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Research Article

Effect of Daily Moderate Red Wine Consumption on Paraoxonase 1

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Abstract

Most diseases caused by oxidative stress, such as cardiovascular diseases, can be mitigated by dietary antioxidant consumption as well as by a healthy lifestyle. Red wine contains a variety of antioxidant components including polyphenolic flavonoids. Polyphenols are a group of compounds with synergistic antioxidant properties associated with induction and upregulation of antioxidant enzymes such as paraoxonase 1 (PON1). PON1 is a calcium-dependent enzyme associated with high-density lipoproteins and may be involved in the antioxidant-mediated prevention of diseases. The aim of this study was to determinate if daily, moderate consumption of red wine for 6 weeks increases the concentration and activity of PON1 in a healthy population.

Method: A descriptive and analytical pilot study was carried out in 45 healthy volunteers. Clinical parameters, lipid profiles, PON1 activities (AREase, LACase, CMPAase, and PONase), and PON1 concentration were evaluated.

Results: Lipid profiles were not changed by the consumption of red wine. A significant increase in the AREase specific enzymatic activity of PON1 (as the quotient of enzymatic activity and PON1 protein concentration) was observed after 6 weeks of red wine consumption.

Conclusions: Our data showed for the first time that the effects of red wine consumption on PON1 specific activities were different among men and women. Further studies are needed to better elucidate the relationship between red wine consumption and PON1 status.

Keywords

Paraoxonase 1, Red wine consumption, Oxidative stress

1. Introduction

Oxidative stress is one of the main causes in the development of chronic diseases such as cardiovascular diseases [1-3]. Most diseases caused by oxidative stress can be prevented by the consumption of dietary antioxidants as well as other healthy lifestyle practices [4,5]. The principal antioxidant groups present in the diet are tocopherols, carotenoids and polyphenolic flavonoids which are found abundantly in pomegranates and red wine [6-10]. Red wine contains a wide variety of components including phenolic compounds such as flavonoids. The main flavonoids present in red wine are flavanols including epicatechin, myricetin, quercetin, anthocyanins and resveratrol which are directly associated with the organoleptic and health-promoting properties of red wine [11-13]. Polyphenols are a wide group of compounds with synergetic antioxidant properties associated with the induction and upregulation of antioxidant enzymes such as paraoxonase 1 (PON1) [14-18].

PON1 is an antioxidant and anti-inflammatory Ca^{2+}-dependent enzyme mainly synthesized in the liver and secreted to the blood, associated with high-density lipoproteins (HDLs) [19]. PON1 is able of hydrolyzing a wide variety of substrates, such as arylesters, organophosphate pesticides and homocysteine thiolactone, which plays an important role in atherothrombosis [20]. PON1 activity varies widely among individuals, and its expression and activity can be modulated by internal and external factors such as sex, single nucleotide polymorphisms, diet, alcohol consumption, smoking and physical activity [21-25].

Studies investigating polyphenol-rich foods have reported that polyphenols play a role in the modulation of metabolic

syndrome, hypertension and cardiovascular diseases [26-27]. These effects seem related to antioxidant activity on HDL metabolism [28-30], most specifically due to an effect on PON1 [31,32]. Considering the influence of the polyphenolic compounds on the expression and activity of PON1, the aim of this study was to determinate if daily, moderate consumption of red wine over 6 weeks increases the concentration and activity of PON1 in healthy individuals.

2. Methods

2.1 Study subjects

A descriptive and analytical pilot study was carried out in a healthy population (n = 45) ranging from 21 to 59 years old. All participants were residents of Nayarit, México and they signed an informed consent to participate. Their clinically healthy status was determined through a clinical history conducted by a general physician. A questionnaire was applied to evaluate general characteristics, lifestyle and dietary habits. This study was conducted according to the principles of the Declaration of Helsinki and was approved by an Ethics Committee (registry number CEBN/07/2018).

Volunteers were asked to maintain their habitual diet and lifestyle for the 6-weeks duration of the study. During the study, the participants ingested 120 ml of red wine (alcohol content 12.5%, Cabernet Sauvignon Malbec) daily for 6 weeks.

