Urinary Resveratrol Metabolites Output: Differential Associations with Cardiometabolic Markers and Liver Enzymes in House-Dwelling Subjects Featuring Metabolic Syndrome

Vanessa Bullón-Vela 1, Itziar Abete 1,2,* , Maria Angeles Zulet 1,2,† , Yifan Xu 3, Miguel A. Martínez-González 2,4, Carmen Sayón-Orea 2,4, Miguel Ruiz-Canela 2,4, Estefanía Toledo 2,4, Vicente Martín Sánchez 5,6, Ramon Estruch 7,8, Rosa María Lamuela-Raventós 2,8, Enrique Almanza-Aguilera 9,10,11, Montserrat Fitó 2,9, Jordi Salas-Salvadó 2,12,13, Andrés Díaz-López 2,12,13, Francisco J. Tínahones 2,14, Josep A. Tur 2,15, Dora Romaguera 2,16, Jadwiga Konieczna 2,16, Xavier Pintó 2,17, Lidia Daimiel 18, Ana Rodriguez-Mateos 3 and José Alfredo Martínez 1,2,18,†

1 Department of Nutrition, Food Science and Physiology, Center for Nutrition Research, University of Navarra, 31008 Pamplona, Spain; mbullon@alumni.unav.es (V.B.-V.); mazulet@unav.es (M.A.Z.); jalfmtz@unav.es (J.A.M.)
2 Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain; mamartinez@unav.es (M.A.M.-G.); msayon@unav.es (C.S.-O.); mcanela@unav.es (M.R.-C.); ETOLEDO@unav.es (E.T.); esacane@clinic.cat (R.E.); lamuela@ub.edu (R.M.L.-R.); mfito@imim.es (M.F.); jordi.salas@urv.cat (J.S.-S.); andres.diaz@urv.cat (A.D.-L.); ffitnahones@uma.es (F.J.T.); pep.tur@uib.es (J.A.T.); dorormaguer@yahoo.es (D.R.); jadzia.konieczna@gmail.com (J.K.); xpinto@bellvitgehospital.cat (X.P.)
3 Department of Nutritional Sciences, School of Life Course Sciences, Faculty of Life Sciences and Medicine, King’s College London, London SE1 9NH, UK; yifan.xu@kcl.ac.uk (Y.X.); ana.rodriguez-mateos@kcl.ac.uk (A.R.-M.)
4 Department of Preventive Medicine and Public Health, University of Navarra, 31008 Pamplona, Spain
5 Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain; vicente.martin@unileon.es
6 Institute of Biomedicine (IBIOMED), University of León, 24071 León, Spain
7 Department of Internal Medicine, IDIBAPS, Hospital Clinic, University of Barcelona, 08036 Barcelona, Spain
8 Department of Nutrition, Food Sciences and Gastronomy, XaRTA, INSA-UB, School of Pharmacy and Food Sciences, Nutrition and Food Safety Research Institute, University of Barcelona, 08028 Barcelona, Spain
9 Cardiovascular Risk and Nutrition Research Group (CARIN), Hospital del Mar Research Institute (IMIM), 08007 Barcelona, Spain; ealmanzaa@outlook.com
10 CIBER Frailty and Healthy Ageing (CIBERFES), Institute of Health Carlos III, 28029 Madrid, Spain
11 Department of Nutrition and Food Safety (INSA-UB), University of Barcelona, Santa Coloma de Gramenet, 08921 Barcelona, Spain
12 Departament de Bioquímica i Biotecnologia, Universitat Rovira i Virgili, Unitat de Nutrició Humana, 43201 Reus, Spain
13 Institut d’Investigació Pere Virgili (IISPV), Hospital Universitari Sant Joan de Reus, 43204 Reus, Spain
14 Department of Endocrinology, Instituto de Investigación Biomédica de Málaga-IBIMA, University of Málaga, Virgen de la Victoria Hospital, 29010 Málaga, Spain
15 Research Group on Community Nutrition & Oxidative Stress, University of Balearic Islands, 07122 Palma de Mallorca, Spain
16 Research Group on Nutritional Epidemiology & Cardiovascular Physiopathology (NUTRECOR), Health Research Institute of the Balearic Islands (IdISBa), University Hospital Son Espases (HUSE), 07120 Palma de Mallorca, Spain
17 Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, 08908 Barcelona, Spain
18 Precision Nutrition Program, IMDEA Food, CEI UAM + CSIC, 28049 Madrid, Spain; lidia.daimiel@imdea.org
* Correspondence: iabetego@unav.es; Tel.: +34-94-842-5600 (ext. 806357)
† On behalf of PREDIMED-Plus Investigators.

Received: 31 July 2020; Accepted: 18 September 2020; Published: 22 September 2020

Abstract: Metabolic syndrome (MetS) components are strongly associated with increased risk of non-alcoholic fatty liver disease (NAFLD) development. Several studies have supported that resveratrol is associated with anti-inflammatory and antioxidant effects on health status. The main objective of this study was to assess the putative associations between some urinary resveratrol phase II metabolites, cardiometabolic, and liver markers in individuals diagnosed with MetS. In this cross-sectional study, 266 participants from PREDIMED Plus study (PREvención con DIeta MEDiterránea) were divided into tertiles of total urinary resveratrol phase II metabolites (sum of five resveratrol conjugation metabolites). Urinary resveratrol metabolites were analyzed by ultra-performance liquid chromatography coupled to triple quadrupole mass spectrometry (UPLC-Q-q-Q MS), followed by micro-solid phase extraction (µ-SPE) method. Liver function markers were assessed using serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). Moreover, lipid profile was measured by triglycerides, very-low-density lipoprotein cholesterol (VLDL-c), and total cholesterol/high-density lipoprotein ratio (total cholesterol/HDL). Linear regression adjusted models showed that participants with higher total urine resveratrol concentrations exhibited improved lipid and liver markers compared to the lowest tertile. For lipid determinations: log triglycerides ($\beta_{T3} = -0.15$, 95% CI; $-0.28$, $-0.02$, p-trend = 0.030), VLDL-c, ($\beta_{T3} = -4.21$, 95% CI; $-7.97$, $-0.46$, p-trend = 0.039), total cholesterol/HDL ratio Moreover, ($\beta_{T3} = -0.35$, 95% CI; $-0.66$, $-0.03$, p-trend = 0.241). For liver enzymes: log AST ($\beta_{T3} = -0.12$, 95% CI; $-0.22$, $-0.02$, p-trend = 0.011, and log GGT ($\beta_{T3} = -0.24$, 95% CI; $-0.42$, $-0.06$, p-trend = 0.002). However, there is no difference found on glucose variables between groups. To investigate the risk of elevated serum liver markers, flexible regression models indicated that total urine resveratrol metabolites were associated with a lower risk of higher ALT (169.2 to 1314.3 nmol/g creatinine), AST (599.9 to 893.8 nmol/g creatinine), and GGT levels (169.2 to 893.8 nmol/g creatinine). These results suggested that higher urinary concentrations of some resveratrol metabolites might be associated with better lipid profile and hepatic serum enzymes. Moreover, urinary resveratrol excreted showed a reduced odds ratio for higher liver enzymes, which are linked to NAFLD.

Keywords: antioxidant; inflammation; liver enzymes; metabolic syndrome; non-alcoholic fatty liver disease; resveratrol

1. Introduction

Metabolic syndrome (MetS) encompasses several clinical conditions, including central obesity, hypertension, dyslipidemia, and insulin resistance leading to an inflammatory state [1], which is frequently accompanied by liver dysfunction [2]. Many clinical studies suggested that non-alcoholic fatty liver disease (NAFLD) is the liver manifestation of MetS [2–4]. NAFLD is characterized by simple hepatic steatosis (excessive triglyceride accumulation) leading to alterations in oxidative and inflammatory pathways. This state promotes non-alcoholic steatohepatitis (NASH), subsequently cirrhosis and hepatic carcinoma in last stages [3,5]. The prevalence of NAFLD increases with rates of obesity and type 2 diabetes mellitus (T2DM), mainly due to unhealthy lifestyle behaviors [2]. It has been suggested that insulin resistance and abnormal lipid profile were strongly involved in NAFLD pathogenesis and prognosis [3]. Hyperinsulinemia increases free fatty acid levels promoting a disrupted flux of triglycerides into hepatocytes [5–7]. In NAFLD, commonly abnormal elevation of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) have presented [3,4]. Liver biopsy is still the gold standard for diagnosing NAFLD, but some limitations regarding high cost and invasive nature hindered it being applicable in epidemiological
In this sense, non-invasive liver markers such as transaminases, fatty liver index (FLI) and hepatic steatosis index (HSI) are recommended in individuals with obesity and MetS as a routine work-up to identify the risk of NAFLD and subjects with worse prognosis [4]. Metabolomics is the technology that analyses metabolites in a biological system, and it has been considered as a potential omics tool to investigate the impact of nutrients, foods, and dietary patterns on human health with application in precision nutrition research [8]. Moreover, metabolite biomarkers related to dietary intake could be useful as potential non-invasive biomarkers of effects and disease risk [8].

