applied sciences

MDPI

Article

The Role of Nutritional Habits and Moderate Red Wine Consumption in PON1 Status in Healthy Population

Fidel Navarro-García [1,2], Néstor Ponce-Ruíz [1], Aurora Elizabeth Rojas-García [1], Gabriela Ávila-Villarreal [3,4], José Francisco Herrera-Moreno [5], Briscia S. Barrón-Vivanco [1], Yael Y. Bernal-Hernández [1], Cyndia Azucena González-Arias [1] and Irma Martha Medina-Díaz [1,*]

1 Laboratorio de Contaminación y Toxicología, Secretaría de Investigación y Posgrado, Universidad Autónoma de Nayarit, Tepic 63000, Mexico; fng95@outlook.com (F.N.-G.); nestor_ruiz@outlook.com (N.P.-R.); aerg81@gmail.com (A.E.R.-G.); bravis13@hotmail.com (B.S.B.-V.); yael010@hotmail.com (Y.Y.B.-H.); cyndia.gonzalez@uan.edu.mx (C.A.G.-A.)
2 Posgrado en Ciencias Biológico Agropecuarias, Universidad Autónoma de Nayarit, Xalisco 63794, Mexico
3 Unidad Académica de Ciencias Químico Biológicas y Farmacéuticas, Universidad Autónoma de Nayarit, Tepic 63000, Mexico; gaby.avila@uan.edu.mx
4 Centro Nayarita de Innovación y Transferencia de Tecnología "Unidad especializada en I+D+i en Calidad de Alimentos y Productos Naturales", Universidad Autónoma de Nayarit, Tepic 63000, Mexico
5 Postdoctoral Research Fellow-CONACyT-Mx, Laboratory of Precision Environmental Health, Department of Environmental Health Sciences, Columbia University, New York, NY 10032, USA; pakotoxic@hotmail.com
* Correspondence: irmartha@hotmail.com

Abstract: Paraoxonase 1 (PON1) plays a role as antioxidant on HDL. Including in diet additionally ingest of polyphenolic compounds can stimulate PON1 transcription and increase its activity. The aim of this study was to evaluate the effect of dietary intake, red wine consumption, and PON1 genotypes (*Q192R*, *L55M* and *C-108T*) on the specific activity of PON1 in a healthy population. A descriptive and analytical pilot study was conducted in Mexican volunteers clinically healthy (n = 45) aged from 21–59 years. Over 6 weeks, the study participants ingested 120 mL of red wine per day. PON1 concentration, PON1 activities, genetic polymorphisms and dietary intake were evaluated. The preliminary fingerprinting of the wine was determined to corroborate the presence of phenolic compounds such as tannins and gallotannins. Neither dietary intake nor PON1 genotypes showed an effect on the specific activity of PON1. However, a significant increase in specific AREase activity after red wine consumption period was observed in the study participants. Our data suggest that the moderate consumption of red wine has a beneficial effect on PON1 specific AREase activity in this healthy Mexican population.

Keywords: PON1; red wine; PON1 genotypes; AREase activity; phenolic compounds; UPLC-MS

check for updates

Citation: Navarro-García, F.; Ponce-Ruíz, N.; Rojas-García, A.E.; Ávila-Villarreal, G.; Herrera-Moreno, J.F.; Barrón-Vivanco, B.S.; Bernal-Hernández, Y.Y.; González-Arias, C.A.; Medina-Díaz, I.M. The Role of Nutritional Habits and Moderate Red Wine Consumption in PON1 Status in Healthy Population. *Appl. Sci.* **2021**, *11*, 9503. https://doi.org/10.3390/app11209503

Academic Editors: Teresa Leszczyńska, Joanna Kapusta-Duch and Ewa Piątkowska

Received: 24 September 2021
Accepted: 9 October 2021
Published: 13 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The Paraoxonase 1 (PON1) gene is a member of the paraoxonase family located on the long arm of human chromosome 7 (7q21-22). PON1 is mainly expressed and synthesized in the liver and secreted into the blood circulation as a high-density lipoprotein (HDL) associated protein [1]. This enzyme has anti-inflammatory, antimicrobial, antioxidant and antiatherogenic properties; and it is involved in the hydrolysis of a wide variety of substrates, such as lactones, arylesters, organophosphate pesticides, and others [2]. PON1 plays a role in HDL protective properties and its status is considered as of the determinants for the development of cardiovascular and other diseases [3]. It has been described that PON1 activity and its concentration in populations can range up to 40-fold and 13 times, respectively [4]. In this regard, it has been shown that PON1 activity and expression is affected by age, sex, lifestyle, drugs, dietary and environmental factors. In addition, some genetic polymorphisms can influence the enzyme concentration by affecting gene and

protein expression, as well as its specific activity. Genetic factors, including polymorphisms, were found to explain more than 60% of phenotypical variance in PON1 activity [5].

The *PON1* gene presents 13 single nucleotide polymorphisms (SNPs) in the promoter region, and five of these have been characterized (*G-909C, G-832A, A-162G, G-126C and C-108T*) [4]. In addition, two polymorphisms in the encoding region have been commonly reported at positions 55 and 192 [6,7]. These SNPs are associated with variations on PON1 concentration and enzymatic activity [8–11]. The *PON1 Q192R* polymorphism has been shown to influence PON1 activity; the Q-isoform catalyzes the faster hydrolysis of diazoxon, a metabolite of the organophosphate diazinon, while the R-isoform hydrolyzes the metabolite paraoxon of the organophosphate parathion more efficiently [12]. Regarding the *PON1 L55M* polymorphism, the L allele is associated with higher PON1 protein and messenger RNA (mRNA) levels compared to the M allele. It has been described that individuals with the QQ and MM isozymes of the *Q192R* and *L55M* polymorphisms exhibit lower enzymatic activity than subjects with the RR and LL variants [5].

The effects of dietary compounds such as carbohydrates, fatty acids, proteins, vitamins and minerals on the PON1 phenotype have been described, [13,14] as well as habits such as physical activity or harmful habits such as: alcoholism, smoking and illicit drug use [4,15,16]. Some studies have evaluated the effect of phenolic compounds such as flavonoids on *PON1* expression and activity in vitro, in vivo and in human populations [17–22]. Furthermore, flavonoids are present in pomegranate and red wine, and these are the principal groups of antioxidants present in the diet [23,24]. In this respect, several studies have been focused on the effects related to the consumption of red wine on the PON1 enzyme, [13,17,25–30] especially in the increased serum paraoxonase activity caused by red wine and moderate wine intake [25,26]. In addition, it is known that red wine has polyphenols compounds commonly divided in flavonoids and nonflavonoids, such as flavonoids like quercetin, nonflavonoid compounds that include phenolic acids (e.g., gallic acid), phenols and stilbenes (e.g., resveratrol) compounds related to cardioprotective effects [27–30]. Specifically, resveratrol can enhance the expression of the *PON1* gene in the HepG2 human cell line [28]. Considering the influence of the phenolic compounds on PON1, the aim of this study was to evaluate the effect of dietary intake, red wine consumption, and three genotypes of *PON1* (*Q192R, L55M* and *C-108T*) on the specific activity of PON1 in a healthy population.

2. Materials and Methods

2.1. Study Subjects

A descriptive and analytical pilot study was carried out in clinically healthy Mexican volunteers (n = 45) aged from 21–59 years. Participants received and signed an informed consent document. This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the State, of Nayarit (registry number CEBN/07/2018). A general medical physician performed a clinical history of each volunteer to determine the health status of the participants. At the same time, through a structured and validated questionnaire we obtained the anthropometric characteristics of the participants, as well as lifestyle, dietary habits and additional data.

Healthy volunteers were asked to maintain their habitual diet and lifestyle during the 6 weeks of the study period. Within this 6-week period, the study participants ingested 120 mL of red wine per day (alcohol content, 12.5%, Cabernet Sauvignon Malbec).