2.2 Sample collection

Blood samples were obtained after the period of fasting (12 hours) by venipuncture using a BD vacutainer with heparin (Becton, Dickinson and Company, Franklin Lakes, NJ), EDTA and dry plastic tubes. Samples were centrifuged at 2500 rpm for 15 min for the separation of plasma and serum and stored at -80°C until analysis.

Plasma samples were obtained every 2 weeks to evaluate PON1 activity. Lipid profiles, oxidized low-density lipoprotein (Oxi-LDL) levels and PON1 concentrations were evaluated at the beginning and end of the period of the study.

2.3 Lipid profile

Intravenous blood samples were obtained in tubes without anticoagulant to determinate total cholesterol, triglycerides and HDL cholesterol (HDL-C) concentrations. All analyses were performed in a certified clinical laboratory. Oxi-LDL concentrations were determined through an OxiSelect™ Human Oxidized LDL enzyme-linked immunosorbent assay (ELISA) kit (Cell Biolabs, Inc., San Diego, CA) following the manufacturer's instructions.

2.4 PON1 concentration

The PON1 concentration was determined by using a commercially available ELISA kit for human PON1 (SEA243Hu, Cloud-Clone Corp., Katy, TX) according to the manufacturer's instructions.

2.5 PON1 activity

Arylesterase (AREase) activity was measured using phenylacetate as a substrate according to [33,34] with some modifications. The rate of phenylacetate hydrolysis was measured by mixing 2.7 ml of buffer (10 mM Tris-HCl, 40 µM eserine hemisulfate, 1 mM $CaCl_2$, pH 8.0) and 20 µl of plasma (1:50). The mixture was incubated for 5 min in the dark at room temperature and then 300 µl of phenylacetate (10 mM) was added and the absorbance change was monitored at 270 nm each minute for 5 min at 37°C. AREase activity was reported in U/ml according to the molar extinction coefficient of phenylacetate ($\varepsilon = 1.31\times10^3$ M^{-1} cm^{-1}). One U of AREase activity is equivalent to 1 µM of phenylacetate hydrolyzed/min/ml.

CMPAase activity was evaluated by the method of [35] through 4-chloromethylphenylacetate (4-CMPA) hydrolysis. The reaction mixture contained 295.2 µl of buffer (20 mM Tris-HCl, 1 mM $CaCl_2$, pH 8.0), 60 µl of plasma diluted (1:40) in buffer, and 304.8 µl of 4-CMPA (3 mM). Absorbance change was monitored at 280 nm each minute for 5 min at 25°C. The activity was expressed in U/ml according to the molar extinction coefficient of 4-CMPA ($\varepsilon = 1.30$ mM^{-1} cm^{-1}).

LACase activity was determined according to [36], with some modifications according to [37]. Plasma (2.5 µl) was mixed with 987.5 µl of buffer (40 mM Tris-HCl, 1 mM $CaCl_2$, pH 8.0) and 10 µl of 100 mM dihydrocoumarin (DHC). The hydrolysis of DHC at 270 nm was measured every 30 seconds for 3 min at 25°C. LACase activity was expressed in U/ml according to the DHC molar extinction coefficient ($\varepsilon = 1{,}295$ M^{-1} cm^{-1}).

The *PONase activity* of salt-stimulated PONase was measured using paraoxon as a substrate according to [33]. The reaction mixture contained 795 µl of buffer (10 mM Tris-HCl, 1 mM $CaCl_2$, 1 M NaCl, pH 8.0), 5 µl of plasma, and 200 µl of substrate (6 mM). The activity was performed spectrophotometrically at 405 nm every minute for 5 min at 37°C. The activity was expressed as U/L. The molar extinction coefficient of *p*-nitrophenol was used ($\varepsilon = 1.8\text{x}10^3$ M^{-1} cm^{-1}).

Internal controls with known activities were used in each set of samples. The reproducibility of the enzymatic analyses was assessed on the triplicate analyses of plasma samples. In each case, the coefficient of variation calculated was 5% or less.

2.6 Statistical analysis

Continuous distribution of the variables was determined from testing skewness and kurtosis. Parametric data are presented as means ± standard deviations while non-parametric data are presented as geometric means at a 95% confidence interval. The Fisher's exact and chi-squared tests were used to evaluate significance of parameters

expressed in frequencies. Normally distributed data was compared with Student's *t*-test, while Mann-Whitney *U* tests and Wilcoxon signed-rank tests were used for non-parametric data. Associations determined in the present study were established using logistic regression with the geometric mean as the cutoff point. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using Stata program version 14.0 (Stata Corporation, College Station, TX).

