Association between alcohol consumption and serum paraoxonase and arylesterase activities: a cross-sectional study within the Bavarian population

Carolina Schwedhelm1,2, Katharina Nimptsch1*, Achim Bub3, Tobias Pischon1 and Jakob Linseisen4
1Molecular Epidemiology Research Group, Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Robert-Rössle-Straße 10, 13125 Berlin, Germany
2Berlin School of Public Health, Charité Universitätsmedizin, Seestrasse 73, 13347 Berlin, Germany
3Department of Physiology and Biochemistry of Nutrition, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Heid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany
4Helmholtz Centre Munich, Institute of Epidemiology 2, Ingolstädt Landstrasse 1, 85764 Neuberger, Germany

(Submitted 20 May 2015 – Final revision received 20 October 2015 – Accepted 12 November 2015)

Abstract
High alcohol consumption is an important risk factor for chronic disease and liver degeneration. Paraoxonase (PON1) and arylesterase (AE) are functions of the enzyme paraoxonase, which is synthesised by the liver. Paraoxonase circulates in plasma bound to HDL and hydrolyses lipid peroxides, protecting lipoproteins against oxidative modification. It has been shown that excessive alcohol consumption leads to a reduction of serum PON1 and AE activities; however, studies investigating the association with low and moderate alcohol consumption are scarce. We investigated the cross-sectional association between alcohol consumption and serum activities of PON1 and AE using data from the population-based Bavarian Food Consumption Survey II survey. PON1 and AE activities were quantified in serum samples of 566 male and female study participants (aged 18–80 years), and dietary intake including alcohol consumption was estimated from three 24-h dietary recalls. The association between alcohol consumption and PON1 and AE activities was analysed using linear regression, adjusted for age, sex and socio-economic status. There was no strong association between alcohol consumption and enzymatic activities of PON1 and AE in the Bavarian population. PON1 activity was seen to be lowest in non-drinkers (0 g/d) and highest in people who consumed 15–30 g of alcohol/d. AE activity increased across alcohol consumption categories, with a mean maximum difference of 14 U/ml (Pfor linear trend 0.04). These associations were attenuated after adjustment for blood concentrations of HDL. The results of this study do not support the hypothesis that alcohol consumption is related to important alterations in PON1 and AE activities.

Key words: Paraoxonase enzyme: Paraoxonase activity: Arylesterase activity: Alcohol consumption

Paraoxonase is an enzyme mainly synthesised in the liver that catalyses the hydrolysis of organophosphates such as pesticides, neurotoxins and arylesterers(1,2). It is widely distributed among tissues in the body, with its higher activity in blood and liver(3,4). Paraoxonase has four known activities: paraoxonase (PON1) (carrying the same name as the enzyme itself), arylesterase (AE), lactonase and dyazoxonase – which are all functions of a single enzyme(5–7) and depend on a substrate-dependent activity polymorphism of the PON1 gene(1). PON1 enzyme circulates in plasma bound to HDL and hydrolyses lipid peroxides(2,5,8,9), protecting lipoproteins against oxidative modification(3). PON1 enzyme also protects against the toxicity of lipopolysaccharides (bacterial endotoxins), and it can possibly prevent or reduce the release of cytokines(9). PON1 activity varies widely among individuals, up to 40-fold(10,11), and it is influenced by genetic, developmental, environmental and pathologic determinants(1,12–14). Low PON1 and AE activities have been associated with a variety of health outcomes(15–17). For instance, low PON1 activity has been suggested as a predictor for coronary events(15), and both low PON1 and AE activities have been associated with an increased risk of vascular dementia(16).