Lifestyle interventions focused on weight loss, exercise, and a healthy diet can improve the histopathological and clinical features of NAFLD [3]. Scientific evidence suggests that the modulation of dietary components can influence NAFLD pathogenesis beyond caloric restriction [9]. In this sense, many epidemiological and clinical data support that the beneficial effects of the Mediterranean diet (MedDiet) on metabolic disturbances linked to NAFLD mainly attributed to higher consumption of bioactive compounds, such as resveratrol and anthocyanins that are present in whole-grain cereals, fruits, vegetables, healthy fatty acids, and moderate intake of wine [10–15]. Resveratrol is a member of the stilbene family that is present in several foods and plants [16,17]. The primary dietary sources are red grapes and red wine, with smaller amounts present in peanuts, berries, and dark chocolate [16,18]. After the intake, resveratrol enters the gastrointestinal tract and then the liver via the hepatic portal system, and is metabolized by phase II enzymes generating sulfate (trans-cis-resveratrol 3-O-sulfate and 4-O-sulfate) and glucuronide (trans-cis-resveratrol 3-O-glucuronide and 4-O-glucuronide) metabolites [19,20]. The gut microbiota can metabolize the resveratrol and conjugated metabolites into dihydro-resveratrol and lunularin [19]. Several human studies have shown that the most abundant resveratrol phase II conjugates are glucuronides and sulfate metabolites in urine and plasma samples [20,21]. Limited information has been reported regarding bioavailability and pharmacokinetics of glycosylated metabolites (piceid), derived from gut microbiota and other stilbenes (piceatannol). Pharmacokinetics studies in human showed that resveratrol is highly metabolized, but it has low bioavailability [20,22,23]. In a study used radiolabeled 14C-resveratrol to evaluate the bioavailability of resveratrol intake in humans, results indicated that 70% of the resveratrol absorption was recovered in urine. Moreover, the rapid sulfate conjugation by the intestine-liver could be the principal influence of their bioavailability [22]. Moreover, the bioavailability and quantity of resveratrol metabolites can be affected by several factors leading to a significant interindividual variability [19,24,25]. Despite its low bioavailability, several studies have reported that resveratrol metabolites exert beneficial effects modulating inflammatory and oxidative pathways related to several chronic diseases, such as cancer, cardiovascular disease (CVD), T2DM, obesity, and NAFLD [21]. NAFLD, most of the studies on resveratrol, and the mechanism of action have been developed in vitro and animal rodents, which used higher resveratrol concentrations, different cell lines, and animal models that often overlap [26]. The protective effects of resveratrol on NAFLD mainly include improvements in principal risk factors, such as blood glucose and insulin levels [27], lipid metabolism [28], and liver damage [29], but results have remained inconclusive. In this regard, knowledge of the underlying effects of resveratrol metabolites on liver markers and risk of NAFLD in individuals diagnosed with MetS is still needed. Thus, our objective was to determine the potential association between some phase II urinary metabolites of resveratrol and cardiometabolic and liver markers related to the risk of NAFLD development.

2. Results

2.1. Participant Characteristics

Baseline sociodemographic, clinical, and liver characteristics stratified by sex are summarized in Table 1. This study included subjects between 55 to 75 years old (65.8 ± 5.1 years) who were overweight or obese (32.2 ± 3.4 kg/m²). Men were more prevalent as former smokers, had higher waist
circumference (109.9 ± 8.5 cm), and visceral fat mass (2850.3 ± 826.2 g) compared to women (all p-values < 0.05). Moreover, men showed higher levels of physical activity (3690 ± 3101.4 Metabolic Equivalent of Task (MET)/min/week) (p > 0.001). There were no differences in taking lipid-lowering and anti-diabetic medications in between genders. Likewise, glucose, homeostatic model assessment for insulin (HOMA-IR), triglycerides, and very-low-density lipoprotein cholesterol (VLDL-c) did not differ among sexes. Nevertheless, women had higher insulin, cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) levels in comparison to men (all p > 0.05). Concerning liver markers, ALT (31.2 ± 24.5 U/L), AST (25.2 ± 16.3 U/L), and FLI (80.4 ± 14.4) were significantly higher in men than women (all p-values > 0.05). Moreover, there was significant difference in the percentage of participants with ALT values above the upper limit normal (ULN) between men and women. Meanwhile, women had higher HSI (44.0 ± 5.1) in comparison to men (p = 0.004). Regarding dietary intake and urine resveratrol metabolites (Table 2), the intake of macronutrients did not differ significantly between genders. However, men had higher energy (2689.4 ± 534.5 kcal/d, p = 0.004) intake and polyunsaturated fatty acid (PUFA) consumption (19.1 ± 7.2 g/d, p = 0.049) compared to women. Nevertheless, women consumed more vegetables (353.0 ± 124.0 g/d, p = 0.024) and had a lower grape intake (5.8 ± 12.3 g/d, p = 0.012). Moreover, men had a much higher alcohol consumption than women (19.7 ± 19.8 g/d, p < 0.001) with statistically significant differences in total red, young red, aged red, and rose wine consumption between sexes.

Table 1. Sociodemographic, clinical and liver characteristics of study participants diagnosed with MetS by sex at baseline.

|                      | All           | Men (n = 153) | Women (n = 113) | p  |
|----------------------|---------------|---------------|----------------|----|
| Age (years)          | 65.8 (5.1)    | 64.6 (5.4)    | 67.5 (3.9)     | <0.001 |
| BMI (kg/m²)          | 32.2 (3.4)    | 31.8 (3.1)    | 32.8 (3.7)     | 0.019  |
| Waist circumference  | 107.3 (9.0)   | 109.9 (8.5)   | 103.9 (8.6)    | <0.001 |
| VAT (g)              | 2403.6 (888.9)| 2850.3 (826.2)| 1831.5 (589.7)| <0.001 |
| SBP (mmHg)           | 144.8 (16.3)  | 144.9 (15.8)  | 144.6 (17.0)   | 0.887  |
| DBP (mmHg)           | 87.5 (8.5)    | 87.9 (8.2)    | 86.9 (8.9)     | 0.338  |
| Type 2 diabetes, n (%) | 100 (37.6)   | 61 (39.9)     | 39 (34.5)      | 0.373  |
| Smoking, n (%)       |               |               |                | <0.001 |
| Never                | 105 (39.5)    | 29 (18.9)     | 76 (67.3)      |       |
| Former               | 124 (46.6)    | 97 (63.4)     | 27 (23.9)      |       |
| Current              | 37 (13.9)     | 27 (17.7)     | 10 (8.8)       |       |
| Lipid-lowering treatment | 93 (36.0)   | 54 (57.5)     | 39 (54.2)      | 0.673  |
| Any anti-diabetic treatment | 69 (25.9) | 43 (28.1)      | 26 (33.0)      | 0.349  |
| Glucose (mmol/L)     | 6.7 (1.9)     | 6.8 (2.1)     | 6.5 (1.6)      | 0.286  |
| HbA1c (%)            | 6.1 (0.9)     | 6.1 (1.0)     | 6.1 (0.8)      | 0.721  |
| Insulin (mU/L)       | 14.0 (9.0)    | 13.0 (7.1)    | 15.6 (11.0)    | 0.020  |
| HOMA-IR              | 4.2 (3.2)     | 3.9 (2.4)     | 4.7 (4.2)      | 0.056  |
| Total cholesterol (mg/dL) | 200.4 (36.5)| 192.2 (34.2)  | 211.4 (36.9)   | <0.001 |
| Triglycerides (mg/dL)| 148.3 (61.8) | 151.1 (69.0)  | 144.6 (50.6)   | 0.402  |
| HDL-c (mg/dL)        | 45.8 (10.0)   | 43.0 (8.9)    | 49.5 (10.1)    | <0.001 |
| LDL-c (mg/dL)        | 125.8 (33.1)  | 119.8 (31.3)  | 133.6 (33.9)   | <0.001 |
| VLDL-c (mg/dL)       | 29.7 (12.4)   | 30.2 (13.8)   | 28.9 (10.1)    | 0.402  |
| ALT (U/L)            | 28.1 (20.6)   | 31.2 (24.5)   | 23.8 (12.3)    | 0.004  |
| AST (U/L)            | 23.7 (13.4)   | 25.2 (16.3)   | 21.8 (7.4)     | 0.042  |
| GGT (U/L)            | 42.1 (41.1)   | 44.9 (41.5)   | 38.5 (40.3)    | 0.209  |
| ALT > ULN, n (%)     | 122 (46.0)    | 55 (36.0)     | 67 (59.8)      | <0.001 |
| AST > ULN, n (%)     | 28 (10.5)     | 12 (7.8)      | 16 (14.2)      | 0.097  |
| GGT > ULN, n (%)     | 51 (19.3)     | 24 (15.9)     | 27 (23.9)      | 0.103  |
| FLI                  | 78.7 (15.1)   | 80.4 (14.4)   | 76.4 (15.9)    | 0.035  |
Table 1. Cont.

|                      | Men                  | Women                 | p \( ^\dagger \) |
|----------------------|----------------------|-----------------------|------------------|
| HSI                  | 43.0 (4.9)           | 42.3 (4.7)            | 44.0 (5.1)       | 0.004 |
| Physical activity    | 3099.9 (2757.8)      | 3690 (3101.4)         | 2301.0 (1954.8)  | <0.001 |

Data were calculated by chi-square or student’s t-test as appropriate. Results are expressed as mean (standard deviation). \( p ^\dagger \) for differences between sexes. Abbreviations: BMI, Body mass index; VAT, visceral adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin; HDL-c, high-density lipoprotein cholesterol; LDL-c, Low-density lipoprotein cholesterol; VLDL, very-low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; FLI, fatty liver index; HSI, hepatic steatosis index; MET, metabolic equivalent task. * Upper limit of normal (ULN) range for ALT (men \( \geq 30 \) UI/L, women \( \geq 19 \) UI/L), AST (men \( \geq 37 \) UI/L, women \( \geq 31 \) UI/L), and GGT (men \( \geq 60 \) UI/L, women \( \geq 40 \) UI/L).

Table 2. Dietary intake and urine resveratrol metabolites in participants diagnosed with MetS by sex at baseline.