3. Results

The study sample consisted of 19 men (42%) and 26 women (58%). No significant differences were observed among the genders in relation to age (Table 1). Over 35% of the participants were overweight (body mass index (BMI) of 25 to 30 kg/m^2), 18% were obese (BMI ≥ 30 kg/m^2), and 44% were in the ideal range (BMI of 18.5 to 25 kg/m^2) according to the [37]. A significant sex difference ($p < 0.02$) was observed in systolic blood pressure; men had higher systolic blood pressures than women. No significant differences were observed in diastolic blood pressure, physical activity, alcohol and drug consumption, or rates of smoking (Table 1).

3.1 Lipid profiles

Lipid profile parameters were within the normal ranges at the beginning and at the end of the red wine consumption period in the study population (Table 2). However, women showed a decrease in their final HDL-C concentration and an increase in the atherogenic index compared to the initial assessment. Also, PON1 concentration fell 13.06% in women after 6 weeks of red wine consumption. No differences were observed in Oxi-LDL concentrations (Table 2).

3.2 PON1 activities

PON1 activities were evaluated before and after red wine consumption, as well as every 2 weeks during the 6-weeks consumption period. Specifically, AREase (Figure 1), CMPAase (Figure 2), LACase (Figure 3), and PONase (Figure 4) activities were measured.

AREase activity before red wine consumption was 141.2 ± 34.8 U/ml, after 2 weeks of red wine consumption the AREase activity decreased in 6.7%. However, at the fourth and sixth weeks of red wine consumption a significant increase in AREase activity was observed compared to the second week but not with the initial measurement (Figure 1A). With respect by sex, an increase in AREase activity (9%) was observed at the sixth week compared to the second week (131.2 ± 20.1 U/ml) in the males (Figure 1B). Women showed decreased (9.6%) AREase activity at the second week compared to the initial activity (145.2 ± 37.5 U/ml), however by the fourth and sixth weeks AREase activity increased 10.2 and 14.5% respectively, compared to the second week (Figure 1C).

Figure 2 shows CMPAase activity. Initial CMPAase activity was 22.04 ± 5.1 U/ml; after 2 and 4 weeks of red wine consumption a decrease (10.2 and 11.9%) in CMPAase activity was observed (19.78 ± 4.6 and 19.41 ± 4.7 U/ml, respectively), but a significant increase was observed (21.01 ± 5.1 U/ml) at the sixth week compared to the second and fourth weeks. The same patterns were observed in both genders (Figures 2B and 2C).

Figure 3 shows LACase activity. After 2 weeks of red wine consumption a significant increase in LACase activity (10.97 ± 1.6 U/ml) was observed, which decreased at the end of the study (9.96 ± 1.5 U/ml) (Figure 3A). Both men and women showed an increase (4.8 and 4.7%) at week 2 (10.8 ± 2.1 U/ml; 11.0 ± 1.4 U/ml, respectively) (Figures 3B and 3C), but only women presented lower activity at the sixth week (by

Table 1: General characteristics of the study population.

	Men (n=19)	Women (n=26)	*p*
Age (years)	28 (25-31)	28 (25-31)	1.00[a]
BMI, kg/m^2	27.4 ± 5.8	24.5 ± 3.8	0.05[b]
Underweight, n (%)	1 (5.3)	---	0.27[c]
Normal, n (%)	6 (31.6)	14 (53.9)	
Overweight, n (%)	7 (36.8)	9 (34.6)	
Obese, n (%)	5 (26.3)	3 (11.5)	
SBP, mmHg	114 ±11	106 ±11	**0.02[b]**
DBP, mmHg (95% CI)	73 (68-78)	67 (64-71)	0.06[a]
Heart rate, bpm (95% CI)	70 (66-74)	72 (69-76)	0.31[a]
Physical activity, n (%)	12 (63.2)	16 (61.5)	0.73[d]
Alcohol consumption, n (%)	19 (100)	24 (92.3)	0.50[c]
Smokers n (%)			
Active	5 (26.3)	3 (11.5)	0.24[c]
Passive	1 (5.3)	5 (19.2)	
Past	6 (31.6)	5 (19.2)	0.07[d]
Drug consumption, n (%)	2 (10.5)	2 (7.7)	

BMI: body mass index. SBP: systolic blood pressure. DBP: diastolic blood pressure. [a]Mann-Whitney *U* test represented as geometric means with confidence intervals (CI) at 95%. [b]Student's *t*-test represented as means with standard deviations (±SD). [c]Fisher's exact test. [d]Chi-squared test.