2.2. Evaluation of Macro- and Micronutrients

Macro- and micronutrient habits were determined prior to carrying the study and the end of the study period according to a frequency-of-consumption questionnaire through using Software Program Evaluation of Nutritional Habits and Nutrient Consumption System (SNUT) from the Mexican National Institute of Public Health and National Institute of Cardiology Ignacio Chávez [31].

2.3. Sample Collection

Venous blood samples were obtained after a 12 h by venipuncture using a BD Vacutainer® tubes with heparin, EDTA and dry plastic tubes. Samples were centrifuged at 1173 rcf for 15 min at 5 °C for separation of the plasma and serum, these were kept cold for aliquoting and then were stored at −80 °C until analysis.

2.4. PON1 Concentration

PON1 concentration was determined by Enzymatic-Linked Immunosorbent Assay (ELISA) from plasma samples using a commercial ELISA kit for human PON1 (SEA243Hu, Cloud-Clone Corp., Katy, TX, USA) according to the manufacturer's instructions.

2.5. PON1 Activity

Arylesterase (AREase) activity was measured using phenylacetate as a substrate [32]. The mix reaction consisted of 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 diluted at 1:50 and was incubated for 5 min in the dark at room temperature following the addition of 300 µL of phenylacetate (10 mM). The change in absorbance was monitored at 270 nm for 5 min at 37 °C in a Spectronic GENESYS^{TM} 10 spectrophotometer from Bio-Thermo Scientific. AREase activity was reported in U/mL according to the molar extinction coefficient of phenylacetate ($\varepsilon = 1.31 \times 10^3$ M^{-1} cm^{-1}). One unit of AREase activity is equivalent to 1 µmol of phenylacetate hydrolyzed/min/mL of plasma.

CMPAase activity was measured through 4-chloromethylphenylacetate (4-CMPA) hydrolysis [33]. The mix reaction consisted of 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 for 5 min at 25 °C in a Spectronic GENESYS^{TM} 10 spectrophotometer from Bio-Thermo Scientific. CMPAase activity was expressed in U/mL according to the molar extinction coefficient of 4-CMPA ($\varepsilon = 1.30$ mM^{-1} cm^{-1}). One unit of CMPAase activity is equal to 1 µmol of 4-CMPA hydrolyzed/min/mL of plasma.

Lactonase (LACase) activity was determined using dihydrocoumarin (DHC) as substrate through the modified method as described by Billecke et al. [34]. The reaction mix was consisted of 987.5 µL of buffer (40 mM Tris-HCl, 1 mM $CaCl_2$, pH 8.0), 10 µL of 100 mM DHC and 2.5 µL of serum. The hydrolysis of DHC was monitored at 270 nm for 3 min at 25 °C using a Spectronic GENESYS^{TM} 10 spectrophotometer from Bio-Thermo Scientific. LACase activity was expressed in U/mL according to the DHC molar extinction coefficient ($\varepsilon = 1295$ M^{-1} cm^{-1}). One unit of LACase activity is equal to 1 µmol of DHC hydrolyzed/min/mL of serum.

Paraoxonase (PONase) activity of salt-stimulated PONase was measured using ethylparaoxon as a substrate [35]. The mix reaction 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 ethyl paraoxon (6 mM). The hydrolysis of ethyl paraoxon at 405 nm was monitored for 5 min at 37 °C using a Spectronic GENESYS^{TM} 10 spectrophotometer from Bio-Thermo Scientific. One unit of PONase activity is equal to 1 µmol of ethyl paraoxon hydrolyzed/min/L of plasma. The molar extinction coefficient of *p*-nitrophenol was used ($\varepsilon = 1.8 \times 10^3$ M^{-1} cm^{-1}), and PONase activity was expressed as U/L.

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

PON1 specific enzymatic activity represents the quotient of enzymatic activity through a specific substrate (AREase; CMPAase; LACase; PONase) and PON1 protein concentration.

2.6. DNA Isolation and PON1 Genotyping

DNA was isolated from whole blood employing a High Pure PCR Template Preparation kit (Roche, Indianapolis, IN, USA) following the manufacturer's instructions. DNA purity and concentration were determined using the NanoDrop system. Real-time PCR

assays of *PON1 T-108C* (rs705379), *PON1 L55M* (rs854560) and *PON1 Q192R* (rs662) were performed with primer-specific fluorescent labeled probes from Applied Biosystems (Foster City, CA, USA). PCR reactions were performed using a StepOne™ real-time PCR system as follows: at 50 °C for 2 min; at 95 °C for 10 min, then 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. A negative control was included on each plate. Allelic discrimination was performed using StepOne™ ver. 2.1 software.

2.7. Wine Phytochemical Composition Analysis (Fingerprinting)

The ultra-performance liquid chromatography (UPLC) method was used to analyze and tentatively characterized the preliminary phytochemical composition of Cabernet Sauvignon Malbec wine used for healthy volunteer participants (Table S3, Supplementary Material). The wine sample was diluted 1:100 with water and filtered using nylon Acrodisc® Syringe filter 25mm 0.2µm. Chromatographic separation was achieved on a WATERS® ACQUITY HSS T3 1.8 µm 2.1 mm × 100 mm column preheated at 40 °C. All the chromatographic mass spectrometric measurements were performed on a Waters ACQUITY H-Class UPLC-MS® system equipped with a quaternary solvent manager, sample manager, flow through needle, high temperature column heater with active preheating, and Quadrupole Dalton (QDa) (Waters Corporation), detector. A single quadrupole mass spectrometer equipped with electrospray ionization (ESI) was used to record the ESI-MS spectra. The MS was operated in positive mode ($[M - H]^+$).The conditions of the electrospray ionization (ESI) source were as follows: ESI in positive mode; capillary voltage, 0.8 kV; fragmentor, 2 V; sampling frequency, 10 Hz. The QDA analysis worked using full scan mode, and the mass range was set at m/z 50–1000 Da. exploratory method for 10 min. The mobile phase consisted at 0.1% formic acid water (A) and acetonitrile (B), using a gradient elution for the sample (of 0–5 min, 10% B; 5–7 min,10–90% B; 7–8 min, 90–10% B; 8–10 min, 10% B). The sample volume injected was 5 µL the flow rate was 0.35 mL/min.

2.8. Statistical Analysis

Statistical analyses were performed using Stata statistical software version 14.0 (College Station, TX, USA). The distribution of continuous variables was determined by means of the Skewness and Kurtosis test. Parametric data are presented as mean and standard deviation (±SD), while nonparametric data are presented as the geometric mean and 95% confidence intervals (95% CI). The Fisher's exact and chi-squared tests were employed to evaluate the significance of the parameters expressed in frequencies. Normally distributed data were compared with Student's *t*-test, while Mann–Whitney *U* and Wilcoxon signed rank tests were utilized for nonparametric data to know differences between two groups. Furthermore, the Kruskal–Wallis and post hoc Dunn's tests for the nonparametric variables were used to know differences between three or more groups. Associations determined in the present study were established using linear and logistic regressions. The statistical significance level was accepted as $p < 0.05$.

3. Results

3.1. Study Subjects

The general characteristics of the study population are presented in Table 1. A total of 58% of participants were women and 42% were men. With respect to body mass index (BMI), 44% were in the normal range (BMI, of 18.2–25 kg/m^2) according to World Health Organization (WHO) criteria [36], 35% of participants presented overweight (BMI, 25–30 kg/m^2), and 21% were obese (BMI $\geq$ 30 kg/m^2). A significant difference ($p = 0.02$) was observed in systolic blood pressure (SBP) between the sexes: males had a higher SBP than women ($p = 0.02$); however, no significant differences were observed in diastolic blood pressure (DBP), physical activity, alcohol and drug consumption, or smoking habits.

Table 1. General characteristics of the study population.