|                      | Men                  | Women                 | p \( ^\dagger \) |
|----------------------|----------------------|-----------------------|------------------|
| Total energy intake  | 2608.6 (539.1)       | 2689.4 (534.5)        | 2499.1 (528.0)   | 0.004 |
| Carbohydrate intake  | 282.9 (75.1)         | 287.0 (73.8)          | 277.5 (76.9)     | 0.310 |
| Protein intake       | 102.5 (22.9)         | 101.3 (23.8)          | 104.1 (21.8)     | 0.330 |
| Fat intake           | 108.5 (27.1)         | 110.9 (26.9)          | 105.3 (27.2)     | 0.171 |
| MUFAs (g/d)          | 55.3 (14.3)          | 56.4 (14.2)           | 53.9 (14.5)      | 0.171 |
| PUFAs (g/d)          | 18.3 (7.2)           | 19.1 (7.2)            | 17.3 (7.1)       | 0.049 |
| Linoleic (g/d)       | 15.4 (6.9)           | 16.1 (6.8)            | 14.6 (7.0)       | 0.075 |
| Linolenic (g/d)      | 1.6 (0.8)            | 1.6 (0.8)             | 1.5 (0.8)        | 0.317 |
| Omega-3 (g/d)        | 0.9 (0.5)            | 0.9 (0.5)             | 0.9 (0.4)        | 0.305 |
| Fiber (g/d)          | 30.0 (9.8)           | 29.3 (10.1)           | 30.9 (9.3)       | 0.175 |
| Total cholesterol    | 375.7 (110.8)        | 382.4 (124.4)         | 366.5 (88.9)     | 0.248 |
| Total vegetables     | 333.0 (124.4)        | 318.2 (123.0)         | 353.0 (124.0)    | 0.024 |
| Total fruits         | 423.9 (220.4)        | 407.3 (221.6)         | 446.3 (217.8)    | 0.154 |
| Grapes intake        | 9.7 (21.8)           | 12.6 (26.4)           | 5.8 (12.3)       | 0.012 |
| Cherries and plums   | 14.4 (19.4)          | 14.9 (19.8)           | 13.6 (18.9)      | 0.599 |
| Nuts intake          | 15.1 (18.1)          | 15.6 (18.1)           | 14.3 (18.2)      | 0.566 |
| Homemade fruit juice | 4.1 (23.6)           | 5.6 (29.4)            | 2.0 (11.5)       | 0.209 |
| Fruit juice bottle   | 12.4 (54.8)          | 12.7 (52.9)           | 12.1 (57.5)      | 0.931 |
| Adherence to MedDiet | 8.8 (2.5)            | 8.8 (2.4)             | 9.0 (2.5)        | 0.484 |
| Alcohol consumption  | 12.9 (17.7)          | 19.7 (19.8)           | 3.5 (7.2)        | <0.001 |
| Total red wine       | 61.4 (105.9)         | 91.3 (120.8)          | 20.8 (62.1)      | <0.001 |
| Young red wine       | 56.7 (105.3)         | 84.1 (121.3)          | 19.6 (61.8)      | <0.001 |
| Aged red wine        | 4.7 (25.6)           | 7.2 (32.5)            | 1.2 (9.5)        | 0.056 |
| Rosé wine            | 10.4 (49.8)          | 16.7 (64.2)           | 1.9 (11.9)       | 0.017 |
| Moscatel wine        | 0.5 (7.7)            | 0.8 (10.1)            | 0.06 (0.7)       | 0.429 |
| White wine           | 7.4 (33.6)           | 10.6 (42.4)           | 3.1 (14.4)       | 0.073 |
| trans-resveratrol-3-O-glucuronide (nmol/g creatinine) | 0.7 (1.6) | 0.7 (0.9) | 0.7 (2.2) | 0.973 |
| trans-resveratrol-4′-O-glucuronide (nmol/g creatinine) | 171.9 (375.8) | 143.7 (314.7) | 210.1 (444.1) | 0.154 |
| trans-resveratrol-3-O-sulfate (nmol/g creatinine) | 0.2 (0.5) | 0.2 (0.6) | 0.1 (0.2) | 0.023 |
| cis-resveratrol-3-O-glucuronide and cis-resveratrol-4′-O-glucuronide (nmol/g creatinine) | 2.0 (5.3) | 2.6 (6.0) | 1.1 (4.1) | 0.023 |

Data were calculated by student’s t-test. Results are expressed as mean (standard deviation). \( p ^\dagger \) for differences between sexes. Abbreviations: MUFAs, monounsaturated fatty acids; PUFAs, Polyunsaturated fatty acids; MedDiet, Mediterranean diet.
Urine resveratrol metabolites (Table 2) showed that men had higher *trans*-resveratrol-3-O-sulfate and *cis*-resveratrol-3-O-glucuronide/*cis*-resveratrol-4′-O-glucuronide urine levels (all *p*-values < 0.05) compared to women.

2.2. Association between Total Urine Resveratrol Metabolites Concentrations, Cardiometabolic Profile, and Liver Markers

The association between total urine resveratrol metabolites and glucose metabolism markers, blood lipid, and liver markers are shown in Tables 3–5, respectively. After adjusting for covariates, there were no significant associations between total urine resveratrol metabolites and glucose metabolism markers (Table 3). Table 4 summarizes values concerning lipid metabolism. No significant associations were observed between total urine resveratrol metabolites and LDL-c and log triglyceride/HDL ratio among tertiles. Although in the adjusted model, participants in the T3 had significantly lower levels of total cholesterol compared to T1 (*β* T3 = 11.67, 95% CI, −22.21 to −1.13), but there was no significant tendency among tertiles (*p*-trend = 0.108). Interestingly, individuals in the highest tertile (T3) of total urine resveratrol metabolites had significantly lower levels of log triglycerides (*β* T3 = −0.15, 95% CI, −0.28 to −0.02), VLDL-c (*β* T3 = −4.21, 95% CI, −7.97 to −0.46), and total cholesterol/HDL ratio (*β* T3 = −0.35, 95%CI, −0.66 to −0.03) after adjustment. Regarding to liver markers (Table 5), compared with those in the first tertile, individuals in the third tertile had 2.4 significant unit decrease in the log GGT (95% CI, −0.42 to −0.06; *p*-trend = 0.002) and lower log AST (*β* T3 = −0.12, 95% CI, −0.22 to −0.02; *p*-trend = 0.011). Nevertheless, the differences of other liver markers (log ALT, HSI and FLI) between levels of urinary resveratrol metabolites were not statistically significant.

### Table 3. Linear regression analysis distributed in tertiles evaluating the associations between total urine resveratrol (independent variable) and glucose metabolism markers (outcome) in participants with MetS.

| Glucose markers | T1 (≤4.6) | T2 (>4.6 to 58.1) | T3 (>58.1 to 2481.2) | p-Trend |
|-----------------|-----------|-------------------|---------------------|---------|
| Glucose (mmol/L) |           |                   |                     |         |
| Crude model     | 0 REF.    | 0.02 (−0.55, 0.60) | 0.11 (−0.46, 0.69) | 0.677   |
| Adjusted model  | 0 REF.    | 0.04 (−0.56, 0.63) | 0.04 (−0.55, 0.63) | 0.933   |
| HbA1c (%)       |           |                   |                     |         |
| Crude model     | 0 REF.    | −0.07 (−0.35, 0.21) | −0.01 (−0.28, 0.27) | 0.842   |
| Adjusted model  | 0 REF.    | −0.07 (−0.36, 0.22) | −0.04 (−0.32, 0.25) | 0.982   |

Insulin sensitivity/resistance markers

| Insulin (mU/L)  | T1 (≤4.6) | T2 (>4.6 to 58.1) | T3 (>58.1 to 2481.2) | p-Trend |
|-----------------|-----------|-------------------|---------------------|---------|
| Crude model     | 0 REF.    | −1.23 (−3.94, 1.48) | −1.15 (−3.86, 1.55) | 0.623   |
| Adjusted model  | 0 REF.    | −0.22 (−2.87, 2.43) | −0.58 (−3.20, 2.03) | 0.672   |
| HOMA-IR         |           |                   |                     |         |
| Crude model     | 0 REF.    | −0.45 (−1.44, 0.54) | −0.46 (−1.44, 0.52) | 0.558   |
| Adjusted model  | 0 REF.    | −0.11 (−1.09, 0.87) | −0.31 (−1.28, 0.65) | 0.534   |
| HOMA-%B         |           |                   |                     |         |
| Crude model     | 0 REF.    | −15.19 (−37.11, 6.73) | −11.00 (−32.79, 10.79) | 0.682   |
| Adjusted model  | 0 REF.    | −11.07 (−32.44, 10.30) | −6.32 (−27.35, 14.71) | 0.904   |
| FGIR            |           |                   |                     |         |
| Crude model     | 0 REF.    | −0.12 (−0.30, 0.06) | −0.07 (−0.25, 0.11) | 0.859   |
| Adjusted model  | 0 REF.    | −0.14 (−0.33, 0.05) | −0.07 (−0.26, 0.11) | 0.922   |

| FIRI            |           |                   |                     |         |
Table 3. Cont.

| Total Urine Resveratrol Metabolites (nmol/g Creatinine) | T1 (<4.6) | T2 (>4.6 to 58.1) | T3 (>58.1 to 2481.2) | p-Trend |
|---------------------------------------------|-----------|-------------------|----------------------|--------|
| n                                          | 89        | 89                | 88                   |        |
| β Coefficient (95% IC)                      | −0.40 (−1.29, 0.49) | −0.42 (−1.30, 0.47) | 0.558                |        |
| Adjusted model                              | −0.10 (−0.98, 0.78) | −0.28 (−1.14, 0.58) | 0.534                |        |

Models were adjusted for sex, age, smoking status, marital status, physical activity, energy intake, and BMI. Abbreviations: HbA1c, glycated hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin; HOMA-%B, HOMA of β-cell function; FGIR, fasting glucose insulin ratio; FIRI, fasting insulin resistance index; REF, reference.

Table 4. Linear regression analysis evaluating the associations between total urine resveratrol (independent variable) and blood lipids (outcome) in participants with MetS.

| Total Urine Resveratrol Metabolites (nmol/g Creatinine) | T1 (<4.6) | T2 (>4.6 to 58.1) | T3 (>58.1 to 2481.2) | p-Trend |
|-------------------------------------------------------|-----------|-------------------|----------------------|--------|
| n                                                     | 89        | 89                | 88                   |        |
| β Coefficient (95% IC)                                 |           |                   |                      |        |
| Blood lipids                                           |           |                   |                      |        |
| Total cholesterol (mg/dL)                              |           |                   |                      |        |
| Crude model                                           | 0 REF.    | −13.10 (−23.77, −2.43) | −13.26 (−23.96, −2.56) | 0.132  |
| Adjusted model                                         | 0 REF.    | −8.93 (−19.54, 1.68)  | −11.67 (−22.21, −1.13) | 0.108  |
| LDL-c (mg/dL)                                          |           |                   |                      |        |
| Crude model                                           | 0 REF.    | −11.36 (−21.15, −1.57) | −8.21 (−18.02, 1.61)  | 0.502  |
| Adjusted model                                         | 0 REF.    | −9.32 (−19.13, 0.48)  | −7.77 (−17.57, 2.02)  | 0.435  |
| HDL-c (mg/dL)                                          |           |                   |                      |        |
| Crude model                                           | 0 REF.    | 0.36 (−2.60, 3.32) | −0.38 (−3.36, 2.59) | 0.674  |
| Adjusted model                                         | 0 REF.    | 1.41 (−1.51, 4.32) | 0.27 (−2.63, 3.18) | 0.761  |
| Log triglyceride (mg/dL)                               |           |                   |                      |        |
| Crude model                                           | 0 REF.    | −0.05 (−0.18, 0.07) | −0.14 (−0.26, −0.02) | 0.032  |
| Adjusted model                                         | 0 REF.    | −0.06 (−0.19, 0.07) | −0.15 (−0.28, −0.02) | 0.030  |
| VLDL-c (mg/dL)                                         |           |                   |                      |        |
| Crude model                                           | 0 REF.    | −1.48 (−5.11, 2.15) | −3.95 (−7.60, −0.30) | 0.043  |
| Adjusted model                                         | 0 REF.    | −1.70 (−5.47, 2.08) | −4.21 (−7.97, −0.46) | 0.039  |
| Log triglyceride/HDL ratio                             |           |                   |                      |        |
| Crude model                                           | 0 REF.    | −0.07 (−0.23, 0.09) | −0.14 (−0.30, 0.02) | 0.122  |
| Adjusted model                                         | 0 REF.    | −0.10 (−0.26, 0.07) | −0.16 (−0.33, 0.002) | 0.106  |
| Total cholesterol/HDL ratio                            |           |                   |                      |        |
| Crude model                                           | 0 REF.    | −0.38 (−0.69, −0.08) | −0.32 (−0.62, −0.01) | 0.304  |
| Adjusted model                                         | 0 REF.    | −0.39 (−0.70, −0.07) | −0.35 (−0.66, −0.03) | 0.241  |