Serum PON1 and AE activities have been suggested as useful markers of liver function status(1,7). Because of the liver damage caused as a consequence of heavy alcohol drinking, it has been hypothesised that excessive alcohol intake would lead to a reduction of serum PON1 and AE activities, which has also been demonstrated in a few studies(2,4,11,18). A case–control study with 328 persons with chronic alcohol dependency and 368 healthy individuals investigated the relationship between PON1 activity and liver damage, where PON1 activity was decreased in alcohol abusers(17). Similarly, a small case–control study described lower AE activity in chronic alcoholic hepatitis patients than in healthy

Abbreviations: AE, arylesterase; BVSII, Bavarian Food Consumption Survey II; PON1, paraoxonase.

* Corresponding author: Dr K. Nimptsch, fax +49 30 9406 4576, email katharina.nimptsch@mdc-berlin.de
control individuals. However, studies investigating light and moderate alcohol consumption in relation to PON1 and AE activities in the general population are scarce and results have been inconsistent. Therefore, we aimed to investigate the cross-sectional association between alcohol consumption and serum activities of PON1 and AE using data from the population-based Bavarian Food Consumption Survey II (BVSII), in which dietary intake was assessed by three 24-h dietary recalls. Furthermore, we performed analyses with and without adjustment for circulating HDL in order to investigate its possible role as a mediator.

Methods
The BVSII is a randomly sampled cross-sectional study of 1050 individuals aged 13–80 years old from Bavaria, Germany. Recruitment took place in 2002–2003. The study protocol comprised a computer-aided personal interview, three 24-h dietary recalls by telephone, as well as blood sampling and anthropometric measures. The overall response rate in the BVSII was 70.9%. All adult participants who had participated in the personal interview and at least one dietary recall (n = 879) were invited to the nearest public health office for blood sampling and anthropometrical measurements. This invitation was followed by 65% (n = 568) of eligible study participants. All participants gave their written informed consent. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were ethically approved by the Bavarian Ministry of Health. The study population for the present analysis consisted of BVSII participants with full information on PON1 and AE activities and with at least two 24-h dietary recalls (mean 2.99 ± 0.07). In all, two participants were excluded because of missing PON1 and AE data, leaving a total of 566 BVSII participants.

Laboratory methods
Venous blood was extracted into serum or EDTA tubes and chilled at 4°C and then processed within 3 h by centrifugation to separate the serum from the blood cells. The samples were kept cold for a maximum of 1 d for transportation and aliquoting and then stored at −80°C until analysis.

Enzymatic activities of PON1 and AE were determined spectrophotometrically in serum samples of the study participants under a controlled temperature of 25°C and a pH of 8, as previously described. Enzymatic activity of PON1 was measured using paraoxon as substrate, and the reaction was recorded at 405 nm. One unit of PON1 activity equals 1 nmol of P-nitrophenol formed/min per ml. The activity of AE was measured using phenylacetate as substrate, and the reaction was monitored at 270 nm. One unit of AE activity equals 1 µmol of phenylacetate hydrolyzed/min per ml. Spontaneous hydrolysis was corrected using blanks without serum and subtracting this activity from the serum analysis samples.

Alcohol consumption assessment
Alcohol consumption was assessed with 24-h dietary recalls (three recalls, two in weekdays and one in a weekend day) conducted by trained interviewers by telephone using the EPIC-SOFT software, program developed to standardize 24-h diet recall interviews from the ten countries participating in a large European multi-centre study. The intake of grams of pure alcohol on each recalled day were obtained through the participants’ type and quantity (number of servings consumed) of alcoholic beverages consumed, which were then multiplied by the ethanol content in each portion of beverage type based on the German Nutrient Database (BLS II.3). The mean pure alcohol consumption per day was then obtained by a weighted conversion of the weekday and weekend 24-h recalls to resemble a full week.

Statistical analysis
To compare participants’ characteristics across alcohol consumption categories, we used generalised linear models for the continuous, non-dietary variables, χ² test for the categorical variables and Kruskal–Wallis test for non-parametrical data for the dietary variables. For the purpose of this study, we defined drinking categories as follows: non-drinkers (0 g/d), low alcohol consumption (0–1.5 g/d or up to 2 drinks/week), low-medium alcohol consumption (1.5–15 g/d or up to 1 drink/d), medium alcohol consumption (15–30 g/d or up to 2 drinks/d) and high alcohol consumption (>30 g/d or >2 drinks/d).