Table 2: Effects of red wine on lipid profiles and paraoxonase 1 concentration.

Parameter	Men n=18			Women n=26			Reference
	Initial	Final	*p*	Initial	Final	*p*	
Total cholesterol (mg/dl)	179.61 (166.02-194.31)	182.94 (170.33-196.47)	0.40[a]	173.08 (±27.6)	173.65 (±31.0)	0.86[b]	101-200
HDL-C (mg/dl)	49.98 (46.79-53.40)	49.15 (45.07-53.59)	0.69[a]	60.53 (±15.59)	56.51 (±16.58)	**<0.01[b]**	45-55
Atherogenic index	3.59 (3.20-4.04)	3.72 (3.34-4.14)	0.27[a]	2.93 (2.62-3.28)	3.15 (2.79-3.56)	**<0.01[a]**	1-4
Triglycerides (mg/dl)	106.42 (83.60-135.48)	102.54 (77.19-136.21)	1.00[a]	88.25 (73.27-106.30)	84.41 (64.49-110.49)	0.96[a]	50-200
LDL (mg/dl)	107.56 (±27.23)	111.02 (±20.82)	0.38[b]	92.86 (±25.02)	98.08 (±22.53)	0.09[b]	92-150
VLDL (mg/dl)	21.20 (16.62-27.04)	20.52 (15.45-27.25)	0.96[a]	17.19 (14.55-20.31)	16.17 (12.84-20.36)	0.60[a]	8-30
Total lipids (mg/dl)	437.87 (385.57-497.27)	440.22 (384.44-504.23)	0.68[a]	398.60 (363.77-436.75)	498.85 (349.58-455.09)	0.83[a]	400-1000
PON1 (µg/ml)	2.96 (±1.39)	2.64 (±1.16)	0.31[b]	3.14 (2.69-3.66)	2.73 (2.38-3.13)	**0.02[a]**	
Oxi-LDL (ng/ml)	164.46 (62.38-433.56)	275.41 (189.70-399.85)	0.93[a]	204.44 (87.85-475.80)	195.42 (106.93-357.14)	0.10[a]	

HDL-C: high-density lipoprotein-associated cholesterol. LDL: low-density lipoprotein. VLDL: very low-density lipoprotein. Oxi-LDL: oxidized LDL. [a]Wilcoxon signed-rank test represented as geometric means with 95% confidence intervals. [b]Student's *t*-test represented as means with standard deviations (±SD).

