)</td>
<td>-0.110</td>
<td>0.037</td>
</tr>
<tr>
<td>Active PAI-1 (µg/l)</td>
<td>0.012</td>
<td>0.826</td>
</tr>
<tr>
<td>Total PAI-1 (µg/l)</td>
<td>0.005</td>
<td>0.923</td>
</tr>
</tbody>
</table>

WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; QUICKI, quantitative insulin sensitivity check index; HOMA-IR, homeostasis model assessment for insulin resistance; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FABP-4, fatty acid-binding protein 4; ox-LDL, oxidised LDL; TAC, total antioxidant capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; hsCRP, high-sensitivity C-reactive protein; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; PAI-1, plasminogen activator inhibitor-1.
Table 4. Associations between the paraoxonase 1 (PON1) SNPs and PON1 activities

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs854572</th>
<th>rs705378</th>
<th>rs662</th>
<th>rs854552</th>
<th>PON1 activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactonase</td>
<td>1·30</td>
<td>0·63</td>
<td>0·69</td>
<td>0·67</td>
<td>0·98 ± 0·65</td>
</tr>
<tr>
<td>Arylesterase</td>
<td>1·08</td>
<td>1·09</td>
<td>0·98</td>
<td>1·09</td>
<td>0·98 ± 0·65</td>
</tr>
<tr>
<td>Diazoxonase</td>
<td>1·19</td>
<td>0·71</td>
<td>0·25</td>
<td>0·55</td>
<td>0·98 ± 0·65</td>
</tr>
<tr>
<td>Paraoxonase</td>
<td>0·98</td>
<td>0·97</td>
<td>0·52</td>
<td>0·60</td>
<td>0·98 ± 0·65</td>
</tr>
</tbody>
</table>

A. I. Rupérez et al.

Discussion

Many previous studies have investigated the association between PON1 genetic variations and the risk for certain diseases or changes in PON1 activities in the context of certain diseases; however, no study has considered both variables simultaneously. To date, this is the first study to demonstrate genetic associations between PON1 polymorphisms and prepubertal childhood obesity. Furthermore, the present study has used both intronic and exonic polymorphisms and four different substrates to measure serum PON1 activities. An association between obesity and the PON1 SNP rs854566 has not been reported previously, and we found that it was negatively associated with childhood obesity and also affected all four of the measurements of PON1 activities. Children carrying the minor allele of SNP rs854566 had higher lactonase, lactonase and arylesterase activities and lower paraoxonase activity. In contrast, the genetic variants Q192R and L55M were not significantly associated with childhood obesity, although they exerted no effects on PON1 activities. To the best of our knowledge, three previous studies have tested for genetic associations between the PON1 gene and obesity. One study conducted on adolescents has reported no association between obesity and the Q192R polymorphism. Another study conducted on obese Portuguese women has shown a positive association between the Q192R genotype and disease risk. Lastly, a recent study conducted on obese Mexican adults has identified a positive association between the 55LL genotype and obesity. However, these findings are inconsistent with those of the present study. It is likely that the complex nature of obesity, which depends on both genetic and environmental factors, is the cause for the unclear relationship between PON1 genetic variations, PON1 activities and childhood obesity. The importance of conducting the present study in prepubertal children should be noted as this population should have lower exposure levels to toxins (e.g., tobacco, alcohol and drugs) than adults and is much less likely to have chronic diseases that might affect PON1 activities.

PON1 activities were not found to be significantly altered in the obese group based on any of the metrics that we used. One study has reported lowered paraoxonase activity in obese adults, whereas no changes in paraoxonase and arylesterase activities have been observed in another study. The previous study that was conducted on children reported only measured paraoxonase and arylesterase activities, which were observed to be lower in fifty-nine obese children (11·95 (SEM 1·61) years) than in fifty-one normal-weight children (12·00 (SEM 3·91) years). However, the fact that this
shown in each cell; black cells correspond to r
was not. In this line, a recent study(27) has shown that aryles-
cholesterol and ApoA1 levels, whereas paraoxonase activity
diazoxonase activities were correlated with HDL-C, total
PON1 function (12). Similarly, lactonase, arylesterase and
onase activity, is more physiologically relevant for determining
and the levels of these biomarkers supports the hypothesis
the lack of any relationship between paraoxonase activity
PON1 in the development of obesity and CVD.
However, we did not observe significant correlations
PON1 activities, rather than the paraox-
onase activity, is more physiologically relevant for determining
PON1 function(12). Similarly, lactonase, arylesterase and
diaoxonase activities were correlated with HDL-C, total
cholesterol and ApoA1 levels, whereas paraoxonase activity
not. In this line, a recent study(27) has shown that ary-
esterase activity is a more powerful indicator of cardiovascular
risk than paraoxonase activity. Considering the present results
and the previously published evidence, we propose that laco-
ngase activity is a good indicator of PON1 function and that it
should be used in further studies investigating the role of
PON1 in the development of obesity and CVD.