	Men (n = 19)	**Women (n = 26)**	***p***
Age, years (95% CI)	28 (25–31)	28 (25–31)	1.00 [a]
BMI, kg/m^2 (±SD)	27.4 (±5.8)	24.5 (±3.8)	0.05 [b]
Underweight (≤18.5), n (%)	1 (5.3)	—	0.27 [c]
Normal (>18.5, ≤25), n (%)	6 (31.6)	14 (53.9)	
Overweight (>25, ≤ 30), n (%)	7 (36.8)	9 (34.6)	
Obese (>30), n (%)	5 (26.3)	3 (11.5)	
SBP, mmHg (±SD)	115 (±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			
Active, n (%)	5 (26.3)	3 (11.5)	0.24 [c]
Passive, n (%)	1 (5.3)	5 (19.2)	
Past, n (%)	6 (31.6)	5 (19.2)	0.07 [d]
Drug consumption, n (%)	2 (10.5)	2 (7.7)	

BMI: Body mass index according to the World Health Organization (WHO). SBP: systolic blood pressure. DBP: diastolic blood pressure. [a] Mann–Whitney *U* test represented as geometric means with 95% confidence intervals (95% CI). [b] Student's *t*-test represented as means with standard deviation (±SD). [c] Fisher's exact test. [d] Chi-squared test.

Regarding PON1 concentration, in women, the PON1 concentration at the beginning of the study was 3.14 μg/mL (range, 2.69–3.66 μg/mL) vs. 2.73 μg/mL (range, 2.38–3.13 μg/mL) after the period of red wine consumption, which implied a significant reduction. No differences in PON1 concentration were found in women with respect to men [initial: 2.96 μg/mL (±1.39) and 2.64 μg/mL (±1.16) after wine consumption].

Nutrient habits in the study population were characterized and described by food frequency questionnaire, results are presented in Table 2. According to our data, no significant differences in macronutrient intakes were observed before and during the red wine consumption period. Although, the participants were requested not to change their dietary habits during the study, a significant decrease ($p < 0.001$) in caloric intake (Table 2) was observed. In addition, significant differences in the intake of vitamins and minerals before and during red wine consumption were observed (Table 3).

Table 2. Initial and final macronutrients intakes.

	Initial Intake	**Final Intake**	***p***
Carbohydrates (%)	46.2 (±7.4)	45.44 (±7.4)	0.53 [a]
Protein (%)	11.0 (9.0–13.5)	10.4 (8.6–12.5)	0.61 [b]
Fat (%)	38.0 (±7.11)	38.2 (±6.9)	0.78 [a]
Calories (kCal)	1856.1 (1571.6–2192.0)	1694.6 (1501.3–1912.7)	**<0.001** [b]

kCal: kilocalories. [a] Student's *t*-test presented as arithmetic mean with standard deviation (±SD). [b] Wilcoxon signed rank test presented as geometric means with 95% confidence intervals (95% CI).

Table 3. Initial and final intake of vitamins and minerals.

Vitamin	Initial Intake	Final Intake	*p*
Vitamin C (mg)	132.6 (105.8–166.1)	83.7 (65.8–106.3)	**<0.001**
Vitamin B1 (mg)	1.3 (1.1–1.6)	1.2 (1.0–1.3	**<0.01**
Vitamin B2 (mg)	1.3 (1.1–1.6)	1.2 (1.0–1.3)	**<0.01**
Niacin (mg)	17.9 (15.1–21.3)	16.9 (15.0–19.0)	**0.01**
Vitamin B5 (mg)	7.9 (6.2–9.9)	7.0 (5.4–9.0)	>0.05
Vitamin B6 (mg)	1.7 (1.5–2.1)	1.6 (1.4–1.8)	**<0.01**
Glycosylated vitamin B6 (mg)	4.1 (2.5–6.6)	4.3 (3.3–5.7)	>0.05
Folate (μg)	481.7 (363.5–638.5)	380.5 (277.9–521.1)	**0.01**
Vitamin B12 (μg)	7.1 (5.6–8.9)	6.1 (5.1–7.3)	**0.001**
Vitamin K (μg)	74.3 (58.5–94.6)	72.2 (59.2–87.9)	>0.05
Retinol (UI)	1721.8 (1325.3–2236.9)	1436.6 (1183.0–1744.5)	**0.01**
Vitamin D (UI)	156.5 (123.7–198.1)	136.9 (114.0–164.4)	**0.02**
Vitamin E (μg)	11.7 (±6.6)	8.7 (±4.0)	**<0.01** [a]
Vitamin E activity (IU)	12.3 (9.8–15.3)	9.8 (8.5–11.4)	**<0.01**
α-Tocopherol (mg)	11.6 (9.4–14.2)	9.4 (8.1–10.9)	**<0.01**
β-Tocopherol (mg)	0.6 (±0.43)	0.5 (±0.2)	**0.04** [a]
γ-Tocopherol (mg)	13.4 (10.5–17.1)	14.8 (12.5–17.4)	>0.05
δ-Tocopherol (mg)	2.3 (1.7–3.0)	2.8 (2.3–3.4)	>0.05
α-Tocopherol (eq/mg)	13.3 (10.8–16.3)	11.1 (9.6–12.8)	**<0.01**
Mineral	**Initial Intake**	**Final Intake**	***p***
Calcium (mg)	538.8 (441.9–656.9)	486.3 (423.6–558.4)	**<0.01**
Iron (mg)	12.6 (10.5–15.0)	11.9 (10.6–13.5)	**0.02**
Magnesium (mg)	280.9 (233.3–338.2)	272.7 (243.3–305.6)	**0.04**
Phosphorus (mg)	1077.4 (896.2–1295.2)	1058.5 (946.1–1184.3)	**0.02**
Potassium (mg)	2840.6 (2401.6–3359.9)	2453.5 (2154.6–2793.9)	**<0.001**
Sodium (mg)	1593.4 (1342.2–1891.5)	1410.9 (1225.1–1624.9)	**<0.01**
Zinc (mg)	13.9 (11.1–17.4)	12.6 (10.3–15.5)	**0.03**
Copper (mg)	1.8 (1.4–2.3)	1.6 (1.3–2.0)	**0.01**
Manganese (mg)	11.8 (8–17.4)	11.9 (8.7–16.1)	>0.05
Selenium (μg)	40.3 (32.3–50.6)	38.9 (32.6–46.5)	>0.05

Wilcoxon signed rank test presented as geometric means with 95% confidence intervals (95% CI). [a] Student's *t*-test presented as arithmetic means with standard deviations (±SD).

3.2. Allele Frequencies and PON1 Genotypes

Allelic frequencies were 0.61 for C allele (*C-108T*), 0.30 for M allele (*L55M*), and 0.46 for Q allele (*Q192R*). The study polymorphisms were in agreement with the Hardy–Weinberg equilibrium.

A strong linkage disequilibrium (D% = 56.36; $p < 0.001$) between *PON1 C-108T* and *PON1 L55M* and between *PON1 Q192R* and *PON1 L55M* (D% = 71.73; $p < 0.001$) was observed, and a marginal linkage disequilibrium (D%; $p = 0.06$) was observed between *PON1 C-108T* and *PON1 Q192R* (Table S1, Supplementary Material).

3.3. Status of PON1 and Genotypes

PON1 specific enzymatic activity in the study participants before and after red wine consumption is shown in Figure 1. The data revealed a significant increase in specific AREase activity, after the red wine consumption period (* $p < 0.05$).

