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

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Abbreviations: PON1, paraoxonase; AE, arylesterase; HDL, high-density lipoprotein; BVSII, Bavarian Food Consumption Survey II; BMI, body mass index; CI, confidence interval; SD, standard deviation; IQR, interquartile range.

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 synthesized 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 BVSII 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-hour dietary recalls. The association between alcohol consumption and PON1 and AE activities was analysed using linear regression, adjusted for age, sex, and socioeconomic 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/day) and highest in people who consumed 15.1-30 grams of alcohol per day. AE activity increased across alcohol consumption categories with a mean maximum difference of 14 U/mL (p for 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.

INTRODUCTION

Paraoxonase is an enzyme mainly synthesized in the liver that catalyses the hydrolysis of organophosphates like pesticides, neurotoxins and arylesters^(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^(3; 5-7) and depend on a substrate-dependent activity polymorphism of the *PON1* gene⁽¹⁾. Paraoxonase enzyme circulates in plasma bound to HDL (high-density lipoprotein) and hydrolyses lipid peroxides^(2; 5; 8; 9), protecting lipoproteins against oxidative modification⁽¹⁾. PON1 also protects against the toxicity of lipopolysaccharides (bacterial endotoxins) and can possibly prevent or reduce the release of cytokines⁽⁹⁾. PON1 activity varies widely among individuals, up to 40 fold^(10; 11) and 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 has been suggested as a predictor for coronary events⁽¹⁵⁾ and both low PON1 and AE have been associated with 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 hypothesized 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 and liver damage, where PON1 activity was decreased in alcohol abusers⁽²⁾. Similarly, a small case-control study described lower AE activity in chronic alcoholic hepatitis patients than in healthy control individuals⁽¹⁸⁾. 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^(19; 20). 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 (BVS II), in which dietary intake was assessed by three 24-hour 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 Bavarian Food Consumption Survey II (BVS II) 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 of a computer-aided personal interview, three 24-hour 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⁽²¹⁾. 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-hour dietary recalls (mean 2.99, SD 0.07). Two participants were excluded due to 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 three hours by centrifugation to separate the serum from the blood cells. The samples were kept cold for a maximum of 1 day 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⁽²²⁾. Enzymatic activity of PON1 was measured using paraoxon as substrate and the reaction was recorded at 405 nm. 1 U 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. 1 U of AE activity equals 1 µmol of phenylacetate hydrolysed/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-hour dietary recalls (3 recalls, two in weekdays and one in a weekend day) conducted by trained interviewers by telephone using the EPIC-Soft software^(23; 24). 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)⁽²⁵⁾. The mean pure alcohol consumption per day was then obtained by a weighed conversion of the weekday and weekend 24-hour recalls to resemble a full week.

Statistical analysis

To compare participants' characteristics across alcohol consumption categories, we used generalized linear models for the continuous, non-dietary variables, chi-square 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 followed: non-drinkers (0 g/day), low alcohol consumption (0.1-5 g/day or up to 2 drinks/week), low-medium alcohol consumption (5.1-15 g/day or up to 1 drink/day), medium alcohol consumption (15.1-30 g/day or up to 2 drinks/day) and high alcohol consumption (>30g/day or more than 2 drinks/day).

