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The effects of alcohol on plasma lipid mediators of inflammation resolution in patients with Type 2 diabetes mellitus.

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Abstract

Background: Type 2 diabetes mellitus is characterized by peripheral insulin resistance and low-grade systemic inflammation. Inflammation resolution is recognised as an important process driven by specialised pro-resolving mediators of inflammation (SPMs) and has the potential to moderate chronic inflammation. Alcohol has the potential to affect synthesis of SPMs by altering key enzymes involved in SPM synthesis and may influence ongoing inflammation associated with Type 2 diabetes mellitus.

Aims: (i) To examine the effects of alcohol consumed as red wine on plasma SPM in men and women with type 2 diabetes in a randomised controlled trial and (ii) compare baseline plasma SPM levels in the same patients with those of healthy volunteers.

Methods: Twenty-four patients with Type 2 diabetes mellitus were randomized to a three-period crossover study with men drinking red wine 300 ml/day (~31 g alcohol/day) and women drinking red wine 230 ml/day (~24 g alcohol/day), or equivalent volumes of dealcoholized red wine (DRW) or water, each for 4 weeks. The SPM 18-hydroxyeicosapentaenoic acid (18-HEPE), E-series resolvins (Rv) (RvE1-RvE3), 17-hydroxydocosahexaenoic acid (17-HDHA), and D-series resolvins (RvD1, 17R-RvD1, RvD2, RvD5), 14-hydroxydocosahexaenoic acid (14-HDHA) and Maresin 1 were measured at the end of each period. A baseline comparison of plasma SPM, hs CRP, lipids and glucose was made with healthy volunteers.

Results: Red wine did not differentially affect any of the SPM measured when compared with DRW or water. Baseline levels of the hs-CRP and the SPM 18-HEPE, 17-HDHA, RvD1 and 17R-RvD1 in patients with Type 2 diabetes mellitus were all significantly elevated compared with healthy controls and remained so after adjusting for age and gender.

Conclusion: Moderate alcohol consumption as red wine does not alter plasma SPM in patients with Type 2 diabetes mellitus. The elevation of SPM levels compared with healthy

volunteers may be a homeostatic response to counter ongoing inflammation.

Key Words: alcohol, red wine, polyphenols, lipid mediators of inflammation resolution, resolvins.

Abbreviations:

18-hydroxyeicosapentaenoic acid (18-HEPE), resolvin (Rv), 17-hydroxydocosahexaenoic acid (17-HDHA), protectin D1 (PD1), maresin-1 (MaR1), 14-hydroxydocosahexaenoic acid (14-HDHA); liquid chromatography-tandem mass spectrometry (LC-MS/MS), dealcoholized red wine (DRW), hemoglobin A1c (HbA1c).

1. INTRODUCTION

Type 2 diabetes mellitus is characterized by peripheral insulin resistance and low-grade systemic inflammation. Plasma levels of inflammation markers C-reactive protein (CRP), and IL-6 have been shown to predate the onset of Type 2 diabetes mellitus in women [1], suggesting that low-grade systemic inflammation plays a significant role in the onset of type 2 diabetes mellitus. In this regard, light-to-moderate alcohol intake has been associated with reduced inflammation [2] as well as lower insulin levels and improved glycaemic control [3] . Further, a meta-analysis of 706,716 individuals showed that light - moderate alcohol consumption associated with a reduced risk of developing Type 2 diabetes mellitus [4] and the Health Professionals Follow-up Study showed moderate alcohol intake associated with reduced inflammatory markers such as fibrinogen, soluble tumour necrosis factor receptor-2, and soluble vascular adhesion molecule-1 in Type 2 diabetes mellitus [5]. These findings suggest that the anti-inflammatory effects of moderate alcohol consumption may provide protection against development of Type 2 diabetes mellitus.