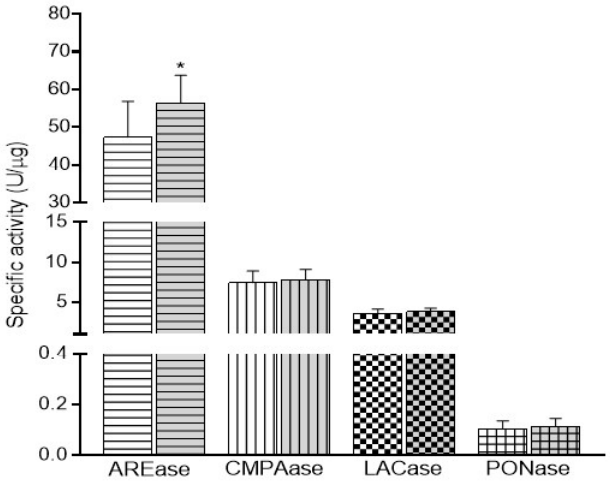

Figure 1. Specific PON1 activities in the study population before (white) and after (gray) the red wine consumption period. Wilcoxon signed rank test presented as geometric means with 95% confidence intervals (95% CI). * $p < 0.05$ with respect initial activity.

Furthermore, differences in AREase, CMPAase and PONase activities among according to genotype before and after red wine consumption were found, except for LACase activity. We only depicted the results obtained for *PON1 Q192R* (Figure 2) in terms of the homogeneity in the allelic frequencies. The remaining data are presented in Table S2 (Supplementary Material).

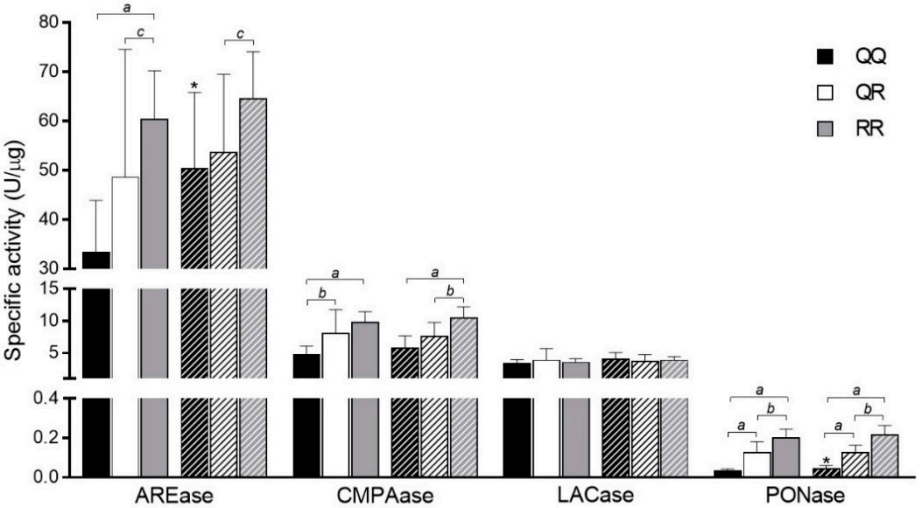

Figure 2. Specific activity of PON1 according to *Q192R* polymorphism. *PON-Q192* homozygote (QQ), *PONQ192/PON1-R192* heterozygote (QR), *PON1-R192* homozygote (RR). Statistical analyses were conducted through the Kruskal–Wallis and Dunn's tests. Data was presented as geometric means with 95% confidence intervals. Solid bars represent activity before red wine consumption and diagonal pattern bars represent activity after red wine consumption. * Statistical difference in comparison to activity before red wine intake. [a] $p < 0.001$, [b] $p < 0.01$ and [c] $p < 0.05$ statistical differences among genotypes.

3.4. PON1 Activities and Nutrient Intake

Logistic regression analyses were conducted to evaluate the association between PON1 specific activities (AREase and PONase) and nutrient intake. The model was adjusted by sex, age, BMI, physical activity, smoking habits, lipid profile parameters and genotypes.

Initial AREase activity was associated negatively with tocopherols (β-, γ- and δ) and polyunsaturated fat intake. In addition, negative associations were found between PONase activity with calcium and potassium, as well as with vitamins B (2,3,6) intake (Table 4). No association was found regarding CMPAase or LACase activities with respect to nutrient intake.

Table 4. Associations between initial PON1 AREase and PONase specific activities and nutrient intake.

AREase			
Nutrient	**OR**	**95% CI**	***p***
Total fiber (g)	1.002	1.0004–1.004	**0.02**
β-tocopherol (mg)	0.085	0.0088–0.813	**0.03**
γ-tocopherol (mg)	0.814	0.668–0.992	**0.04**
δ-tocopherol (mg)	0.517	0.281–0.950	**0.03**
Polyunsaturated fat (g)	0.906	0.823–0.998	**0.04**
PONase			
Nutrient	**β**	**95% CI**	***p***
Total protein (g)	−0.008	−0.0139–−0.0013	**0.02**
Animal protein (g)	−0.012	−0.0214–−0.0032	**0.01**
Calcium (mg)	−0.0007	−0.0014–−0.00006	**0.03**
Potassium (mg)	−0.0001	-0.0003–-4.6×10^{-6}	**0.04**
Vitamin B2 (mg)	−0.302	−0.581–−0.0234	**0.03**
Vitamin B3 (mg)	−0.023	−0.045–−0.0024	**0.03**
Vitamin B6 (mg)	−0.217	−0.416–−0.0178	**0.03**
Animal fat (g)	−0.008	−0.016–−0.0003	**0.04**
Saturated fat (g)	−0.017	−0.032–−0.0011	**0.04**

A Results are expressed as odds ratio (OR) and 95% confidence interval (95% CI) and β: beta coefficient. AREase associations were conducted by logistic regression analysis. Geometric mean (47.38 U/μg) of dichotomized specific AREase activity was used as the cutoff point. PONase associations were performed by linear regression analysis with specific PONase activity logarithmic transformation.

3.5. Wine's Phytochemical Composition

The chromatogram was analyzed and compared using online databases to identify the principal compounds from the Cabernet Sauvignon Malbec wine used in healthy volunteer participants [37,38]. It is well known that wine is a rich source of phenolic compounds, and the polyphenolic profile in wine changes due to different conditions including grape variety, environmental factors in the vineyard, storage conditions and management, for this reason we decided to conduct this fast analysis to confirm the presence of these compounds [39–41]. For this reason, we determined a preliminary finger-printing using a UPLC-QDa-MS, looking for information to link its effects. Phenolic compounds present in wine can be divided in two major groups according to their carbon skeletons: flavonoids and nonflavonoids. The main nonflavonoid phenolics include cinnamic acids (caffeic, p-coumaric, and ferulic acids), benzoic acids (gallic, vanillic, and syringic acids), and stilbenes (resveratrol).The chromatogram Figure S1 (Supplementary Material) and mass spectrums analysis in Figure S2 showed the presence of polyphenolic such as gallotannins, stilbenes, and tannin fragments (Table S3 Supplementary Material) [42]. According to our findings we can classify the wine used as richer in nonflavonoids compounds like benzoic acids derivatives and stilbenes such as resveratrol, and resveratrol fragments.

4. Discussion

Several studies have documented the capacity of polyphenols to regulate PON1 transcription and its antioxidant effects. Polyphenolic compounds and flavonoids are found abundantly in some foods and in beverages such as red wine [13,25,29]. In the present study, the effect of dietary habits (macro- and micronutrients) and red wine on PON1 status in healthy population was evaluated.

Our results showed that more than one half of the study participants were overweight or obese and that one-tenth of participants consumed tobacco or illicit drugs. Epidemiological studies have demonstrated that nutritional habits entertain a relationship with the onset of metabolic diseases [43]. A low intake of vegetables and fruits and a high intake of saturated fats predisposes to metabolic diseases. In this study, we requested that the participants did not change the normal diet that they had; however, we observed a significant decrease (8.7%; $p < 0.001$) in caloric intake. Some studies have reported that alcohol consumption increases or decreases the blood levels of leptin, the hormone responsible for the sensation of satiety [44–46]. The effects of alcohol on leptin depend on the quantity, frequency of ingestion, and type of alcoholic beverage [47–49]. However, the effects of alcohol on the gastrointestinal signaling pathways have not been completely described given to that the mechanisms involved in satiety and food intake are complex [50,51]. This also might explain the results observed in the intake of vitamins and minerals in our study population.