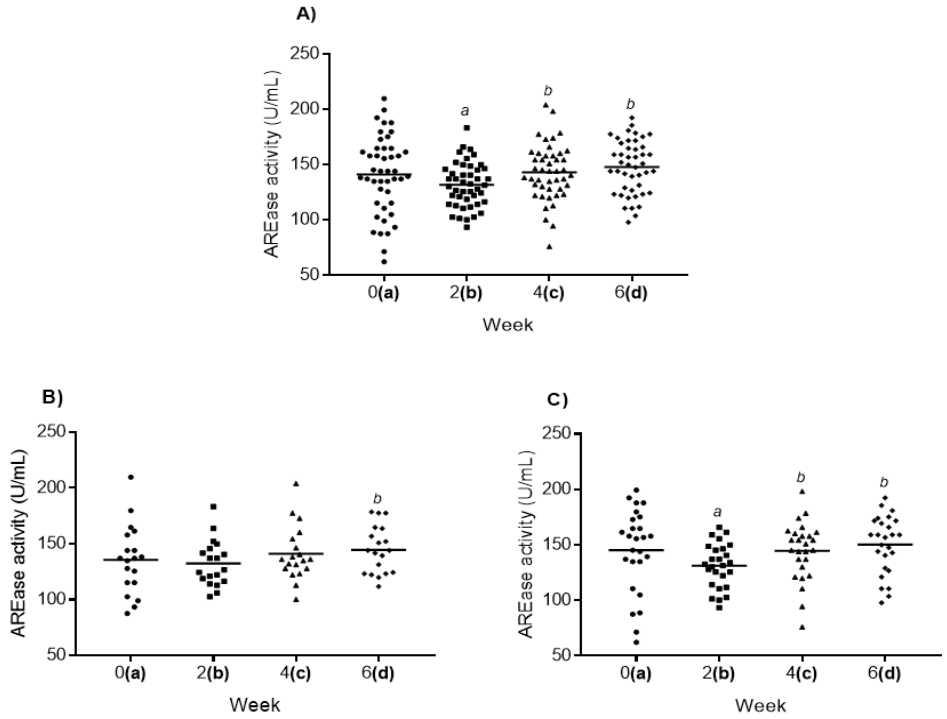

Figure 1: AREase activity in the study population overall (A), the men (B), and the women (C). Comparisons between weeks were made using Student's *t*-test. Statistical significance level was accepted as $p<0.05$. Week 0 (a), week 2 (b), week 4 (c), and week 6 (d) of daily wine consumption.

4.8%) compared to the initial activity.

Figure 4 shows PONase activity. No differences were observed in the study population between week 0 (355.9 ± 188.6 U/L) and week 6 (349.9 ± 181.9 U/L) (Figure 4A). Men presented higher activity at the sixth week (352.5 ± 180.2 U/L) (Figure 4B) compared to the second and fourth weeks. On the contrary, women showed a decrease (7.3%) in PONase activity at the second week (345.0 ± 181.9 U/L) (Figure 4C).

Specific enzymatic activity is presented as the quotient of enzymatic activity and PON1 protein concentration in Figure 5. Initial specific AREase activity was 47.4 U/µg which significantly increased after 6 weeks of red wine consumption to 56.4 U/µg (Figure 5A). No increases in CMPAase (Figure 5B), LACase (Figure 5C), or PONase (Figure 5D) were observed.

Associations between initial lipid profile parameters and Oxi-LDL concentrations were made using geometric

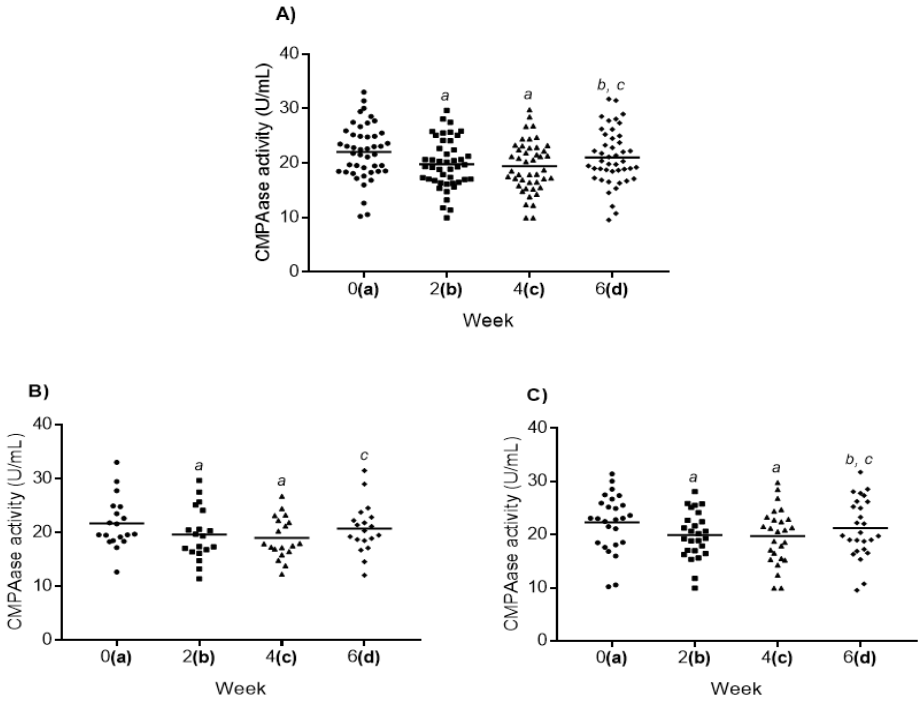

Figure 2: CMPAase activity in the study population overall (A), the men (B), and the women (C). Comparisons between weeks were made using Student's *t*- test. Statistical significance level was accepted as $p<0.05$. Week 0 (a), week 2 (b), week 4 (c), and week 6 (d) of daily wine consumption.