Inflammation resolution is recognised as an important process that is driven by specialised pro-resolving mediators of inflammation (SPMs) and has the potential to moderate chronic inflammation [6, 7]. The SPM's include lipoxins from arachidonic acid (AA) and resolvins, protectins and maresins derived from the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [6] . SPM synthesis from EPA is via metabolism by cyclooxygenase-2 (COX-2) or cytochrome P450 (CYP450) to 18-hydroxyeicosapentaenoic acid (18-HEPE) with further metabolism by 5- lipoxygenase (5-LOX) and 15-lipoxygenase (15-LOX) to form the E-series resolvins (RvE1-RvE3). SPM synthesis from DHA occurs by metabolism by COX-2/15-LOX to 17-hydroxydocosahexaenoic acid (17-HDHA) that is

further metabolised to Protectin D1 (PD1), and via 5-LOX to the D-series resolvins (such as RvD1, 17R-RvD1, RvD2 and RvD5). DHA metabolism by 12-lipoxygenase (12-LOX) leads to another group of SPM, the Maresins (MaR-1) and 14-hydroxydocosahexaenoic acid (14-HDHA) [6, 7]. SPMs halt neutrophil infiltration and enhance macrophage uptake of apoptotic cells and debris, promoting a return to homeostasis after an inflammatory event [6, 7]. Alcohol has the potential to affect synthesis of SPMs by altering the availability of fatty acid substrates, [8] and the activity of, COX-2 [9] and 5-lipoxygenase [10] that are key enzymes involved in SPM synthesis and is also known to affect cytochrome P450 enzymes [11] and CYP450 metabolism to eicosanoids[12-14].

In a controlled trial in persons with Type 2 diabetes mellitus, that compared red wine with dealcoholized red wine (DRW) and water, we showed that red wine increased awake systolic and diastolic blood pressure and heart rate relative to water and DRW, without affecting glycemic control [15]. In a trial of similar design in healthy men we showed that consumption of alcohol as red wine increased plasma SPM levels [13]. The finding that alcohol stimulated plasma SPM in healthy volunteers suggested that alcohol may also affect SPM in diabetic patients who are known to have low grade systemic inflammation.

In this report we aimed to examine the effects of red wine on plasma SPM in plasma samples collected from the same randomised controlled trial of red wine in patients with Type 2 diabetes mellitus [15]. In addition, we compared plasma SPM levels in patients with Type 2 diabetes mellitus with those of healthy volunteers.

2. PATIENTS AND METHODS

2.1 Effects of red wine on plasma SPM in Type 2 diabetes mellitus

2.1.1 Study volunteers

Men and postmenopausal women with Type 2 diabetes mellitus were recruited from the general population by newspaper advertisement and community screening. Participants were aged 40–70 years and regular drinkers. Inclusion criteria for alcohol consumption in women was 2–3 standard drinks/day (20–30 g alcohol/day) and in men 3–4 standard drinks/day (30–40 g alcohol/day). Exclusion criteria included type 1 diabetes mellitus, recent (<3 months) symptomatic heart disease, angina pectoris, history of myocardial infarction or stroke, peripheral vascular disease, major surgery 3 months or less, BP > 170/ 100 mmHg, liver or renal disease (plasma creatinine >120 mmol/l), haemoglobin A1c (HbA1c) more than 8.5% (>69 mmol/mol), and current smokers or ex-smokers (<2 years). Antihypertensive or lipid-lowering medication usage were not exclusion criteria.

2.1.2 Study design

After a 4-week run-in period of usual alcohol intake, 24 participants (19 men and 5 women) with Type 2 diabetes mellitus were randomised to a 12 week three-period cross-over study of Latin square design. The three study periods each of 4 weeks were, red wine with women drinking 230 ml/day (~24 g alcohol/day) and men drinking 300 ml/day (~31 g alcohol/day), or the equivalent volumes of dealcoholized red wine (DRW) or the consumption of water only. Participants were asked to consume each beverage with the evening meal and to abstain from all alcohol during the DRW and water only periods. The water only group controlled for any effect of phenolic compounds in red wine or DRW. There was no washout between each study period. The red wine was a Shiraz Cabernet blend containing 13% v/v alcohol. The red wine and DRW were from Orlando Wyndham, Rowland Flat, South Australia. Volunteers

were assigned to each study period sequence via block randomization using computer-generated random numbers, devised by the statistician.

The trial was conducted in the Clinical Trials Unit of the Medical School at Royal Perth Hospital according to the Declaration of Helsinki guidelines and approved by the Royal Perth Hospital Ethics Committee. Written informed consent was obtained from all participants. The trial is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615000133538).