Models were adjusted for sex, age, smoking status, marital status, physical activity, energy intake, and BMI. Abbreviations: LDL-c, Low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; VLDL, very low-density lipoprotein cholesterol; Triglyceride/HDL ratio, triglyceride/high density lipoprotein cholesterol ratio; Low density lipoprotein cholesterol/high density lipoprotein cholesterol; total cholesterol/HDL, total cholesterol/high density lipoprotein cholesterol; REF, reference.
Table 5. Linear regression analysis evaluating the associations between total urine resveratrol (independent factor) and liver status markers (dependent factor) in participants with MetS.

| Total Urine Resveratrol Metabolites (nmol/g Creatinine) | T1 (≤4.6) | T2 (>4.6 to 58.1) | T3 (>58.1 to 2481.2) | p-Trend |
|--------------------------------------------------------|-----------|-------------------|----------------------|---------|
| n                                                      | 89        | 89                | 88                   |         |
| Liver markers                                          |           |                   |                      |         |
| Log ALT (U/L)                                          |           |                   |                      |         |
| Crude model                                            | 0 REF.    | 0.03 (−0.12, 0.18)| −0.10 (−0.25, 0.05)  | 0.074   |
| Adjusted model                                         | 0 REF.    | 0.03 (−0.11, 0.18)| −0.12 (−0.27, 0.02)  | 0.028   |
| Log AST (U/L)                                          |           |                   |                      |         |
| Crude model                                            | 0 REF.    | 0.003 (−0.10, 0.10)| −0.09 (−0.19, 0.01)  | 0.040   |
| Adjusted model                                         | 0 REF.    | −0.01 (−0.11, 0.09)| −0.12 (−0.22, −0.02) | 0.011   |
| Log GGT (U/L)                                          |           |                   |                      |         |
| Crude model                                            | 0 REF.    | 0.02 (−0.15, 0.20)| −0.23 (−0.41, −0.06) | 0.002   |
| Adjusted model                                         | 0 REF.    | 0.01 (−0.17, 0.19)| −0.24 (−0.42, −0.06) | 0.002   |
| HSI *                                                  |           |                   |                      |         |
| Crude model                                            | 0 REF.    | −0.28 (−1.74, 1.18)| −0.59 (−2.45, 1.27)  | 0.893   |
| Adjusted model                                         | 0 REF.    | 0.14 (−1.35, 1.63)| 0.11 (−1.37, 1.59)   | 0.948   |
| FLI ¶                                                  |           |                   |                      |         |
| Crude model                                            | 0 REF.    | −0.97 (−5.46, 3.52)| −2.55 (−7.07, 1.98)  | 0.294   |
| Adjusted model                                         | 0 REF.    | −1.39 (−5.97, 3.18)| −2.54 (−7.10, 2.02)  | 0.346   |

Models were adjusted for sex, age, smoking status, marital status, physical activity, energy intake, and BMI. * Adjusted for all variables except for sex and BMI. ¶ Adjusted for all variables except for BMI. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HSI, hepatic steatosis index; FLI, fatty liver index; REF, reference.

2.3. Risk of Higher Liver Enzymes and Total Urine Resveratrol Metabolites

We tested the associations of total urine resveratrol metabolites and the risk of higher ALT (A), AST (B), and GGT (C) levels (Figure 1). Cubic splines analyses indicated that participants who had total urinary resveratrol concentration threshold had a lower odds ratio for liver enzymes above the ULN. Urinary resveratrol metabolites concentration threshold: ALT (169.2 to 1314.3 nmol/g creatinine), AST (559.9 to 893.8 nmol/g creatinine), and GGT (169.2 to 893.8 nmol/g creatinine).

Figure 1. Cont.
Figure 1. The odds ratio for liver enzymes levels above the upper limit of normal (ULN) for total urine resveratrol concentration in nmol/g creatinine. ULN range for ALT (men ≥ 30 UI/L, women ≥ 19 UI/L) (A), AST (men ≥ 37 UI/L, women ≥ 31 UI/L) (B), and GGT (men ≥ 60 UI/L, women ≥ 40 UI/L) (C). The smooth line represents the estimation of higher ALT, AST, and GGT levels when using zero as the reference value for total urine resveratrol metabolite (4 knots for ALT and GGT, 3 knots for AST) whereas the dashed lines indicate 95% CIs.

3. Discussion

In this research, higher urine concentrations of some resveratrol phase II metabolites (total sum of trans-resveratrol-3-O-glucuronide, trans-resveratrol-4′-O-glucuronide, cis-resveratrol-3-O-glucuronide, cis-resveratrol-4′-O-glucuronide, and trans-resveratrol-3-O-sulfate) have associated with favorable lipid and liver markers in individuals diagnosed with MetS. Indeed, cubic spline models suggest that total urinary resveratrol excretion was associated with a lower risk of higher levels of liver enzymes related with increased risk of NAFLD (concentration threshold for ALT = 169.2 to 1314.3 nmol/g creatinine, AST = 559.9 to 893.8 nmol/g creatinine and GGT = 169.2 to 893.8 nmol/g creatinine), even after adjustment for potential factors. MetS components increase the risk of NAFLD development [2–4]. In general, our population had an abnormal metabolic profile as characteristic of MetS where women showed higher cholesterol and LDL-c levels compared to men. Meanwhile, men exhibited higher levels of ALT, AST, and FLI. Epidemiological studies evidenced that age and sex affect NAFLD prevalence [3]. Ageing involves changes in sex hormones levels, fat redistribution that increases the risk of CVD and NAFLD, especially in post-menopausal women [30,31]. Healthy dietary patterns, such MedDiet, include foods that not only might improve weight modulation, but also have several bioactive compounds like (poly)phenols with anti-inflammatory and antioxidant properties, which show beneficial metabolic effects [9,10,32,33]. Some molecules, such as anthocyanidins and resveratrol, might involve in the metabolic process involved in NAFLD [15,29]. Scientific evidence suggested that resveratrol is a multi-targeted compound for chronic diseases [21]. However, variations in the study design, small samples sizes, diverse analytical methods, and other factors trigger heterogeneous conclusions. Consequently, results should be interpreted cautiously [24,29,33,34]. Interestingly, our findings showed that individuals in the highest tertile of total urinary resveratrol metabolites had lower levels of triglycerides, VLDL-c and total cholesterol/HDL ratio compared with those in the lowest tertile. Previously, the PREDIMED study (PREvención con Díeta MEDiterránea) evaluated the association of cardiovascular risk factors and total urinary resveratrol metabolites [35]. Authors demonstrated that increased urinary resveratrol excretions were associated with higher HDL-c, lower triglycerides concentrations and decreased heart rate, but did not found associations with blood pressure [35]. While in our results, no differences were found in the HDL-c concentrations. The discrepancies between our results related to the lipid metabolism could be partly explained for the T2DM status attributable to the synergetic effects of anti-diabetic drug and resveratrol as well as lipid-lowering medication [28]. It is important to mention that incidence of T2DM in our population was lower compared to Zamora et al., reported [35]. Moreover, when we adjusted the regression models considering lipid-powering
and anti-diabetic treatment, our results did not change (data not shown). Another difference between both studies is that we quantified slightly different resveratrol metabolites. We quantified trans-resveratrol-3-O-glucuronide, trans-resveratrol-4′-O-glucuronide, cis-resveratrol-3-O-glucuronide, cis-resveratrol-4′-O-glucuronide and trans-resveratrol-3-O-sulfate while Zamora-Ros et al. quantified (trans-cis-resveratrol-3-O-glucuronide, cis-resveratrol-4′-O-glucuronide, trans-cis resveratrol-4′-O-sulfate, trans-cis resveratrol-3-O-sulfate). Furthermore, we used authentic glucuronide and sulfated standards to quantify each metabolite. In contrast, Zamora-Ros et al. used the resveratrol aglycone to quantify all glucuronide and sulfated metabolites, which can lead to errors in the quantification of glucuronide and sulfated metabolites [36]. The lack of commercially available glucuronide and sulfate resveratrol standards is still an issue that hampers advancements in the quantification of total resveratrol metabolite. The lipophilic nature of resveratrol could facilitate the entry into the surface of albumin and lipoprotein, and these properties could confer benefits on lipid profile, avoiding the oxidation of LDL [28,37,38]. A study found that resveratrol metabolites, including trans-cis-resveratrol-3-O-glucuronide, and cis-resveratrol-3-O-glucoside, as well as free trans-resveratrol, were incorporated into the LDL of human participants after intake of moderate red wine, which could suggest the cardioprotective role of resveratrol on atherogenic markers and oxidative stress [37]. In line with our findings, a meta-analysis indicated that more prolonged resveratrol supplementation (≥6 months) with doses ranged from 8.1 to 3000 mg/d might improve triglyceride levels in subjects with T2DM [28]. However, in a prospective cohort study, Semba et al. did not find significant differences in lipid profile and inflammatory cytokines across groups of total urinary resveratrol [39]. It is essential to highlight that our participants had MetS, which are closely related to NAFLD due to deregulation of the de novo lipogenesis (DNL), insulin resistance, and hepatic triglyceride accumulation [5,6]. Thus, these findings suggested that resveratrol could improve liver parameters. Several mechanisms of resveratrol action on lipid metabolism include the activation of AMP-activated kinase (AMPK), which inhibit sterol regulatory element-binding protein 1 (SREBP-1) activity, which plays a crucial role in the DNL [28,40]. Moreover, the regulation of hepatic enzyme 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) related to cholesterol synthesis [38], and the overexpression of the paraoxonase 1 (PON 1) that it has shown cardioprotective effects [41]. In fact, disrupted SREBP-1 levels, increased HMG-CoA expression and decreased PON 1 activity have evidenced in NAFLD promoting a dysregulation of lipid metabolism [28,38,40,42]. This environment stimulates the accumulation of lipids into hepatocytes (liver steatosis) increasing risk to develop NAFLD [7,43]. In the current study, there were no statistically significant differences in glucose metabolism markers among tertiles of total urine resveratrol metabolite, but so far the effect of resveratrol on glucose metabolism is unclear [44]. A randomized controlled trial did not show significant effects in HOMA-IR and fasting glucose levels after four weeks of supplementation with 150 mg of trans-resveratrol in subjects who were overweight [45]. In contrast, a recent meta-analysis has shown that resveratrol supplementation (≥100 mg/d) might reduce levels of insulin and glucose in individuals diagnosed with T2DM [27]. Regarding liver parameters, 46% of the participants had ALT values above ULN, and higher total urinary resveratrol metabolites were significantly associated with lower AST and GGT levels. We also observed in the cubic spline analyses that total urinary resveratrol metabolite (concentration threshold) reduced the probability of having higher liver transaminases (ALT, AST and GGT). In this respect, clinical trials studies focus on resveratrol effects on NAFLD individuals are scarce with ambiguous results [46,47]. For instance, Chen et al. showed that resveratrol supplementation (600 mg for 3 months) could decrease levels of liver transaminases, LDL-c, total cholesterol and HOMA-IR in, but did not found a significant reduction in liver steatosis. However, another study indicated that lifestyle changes focused on a healthy diet and physical activity in addition to 500 mg/d (12 weeks) of resveratrol supplementation only had beneficial effects on improvements in ALT levels and hepatic steatosis [48]. In contrast, a study showed that insulin resistance markers and hepatic steatosis remained unchanged after resveratrol supplementation [49]. Contrary to our study, Cachay et al. only included men in their study design. In fact, it has suggested that stilbene glucuronidation is more efficient in women compared to men [50].
Moreover, there are significant differences in the doses. Cachay et al. used up to 20 times higher the amount compared to other research groups. In this sense, our contrasting results can be explained by the fact that chronic higher resveratrol doses might promote saturation in absorption sites [51]. Our findings suggested that inter-individual heterogeneities might play a key role in the effectiveness of resveratrol metabolites in individuals with MetS who are overweight or obese. However, it should be noted that the studies, as mentioned above, are different in study design, populations, and several other aspects that could potentially affect results and the interpretation of their conclusions.