The association between alcohol consumption and PON1 and AE enzymatic activities was investigated using multivariable linear regression models with robust variance. Alcohol consumption was analysed as a categorical variable, as well as continuously. Trends across alcohol categories were calculated by treating the middle value for each alcohol category (median for high alcohol consumption group) as a continuous variable and examining significance using Wald’s test. If the categorical analysis was indicative of a non-linear relationship, we additionally examined quadratic trends using likelihood ratio tests.

We examined the impact of several potentially confounding factors in our analysis, including age, sex, socio-economic status, sports activity, body fatness and dietary intake. Covariates were selected according to clinical relevance and univariate significance testing based on a previously described model-fitting procedure. The final multivariable models were adjusted for age, sex and socio-economic status. In separate models, we additionally adjusted for HDL, as paraoxonase enzyme is mainly transported bound to HDL. As a sensitivity analysis, we ran all models excluding participants with chronic diseases that have been related to PON1 or AE activities in previous studies (arterial hypertension, history of myocardial infarction or stroke. CVD was considered as individuals having at least one of the following: mean arterial hypertension, history of myocardial infarction or stroke. Furthermore, we ran models with exclusion of heavy drinkers (>70 g ethanol/d, n 9). Moreover, we tested for statistical interaction in the association between alcohol consumption and PON1 and AE by sex, age, smoking status and obesity (BMI ≤30 kg/m²) using cross-product terms.

Results
Characteristics of BVSII study participants by alcohol consumption categories are shown in Table 1. Comparing the
Table 1. Characteristics by alcohol consumption groups (Frequencies† and percentages; mean values‡ and standard deviations; medians§ and interquartile ranges (IQR))

<table>
<thead>
<tr>
<th>Alcohol consumption (g/d)</th>
<th>0 (n 92)</th>
<th>0.1–5 (n 161)</th>
<th>5.1–15 (n 136)</th>
<th>15.1–30 (n 94)</th>
<th>&gt;30 (n 83)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>30.0 32.6</td>
<td>39.0 24.2</td>
<td>47.0 34.6</td>
<td>57.0 60.6</td>
<td>690 831</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>39.0 42.4</td>
<td>98.0 60.9</td>
<td>78.0 57.4</td>
<td>37.0 39.4</td>
<td>410 49.4</td>
<td>0.001*</td>
</tr>
<tr>
<td>Former</td>
<td>22.0 23.9</td>
<td>35.0 21.7</td>
<td>30.0 22.1</td>
<td>27.0 28.7</td>
<td>190 22.9</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>31.0 33.7</td>
<td>28.0 17.4</td>
<td>28.0 20.6</td>
<td>30.0 31.9</td>
<td>230 27.7</td>
<td></td>
</tr>
<tr>
<td>SES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002*</td>
</tr>
<tr>
<td>1 (lowest)§</td>
<td>24.0 26.1</td>
<td>25.0 15.5</td>
<td>15.0 11.0</td>
<td>11.0 11.7</td>
<td>30 36</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23.0 25.0</td>
<td>45.0 28.0</td>
<td>36.0 26.5</td>
<td>13.0 13.8</td>
<td>200 24.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25.0 27.2</td>
<td>48.0 28.6</td>
<td>48.0 35.3</td>
<td>31.0 33.0</td>
<td>260 31.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15.0 16.3</td>
<td>29.0 17.4</td>
<td>29.0 21.3</td>
<td>25.0 26.6</td>
<td>230 27.7</td>
<td></td>
</tr>
<tr>
<td>5 (highest)</td>
<td>5.0 5.4</td>
<td>17.0 10.6</td>
<td>8.0 5.9</td>
<td>14.0 14.9</td>
<td>110 13.3</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.5 ±6.1</td>
<td>47.3 ±4.4</td>
<td>48.0 ±5.5</td>
<td>51.2 ±14.7</td>
<td>506 ±15.7</td>
<td>0.001*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ±6.6</td>
<td>26.5 ±4.5</td>
<td>26.5 ±5.1</td>
<td>263 ±42</td>
<td>274 ±43</td>
<td>0.097</td>
</tr>
<tr>
<td>Physical activity (MET·h/d)</td>
<td>1.9 ±3.1</td>
<td>2.2 ±4.0</td>
<td>2.0 ±3.1</td>
<td>2.5 ±3.3</td>
<td>21 ±3.1</td>
<td>0.070</td>
</tr>
<tr>
<td>Plasma HDL (mmol/l)†‡</td>
<td>1.14 ±0.21</td>
<td>1.22 ±0.20</td>
<td>1.21 ±0.22</td>
<td>1.21 ±0.21</td>
<td>1.22 ±0.19</td>
<td>0.005</td>
</tr>
<tr>
<td>Median ±IQR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ/d)</td>
<td>6535 ±5389–8268</td>
<td>7422 ±6054–8987</td>
<td>7962 ±6406–9381</td>
<td>8899 ±7364–10 652</td>
<td>9845 ±8590–11 380</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>1562 ±1288–1760</td>
<td>1774 ±1447–2148</td>
<td>1903 ±1531–2242</td>
<td>2127 ±1760–2546</td>
<td>2353 ±2059–2720</td>
<td></td>
</tr>
</tbody>
</table>