The antioxidant compounds that are contained in some foods and red wine also regulate and protect PON1 from oxidation and could act synergistically or antagonistically with the enzyme [52]. Our data revealed a significant increase in the AREase specific activity by 18.97% (quotient of activity/PON1 protein concentration), after red wine consumption (* $p < 0.05$). We observed differences in the activities of PON1 according to *Q192R* genotype. The genotype distribution found in the current study was not different from that found in other Mexican populations [10,53–55]. Few studies have reported PON1 specific activities. In this regard Mota et al. [56] reported specific PON1 AREase (0.15 ± 0.06 U/ng) and PONase (0.56 ± 0.23 U/μg) activities in an Iranian population; and found a lower concentration of PON1, but higher AREase and PONase activities than the present study. González et al. [55] reported that specific PON1 LACase activity is not influenced by PON1 polymorphism genetics. These results are in agreement with those found in this study, in which we did not observe in the LACase activity among the *Q192R* genotypes either before or after red wine consumption.

The relationship among the compounds contained in foods and the induction of PON1 is being thoroughly revised. Recent evidence deriving from in vitro, in vivo, and clinical observational studies suggests that PON1 activity is affected by the specific food profile intake [14,57,58]. Our results show that total fiber consumption increases PON1 specific AREase activity; contrariwise, Rantala et al. [59] reported decreases in PONase activity in healthy Finnish women with a high-fiber diet.

Moreover, the beneficial antioxidant effects of vitamin E and its active compounds (primarily α-, β-, γ- and δ-tocopherols) in human health are well known [60]. However, our results have shown a negative association between specific AREase activity and the consumption of β-, γ- and δ-tocopherols. Nadeem et al. [61] reported failure in the antioxidant protection of tocopherols (α- and γ-) on HDLs in vitro. As well, Wade et al. [62] observed a decrease of 15.9 and 13.2% in PON1 AREase activity associated with HDL2 and HDL3, respectively, in healthy Irish volunteers whose uptake was of 400 IU of α-tocopherol for 6 weeks.

Regarding the intake of saturated and polyunsaturated fats, a negative association in both specific AREase and PONase activities was found. These results are in agreement with several in vivo and epidemiological studies [63–66]. With respect to total and animal protein intakes, our results revealed increases in specific AREase and PONase activities. Haraguchi et al. [67] reported higher PONase activity in whey-protein-fed rats than in casein-fed rats. Increases have been reported in AREase and PONase activity with taurine supplementation [68]. Recently, our study group observed, in a Mexican population, that protein intake exerted positive effects on PON1 CMPAase activity ($\beta = 0.0093$; $p = 0.02$) [69].

The antioxidant effects of trace elements such as, zinc, selenium, copper, and manganese are widely described, [70–72] given the fact that nutritional supplementation containing mixtures of vitamins and these trace elements have shown positive effects on PONase activity [73,74]. However, the effects of minerals alone remain scarcely studied. In the present study, we found, in a healthy population, that calcium and potassium consumption decreased PONase activity. Ardalić et al. [75] reported that calcium mixed with zinc, iron, folic acid, and vitamins B1, B2, B3, B12, C, D and E had no effects on PON1 activity in healthy pregnant Serbian women. Ponce-Ruíz et al. [67] reported that potassium intake influenced LACase activity in the control group ($\beta = 0.00002$; $p < 0.01$). Our results have shown negative effects of vitamins B1 and B2 on specific PONase activity; these results support the findings by Ponce-Ruíz et al. [69] who observed a negative influence of vitamin B1 on AREase activity. We found a negative influence of vitamin B6 on PONase activity, contrary to Tas et al. [76] who showed increases of 26.1% in AREase and 25.9% in PONase in healthy subjects. A study of a habitual diet conducted in healthy volunteers demonstrated a negative correlation of PON1 activity with a high intake of vegetables and fats; [77,78] support our findings on PONase activity, given that the natural sources of tocopherols and vitamins B1, B2 and B6 are mainly animal-derived products and vegetables [79,80].

Our results suggest that compounds found in the red wine like benzoic acids derivatives, stilbenes such as resveratrol, as well as genotype and dietary habits exert an influence on both the phenotype and concentration of PON1, including macro- and micronutrients, suggesting the relevance of taking into account the internal and external factors that could exert an effect on PON1 status in human populations. In conclusion, our data acknowledge that the moderate consumption of red wine, for this study 120 mL specifically has a beneficial effect on PON1 specific AREase activity in this healthy Mexican population.

5. Limitations of the Study

An important limitation of this study is the small sample size; however, one strength is that the subjects were their own control. A large-scale longitudinal study to evaluate the relationship between the status of PON1 and red wine and dietary intake are required.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11209503/s1, Figure S1. Preliminary fingerprinting: Total Ion Chromatogram, and 3D Chromatogram for Cabernet Sauvignon Malbec wine sample by UPLC-QDa., Figure S2. Mass spectrum for flavagallol and trans-resveratrol. Tables: Table S1. Linkage disequilibrium among PON1 polymorphisms, Table S2. Allele and genotype frequencies of *PON1*, Table S3: UPLC-MS. Data of the compounds detected from Malbec wine Retention times and MS fragmentation data in positive ion mode for wine Cabernet Sauvignon Malbec [81–85].

Author Contributions: Author contributions to the paper were as follows: methodology, F.N.-G., N.P.-R., G.Á.-V. and J.F.H.-M.; formal analysis, A.E.R.-G., B.S.B.-V., F.N.-G., G.Á.-V. and I.M.M.-D.; investigation, Y.Y.B.-H. and C.A.G.-A.; writing—original draft preparation, A.E.R.-G., G.Á.-V. and I.M.M.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Strengthening Research [UAN-2018] and CONACyT [Grant SSA/IMSS/ISSSTE-233745].