There is a lack of epidemiological research in evaluating the effects of resveratrol from dietary consumption and health outcomes [21,27,28,39]. On the other hand, the majority of clinical trials assessed the effects of resveratrol supplementation using diverse resveratrol dosage and frequency of intake and heterogeneous treatment lengths. Therefore, it is difficult to interpret results and establish an effective dose and treatment, especially for the use of higher amounts, which is not applicable in a normal dietary context. Resveratrol is mainly found in wine, grapes, and grape juice [18,52,53]. In our data, wine consumption was correlated with urinary resveratrol metabolites (data not shown). However, resveratrol content can vary in the same type of fruit, climate, and grape variety for wine [53,54]. The bioavailability of resveratrol is poor, resulting from low water solubility (<0.05 mg) that can vary according to the matrix (wine, grapes, supplements, others) [20,24,54]. Rotches-Ribalta et al. evaluated resveratrol metabolites profiles after a moderate intake of red wine and grape extract tablets in healthy men [20]. Investigators found differences in the quantification of some resveratrol metabolites due to the different resveratrol composition of both sources [20]. A large number of human and animal studies suggested that bioactive phytochemicals have therapeutic effects on chronic diseases, but several factors may affect their biological response [19,25]. The main determinants of inter-individual variation could be attributed to the gut microbiota, sex, age, lifestyle, genetics, and others [25]. In this line, it seems essential to consider that resveratrol metabolites could have beneficial effects on specific population groups, where inter-individual variances in their metabolism could confer to these discrepancies [21,25,27,28,39]. Consequently, conflicting views about the effect on the metabolic profile of resveratrol in a supplementation or food form are still unclear.

Current recommendations for NAFLD prevention, treatment, and follow-up encourage lifestyle modifications to focus on habitual physical activity and healthy dietary patterns. From a dietary point of view, it is a challenge to promote healthy diets focused on foods with high content in bioactive compounds. The MedDiet could be a preferable option to be considered since its dietary components are rich in antioxidants, which are pivotal factors for the prevention and management of NAFLD [9–15]. In this context, well-design epidemiological and clinical trial studies to investigate the effects of dietary resveratrol on health outcomes are crucial.

The strength of this study is the large sample size of patients with detailed clinical and biochemical data. Moreover, the use of metabolomics has been considered a reliable and innovative technique for food science and precision nutrition studies [8]. In the present study, we performed an analytical method to accurately identify and quantify resveratrol metabolites using authentic standards. However, our study has some limitations. First, for total urine analyses, not all phase II metabolites were included, and we did not use glucosides and gut microbial metabolites, which can lead to underestimation of total resveratrol metabolite levels and, therefore, influence our conclusions. Hence, data are not representative of total resveratrol intake, and we included the main resveratrol phase II metabolites reported in human studies [20,21,53] and also considering the limited availability of authentic standards. Second, liver biopsy was not performed for diagnosing NAFLD participants. In this sense, non-invasive markers were acceptable to identify patients with metabolic features at risk of developing NAFLD [4,55]. Finally, this study has a cross-sectional design, and the findings cannot infer causality. Likewise, results cannot be generalized to other ethnic or age groups, because the participants were elderly diagnosed with MetS at high CVD risk. However, type I and II errors cannot be discarded, despite that, the results are plausible and with clinical relevance.
4. Materials and Methods

4.1. Study Population

Participants were volunteers from the PREDIMED-Plus study, a parallel-group multi-center randomized trial (https://www.predimedplus.com/). Details of the study design have been previously described [56]. In brief, PREDIMED-Plus study was designed to investigate the effects of an energy-reduced Mediterranean diet and a weight-loss intervention by the promotion of physical activity and behavioral support on cardiovascular endpoints [57]. Individuals were men and women (65 to 75 years) who were overweight or obese and met at least three components of the MetS [58]. The study excluded participants with excessive alcohol consumption or addiction, several medical conditions (active cancer or history of malignancy, history of previous CVD, cirrhosis or liver injury, cytotoxic agents, therapy with immunosuppressive drugs, or treatment with systemic corticosteroids [56]. All participants gave their informed consent to participate in the study. This clinical trial was conducted following the Declaration of Helsinki, and the protocol was approved by institutional ethics committees of all participant centers (http://www.isrctn.com/ISRCTN89898870). This study is a cross-sectional study using baseline database from the Navarra-Nutrition node. A total of 266 participants with feasible data in the form of spot urine specimen were included in the present study.

4.2. Sociodemographic, Clinical, Anthropometric, and Body Composition Variables

Sociodemographic characteristics, lifestyle data, and medical history were collected during the baseline interview according to the study protocol [56]. Smoking habit was classified into never, former, and current smoker. Diabetes was defined according to the criteria of the American Diabetes Association guidelines [59]. Anthropometric variables were measured by trained dietitians using standardized procedures and calibrated equipment [56]. Height (in centimeters) and weight (in kilograms) were measured to calculate body mass index (BMI) (kg/m²). Visceral fat mass was estimated using the dual-energy X-ray absorptiometry (Lunar iDXA™, software version 6.0, Madison, WI, USA) performed by trained study staff. We used a validated Registre Gironi del Cor (REGICOR) questionnaire to assess physical activity (Metabolic Equivalent of Task (MET)-minute/week, as described in detail elsewhere [60–62].

4.3. Dietary Record

A validated 143-item semi-quantitative food frequency questionnaire was administered in a face to face interviews by a trained nutritionist to explore dietary intake over the previous 12 months [63]. Furthermore, adherence to MedDiet was assessed by a 17-point score questionnaire, which is a version of the 14-point score performed in the PREDIMED study [56,64,65]. The 17-point score questionnaire includes additional questions to the 14-point score and more restrictive cut-offs for some caloric-dense foods [56,65].

4.4. Urine and Plasma Collection, and Biochemical Determinations

The first spot urine was taken in the morning, and blood samples were obtained after 12 h overnight fasting. Biological specimens were stored frozen at a −80 °C according to approved protocols by trained technicians [56]. Biochemical analyses including glucose, hemoglobin A1c (HbA1c), triglyceride, HDL-c, total cholesterol, ALT, and AST were performed on fasting plasma by using specific kits according to manufacturer’s protocols [56]. The insulin was measured using specific ELISA kits in a Triturus autoanalyzer (Grifols, Barcelona, Spain). The Friedewald formula was used to calculate LDL-c and the VLDL-c [66].
4.5. Urine Resveratrol Metabolites Measurements

Standards of trans-resveratrol-3-O-glucuronide, trans-resveratrol-4′-O-glucuronide, cis-resveratrol-3-O-glucuronide, cis-resveratrol-4′-O-glucuronide, and trans-resveratrol-3-O-sulfate were obtained from Toronto Research Chemicals (Toronto, ON, Canada). The resveratrol metabolites were extracted and quantified using a modified method developed by Feliciano et al. (2016) [67]. The analytical method was validated according to the Food and Drug Administration (FDA) guidelines. Briefly, 600 µL of diluted urine samples (urine:water, 1:10) were thawed on ice and centrifuged at 15,000 × g for 15 min at 4 °C. Then the supernatant (350 µL) was transferred to a microtube and acidified with 4% phosphoric acid. The mixture (600 µL) was loaded onto Oasis 96-well reversed-phase hydrophilic-lipophilic balanced (HLB) sorbent µ-SPE plates (Waters, Eschborn, Germany) and eluded with 60 µL of methanol after washing. Isotope labelled standards (±)-Catechin-2,3,4-13C3 (0.54 mg/mL, Sigma-Aldrich, Steinheim, Germany) and ferulic acid-1,2,3-13C3 (0.99 mg/mL, Sigma-Aldrich, Steinheim, Germany) were spiked in samples before µ-SPE to indicate the recovery rate. Taxifolin (0.25 mg/mL, Sigma-Aldrich, Steinheim, Germany) were used as internal standard. The identification and quantification of resveratrol metabolites was performed on a Shimadzu Triple Quadrupole Mass Spectrometer (LCMS8060, SHIMADZU, Kyoto, Japan) through an electro-spray interface (ESI) source. Eluded samples (5 µL) were injected through a Raptor Biphenyl column 2.1 × 50 mm, 1.8 µm (Restek, Bellefonte, PA, USA) with a compatible Raptor Biphenyl Guard Cartridges 5 × 2.1 mm (Restek, Bellefonte, PA, USA) in the UPLC system. The mobile phases consisted of solvent A: water (HPLC grade, Sigma-Aldrich, Steinheim, Germany) with 0.1% formic acid (LC-MS grade, Thermo Fisher Scientific, Loughborough, UK), and solvent B: acetonitrile (HPLC grade, Sigma-Aldrich, Steinheim, Germany) with 0.1% formic acid. A fourteen-minute gradient joined by a two minutes equilibration was applied to the run under a flow rate of 0.5 mL/min at 30 °C. The gradient was as follows (t(min), %B): (0, 1), (1, 1), (4, 12), (8, 12) (8.1, 15), (11, 15), (11.5, 30), (12, 99), (14, 99), (14.1, 1), (16, 1). The MS/MS parameters and transitions of the target compounds were obtained in optimization run. The resveratrol metabolites in samples were identified by comparing retention times with standards in corresponding to the multiple reaction monitoring (MRM) transitions and quantified by calibration curves made from standard mixes. One pair of isomers cis-resveratrol-3-O-glucuronide and cis-resveratrol-4′-O-glucuronide were quantified together as they appear in the same retention time. The identification of each metabolite was based on retention time of its corresponding pure standard following the same conditions and reference ion ratios based on the MS optimizations. Urinary resveratrol metabolites were normalized for urine creatinine concentrations.