The association between alcohol consumption and PON1 and AE enzymatic activities was investigated using multivariable linear regression models with robust variance⁽²⁶⁾. Alcohol consumption was analysed as 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, socioeconomic status, sports activity, body fatness and dietary intake. Covariables were selected according to clinical relevance and univariate significance testing based on a previously-described model-fitting procedure⁽²⁷⁾. The final multivariable models were adjusted for age, sex and socioeconomic status. In separate models, we additionally adjusted for HDL, since 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 in previous studies^(1; 12-14) (type 2 diabetes, n=40; asthma, n=34; cardiovascular diseases, n=136; inflammatory bowel disease, n=7). Cardiovascular disease was considered as individuals having at least one of the following: arterial hypertension, history of myocardial infarction, or stroke. Furthermore, we ran models with exclusion of heavy drinkers (>70g ethanol/day, 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 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 socioeconomic status and had a higher caloric intake. Body-mass-index (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 (Figure 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 socioeconomic status), 1 gram 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 (Figure 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 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 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 388 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 per day in the past 6 months or longer, equivalent to low-medium/medium intake in our study) had higher PON1 activity than abstainers (n=12, less than 1 drink per day in the past 6 months or longer)⁽⁴⁾, which is in line with our observations of lower PON1 in non-drinkers than in the medium alcohol consumption category. Regarding intervention studies with alcohol, our PON1 findings are consistent with a study of 14 healthy male individuals⁽²⁸⁾, in whom intake of red wine for three weeks (40 grams of ethanol per day or around 4 drinks per day) 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 grams of alcohol per day (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 (around 3-4 drinks/day) for three 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 in Germans, with detailed characterization 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, standardized 24-hour 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 food frequency questionnaires⁽³¹⁾. It is also an advantage that the 24-hour dietary recalls were conducted relatively close in time (up to six weeks) to the blood draw. However, similar to other self-reported methods, assessment of alcohol consumption through 24-hour 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-hour dietary recalls were compared against a 5-day estimated dietary record and a fairly good Spearman 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-hour dietary recall is that it is dependent on the memory of the participant. Thus, it is impossible to rule out under- or over-reporting and a resulting misclassification in the alcohol consumption groups. This would likely be non-differential misclassification due to an independent behaviour from PON1 and AE activities, which could potentially bias the results towards the null. We also cannot discard the possibility of intentional misclassification as non-drinkers (response bias)⁽³⁵⁾. For instance, it has been suggested that persons with chronic alcohol dependency and women during pregnancy are more likely to underreport alcohol consumption⁽³⁶⁾. Biochemical indicators of dietary intake are an objective tool to assess validity of reported intake. In the case of alcohol consumption, urinary ethyl glucuronide would be of value and is a limitation of this study that such data was not available. Furthermore, it is a general limitation of our study that the data collection for the BVSII study took place 12-13 years ago, although we would expect to obtain similar results in a present-day analysis. Another limitation is that there is no universal definition for low, medium and high alcohol consumption, which may complicate comparability between studies. Finally, statistical power in the present analysis was limited and therefore we cannot exclude that we missed to detect small associations.

CONCLUSION

In conclusion, our study does not support the hypothesis that alcohol consumption is related to important PON1 and AE activities alterations. The observed association between alcohol consumption and PON1 activity was weak and suggestive 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 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.

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CONFLICT OF INTEREST

None.

AUTHORSHIP

Carolina Schwedhelm carried the study out, analysed and interpreted the data. Katharina Nimptsch added substantial contributions to the conception and design, made the data availability possible, and together with Tobias Pischon revised the article critically for important intellectual content.

Jakob Linseisen was responsible for the concept and design of the BVSII and critically appraised the manuscript. Achim Bub 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.

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TABLES

TABLE 1: CHARACTERISTICS BY ALCOHOL CONSUMPTION GROUPS (FREQUENCIES AND PERCENTAGES; MEANS AND STANDARD DEVIATIONS; MEDIANS AND INTERQUARTILE RANGES)