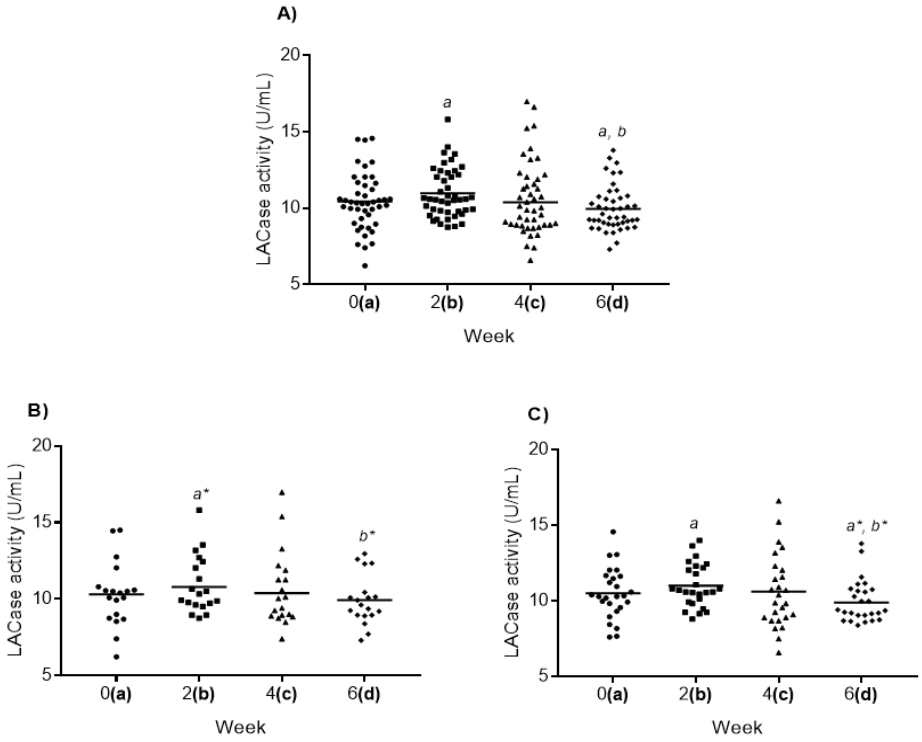

Figure 3: LACase activity in the study population overall (A), the men (B), and the women (C). Comparisons between weeks were made using Student's *t*-test and *Wilcoxon's signed rank tests. Statistical significance level was accepted as $p<0.05$. Week 0 (a), week 2 (b), week 4 (c), and week 6 (d) of daily wine consumption.

means as the cutoff points (Table 3). Our results show that an increase in HDL-C concentration was associated with a 5% decrease in Oxi-LDL concentration. Also, the triglycerides, very low-density lipoprotein, and total lipids showed a significant association with Oxi-LDL (Table 3). The logistic regression analysis showed an association between Oxi-LDL concentrations and the atherogenic index in women (odds ratio = 1.003, 95% confidence interval = 1.000-1.005, $p = 0.03$), but not in men (odds ratio = 0.999, 95% confidence interval = 0.997-1.002, $p = 0.73$).