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Ethics Committee of the State, of Nayarit (registry number CEBN/07/2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: We are grateful for the support received from general physicians Consuelo Rojas García and Violeta Hermosillo Medina, and QFB. Guadalupe Yáñez Ibarra for the support on mass spectrometric analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Furlong, C.E.; Marsillach, J.; Jarvik, G.P.; Costa, L.G. Paraoxonases-1, -2 and -3: What are their functions? *Chem. Biol. Interact.* **2016**, *259*, 51–62. [CrossRef] [PubMed]
2. Fridman, O.; Fuchs, A.G.; Porcile, R.; Morales, A.V.; Gariglio, L.O. Paraoxonasa: Sus múltiples funciones y regulación farmacológica. *Arch. Cardiol. Mex.* **2011**, *81*, 251–260. [PubMed]
3. Mackness, B.; Turkie, W.; Mackness, M. Paraoxonase-1 (PON1) promoter region polymorphisms, serum PON1 status and coronary heart disease. *Arch. Med. Sci.* **2013**, *9*, 8–13. [CrossRef] [PubMed]
4. Mackness, M.; Mackness, B. Human paraoxonase-1 (PON1): Gene structure and expression, promiscuous activities and multiple physiological roles. *Gene* **2015**, *567*, 12–21. [CrossRef] [PubMed]
5. Rainwater, D.L.; Rutherford, S.; Dyer, T.D.; Rainwater, E.D.; Cole, S.A.; VandeBerg, J.L.; Almasy, L.; Blangero, J.; MacCluer, J.W.; Mahaney, M.C. Determinants of variation in human serum paraoxonase activity. *Heredity* **2009**, *102*, 147–154. [CrossRef]
6. Hassett, C.; Richter, R.J.; Humbert, R.; Chapline, C.; Crabb, J.W.; Omiecinski, C.J.; Furlong, C.E. Characterization of cDNA clones encoding rabbit and human serum paraoxonase: The mature protein retains its signal sequence. *Biochemistry* **1991**, *30*, 10141–10149. [CrossRef]
7. Humbert, R.; Adler, D.A.; Disteche, C.M.; Hassett, C.; Omiecinski, C.J.; Furlong, C.E. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat. Genet.* **1993**, *3*, 73–76. [CrossRef]
8. Adkins, S.; Gan, K.N.; Mody, M.; La Du, B.N. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: Glutamine or arginine at position 191, for the respective A or B allozymes. *Am. J. Hum. Genet.* **1993**, *52*, 598–608.
9. Garin, M.C.; James, R.W.; Dussoix, P.; Blanché, H.; Passa, P.; Froguel, P.; Ruiz, J. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J. Clin. Investig.* **1997**, *99*, 62–66. [CrossRef]
10. Rojas-García, A.E.; Solís-Heredia, M.J.; Piña-Guzmán, B.; Vega, L.; López-Carrillo, L.; Quintanilla-Vega, B. Genetic polymorphisms and activity of PON1 in a Mexican population. *Toxicol. Appl. Pharmacol.* **2005**, *205*, 282–289. [CrossRef]
11. Turgut Cosan, D.; Colak, E.; Saydam, F.; Yazici, H.U.; Degirmenci, I.; Birdane, A.; Colak, E.; Gunes, H.V. Association of paraoxonase 1 (PON1) gene polymorphisms and concentration with essential hypertension. *Clin. Exp. Hypertens.* **2016**, *38*, 602–607. [CrossRef]
12. Davies, H.G.; Richter, R.J.; Keifer, M.; Broomfield, C.A.; Sowalla, J.; Furlong, C.E. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. *Nat. Genet.* **1996**, *14*, 334–336. [CrossRef]
13. Lou-Bonafonte, J.M.; Gabás-Rivera, C.; Navarro, M.A.; Osada, J. PON1 and Mediterranean Diet. *Nutrients* **2015**, *7*, 4068–4092. [CrossRef]
14. Lou-Bonafonte, J.M.; Gabás-Rivera, C.; Navarro, M.A.; Osada, J. The Search for Dietary Supplements to Elevate or Activate Circulating Paraoxonases. *Int. J. Mol. Sci.* **2017**, *18*, 416. [CrossRef]
15. Otocka-Kmiecik, A.; Lewandowski, M.; Szkudlarek, U.; Nowak, D.; Orlowska-Majdak, M. Aerobic training modulates the effects of exercise-induced oxidative stress on PON1 activity: A preliminary study. *Sci. World J.* **2014**, *2014*, 230271. [CrossRef]
16. Abdel-Salam, O.M.; Youness, E.R.; Khadrawy, Y.A.; Sleem, A.A. Acetylcholinesterase, butyrylcholinesterase and paraoxonase 1 activities in rats treated with cannabis, tramadol or both. *Asian Pac. J. Trop. Med.* **2016**, *9*, 1089–1094. [CrossRef]
17. Gouédard, C.; Barouki, R.; Morel, Y. Dietary Polyphenols Increase Paraoxonase 1 Gene Expression by an Aryl Hydrocarbon Receptor-Dependent Mechanism. *Mol. Cell Biol.* **2004**, *24*, 5209–5222. [CrossRef] [PubMed]
18. Curtin, B.F.; Seetharam, K.I.; Dhoieam, P.; Gordon, R.K.; Doctor, B.P.; Nambiar, M.P. Resveratrol induces catalytic bioscavenger paraoxonase 1 expression and protects against chemical warfare nerve agent toxicity in human cell lines. *J. Cell Biochem.* **2008**, *103*, 1524–1535. [CrossRef] [PubMed]
19. Rock, W.; Rosenblat, M.; Miller-Lotan, R.; Levy, A.P.; Elias, M.; Aviram, M. Consumption of wonderful variety pomegranate juice and extract by diabetic patients increases paraoxonase 1 association with high-density lipoprotein and stimulates its catalytic activities. *J. Agric. Food Chem.* **2008**, *56*, 8704–8713. [CrossRef] [PubMed]
20. Boesch-Saadatmandi, C.; Egert, S.; Schrader, C.; Coumoul, X.; Barouki, R.; Muller, L.J.; Wolffram, S.; Rimbach, G. Effect of quercetin on paraoxonase 1 activity—Studies in cultured cells, mice and humans. *J. Physiol. Pharmacol.* **2010**, *61*, 99–105.
21. Rosenblat, M.; Volkova, N.; Attias, J.; Mahamid, R.; Aviram, M. Consumption of polyphenolic-rich beverages (mostly pomegranate and black currant juices) by healthy subjects for a short term increased serum antioxidant status, and the serum's ability to attenuate macrophage cholesterol accumulation. *Food Funct.* **2010**, *1*, 99–109. [CrossRef]
22. Martini, D.; Del Bo, C.; Porrini, M.; Ciappellano, S.; Riso, P. Role of polyphenols and polyphenol-rich foods in the modulation of PON1 activity and expression. *J. Nutr. Biochem.* **2017**, *48*, 1–8. [CrossRef] [PubMed]
23. Fernandes, I.; Pérez-Gregorio, R.; Soares, S.; Mateus, N.; de Freitas, V. Wine Flavonoids in Health and Disease Prevention. *Molecules* **2017**, *22*, 292. [CrossRef] [PubMed]
24. Rios-Corripio, G.; Guerrero-Beltrán, J.A. Antioxidant and physicochemical characteristics of unfermented and fermented pomegranate (*Punica granatum* L.) beverages. *J. Food Sci. Technol.* **2019**, *56*, 132–139. [CrossRef] [PubMed]
25. Sarandöl, E.; Serdar, Z.; Dirican, M.; Şafak, Ö. Effects of red wine consumption on serum paraoxonase/arylesterase activities and on lipoprotein oxidizability in healthy-men. *J. Nutr. Biochem.* **2003**, *14*, 507–512. [CrossRef]