	Alcohol consumption (g/day)					<i>p</i> value
	0 n=92	0.1-5 n=161	5.1-15 n=136	15.1-30 n=94	> 30 n=83	
	Frequency n (%)†					
Sex (male)	30.0 (32.6)	39.0 (24.2)	47.0 (34.6)	57 (60.6)	69.0 (83.1)	<0.001*
Smoking status						0.01*
Never	39.0 (42.4)	98.0 (60.9)	78.0 (57.4)	3.70 (39.4)	41.0 (49.4)	
Former	22.0 (23.9)	35.0 (21.7)	30.0 (22.1)	27.0 (28.7)	19.0 (22.9)	
Current	31.0 (33.7)	28.0 (17.4)	28.0 (20.6)	30.0 (31.9)	23.0 (27.7)	
SES						0.002*
1 (lowest)	24.0 (26.1)	25.0 (15.5)	15.0 (11.0)	11.0 (11.7)	3.0 (3.6)	
2	23.0 (25.0)	45.0 (28.0)	36.0 (26.5)	13.0 (13.8)	20.0 (24.1)	
3	25.0 (27.2)	46.0 (28.6)	48.0 (35.3)	31.0 (33.0)	26.0 (31.3)	
4	15.0 (16.3)	28.0 (17.4)	29.0 (21.3)	25.0 (26.6)	23.0 (27.7)	
5 (highest)	5.0 (5.4)	17.0 (10.6)	8.0 (5.9)	14.0 (14.9)	11.0 (13.3)	
	Mean (SD)‡					
Age (years)	46.5 (16.1)	47.3 (14.4)	48.0 (15.6)	51.2 (14.7)	50.6 (15.7)	0.01*
BMI (kg/m ²)	27.3 (6.6)	26.5 (4.5)	26.5 (5.1)	26.3 (4.2)	27.4 (4.3)	0.97
Physical activity (MET*H/day)	1.9 (3.1)	2.2 (4.0)	2.0 (3.1)	2.5 (3.3)	2.1 (3.1)	0.70
Plasma HDL (mg/dL) ‡	44.0 (8.2)	47.0 (7.9)	46.8 (8.4)	46.7 (8.2)	47.0 (7.4)	0.05
	Median (IQR)§					
Caloric intake (kcal/d)	1562 (1288- 1976)	1774 (1447- 2148)	1903 (1531- 2242)	2127 (1760- 2546)	2353 (2053- 2720)	<0.001*

BMI indicates body mass index; SES, socioeconomic status; SD, standard deviation; IQR, interquartile range.

† Frequency values with percentage are shown for covariates. *p* values were obtained by Chi-square test.

‡ Mean values with standard deviation are shown for covariates. *p* values were obtained by linear trend derived from generalized linear models.

§ Median values with interquartile range are shown for covariates. *p* values were obtained by trend based on Kruskal-Wallis test.

* *p* < 0.05

TABLE 2: LEAST SQUARES MEAN (95% CI) CONCENTRATIONS OF PON1 AND AE ACTIVITIES ACCORDING TO ALCOHOL CONSUMPTION CATEGORIES AMONG ADULTS IN THE BVSII†.

	Alcohol consumption (g/day)					<i>P</i> <i>trend</i> §	<i>P</i> for <i>quadratic</i> <i>trend</i>	Continuous model‡
	0 n=92	0.1-5 n=161	5.1-15 n=136	15.1-30 n=94	> 30 n=83			
PON1 (U/mL)	Mean (95% CI) % difference							β (95%CI)
Crude model	105 (94;117) -4.5%	110 (101;119) Ref.	109 (99;120) -0.9%	121 (106;135) +10.0%	112 (99;125) +1.8%	0.31	0.46	0.19 (-0.09;0.47)
Multivariable model¶	105 (92;117) -2.9%	108 (98;118) Ref.	107 (96;118) -0.9%	119 (104;134) +10.2%	110 (97;124) +1.9%	0.39	0.54	0.21 (-0.11;0.52)
Multivariable model plus HDL¶	108 (95;121) -0.9%	109 (99;119) Ref.	107 (97;118) -1.8%	119 (104;133) +9.2%	108 (95;122) -0.9%	0.69	0.54	0.13 (-0.19;0.45)
AE (U/mL)	Mean (95% CI) % difference							β (95%CI)
Crude model	160 (152;168) -1.8%	163 (156;170) Ref.	165 (158;173) +1.2%	165 (157;172) +1.2%	171 (161;181) +4.9%	0.14	-	0.16 (-0.04;0.36)
Multivariable model¶	158 (150;166) -0.6%	159 (152;166) Ref.	161 (153;169) +1.3%	163 (155;172) +2.5%	172 (161;183) +8.2%	0.04*	-	0.26 (-0.02;0.49)
Multivariable model plus HDL¶	163 (155;171) +1.2%	161 (154;167) Ref.	162 (155;170) +0.6%	163 (155;171) +1.2%	169 (158;179) +5.0%	0.28	-	0.13 (-0.10;0.37)

PON1 indicates paraoxonase activity; AE, arylesterase activity; HDL, high-density lipoprotein.

† Mixed method linear regression models were used.

‡ Continuous model (alcohol consumption in grams of pure alcohol/day).

§ *p-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 socioeconomic status.