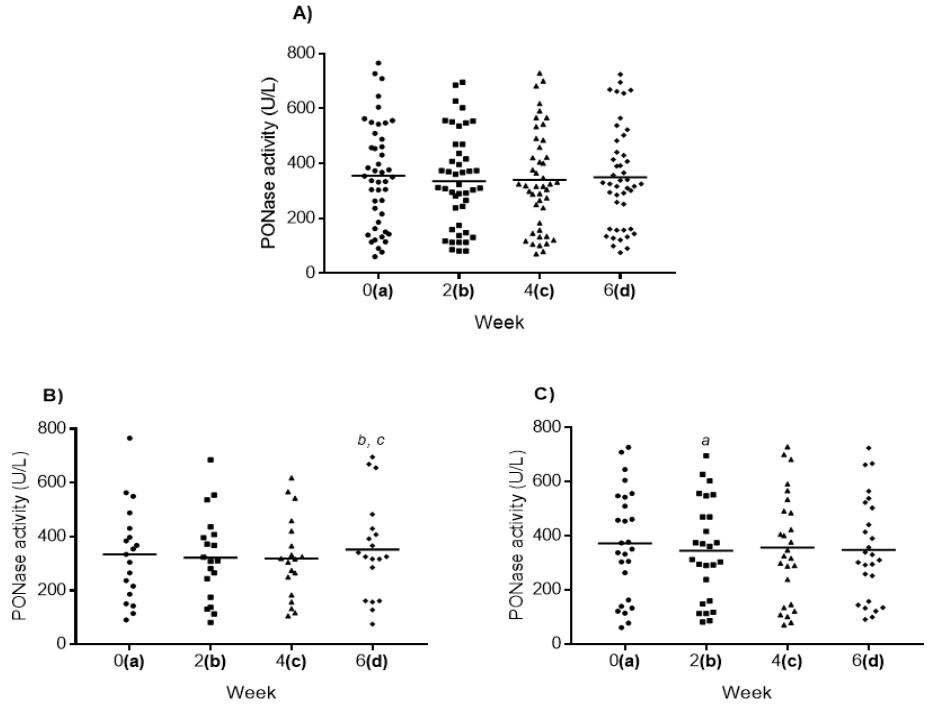

Figure 4: PONase activity in the study population overall (A), the men (B), and the women (C). Comparisons between weeks were made using Student's *t*-test. Statistical significance level was accepted as $p<0.05$. Week 0 (a), week 2 (b), week 4 (c), and week 6 (d) of daily wine consumption.

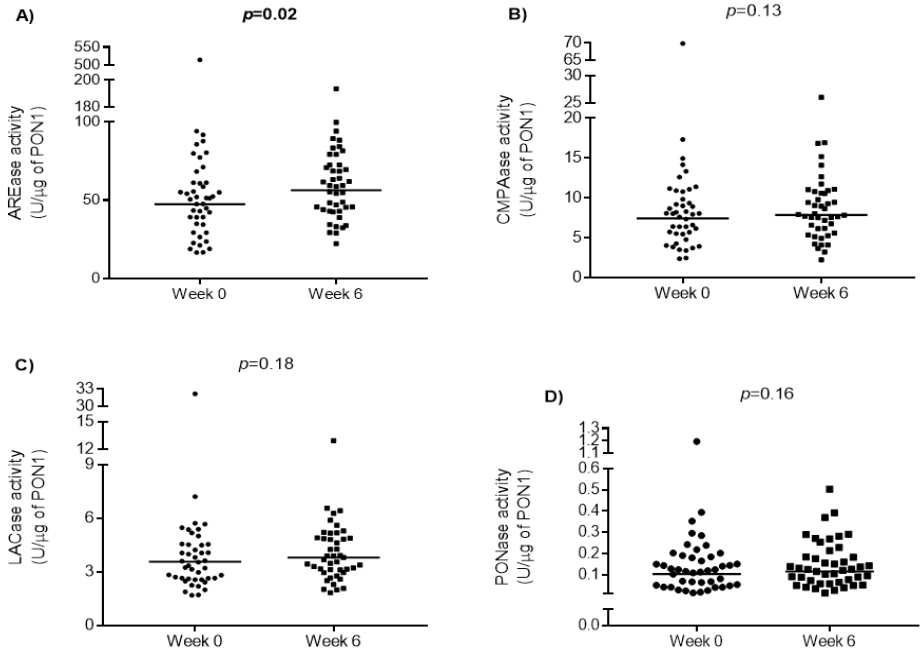

Figure 5: Specific activities of PON1 including AREase (A), CMPAase (B), LACase (C), and PONase (D) after 6 weeks of red wine consumption. Comparisons made by Wilcoxon's signed rank test.

Table 3. Associations between oxidized low-density lipoprotein concentrations and lipid profiles.

Parameter	Oddss Ratio	95% Confidence Intervals	*p*
HDL-C (mg/dl)	0.945	0.894-0.999	0.03
TG (mg/dl)	1.020	1.003-1.037	<0.01
VLDL (mg/dl)	1.108	1.015-1.209	<0.01
TL (mg/dl)	1.008	1.001-1.015	0.01
HDL-C: high-density lipoprotein-associated cholesterol. TG: triglycerides. VLDL: very low-density lipoprotein. TL: Total lipids. Associations were made by logistic regression analysis. Geometric mean (187.03 ng/ml) of dichotomized Oxi-LDL was used as the cutoff point.			