26. Leckey, L.C.; Garige, M.; Varatharajalu, R.; Gong, M.; Nagata, T.; Spurney, C.F.; Lakshman, R.M. Quercetin & ethanol attenuate the progression of atherosclerotic plaques with concomitant up regulation of paraoxonase1 (PON1) gene expression and PON1 activity in LDLR$^{-/-}$ mice. *Alcohol. Clin. Exp. Res.* **2010**, *34*, 1535–1542.
27. Lakshman, R.; Garige, M.; Gong, M.; Leckey, L.; Varatharajalu, R.; Zakhari, S. Is alcohol beneficial or harmful for cardioprotection? *Genes Nutr.* **2010**, *5*, 111–120. [CrossRef]
28. Gupta, N.; Kandimalla, R.; Priyanka, K.; Singh, G.; Gill, K.D.; Singh, S. Effect of Resveratrol and Nicotine on PON1 Gene Expression: In Vitro Study. *Indian J. Clin. Biochem.* **2014**, *29*, 69–73. [CrossRef]
29. Rizzi, F.; Conti, C.; Dogliotti, E.; Terranegra, A.; Salvi, E.; Braga, D.; Ricca, F.; Lupoli, S.; Mingione, A.; Pivari, F.; et al. Interaction between polyphenols intake and PON1 gene variants on markers of cardiovascular disease: A nutrigenetic observational study. *J. Transl. Med.* **2016**, *14*, 186. [CrossRef]
30. Schwedhelm, C.; Nimptsch, K.; Bub, A.; Pischon, T.; Linseisen, J. Association between alcohol consumption and serum paraoxonase and arylesterase activities: A cross-sectional study within the Bavarian population. *Br. J. Nutr.* **2016**, *115*, 730–736. [CrossRef]
31. Hernández-Ávila, M.; Romieu, I.; Parra, S.; Hernández-Ávila, J.; Madrigal, H.; Willett, W. Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. *Salud Publica Mex.* **1998**, *40*, 133–140. [CrossRef] [PubMed]
32. Eckerson, H.W.; Wyte, C.M.; La Du, B.N. The human serum paraoxonase/arylesterase polymorphism. *Am. J. Hum. Genet.* **1983**, *35*, 1126–1138. [PubMed]
33. Richter, R.J.; Jarvik, G.P.; Furlong, C.E. Determination of paraoxonase 1 status without the use of toxic organophosphate substrates. *Circ. Cardiovasc. Genet.* **2008**, *1*, 147–152. [CrossRef] [PubMed]
34. Billecke, S.; Draganov, D.; Counsell, R.; Stetson, P.; Watson, C.; Hsu, C.; La Du, B.N. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. *Drug Metab. Dispos.* **2000**, *28*, 1335–1342.
35. Furlong, C.E.; Richter, R.J.; Seidel, S.L.; Motulsky, A.G. Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon. *Am. J. Hum. Genet.* **1988**, *43*, 230–238.
36. WHO. Obesity and Overweight. Available online: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed on 27 November 2020).
37. Spectra Search Mass Spectrum. Available online: https://foodb.ca/spectra/ms/search (accessed on 24 September 2021).
38. Spectra Search Mass Spectrum. Available online: https://hmdb.ca/spectra/ms/search (accessed on 20 August 2021).
39. Ragusa, A.; Centonze, C.; Grasso, M.E.; Latronico, M.F.; Mastrangelo, P.F.; Sparascio, F.; Maffia, M. HPLC analysis of phenols in Negroamaro and primitivo red wines from salento. *Foods* **2019**, *8*, 45. [CrossRef]
40. Granato, D.; Katayama, F.C.U.; De Castro, I.A. Phenolic composition of South American red wines classified according to their antioxidant activity, retail price and sensory quality. *Food Chem.* **2011**, *129*, 366–373. [CrossRef]
41. Giovinazzo, G.; Grieco, F. Functional Properties of Grape and Wine Polyphenols. *Plant Foods Hum. Nutr.* **2015**, *70*, 454–462. [CrossRef]
42. Hornedo-Ortega, R.; González-Centeno, M.R.; Chira, K.; Jourdes, M.; Teissedre, P.-L. Phenolic Compounds of Grapes and Wines: Key Compounds and Implications in Sensory Perception. *Front. Cell Neurosci.* **2018**, *23*, 373. [CrossRef] [PubMed]
43. Mazidi, M.; Pennathur, S.; Afshinnia, F. Link of dietary patterns with metabolic syndrome: Analysis of the National Health and Nutrition Examination Survey. *Nutr. Diabetes* **2017**, *7*, e255. [CrossRef] [PubMed]
44. Roth, M.J.; Baer, D.J.; Albert, P.S.; Castonguay, T.W.; Dorgan, J.F.; Dawsey, S.M.; Brown, E.D.; Hartman, T.J.; Campbell, W.S.; Giffen, C.A.; et al. Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. *J. Natl. Cancer Inst.* **2003**, *95*, 1722–1725. [CrossRef]
45. Otaka, M.; Konishi, N.; Odashima, M.; Jin, M.; Wada, I.; Matsuhashi, T.; Ohba, R.; Watanabe, S. Effect of alcohol consumption on leptin level in serum, adipose tissue, and gastric mucosa. *Dig. Dis. Sci.* **2007**, *52*, 3066–3069. [CrossRef]
46. Bach, P.; Koopmann, A.; Kiefer, F. The Impact of Appetite-Regulating Neuropeptide Leptin on Alcohol Use, Alcohol Craving and Addictive Behavior: A Systematic Review of Preclinical and Clinical Data. *Alcohol Alcohol.* **2021**, *56*, 149–165. [CrossRef] [PubMed]
47. Rojdmark, S.; Calissendorff, J.; Brismar, K. Alcohol ingestion decreases both diurnal and nocturnal secretion of leptin in healthy individuals. *Clin. Endocrinol.* **2001**, *55*, 639–647. [CrossRef]
48. Yeomans, M.R. Alcohol, appetite and energy balance: Is alcohol intake a risk factor for obesity? *Physiol. Behav.* **2010**, *100*, 82–89. [CrossRef] [PubMed]
49. Schrieks, I.C.; Stafleu, A.; Griffioen-Roose, S.; de Graaf, C.; Witkamp, R.F.; Boerrigter-Rijneveld, R.; Hendriks, H.F.J. Moderate alcohol consumption stimulates food intake and food reward of savoury foods. *Appetite* **2015**, *89*, 77–83. [CrossRef] [PubMed]
50. Kraus, T.; Schanze, A.; Gröschl, M.; Bayerlein, K.; Hillemacher, T.; Reulbach, U.; Kornhuber, J.; Bleich, S. Ghrelin levels are increased in alcoholism. *Alcohol Clin. Exp. Res.* **2005**, *29*, 2154–2157. [CrossRef]
51. Hennink, S.D.; Maljaars, P.W.J. Fats and satiety. In *Satiation, Satiety and the Control of Food Intake. Theory and Practice*; Blundell, J.E., Bellisle, F., Eds.; Woodhead Publishing Limited: Sawston, UK, 2013; pp. 143–165.
52. Aviram, M.; Kaplan, M.; Rosenblat, M.; Fuhrman, B. Dietary Antioxidants and Paraoxonases against LDL Oxidation and Atherosclerosis Development. In *Atherosclerosis: Diet and Drugs*; von Eckardstein, A., Ed.; Springer: Berlin/Heidelberg, Germany, 2005; pp. 263–300.