Although the protective effects of SNP rs854566 on child-
hood obesity need to be validated, it should be noted that
this allele was associated with increased lactonase activity,
which itself was positively correlated with HDL-C, ApoA1, reti-
nol and α-tocopherol levels and negatively correlated with
fatty acid-binding protein 4 and MPO levels. These relation-
ships support a protective role for SNP rs854566 by linking
the antioxidant and anti-inflammatory functions of PON1
with protection from the development of obesity and CVD.
Indeed, it is known that HDL has anti-inflammatory and anti-
oxidant functions in vivo(2), and it is believed from previous
studies carried out in animal models that PON1 contributes
to these functions(2,28). However, although lactonase activity
is most probably responsible for the antioxidant effects
PON1(10,11,29), we did not observe significant correlations
between ox-LDL levels. The present findings are consistent with
those of Carlson et al.(29), who reported no association
between ox-LDL levels and aryesterase activity.
PON1 is expressed in the interstitial space of adipose
tissue(3). Mature adipocytes have been reported to express
cluster of differentiation 36 (CD36), which can recognise
and bind to ox-LDL; these molecules are then endocytosed(31)
and can modulate adipocyte tissue mass by stimulating adipocyte pro-
liferation and differentiation(32). However, the function of PON1
in adipose tissue is unknown. Although we did not identify any
relationship between PON1 activities and ox-LDL levels in the
plasma, more studies should be performed to describe the
effect of PON1 on ox-LDL levels in adipose tissue and to deter-
mine whether PON1 plays a role in adipocyte differentiation
and proliferation during the development of obesity.

Several limitations in the present study should be noted. The
negative association between the PON1 SNP rs854566
and childhood obesity identified in the present study requires further validation in a larger population. Another potential limitation is that the measurements were only carried out under fasting conditions at a single time point. We, therefore, were unable to determine the variability and prognostic value of changing levels over time and of the impact of dietary or therapeutic interventions on serum PON1 activities.

Conclusion
The present results suggest a protective role for SNP rs854566 with respect to obesity. This SNP also exhibits strong associations with serum PON1 activities, namely increased diazoxonase, lactonase and arylesterase activities. Despite these results, serum PON1 activities were not found to be significantly different in prepubertal obese children. Nevertheless, we demonstrate that lactonase activity is a reliable indicator of PON1 function and should be used in future studies investigating the role of PON1 in the development of obesity and CVD. Future in vivo and in vitro studies on the function of PON1 in adipose tissue may help us to identify potential antioxidant and protective roles for PON1 in the protection against the development of obesity and its co-morbidities.

Supplementary material
To view supplementary material for this article, please visit [link to supplementary material].

Acknowledgements
The authors thank the children and parents who participated in the study. The present study was funded by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I + D + I), the Instituto de Salud Carlos III-Fondo de Investigación Sanitaria (FIS) (PI 020826, PI051968), Redes temáticas de investigación cooperativa (RETI) (Red SAMID RD08/0072/0028), Junta de Andalucía, the Consejería de Innovación y Ciencia (P06-CTS 2203), the Consejería de Salud (0098/2005) and the Ministerio de Ciencia e Innovación, Campus of Excelencia Internacional de Granada. GREIB-CTS461. The authors’ contributions were as follows: F. G., A. G. and C. M. A. conceptualised and designed the study; M. G.-C., R. L., R. T. and R. C. were involved in the data and sample collection; J. O. carried out the biomarker analysis; A. I. R. and O. L.-G. conducted the enzymatic analysis; A. I. R. performed the statistical analysis; A. I. R. and C. M. A. wrote the manuscript.

The authors declare no conflicts of interest that could be perceived as affecting the impartiality of this research.

References