4. Discussion

The beneficial health effects of red wine have been mainly attributed to its antioxidants effects on lipids. However, the antioxidant effect of red wine is dependent partly on its flavonoids content, the dosage or time course of red wine consumption, and other internal and external factors [6, 38]. In this work, the study population consisted of men and women of reproductive age (average of 28 years) of whom 63.2% of the men and 46.1% of the women were overweight or obese. This is important because a high BMI is an important factor for the development of various chronic diseases such as alterations in the circulatory system and diabetes [39]. Regarding the consumption of tobacco and alcohol, 100% of the men and 92.3% of the women in our study reported consuming alcohol regularly, while 26.3% of the men and 11.5% of the women used tobacco. These data are relevant to the national average of ethanol consumption in México in the last 3 years reported by [40].

While some studies have focused on investigating the effects of low and moderate alcohol consumption on PON1, few have explored the relationship between alcohol consumption, PON1 activity/concentration, and lipid profiles as we have. Our data showed that consuming 120 ml of red wine (12 g of alcohol) daily for 6 weeks did not alter lipid profiles in men or their PON1 Oxi-LDL or PON1 concentrations. In women, to our surprise, alcohol increased LDL-C and atherogenic indices while it decreased HDL-C and PON1 concentrations. However, all values of HDL-C, LDL-C, and the atherogenic index were within the normal ranges.

Our results do not agree with the literature, where most studies found positive effects of alcohol on HDL and its apolipoproteins [41,42]. One study conducted on moderate alcohol consumption showed that HDL-C changes are dependent on time; there were no changes after 5 days, however HDL-C increased after 10 (6.8%), 15 (8.9%), and 20 (11.7%) days of consumption, although they did not show any changes in PON1 concentration [43]. In addition, [44] conducted a cross-sectional study with different levels of alcohol intake. They found that PON1 activity was positively correlated with HDL-C and apoA-I when 10-30 g/d of alcohol was ingested, but it was not observed at higher alcohol levels. The authors also observed that PON1 activity was related to alcohol consumption independently of clinical covariates, such as lipid concentrations, including HDL-C. It is important to consider that, although studies have been conducted with different beverage types, it has not been shown that beverage type affects lipoprotein levels different [45]. Furthermore, there are other factors to consider such as nutritional fat intake, since high consumption of dietary fat can modulate serum lipid concentrations [46].

Gender-related differences in kinetics are also considered important factors in clinical and toxicological studies [47-49], ethanol kinetics included. Women have lower gastric alcohol dehydrogenase activity, resulting in higher blood ethanol levels after consuming similar amounts of ethanol [50,51]. Furthermore, [52] reported consumption of 150 ml/d of red wine over 2 years increased HDL-C levels in women significantly. However, in men this effect was not observed. In the present study, our data did not show significant changes in lipid profiles in either gender.

Lastly, we found moderate red wine consumption decreased the enzymatic activity of PON1. However, when the specific activity (the quotient of enzymatic activity and PON1 protein concentration) was considered, a significant increase in AREase activity was observed (56.4 U/μg) after 6 weeks of red wine consumption. No differences were observed for other PON1 activities. Few studies have explored the relationship between alcohol consumption and PON1 activity, with variable results [53] reported that daily moderate alcohol consumption increased serum PONase activity. However, [48] reported decreased AREase activity and no changes in PONase activity after red wine consumption in healthy men [54] reported no differences in total antioxidant activity in healthy and overweight subjects after 4 weeks of red wine intake.

5. Conclusion

While much has been reported about the health effects attributed to moderate red wine consumption, less is known about its effects on PON1 specific activities in healthy women and men. Here, we show for the first time a differential effect of red wine consumption on PON1 specific activities among men and women. Further studies are needed to clarify the impact of red wine consumption on PON1 status. More research into the antioxidant properties of specific types of grapes is also warranted.

Our study had both limitations and strengths. One limitation of this study was the low sample size, however its design allowed for the observation of intra-individual changes as well as between-subject variations, especially gender differences. Strength of this study is that the administered dose of red wine is achievable in a normal diet.

6. Acknowledgment

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