2.1.3 Measurements

Measurements were at the end of the 4-week run-in period and at the end of each 4-week study period following an overnight fast. Compliance with alcohol intake during the study was recorded using 7-day retrospective diaries completed at weekly visits to the Clinical Trials Unit. Plasma γ-glutamyl transpeptidase (γ-GT) was measured as a biomarker of change in alcohol intake during the 4 week run-in period and at the end of each 4 week study period. Twenty four-hour urinary 4-O-methylgallic acid a biomarker of polyphenol content of red wine was determined at the same time points by gas chromatography–mass spectrometry using established methods [16].

2.1.4 Biochemical analyses

A fasted blood sample was collected into EDTA and reduced glutathione (Sigma –Aldrich) 1mg/ml at the end of the 4-week run-in period and at the end of each 4-week study period for measurement of plasma SPM and F_2-isoprostanes. The blood samples were collected on ice, centrifuged immediately and the plasma aliquoted and stored at -80^{o}C until assay. All samples from each study participant were assayed in the one batch.

SPM included 18-hydroxyeicosapentaenoic acid (18-HEPE), resolvin E1 (RvE1), resolvin E2 (RvE2), resolvin E3 (RvE3) and 18R-resolvin E3 (18R-RvE3), 17-hydroxydocosahexaenoic acid (17-HDHA), resolvin D1 (RvD1), 17R-resolvin D1 (17R-RvD1), resolvin D2 (RvD2), resolvin D5 (RvD5), protectin D1 (PD1), maresin-1 (MaR1) and 14-hydroxydocosahexaenoic acid (14-HDHA). SPM were analysed and quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Thermo Scientific TSQ Quantum Ultra triple quadrupole LC-MS system (Thermo Scientific) equipped with an electrospray ionization source operated in the negative-ion mode as previously described [17, 18]. The retention time of standards, their mass spectrum, and parent and product ions and collision energy used to identify individual SPMs were as previously reported (see Supplemental Data in reference) [18]. Instrument control and data acquisition were performed with Xcalibur 2.0.7 software. The limit of detection and limit of quantitation for the precursor's 18-HEPE, 17-HDHA, and 14- HDHA, were 8 and 15 pg/mL, respectively, and for all other SPM, 15 pg/mL and 20 pg/mL, respectively.

Plasma F_2-isoprostanes that are markers of lipid peroxidation were measured as by gas chromatography mass spectrometry using methods established in our laboratory [13].C-reactive protein (CRP) was measured using a high-sensitivity monoclonal antibody assay (Dade Behring Marburg GmbH, Marburg, Germany). γGT was measured with Roche kit (Roche DiagnosticsGmbH, Mannheim, Germany)

2.2 Comparison of plasma SPM in patients with Type 2 diabetes mellitus and healthy volunteers

Plasma SPM in patients with Type 2 diabetes mellitus (n=24) were compared with those of 22 healthy volunteers who were regular drinkers recruited from the general population for a study of similar design that examined the effect of consuming red wine on blood pressure

[13]. The study in healthy volunteers was conducted in the Clinical Trials Unit of the Medical School at Royal Perth Hospital according to the Declaration of Helsinki guidelines with ethics approval for recruitment of the from the University of Western Australia Human Ethics Committee. Written informed consent was obtained from all participants. The healthy volunteers were male non-smokers, aged between 38-62y, not diabetic and had no history of chronic disease. Although a similar spectrum of SPM was measured in the 22 healthy volunteers we could only detect measurable levels of 18-HEPE, 17-HDHA, 17R-RvD1, RvD1, and 14-HDHA in plasma. Therefore we compared these plasma SPM as well as plasma glucose, total cholesterol, triglycerides, HDL- and LDL-cholesterol, and hs-CRP from two baseline fasted blood samples with measurements from patients with Type 2 diabetes mellitus. The plasma SPM from the 2 visits were averaged and compared with plasma SPM obtained from the patients with Type 2 diabetes mellitus at baseline. The samples from healthy volunteers and patients with Type 2 diabetes mellitus were collected and stored under the same standardised conditions. The SPM from healthy volunteers and patients with Type 2 diabetes mellitus were assayed within 6 months. The inter-assay coefficient of variation was 9%.