SES, socio-economic status; MET, metabolic equivalent task.
*P < 0.05.
† P values were obtained by χ² test.
‡ P values were obtained by linear trend derived from generalised linear models.
§ P values were obtained by trend based on Kruskal–Wallis test.
upper alcohol consumption categories with the lower categories, study participants were of older age, more likely to be male, less likely to belong to the lowest socio-economic status and had a higher energy intake. BMI and physical activity did not differ substantially by alcohol consumption categories. Circulating HDL-cholesterol was the lowest in non-drinkers and of similar magnitude across the other alcohol consumption categories.

**Regression models**

We did not observe strong differences in PON1 activity across alcohol consumption categories (Table 2). Mean PON1 activities across alcohol consumption categories were suggestive of a non-linear association, with lowest PON1 activities observed in non-drinkers and highest PON1 activities in the medium alcohol consumption category. However, tests for quadratic trend were non-significant. The results were not substantially different between the crude model, the multivariable adjusted model and the model that additionally adjusted for HDL. Results were also not substantially changed after exclusion of study participants with chronic diseases or heavy drinkers (Fig. 1). We observed no statistically significant interactions by sex, age, smoking status or obesity (data not shown).

AE activity increased across alcohol consumption categories, with a borderline statistically significant linear trend \((P=0.04)\). On a continuous scale in the multivariable model (adjusted for sex, age and socio-economic status), about 1 g higher consumption of alcohol was associated with 0.26 U/ml higher activity of AE (95% CI =0.02, 0.49) (Table 2). After adjusting for HDL, the main transport protein of paraoxonase enzyme, the association was attenuated. In sensitivity analyses excluding participants with chronic diseases and heavy alcohol use, mean concentrations and continuous effect estimates were similar to the main analysis, although no statistically significant linear trends were observed (Fig. 2). We observed no statistically significant interactions by sex, age, smoking status or obesity (data not shown).

**Discussion**

In this study, we found a weak, non-significant, non-linear association between alcohol consumption and serum PON1 activity, with lowest activities in non-drinkers and highest activities in people with medium alcohol consumption. In addition, we observed a borderline statistically significant positive association between alcohol consumption and AE activity, which was, however, attenuated and no longer statistically significant after adjusting for HDL. These results suggest that the positive association between alcohol consumption and AE is partly explained by HDL-cholesterol, paraoxonase’s main transport protein.