4.6. Glucose Homeostasis and Liver Markers Measurements

Glucose homeostasis markers, such as insulin resistance and insulin sensitivity, were calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) [68], as well as the homeostasis model assessment for β-cell function (HOMA-%B) [69], the fasting glucose insulin ratio (FGIR) [70], and the fasting insulin resistance index (FIRI) [70]. Moreover, non-invasive liver markers such as the hepatic steatosis index (HSI) [55,71] and the fatty liver index (FLI) [72] were also determined estimated considering clinical, biochemical and anthropometric data. Formulas followed for all these determinations were as follows:

\[ HOMA - IR = \frac{\text{Insulin}(\text{mU/L}) \times \text{glucose} \ (\text{mmol/L})}{22.5} \]

\[ HOMA - %B = \frac{\text{Insulin}(\text{mU/L})}{\text{glucose} \ (\text{mmol/L})} - 3.5 \]

\[ \text{FGIR} = \frac{\text{glucose} \ (\text{mmol/L})}{\text{Insulin}(\text{mU/L})} \]

\[ \text{FIRI} = \frac{\text{Insulin}(\text{mU/L}) \times \text{glucose} \ (\text{mmol/L})}{25} \]

\[ \text{HSI} = 8 \times \text{ALT/AST ratio} + \text{BMI} (+2, \text{if diabetes}; +2, \text{if female}) \]
4.7. Statistical Analysis

Descriptive statistics were shown as means and standard deviation (SD) for continuous variables, and n (%) for categorical variables. A chi-squared test for categorical variables and Student’s t-test were used to compare baseline characteristics of participants by sex. Participants were categorized according to tertiles of some urinary resveratrol phase II metabolites excretion (T1 = ≤4.6 nmol/g; T2 = >4.6 to 58.1 nmol/g; T3 = >58.1 to 2481.3 nmol/g creatinine). Unadjusted and adjusted linear regression models were used to analyze the relationship between total urine resveratrol metabolite and cardiometabolic profile and NAFLD risk markers.

The normality of the residuals was tested in order to assess the validity of the regression models. Variables such as triglycerides, Triglyceride/HDL ratio, ALT, AST, and GGT were markedly skewed and were log-transformed. Linear regression analysis was adjusted for sex (except for covariates that include sex), age, smoking status (never, former, current), marital status (single, married, widow, divorced, separated, others), physical activity (MET-min/week), energy intake (kcal/d), and BMI (except for covariates that include BMI). Tests of linear trend were performed assigning the median value of each tertile of total resveratrol urine metabolite and then using it as a continuous variable.

We applied flexible cubic spline models to evaluate the association of total urinary resveratrol metabolites (continuous variable) with liver enzymes above the ULN. For ALT (men ≥ 30 UI/L, women ≥ 19 UI/L) [73], AST (men ≥ 37 UI/L, women ≥ 31 UI/L) [74], and GGT (men ≥ 60 UI/L, women ≥ 40 UI/L) [75]. Models were adjusted by all variables previously mentioned except for sex and included total sleeping hours (h/d). In the cubic spline analysis for total urinary resveratrol metabolites, we used 0 as a reference, with 4 knots (ALT and GGT) and 3 knots (AST). Statistical tests were two-tailed, and the significance level was p < 0.05. All statistical analyses were conducted with STATA version 16.0, StataCorp LP, College Station, TX, USA.

5. Conclusions

Current data showed that high urinary levels of some resveratrol phase II metabolites were associated with better blood lipid profile and liver enzymes in individuals diagnosed with MetS. Moreover, urinary resveratrol concentration threshold is associated with a reduced risk of higher liver enzymes. These results suggested that some resveratrol metabolites might have associated with benefits on risk factors linked to NAFLD development. Further studies are warranted to elucidate the impact and effectiveness of resveratrol in liver outcomes in individuals with MetS.

Author Contributions: Conceptualization, V.B.-V., I.A., M.A.Z., A.R.-M., and J.A.M.; performed experiments, V.B.-V. and Y.X.; formal analysis, V.B.-V., I.A., M.A.Z., Y.X., A.R.-M., and J.A.M.; investigation, V.B.-V., I.A., M.A.Z., Y.X., M.A.M.-G., C.S.-O., M.R.-C., E.T., V.M.S., R.E., R.M.-R., E.A.-A., M.F., J.S.-S., A.D.-L., F.J.T., J.A.T., D.R., J.K., X.P., L.D., A.R.-M., J.A.M.; writing—original draft preparation, V.B.-V., I.A., M.A.Z., Y.X., A.R.-M., and J.A.M.; writing—review and editing, V.B.-V., I.A., M.A.Z., Y.X., A.R.-M., J.A.M., M.A.M.-G., C.S.-O., M.R.-C., E.T., V.M.S., R.E., R.M.-R., E.A.-A., M.F., J.S.-S., A.D.-L., F.J.T., J.A.T., D.R., J.K., X.P., L.D.; supervision, I.A., M.A.Z., A.R.-M., J.A.M.; All authors have read and agreed to the published version of the manuscript.

Funding: The PREDIMED-Plus trial was supported by the European Research Council (Advanced Research grant 2014–2019; agreement #340918; granted to Martínez-González); the official Spanish institutions for funding scientific biomedical research, CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn) and Instituto de Salud Carlos III (ISICIII) through the Fondo de Investigación para la Salud (FIS) that is co-funded by the European Regional Development Fund (coordinated FIS projects led by Salas-Salvadó and Vidal, including the following projects: PI13/00673, PI13/00492, PI13/00272, PI13/01213, PI13/00462, PI13/00235, PI13/02184, PI13/00728, PI13/01090, PI13/01056, PI14/00722, PI14/00636, PI14/00618, PI14/00696, PI14/01206, PI14/01919, PI14/00853, PI14/01374, PI14/00972, PI14/00728, PI14/01471, PI16/00473, PI16/00626, PI16/01873, PI16/00194, PI16/00501, PI16/00533, PI16/00381, PI16/00366, PI16/01522, PI16/01120, PI17/00764, PI17/01183, PI17/00855, PI17/01347, PI17/00525, PI17/01827, PI17/00532, PI17/00215, PI17/01441, PI17/00508, PI17/01732, PI17/00926, PI19/00957, PI19/00386, PI19/00309, PI19/01032, PI19/00576, PI19/00017, PI19/01226, PI19/00781, PI19/01560, PI19/01332), and the Especial Action Project “Implementación y evaluación de una intervención intensiva sobre la actividad física Cohorte PREDIMED-Plus” (Salas-Salvadó); the Recercaixa (grant number 2013ACUP00194) (Salas-Salvadó). Moreover, J.
Salas-Salvador, gratefully acknowledges the financial support by ICREA under the ICREA Academia program; the SEMERGEN grant; International Nut and Dried Fruit Council–FESNAD (Long-term effects of an energy-restricted Mediterranean diet on mortality and cardiovascular disease 2014–2015; No. 201302) (Martinez-Gonzalez); Department of Health of the Government of Navarra (61/2015), the Fundacio La Marató de TV (Ref. 201630.10); the AstraZeneca Young Investigators Award in Category of Obesity and T2D 2017 (Romaguera); grants from the Consejeria de Salud de la Junta de Andalucia (PI10458/2013; PS0358/2016; PI1317/2018), the PROMETEO/2017/017 grant from the Generalitat Valenciana, the SEMERGEN grant; grant of support to research groups 35/2011 (Balearic Islands Gov; FEDER funds) (Tur and Bouz). J.K. is contracted for the “FOLIUM” program within the FUTURMed project. Talent for the medicine within the future from the Fundación Instituto de Investigación Sanitaria Illes Balears (financed by 2017 annual plan of the sustainable tourism tax and at 50% with charge to the ESF Operational Program 2014–2020 of the Balearic Islands). V.B.-V. received a grant from the Center for Nutrition Research of the University of Navarra.

Acknowledgments: The authors are especially grateful to all the participants and staff of the PREDIMED Plus study and special thanks to Maite Dominguez for the technical assistance.