* *p* < 0.05

FIGURE 1: Least squares mean (95%CI) PON1 activity according to alcohol consumption categories. All models were adjusted for sex, age, and socioeconomic status. Multivariable model, n=566; Excluding heavy drinkers (>70g alcohol/d), n=537; Excluding chronic diseases (type 2 diabetes, asthma, cardiovascular diseases, inflammatory bowel disease), n=339. Mixed method linear regression models were used. *P-quad* was obtained through log-likelihood ratio testing to examine quadratic trends.

FIGURE 2: Least squares mean (95%CI) AE activity according to alcohol consumption categories. All models were adjusted for sex, age, and socioeconomic status. Multivariable model, n=566; Excluding heavy drinkers (>70g alcohol/d), n=537; Excluding chronic diseases (type 2 diabetes, asthma, cardiovascular diseases, inflammatory bowel disease), n=339. Mixed method linear regression models were used. *P-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 < 0.05.

Figure 1

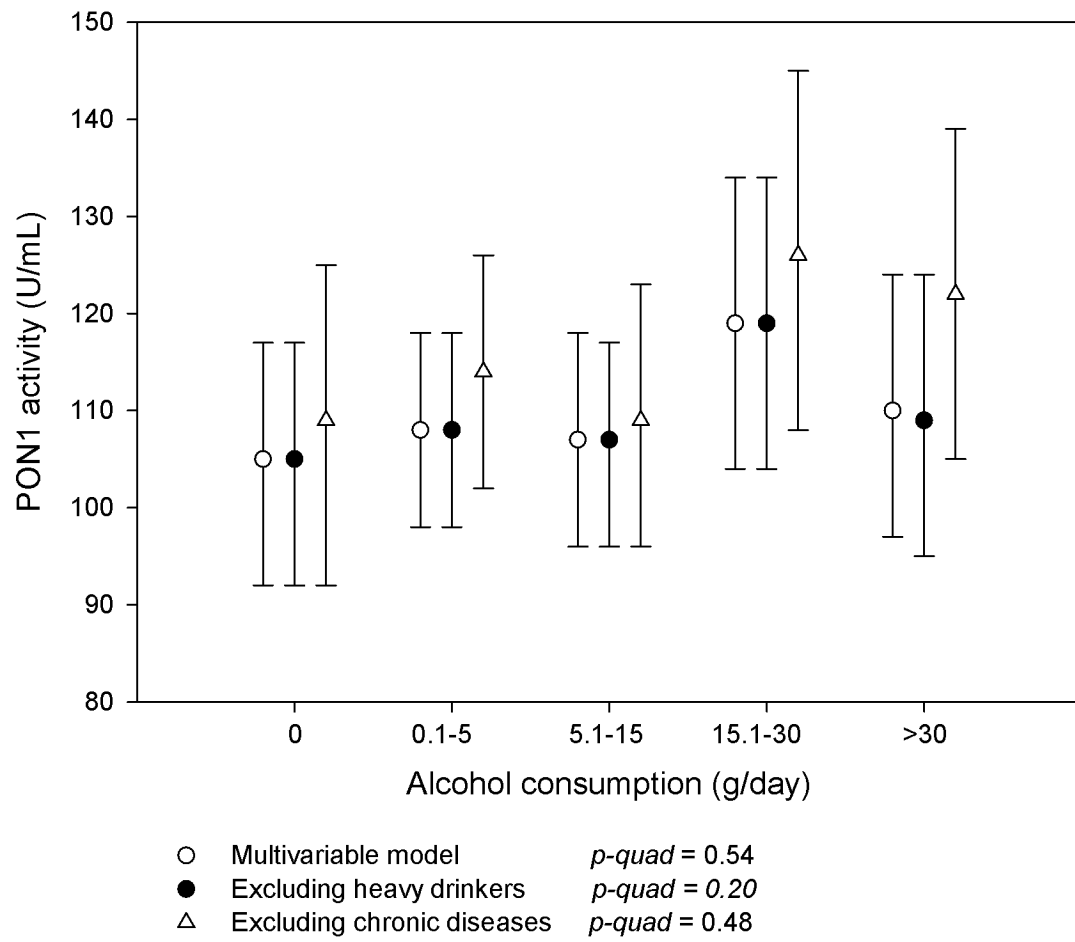


Figure 2