So far, observational studies on PON1 and AE activities have been small and investigated the association with larger quantities of alcohol consumption, usually among people with alcohol dependency, rather than intake in the general population. The present study is one of the few population-based studies investigating alcohol consumption in relation to PON1 and AE activities. Our observation of no statistically significant association between alcohol consumption and PON1 activity is generally supported by two previously conducted population-based studies: in a study of 918 individuals in France, alcohol drinking was not associated with PON1 activity. However, alcohol drinking was analysed as a binary variable (yes/no)\(^{(20)}\).

In a second population-based study on 368 individuals in Spain, no linear association between alcohol consumption and PON1 activity was observed\(^{(19)}\). Furthermore, in a small cross-sectional study, light drinkers (\(n = 12\), 1–3 drinks/d in the past 6 months or longer, equivalent to low-medium/medium intake in our study) had higher PON1 activity than abstainers (\(n = 12\), <1 drink/d in the past 6 months or longer)\(^{(49)}\), which is in line with our observations of lower PON1 activity in non-drinkers than in the medium alcohol consumption category. With regard to intervention studies with alcohol, our PON1 activity findings are consistent with a study of fourteen healthy male individuals\(^{(28)}\), in whom intake of red wine for 3 weeks (40 g of ethanol/d or about 4 drinks/d) did not trigger a significant change in PON1 activity. In contrast, in two further intervention studies\(^{(29,30)}\), PON1 activity was higher after an intervention of about 40 g of alcohol/d (for 3–4 weeks), which is equivalent to high alcohol consumption in our study.

Our finding of a weak positive association between alcohol consumption and AE activity is in line with the population-based study from France, in which alcohol consumption was analysed as a binary exposure\(^{(20)}\). Contrary to our observation, a decrease in AE activity was observed after a red wine intervention (about 3–4 drinks/d) for 3 weeks in a study by Sarandol et al.\(^{(28)}\). To our understanding, these studies did not adjust for HDL, and we are not aware of any other population-based or intervention study investigating low or moderate alcohol consumption in relation to AE activity.

**Strengths and limitations**

The BVSII is the first population-based study that measured PON1 and AE activity in Germans, with detailed characterisation of lifestyle, anthropometric and medical information, and a wide array of biomarkers including HDL available. A strength of the present study is that alcohol consumption was assessed through the application of three interactive, standardised 24-h dietary recalls. A main advantage of this method is that it is a more precise, quantitative dietary assessment method in comparison with other tools such as FFQ\(^{(31)}\). It is also an advantage that the 24-h dietary recalls were conducted relatively close in time (up to 6 weeks) to the blood draw. However, similar to other self-reported methods, assessment of alcohol consumption through 24-h recalls is prone to errors. The dietary assessment tool used in this study (EPIC-SOFT) has been validated. In a study including 127 men and women, two non-consecutive 24-h dietary recalls were compared against a 5-d estimated dietary record, and a fairly good Spearman’s correlation coefficient of 0.60 was observed for alcohol intake\(^{(32)}\). However, alcohol is an episodically consumed food group with substantial weekly\(^{(33)}\) and seasonal variation\(^{(34)}\), complicating measurement of the usual intake. A general limitation of the 24-h dietary recall is that it is dependent on the memory of the participant. Thus, it is impossible to rule out...
<table>
<thead>
<tr>
<th>Alcohol consumption (g/d)</th>
<th>0 (n 92)</th>
<th>0·1–5 (n 161)</th>
<th>5·1–15 (n 136)</th>
<th>15·1–30 (n 94)</th>
<th>&gt;30 (n 83)</th>
<th>P for quadratic trend</th>
<th>Continuous model‡</th>
<th>β</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 (U/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model†</td>
<td>105</td>
<td>94, 117</td>
<td>−4·5</td>
<td>110</td>
<td>101, 119</td>
<td>Ref.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable model¶</td>
<td>107</td>
<td>96, 118</td>
<td>−0·9</td>
<td>119</td>
<td>104, 134</td>
<td>+10·2</td>
<td>0·39</td>
<td>0·54</td>
<td>−0·21, 0·52</td>
</tr>
<tr>
<td>Multivariable model plus HDL¶</td>
<td>107</td>
<td>97, 118</td>
<td>−1·8</td>
<td>119</td>
<td>104, 133</td>
<td>+9·2</td>
<td>0·69</td>
<td>0·54</td>
<td>−0·13, 0·45</td>
</tr>
<tr>
<td>AE (U/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model†</td>
<td>160</td>
<td>152, 168</td>
<td>−1·8</td>
<td>163</td>
<td>156, 170</td>
<td>Ref.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable model¶</td>
<td>158</td>
<td>150, 166</td>
<td>−0·6</td>
<td>159</td>
<td>152, 166</td>
<td>Ref.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable model plus HDL¶</td>
<td>163</td>
<td>155, 171</td>
<td>+1·2</td>
<td>163</td>
<td>155, 171</td>
<td>+1·2</td>
<td>0·14</td>
<td>–</td>
<td>0·16, 0·36</td>
</tr>
</tbody>
</table>