2.3 Statistical analysis

The study of the effects of red wine in patients with Type 2 diabetes mellitus was initially powered to detect changes in the primary outcome of blood pressure [15]. A follow-up power calculation estimated that recruitment of 20-25 participants would give 80% power to detect a 20% difference in plasma 18-HEPE based upon repeated measures ANOVA analysis. Comparison of plasma SPMs for each beverage used linear mixed models with restricted maximum likelihood estimation and a STATA 15 statistical package. A covariate was entered into each of the regression analyses to test for the effects of the randomization order on each of the outcome variables. Spearman correlations were used to examine the

relationship between fasting glucose, HbA1c, insulin and hs-CRP in the patients with Type 2 diabetes mellitus and plasma SPM. The comparison between SPM in patients with Type 2 diabetes mellitus and healthy volunteers used general linear models adjusting for age, gender and alcohol consumption.

3. RESULTS

3.1 The effects of red wine on plasma SPM and F_2-isoprostanes in Type 2 diabetes mellitus

Twenty-four patients with Type 2 diabetes mellitus (19 men and 5 women) were studied and 23 (19 men and 4 women) had plasma SPM measured. The baseline characteristics of the patients are shown in Table 1. They were middle aged (59.3 years), overweight (BMI 29.3 kg/m^2), with blood pressure 130 ± 2 /78 ± 1 mmHg and regularly consuming a moderate amount of alcohol (37 g/day). Duration of diabetes was 4.0 ± 2.3 years.

Urinary 4-O-methyl gallic acid was measured as a marker of red wine polyphenol intake and was 1074 ± 209 µg/day at baseline; 454 ± 156 µg/day after water; 1238 ± 217 µg/day after DRW and 1550 ± 227µg/day after RW, indicating that there was good compliance with the treatment polyphenol intake during the periods of DRW and RW consumption. γGT measured as a marker of alcohol intake was 30.51 (23.50, 39.62) U/L after red wine compared with 26.55 (20.66, 34.13) U/L after water and 28.39 (21.72, 37.10) U/L after DRW indicating good compliance with the intervention.

At baseline the precursors 18-HEPE, 17-HDHA, the E-series resolvins (RvE1, RvE2, and 18R-RvE3); D-series resolvins (RvD1, 17R-RvD1, RvD2), PD1, MaR1 and 14-HDHA were all detected in plasma. RvE3 and RvD5 were below the limit of quantitation. Drinking RW or DRW did not significantly affect plasma 18-HEPE, the E -series resolvins, 17-HDHA or the D-series resolvins (Table 2). Similarly, there were no significant effects of drinking RW or DRW on PD1, MaR1 or 14-HDHA (Table 2). Plasma F_2-isoprostanes were 1382 ± 90 pmol/L at baseline, 1194 ± 78 pmol/L after water, 1214 ± 50 pmol/L after DRW and 1305 ± 60 after RW and not significantly different between the treatment periods.

Spearman correlations were used to examine possible relationship between fasting glucose, insulin and hs-CRP and the plasma SPM at baseline and after each intervention. There were no consistent significant correlations observed for any of the plasma SPM with fasting glucose, HbA1c, insulin or hs-CRP in the patients with Type 2 diabetes mellitus.

3.2 Comparison of plasma SPM in patients with Type 2 diabetes mellitus and healthy volunteers

The patients with Type 2 diabetes mellitus were on average 5 years older with elevated fasting glucose and triglycerides compared with the healthy volunteers (Table 1). Self-reported alcohol consumption was higher in the healthy volunteers (P=0.03) (Table 1). The gender distribution also differed between the two groups with patients with Type 2 diabetes mellitus comprising 19 males and 5 females whereas the healthy volunteers were all men (Table 1). BMI, blood pressure, total cholesterol, HDL- and LDL-cholesterol and γGT were not different between the groups (Table 1). Urinary 4-O-methyl gallic acid was 1074 ± 209 µg/day in the diabetics and not different to healthy volunteers (767 ± 223 µg/day, P=0.28) Hs-CRP was elevated in the patients with Type 2 diabetes mellitus (1.58 mg/L, CI 1.01, 2.47mg/L) compared with healthy volunteers (0.85mg/L, CI 0.45, 1.24mg/L; *P*=0.05). The healthy volunteers had measureable levels of 18-HEPE, 17-HDHA, RvD1, 17R-RvD1 and 14-HDHA. In contrast, the E-series resolvins PD1 and MaR1were not detected. The levels of 18-HEPE and 17-HDHA were significantly elevated (P<0.0001) in the patients with Type 2 diabetes mellitus compared with healthy volunteers and remained so after adjusting for age, gender, alcohol consumption (Figure 1). The levels of RvD1 and 17R-RvD1 were also significantly elevated in patients with Type 2 diabetes mellitus compared with healthy volunteers (Figure 1). Plasma 14-HDHA was 1218 ± 241 pg/mL in the patients with Type 2 diabetes mellitus compared with healthy volunteers 1847 ± 254 pg/mL (*P*=0.14). Plasma F_2-