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

References

1. Eckel, R.H.; Alberti, K.; Grundy, S.M.; Zimmet, P.Z. The metabolic syndrome. *Lancet* **2010**, *375*, 181–183. [CrossRef]
2. Younossi, Z.M. Non-alcoholic fatty liver disease—A global public health perspective. *J. Hepatol.* **2019**, *70*, 531–544. [CrossRef] [PubMed]
3. Chalasani, N.; Younossi, Z.; Lavine, J.E.; Charlton, M.; Cusi, K.; Brunt, E.M.; Sanyal, A.J. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* **2018**, *67*, 328–357. [CrossRef]
4. European Association for the Study of the Liver; European Association for the Study of Diabetes; European Association for the Study of Obesity. EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J. Hepatol.* **2016**, *64*, 1388–1402. [CrossRef]
5. Bullón-Vela, M.V.; Abete, I.; Martínez, J.A.; Zulet, M.A. Obesity and Nonalcoholic Fatty Liver Disease: Role of Oxidative Stress. In *Obesity: Oxidative Stress and Dietary Antioxidants*; Moral, A.M.d., Garcia, M.A.C., Eds.; Academic Press: London, UK, 2018; pp. 111–133. ISBN 9780128125045.
6. Khan, R.S.; Bril, F.; Cusi, K.; Newsome, P.N. Modulation of Insulin Resistance in Nonalcoholic Fatty Liver Disease. *Hepatology* **2019**, *70*. [CrossRef] [PubMed]
7. Kawano, Y.; Cohen, D.E. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J. Gastroenterol.* **2013**, *48*, 434–441. [CrossRef] [PubMed]
8. Tebani, A.; Bekri, S. Paving the way to precision nutrition through metabolomics. *Front. Nutr.* **2019**, *6*, 41. [CrossRef]
9. Zelber-Sagi, S.; Salomone, F.; Mlynarsky, L. The Mediterranean dietary pattern as the diet of choice for non-alcoholic fatty liver disease: Evidence and plausible mechanisms. *Liver Int.* **2017**, *37*, 936–949. [CrossRef]
10. Martínez-González, M.A.; Bastarrika, G. Mediterranean diet as the ideal model for preventing non-alcoholic fatty liver disease (NAFLD). *Hepatobiliary Surg. Nutr.* **2020**, *9*, 379–381. [CrossRef]
11. Godos, J.; Federico, A.; Dallio, M.; Scazzina, F. Mediterranean diet and nonalcoholic fatty liver disease: Molecular mechanisms of protection. *Int. J. Food Sci. Nutr.* **2017**, *68*, 18–27. [CrossRef]
12. Bullón-Vela, V.; Abete, I.; Tur, J.A.; Pintó, X.; Corbella, E.; Martínez-González, M.A.; Toledo, E.; Corella, D.; Macías, M.; Tjahoes, F.; et al. Influence of lifestyle factors and staple foods from the Mediterranean diet on non-alcoholic fatty liver disease among older individuals with metabolic syndrome features. *Nutrition* **2020**, *71*, 110620. [CrossRef] [PubMed]
13. Silva Figueiredo, P.; Inada, A.; Ribeiro Fernandes, M.; Granja Arakaki, D.; Freitas, K.; Avellaneda Guimarães, R.; Aragão do Nascimento, V.; Aiko Hiane, P. An Overview of Novel Dietary Supplements and Food Ingredients in Patients with Metabolic Syndrome and Non-Alcoholic Fatty Liver Disease. *Molecules* **2018**, *23*, 877. [CrossRef] [PubMed]
14. Chiva-Blanch, G.; Arranz, S.; Lamuela-Raventos, R.M.; Estruch, R. Effects of wine, alcohol and polyphenols on cardiovascular disease risk factors: Evidences from human studies. *Alcohol Alcohol.* **2013**, *48*, 270–277. [CrossRef] [PubMed]
15. Valenti, L.; Riso, P.; Mazzocchi, A.; Porrini, M.; Fargion, S.; Agostoni, C. Dietary Anthocyanins as Nutritional Therapy for Nonalcoholic Fatty Liver Disease. *Oxidative Med. Cell. Longev.* 2013, 1, 1–8. [CrossRef]

16. Burns, J.; Yokota, T.; Ashihara, H.; Lean, M.E.J.; Crozier, A. Plant foods and herbal sources of resveratrol. *J. Agric. Food Chem.* 2002, 50, 3337–3340. [CrossRef] [PubMed]

17. Jeandet, P.; Delaunois, B.; Conreux, A.; Donnez, D.; Nuzzo, V.; Cordelier, S.; Clément, C.; Courrot, E. Biosynthesis, metabolism, molecular engineering, and biological functions of stilbene phytoalexins in plants. *BioFactors* 2010, 36, 331–341. [CrossRef]

18. Noh, H.; Freising, H.; Assi, N.; Zamora-Ros, R.; Achaintre, D.; Affret, A.; Mancini, F.; Boutron-Ruault, M.-C.; Flögel, A.; Boeing, H.; et al. Identification of Urinary Polyphenol Metabolite Patterns Associated with Polyphenol-Rich Food Intake in Adults from Four European Countries. *Nutrients* 2017, 9, 796. [CrossRef]

19. Springer, M.; Moco, S. Resveratrol and its human metabolites—Effects on metabolic health and obesity. *Nutrients* 2019, 11, 143. [CrossRef]

20. Rotches-Ribalta, M.; Andres-Lacueva, C.; Estruch, R.; Escribano, E.; Urpi-Sarda, M. Pharmacokinetics of resveratrol metabolic profile in healthy humans after moderate consumption of red wine and grape extract tablets. *Pharmacol. Res.* 2012, 66, 375–382. [CrossRef]

21. Ramírez-Garza, S.L.; Laveriano-Santos, E.P.; Marhuenda-Muñoz, M.; Storniolo, C.E.; Tresserra-Rimbau, A.; Vallverdú-Queralt, A.; Lamuela-Raventós, R.M. Health effects of resveratrol: Results from human intervention trials. *Nutrients* 2018, 10, 1892. [CrossRef]

22. Walle, T.; Hsieh, F.; DeLegge, M.H.; Oatis, J.E.; Walle, U.K. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab. Dispos.* 2004, 32, 1377–1382. [CrossRef]

23. Jeandet, P.; Sobarzo-Sánchez, E.; Silva, A.S.; Clément, C.; Nabavi, S.F.; Battino, M.; Rasekhian, M.; Belwal, T.; Habtemariam, S.; Kofás, M.; et al. Whole-cell biocatalytic, enzymatic and green chemistry methods for the production of resveratrol and its derivatives. *Biotechnol. Adv.* 2020, 39, 107461. [CrossRef]

24. Gambini, J.; Inglès, M.; Olaso, G.; Lopez-Grueso, R.; Bonet-Costa, V.; Gimeno-Mallench, L.; Mas-Bargues, C.; Abdelaziz, K.M.; Gomez-Cabrera, M.C.; Vina, J.; et al. Properties of Resveratrol: In Vitro and In Vivo Studies about Metabolism, Bioavailability, and Biological Effects in Animal Models and Humans. *Oxidative Med. Cell. Longev.* 2015, 2015, 13. [CrossRef] [PubMed]

25. Manach, C.; Milenkovic, D.; Van de Wiele, T.; Rodriguez-Mateos, A.; de Roos, B.; García-Conesa, M.T.; Landberg, R.; Gibney, E.R.; Heinonen, M.; Tomás-Barberán, F.; et al. Addressing the inter-individual variation in response to consumption of plant food bioactives: Towards a better understanding of their role in healthy aging and cardiometabolic risk reduction. *Mol. Nutr. Food Res.* 2017, 61, 1600557. [CrossRef]

26. Charytoniuk, T.; Drygalski, K.; Konstantynowicz-Nowicka, K.; Berk, K.; Chabowski, A. Alternative treatment methods attenuate the development of NAFLD: A review of resveratrol molecular mechanisms and clinical trials. *Nutrition* 2017, 34, 108–117. [CrossRef] [PubMed]

27. Zhu, X.; Wu, C.; Qiu, S.; Yuan, X.; Li, L. Effects of resveratrol on glucose control and insulin sensitivity in subjects with type 2 diabetes: Systematic review and meta-analysis. *Nutr. Metab.* 2017, 14, 1–10. [CrossRef]

28. Zhao, H.; Song, A.; Zhang, Y.; Shu, L.; Song, G.; Ma, H. Effect of Resveratrol on Blood Lipid Levels in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis. *Obesity* 2019, 27, 94–102. [CrossRef] [PubMed]

29. Panwu, S.; Bhatnagar, A. Resveratrol: From enhanced biosynthesis and bioavailability to multitargeting chronic diseases. *Biomed. Pharmacother.* 2019, 109, 2237–2251. [CrossRef] [PubMed]

30. Suzuki, A.; Abdelmalek, M.F. Nonalcoholic Fatty Liver Disease in Women. *Women’s Health* 2009, 5, 191–203. [CrossRef]

31. Vela, M.V.B.; Abete, I.; Zulet, M.D.L.; Tur, J.A.; Pintó, X.; Corbella, E.; González, M.A.M.; Corella, D.; González, M.M.; Ros, E.; et al. Risk factors differentially associated with non-alcoholic fatty liver disease in males and females with metabolic syndrome. *Rev. Española Enferm. Dig.* 2019, 112, 111–133. [CrossRef]

32. Ros, E.; Martinez-González, M.A.; Estruch, R.; Salas-Salvadó, J.; Fitó, M.; Martínez, J.A.; Corella, D. Mediterranean Diet and Cardiovascular Health: Teachings of the PREDIMED Study. *Adv. Nutr.* 2014, 5, 3305–3365. [CrossRef] [PubMed]