Ref., referent values.
† Mixed method linear regression models were used.
‡ Continuous model (alcohol consumption in grams of pure alcohol per d).
§ P<0·05.
¶ P for quadratic trend was calculated by using the middle values (median for highest category) of each alcohol consumption category and was treated as a continuous variable.
|| P for quadratic trend was obtained through log-likelihood ratio testing to examine quadratic trends.
†† Multivariable model was adjusted for sex, age and socio-economic status.
For instance, it has been suggested that persons with chronic urinal glucuronide would be of value and is a limitation of validity of reported intake. In the case of alcohol consumption, indicators of dietary intake are an objective tool to assess the trends.

*of intentional misclassification towards the null. We also cannot discard the possibility of intentional misclassification from PON1 and AE activities, which could potentially bias differential misclassification across alcohol consumption groups. This would likely be a non-linear relationship between alcohol consumption and AE activity. As the evidence on this topic is still scarce, more large-scale population-based longitudinal studies with multiple exposure measurements are warranted, which would allow elucidating temporal associations. A better understanding of the behaviour of serum PON1 and AE activities in response to alcohol intake could be useful for future epidemiological studies relating PON1 and AE activities to physiological conditions and diseases and at later stages of research and evidence, for public health recommendations regarding the management and prevention of physiological disorders.

**Conclusion**

In conclusion, our study does not support the hypothesis that alcohol consumption is related to important PON1 and AE activity alterations. The observed association between alcohol consumption and PON1 activity was weak and non-linear (lowest activity in non-drinkers, highest in moderate drinkers), whereas a weak positive linear association was observed between alcohol consumption and AE activity. As the evidence on this topic is still scarce, more large-scale population-based longitudinal studies with multiple exposure measurements are warranted, which would allow elucidating temporal associations. A better understanding of the behaviour of serum PON1 and AE activities in response to alcohol intake could be useful for future epidemiological studies relating PON1 and AE activities to physiological conditions and diseases and at later stages of research and evidence, for public health recommendations regarding the management and prevention of physiological disorders.

**Acknowledgements**

The authors acknowledge the cooperation of all study participants. The authors thank Georg Karg, Kurt Gedrich and Stefanie Himmerich for their major contribution in the setup and conduct of the study.

This work was supported by funds of the Bavarian Ministry of Environment, Health and Consumer Protection and the Kurt-Eberhard-Bode-Stiftung. The funder had no role in the design, analysis or writing of this article.

C. S. carried out the study, and analysed and interpreted the data. K. N. added substantial contributions to the conception and design, made the data availability possible and together with T. P. revised the article critically for important intellectual content. J. L. was responsible for the concept and design of the BVSI study and critically appraised the manuscript. A. B. was responsible for the measurement of PON1 and AE and critically appraised the manuscript. All authors gave their final approval of the version to be published.

The authors declare that there are no conflicts of interest.

**References**