53. Bernal-Hernández, Y.Y.; Medina-Díaz, I.M.; Barrón-Vivanco, B.S.; Robledo-Marenco, M.L.; Girón-Pérez, M.I.; Pérez-Herrera, N.E.; Quintanilla-Vega, B.; Cerda-Flores, R.; Rojas-García, A.E. Paraoxonase 1 and its relationship with pesticide biomarkers in indigenous Mexican farmworkers. *J. Occup. Environ. Med.* **2014**, *56*, 281–290. [CrossRef]
54. García-González, I.; Mendoza-Alcocer, R.; Pérez-Mendoza, G.J.; Rubí-Castellanos, R.; González-Herrera, L. Distribution of genetic variants of oxidative stress metabolism genes: Paraoxonase 1 (PON1) and Glutathione S-transferase (GSTM1/GSTT1) in a population from Southeastern Mexico. *Ann. Hum. Biol.* **2016**, *43*, 554–562. [CrossRef]
55. González, F.E.M.; Ponce-Ruíz, N.; Rojas-García, A.E.; Bernal-Hernández, Y.Y.; Mackness, M.; Ponce-Gallegos, J.; Cardoso-Saldaña, G.; Jorge-Galarza, E.; Torres-Tamayo, M.; Medina-Díaz, I.M. PON1 concentration and high-density lipoprotein characteristics as cardiovascular biomarkers. *Arch. Med. Sci. Atheroscler. Dis.* **2019**, *4*, e47–e54. [CrossRef]
56. Mota, A.; Hemati-dinarvand, M.; Akbar Taheraghdam, A.; Reza Nejabati, H.; Ahmadi, R.; Ghasemnejad, T.; Hasanpour, M.; Valilo, M. Association of Paraoxonse1 (PON1) Genotypes with the Activity of PON1 in Patients with Parkinson's Disease. *Acta Neurol. Taiwan* **2019**, *28*, 66–74.
57. Costa, L.G.; Giordano, G.; Furlong, C.E. Pharmacological and dietary modulators of paraoxonase 1 (PON1) activity and expression: The hunt goes on. *Biochem. Pharmacol.* **2011**, *81*, 337–344. [CrossRef]
58. Costa, L.G.; Vitalone, A.; Cole, T.B.; Furlong, C.E. Modulation of paraoxonase (PON1) activity. *Biochem. Pharmacol.* **2005**, *69*, 541–550. [CrossRef] [PubMed]
59. Rantala, M.; Silaste, M.L.; Tuominen, A.; Kaikkonen, J.; Salonen, J.T.; Alfthan, G.; Aro, A.; Kesäniemi, Y.A. Dietary modifications and gene polymorphisms alter serum paraoxonase activity in healthy women. *J. Nutr.* **2002**, *132*, 3012–3017. [CrossRef] [PubMed]
60. Rizvi, S.; Raza, S.T.; Ahmed, F.; Ahmad, A.; Abbas, S.; Mahdi, F. The role of vitamin E in human health and some diseases. *Sultan Qaboos Univ. Med. J.* **2014**, *14*, e157–e165. [PubMed]
61. Nadeem, N.; Woodside, J.V.; Kelly, S.; Allister, R.; Young, I.S.; McEneny, J. The two faces of alpha- and gamma-tocopherols: An in vitro and ex vivo investigation into VLDL, LDL and HDL oxidation. *J. Nutr. Biochem.* **2012**, *23*, 845–851. [CrossRef]
62. Wade, L.; Nadeem, N.; Young, I.S.; Woodside, J.V.; McGinty, A.; McMaster, C.; McEneny, J. α-Tocopherol induces proatherogenic changes to HDL2 & HDL3: An in vitro and ex vivo investigation. *Atherosclerosis* **2013**, *226*, 392–397.
63. Durrington, P.N.; Mackness, B.; Mackness, M.I. The hunt for nutritional and pharmacological modulators of paraoxonase. *Arterioscler. Thromb. Vasc. Biol.* **2002**, *22*, 1248–1250. [CrossRef]
64. Thomàs-Moyà, E.; Gianotti, M.; Proenza, A.M.; Llado, I. Paraoxonase 1 response to a high-fat diet: Gender differences in the factors involved. *Mol. Med.* **2007**, *13*, 203–209. [CrossRef]
65. Hoefel, A.L.; Hansen, F.; Rosa, P.D.; Assis, A.M.; da Silveira, S.L.; Denardin, C.C.; Pettenuzzo, L.; Augusti, P.R.; Somacal, S.; Emanuelli, T.; et al. The effects of hypercaloric diets on glucose homeostasis in the rat: Influence of saturated and monounsaturated dietary lipids. *Cell Biochem. Funct.* **2011**, *29*, 569–576. [CrossRef]
66. Kim, D.S.; Burt, A.A.; Ranchalis, J.E.; Richter, R.J.; Marshall, J.K.; Nakayama, K.S.; Jarvik, E.R.; Eintracht, J.F.; Rosenthal, E.A.; Furlong, C.E.; et al. Dietary cholesterol increases paraoxonase 1 enzyme activity. *J. Lipid Res.* **2012**, *53*, 2450–2458. [CrossRef] [PubMed]
67. Haraguchi, F.K.; Pedrosa, M.L.; Paula, H.; Santos, R.C.; Silva, M.E. Evaluation of biological and biochemical quality of whey protein. *J. Med. Food.* **2010**, *13*, 1505–1509. [CrossRef]
68. Dirican, M.; Tas, S.; Sarandöl, E. High-dose taurine supplementation increases serum paraoxonase and arylesterase activities in experimentalhypothyroidism. *Clin. Exp. Pharmacol. Physiol.* **2007**, *34*, 833–837. [CrossRef] [PubMed]
69. Ponce-Ruíz, N.; Murillo-González, F.E.; Rojas-García, A.E.; Bernal-Hernández, Y.Y.; Mackness, M.; Ponce-Gallegos, J.; Barrón-Vivanco, B.S.; Hernández-Ochoa, I.; González-Arias, C.A.; Ortega-Cervantes, L.; et al. Phenotypes and concentration of PON1 in cardiovascular disease: The role of nutrient intake. *Nutr. Metab. Cardiovasc. Dis.* **2019**, *30*, 40–48. [CrossRef] [PubMed]
70. Powell, S.R. The antioxidant properties of zinc. *J. Nutr.* **2000**, *130*, 1447S–1454S. [CrossRef]
71. Sánchez, C.; López-Jurado, M.; Aranda, P.; Llopis, J. Plasma levels of copper, manganese and selenium in an adult population in southern Spain: Influence of age, obesity and lifestyle factors. *Sci. Total Environ.* **2010**, *408*, 1014–1020. [CrossRef]
72. Azadmanesh, J.; Borgstahl, G.E.O. A Review of the Catalytic Mechanism of Human Manganese Superoxide Dismutase. *Antioxidants* **2018**, *7*, 25. [CrossRef]
73. Begcevic, I.; Simundic, A.M.; Nikolac, N.; Dobrijevic, S.; Rajkovic, M.G.; Tesija-Kuna, A. Can cranberry extract and vitamin C + Zn supplements affect the In vivo activity of paraoxonase 1, antioxidant potential, and lipid status? *Clin. Lab.* **2013**, *59*, 1053–1060. [CrossRef]
74. Manolescu, B.N.; Berteanu, M.; Cinteza, D. Effect of the nutritional supplement ALAnerv® on the serum PON1 activity in post-acute stroke patients. *Pharmacol. Rep.* **2013**, *65*, 743–750. [CrossRef]
75. Ardalić, D.; Stefanović, A.; Kotur-Stevuljević, J.; Vujović, A.; Spasić, S.; Spasojević-Kaliomanvska, V.; Jelić-Ivanović, Z.; Mandić-Marković, V.; Miković, Z.; Cerović, N. The influence of maternal smoking habits before pregnancy and antioxidative supplementation during pregnancy on oxidative stress status in a non-complicated pregnancy. *Adv. Clin. Exp. Med.* **2014**, *23*, 575–583. [CrossRef] [PubMed]
76. Taş, S.; Sarandöl, E.; Dirican, M. Vitamin B6 supplementation improves oxidative stress and enhances serum paraoxonase/arylesterase activities in streptozotocin-induced diabetic rats. *Sci. World J.* **2014**, *2014*, 351598. [CrossRef] [PubMed]

77. Freese, R.; Alfthan, G.; Jauhiainen, M.; Basu, S.; Erlund, I.; Salminen, I.; Aro, A.; Mutanen, M. High intakes of vegetables, berries, and apples combined with a high intake of linoleic or oleic acid only slightly affect markers of lipid peroxidation and lipoprotein metabolism in healthy subjects. *Am. J. Clin. Nutr.* **2002**, *76*, 950–960. [CrossRef] [PubMed]
78. Kleemola, P.; Freese, R.; Jauhiainen, M.; Pahlman, R.; Alfthan, G.; Mutanen, M. Dietary determinants of serum paraoxonase activity in healthy humans. *Atherosclerosis* **2002**, *160*, 425–432. [CrossRef]
79. Shahidi, F.; de Camargo, A.C. Tocopherols and Tocotrienols in Common and Emerging Dietary Sources: Occurrence, Applications, and Health Benefits. *Int. J. Mol. Sci.* **2016**, *17*, 1745. [CrossRef] [PubMed]
80. Mielgo-Ayuso, J.; Aparicio-Ugarriza, R.; Olza, J.; Aranceta-Bartrina, J.; Gil, A.; Ortega, R.M.; Serra-Majem, L.; Varela-Moreiras, G.; González-Gross, M. Dietary Intake and Food Sources of Niacin, Riboflavin, Thiamin and Vitamin B6 in a Representative Sample of the Spanish Population. The Anthropometry, Intake, and Energy Balance in Spain (ANIBES) Study dagger. *Nutrients* **2018**, *10*, 846. [CrossRef] [PubMed]
81. Spectra Search Mass Spectrum. Available online: https://hmdb.ca/metabolites/HMDB0033908 (accessed on 21 August 2021).
82. Spectra Search Mass Spectrum. Available online: https://hmdb.ca/metabolites/HMDB0003747 (accessed on 21 August 2021).
83. Spectra Search Mass Spectrum. Available online: https://hmdb.ca/metabolites/HMDB0005807 (accessed on 21 August 2021).
84. Spectra Search Mass Spectrum. Available online: https://hmdb.ca/spectra/ms_ms/252054 (accessed on 22 August 2021).
85. Spectra Search Mass Spectrum. Available online: https://foodb.ca/compounds/FDB006783 (accessed on 25 August 2021).