33. Tejada, S.; Capó, X.; Mascaro, C.M.; Monserrat-Mesquida, M.; Quetglas-Llabrés, M.M.; Pons, A.; Tur, J.A.; Sureda, A. Hepatoprotective effects of resveratrol in non-alcoholic fatty liver disease. *Curr. Pharm. Des.* 2020, 26. [CrossRef] [PubMed]
34. Theodotou, M.; Fokianos, K.; Mouzouridou, A.; Konstantinou, C.; Aristotelous, A.; Prodromou, D.; Chrysikou, A. The effect of resveratrol on hypertension: A clinical trial. Exp. Ther. Med. 2017, 13, 295–301. [CrossRef]
35. Zamora-Ros, R.; Urpi-Sarda, M.; Lamuela-Raventós, R.M.; Martinez-González, M.A.; Salas-Salvadó, J.; Arós, F.; Fitó, M.; Lapetra, J.; Estruch, R.; Andres-Lacueva, C. High urinary levels of resveratrol metabolites are associated with a reduction in the prevalence of cardiovascular risk factors in high-risk patients. Pharmacol. Res. 2012, 65, 615–620. [CrossRef] [PubMed]
36. Almeida, L.; Vaz-da-Silva, M.; Falcão, A.; Soares, E.; Costa, R.; Loureiro, A.I.; Fernandes-Lopes, C.; Rocha, J.F.; Nunes, T.; Wright, L.; et al. Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. Mol. Nutr. Food Res. 2009, 53, 7–15. [CrossRef]
37. Urpi-Sardà, M.; Jäuregui, O.; Lamuela-Raventós, R.M.; Jaeger, W.; Miksits, M.; Covas, M.-I.; Andres-Lacueva, C. Uptake of Diet Resveratrol into the Human Low-Density Lipoprotein. Identification and Quantification of Resveratrol Metabolites by Liquid Chromatography Coupled with Tandem Mass Spectrometry. Anal. Chem. 2005, 77, 3149–3155. [CrossRef]
38. Khalil, A.; Berrougui, H. Editorial: Mechanism of action of resveratrol in lipid metabolism and atherosclerosis. Clin. Lipidol. 2009, 4, 527–531. [CrossRef]
39. Semba, R.D.; Ferrucci, L.; Bartali, B.; Urpi-Sarda, M.; Zamora-Ros, R.; Sun, K.; Cherubini, A.; Bandinelli, S.; Andres-Lacueva, C. Resveratrol levels and all-cause mortality in older community-dwelling adults. JAMA Intern. Med. 2014, 174, 1777–1784. [CrossRef]
40. Choi, Y.-J.; Suh, H.-R.; Yoon, Y.; Lee, K.-J.; Kim, D.G.; Kim, S.; Lee, B.-H. Protective effect of resveratrol derivatives on high-fat diet induced fatty liver by activating AMP-activated protein kinase. Arch. Pharmacal Res. 2014, 37, 1169–1176. [CrossRef]
41. Gouédard, C.; Barouki, R.; Morel, Y. Induction of the paraoxonase-1 gene expression by resveratrol. Arterioscler. Thromb. Vasc. Biol. 2004, 24, 2378–2383. [CrossRef]
42. Armutcu, F.; Ak yol, S.; Ucar, F.; Erdogan, S.; Ak yol, O. Chapter Three—Markers in Nonalcoholic Steatohepatitis, 1st ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2013; Volume 61, ISBN 9780124076808.
43. Min, H.K.; Kapoor, A.; Fuchs, M.; Mirshahi, F.; Zhou, H.; Maher, J.; Kellum, J.; Warnick, R.; Contos, M.J.; Sanyal, A.J. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. Cell Metab. 2012, 15, 665–674. [CrossRef]
44. Wong, R.H.X.; Howe, P.R.C. Resveratrol counteracts insulin resistance—Potential role of the circulation. Molecules 2020, 25, 4340.
52. Zamora-Ros, R.; Rothwell, J.A.; Achaintre, D.; Ferrari, P.; Boutron-Ruault, M.C.; Mancini, F.R.; Affret, A.; Kühn, T.; Katzke, V.; Boeing, H.; et al. Evaluation of urinary resveratrol as a biomarker of dietary resveratrol intake in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Br. J. Nutr. 2017, 117, 1596–1602. [CrossRef]

53. Zamora-Ros, R.; Urpi-Sardà, M.; Lamuela-Raventós, R.M.; Estruch, R.; Martínez-González, M.A.; Bulló, M.; Arós, F.; Cherubini, A.; Andres-Lacueva, C. Resveratrol metabolites in urine as a biomarker of wine intake in free-living subjects: The PREDIMED Study. Free Radic. Biol. Med. 2009, 46, 1562–1566. [CrossRef] [PubMed]

54. Ortuño, J.; Covas, M.I.; Farre, M.; Pujadas, M.; Fito, M.; Khymenets, O.; Andres-Lacueva, C.; Roset, P.; Joglar, J.; Lamuela-Raventos, R.M.; et al. Matrix effects on the bioavailability of resveratrol in humans. Food Chem. 2010, 120, 1123–1130. [CrossRef]

55. Bugianesi, E.; Rosso, C.; Cortez-Pinto, H. How to diagnose NAFLD in 2016. J. Hepatol. 2016, 65, 643–644. [CrossRef] [PubMed]

56. Martínez-González, M.A.; Buil-Cosiales, P.; Corella, D.; Bulló, M.; Fitó, M.; Vioque, J.; Romaguer, D.; Martínez, J.A.; Wärnberg, J.; López-Miranda, J.; et al. Cohort Profile: Design and methods of the PREDIMED-Plus randomized trial. Int. J. Epidemiol. 2019, 48, 387–388. [CrossRef]

57. Salas-Salvadó, J.; Díaz-López, A.; Ruiz-Canela, M.; Basora, J.; Fitó, M.; Corella, D.; Serra-Majem, L.; Wärnberg, J.; Romaguer, D.; Estruch, R.; et al. Effect of a Lifestyle Intervention Program With Energy-Restricted Mediterranean Diet and Exercise on Weight Loss and Cardiovascular Risk Factors: One-Year Results of the PREDIMED-Plus Trial. Diabetes Care 2018, dc180836. [CrossRef]

58. Alberti, K.G.M.M.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato, K.A.; Fruchart, J.; James, W.P.T.; Loria, C.M.; Smith, S.C. Harmonizing the Metabolic Syndrome. Circulation 2009, 120, 1640–1645. [CrossRef] [PubMed]

59. Atroshenko, S.A.; Korolyov, I.A.; Didenko, N. Standards of Medical Care in Diabetes—2014. Diabetes Care 2014, 37, S14–S80. [CrossRef]

60. Rosique-Esteban, N.; Díaz-López, A.; Martínez-González, M.A.; Corella, D.; Goday, A.; Martínez, J.A.; Romaguer, D.; Vioque, J.; Arós, F.; García-Rios, A.; et al. Leisure-time physical activity, sedentary behaviors, sleep, and cardiometabolic risk factors at baseline in the PREDIMED-PLUS intervention trial: A cross-sectional analysis. PLoS ONE 2017, 12, e0172253. [CrossRef]

61. Rosique-Esteban, N.; Babio, N.; Díaz-López, A.; Romaguer, D.; Alfredo Martínez, J.; Sanchez, V.M.; Schröder, H.; Estruch, R.; Vidal, J.; Buil-Cosiales, P.; et al. Leisure-time physical activity at moderate and high intensity is associated with parameters of body composition, muscle strength and sarcopenia in aged adults with obesity and metabolic syndrome from the PREDIMED-Plus study. Clin. Nutr. 2019, 38, 1324–1331. [CrossRef]

62. Martínez-Gonzalez, M.A.; Lopez-Fontana, C.; Varo, J.J.; Sanchez-Villegas, A.; Martínez, J.A. Validation of the Spanish version of the physical activity questionnaire used in the Nurses’ Health Study and the Health Professionals’ Follow-up Study. Public Health Nutr. 2005, 8, 920–927. [CrossRef]

63. Fernández-Ballart, J.D.; Piñol, J.L.; Zazpe, I.; Corella, D.; Carrasco, P.; Toledo, E.; Perez-Bauer, M.; Martínez-González, M.A.; Salas-Salvadó, J.; Martin-Moreno, J.M. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br. J. Nutr. 2010, 103, 1808–1816. [CrossRef] [PubMed]

64. Schröder, H.; Fitó, M.; Estruch, R.; Martínez-González, M.A.; Corella, D.; Salas-Salvadó, J.; Lamuela-Raventós, R.; Ros, E.; Salaverría, I.; Fiol, M.; et al. A Short Screener Is Valid for Assessing Mediterranean Diet Adherence among Older Spanish Men and Women. J. Nutr. 2011, 141, 1140–1145. [CrossRef] [PubMed]

65. Sayón-Orea, C.; Razquin, C.; Bulló, M.; Corella, L.; Fitó, M.; Romaguer, R.; Vioque, J.; Alonso-Gómez, Ángel, M.; Wärnberg, J.; Martinez, J.A.; et al. Effect of a Nutritional and Behavioral Intervention on Energy-Reduced Mediterranean Diet Adherence Among Patients With Metabolic Syndrome. JAMA 2019, 322, 1486. [CrossRef] [PubMed]

66. Bairaktari, E.T.; Seferiadis, K.I.; Elisaf, M.S. Evaluation of Methods for the Measurement of Low-Density Lipoprotein Cholesterol. J. Cardiovasc. Pharmacol. Ther. 2005, 10, 45–54. [CrossRef] [PubMed]
67. Feliciano, R.P.; Mecha, E.; Bronze, M.R.; Rodriguez-Mateos, A. Development and validation of a high-throughput micro solid-phase extraction method coupled with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry for rapid identification and quantification of phenolic metabolites in human plasma and urine. *J. Chromatogr. A* 2016, 1464, 21–31. [CrossRef]

68. Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985, 28, 412–419. [CrossRef]

69. Song, Y.; Manson, J.E.; Tinker, L.; Howard, B.V.; Kuller, L.H.; Nathan, L.; Rifai, N.; Liu, S. Insulin sensitivity and insulin secretion determined by homeostasis model assessment and risk of diabetes in a multiethnic cohort of women: The women’s health initiative observational study. *Diabetes Care* 2007, 30, 1747–1752. [CrossRef]

70. Silfen, M.E.; Manibo, A.M.; McMahon, D.J.; Levine, L.S.; Murphy, A.R.; Oberfield, S.E. Comparison of simple measures of insulin sensitivity in young girls with premature adrenarche: The fasting glucose to insulin ratio may be a simple and useful measure. *J. Clin. Endocrinol. Metab.* 2001, 86, 2863–2868. [CrossRef]

71. Lee, J.-H.; Kim, D.; Kim, H.J.; Lee, C.-H.; Yang, J.I.; Kim, W.; Kim, Y.J.; Yoon, J.-H.; Cho, S.-H.; Sung, M.-W.; et al. Hepatic steatosis index: A simple screening tool reflecting nonalcoholic fatty liver disease. *Dig. Liver Dis.* 2010, 42, 503–508. [CrossRef]

72. Bedogni, G.; Bellentani, S.; Miglioli, L.; Masutti, F.; Passalacqua, M.; Castiglione, A.; Tiribelli, C. The Fatty Liver Index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol.* 2006, 6, 33. [CrossRef]

73. Prati, D.; Taioli, E.; Zanella, A.; Della Torre, E.; Butelli, S.; Del Vecchio, E.; Vianello, L.; Zanuso, F.; Mozzi, F.; Milan, S.; et al. Updated Definitions of Healthy Ranges for Serum Alanine Aminotransferase Levels. *Ann. Intern. Med.* 2002, 137, 1. [CrossRef][PubMed]

74. Aragon, G.; Younossi, Z.M. When and how to evaluate mildly elevated liver enzymes in apparently healthy patients. *Cleve. Clin. J. Med.* 2010, 77, 195–204. [CrossRef][PubMed]

75. Nivukoski, U.; Niemelä, M.; Bloigu, A.; Bloigu, R.; Aalto, M.; Laatikainen, T.; Niemelä, O. Impacts of unfavourable lifestyle factors on biomarkers of liver function, inflammation and lipid status. *PLoS ONE* 2019, 14, e0218463. [CrossRef][PubMed]

Sample Availability: Samples of the compounds are available from the authors.

© 2020 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 (http://creativecommons.org/licenses/by/4.0/).