isoprostanes were 1162 ± 51 pmol/L in healthy volunteers and not different to patients with Type 2 diabetes mellitus 1288 ± 66 pmol/L.

4. DISCUSSION

We have shown for the first time that patients with Type 2 diabetes mellitus have measurable plasma levels of the SPM 18-HEPE, 17-HDHA, E- and D- series resolvins, PD1, MaR1 and 14-HDHA. There is no evidence that the alcohol or polyphenols in red wine affect the levels of these SPM in Type 2 diabetes. Comparison of the baseline levels of plasma SPM with those of healthy volunteers showed that plasma 18-HEPE, 17-HDHA, RvD1 and 17R-RvD1 were all significantly elevated in patients with Type 2 diabetes mellitus. The elevation of plasma SPM in patients with Type 2 diabetes mellitus was coincident with a significant elevation of plasma Hs-CRP compared with healthy volunteers.

The results from this study contrast that of our recent trial in healthy men that showed red wine significantly increased the levels of plasma SPM 18-HEPE, RvD1 and 17R-RvD1 compared with DRW and water [13]. The two studies reported similar effects of red wine on blood pressure, heart rate, γGT and HDL-cholesterol with no evident effect of DRW on any of the measured parameters. Possible reasons for the differences between the two studies include the diverse study populations (patients with Type 2 diabetes mellitus compared with healthy volunteers), the lower alcohol intake employed in this trial (24-31g/day in the current study compared with 41g/day in the healthy men) and the possible influence of concurrent antihypertensive and lipid lowering treatment in the patients with Type 2 diabetes mellitus.

There was also no significant effect of consumption of red wine on plasma F_2-isoprostanes, this contrasts our results healthy men given red wine and other studies where alcohol was given at a dose ~40g/day.[12, 13, 19, 20].

Baseline levels of 18-HEPE, 17-HDHA in the patients with Type 2 diabetes mellitus were 2-3 fold higher and the downstream SPM RvD1 and 17R-RvD1were significantly elevated compared with SPM of healthy volunteers. All of these SPM promote inflammation resolution. In animal models of Type 2 diabetes RvD1 has been shown to improve insulin resistance [21] and improve wound healing [22]. The precursors to the E- and D-series resolvins, 18-HEPE and 17-HDHA, are also biologically active. 18-HEPE has been shown to reduce maladaptive cardiac remodelling in mice [23]. 17-HDHA modulates macrophage function, alleviates experimental colitis [24], and can mediate B-cell differentiation to antibody secreting cells [25]. Possible mechanisms for increased SPM in Type 2 diabetes include increased levels of n-3 fatty acid substrate for SPM synthesis [26] and heightened expression of the enzymes COX-2, that leads to synthesis of 18-HEPE and 17-HDHA [27] and 5-LOX that is involved in synthesis of the E- and D- series resolvins [26].

Although the two studies were not concurrent the samples from both trials were collected under carefully standardised conditions and stored appropriately for analysis. Furthermore, although the groups were not matched for age and gender, adjusting for these differences in the analysis did not affect the observed differences in plasma SPM. It is possible that use of aspirin and statins, both of which have been shown to increase SPM levels [28, 29], could explain the higher plasma SPM levels in the patients with Type 2 diabetes mellitus compared with the healthy controls. In our study, 25% of the patients with Type 2 diabetes mellitus reported taking these agents. However, adjusting for use of these medications did not account for baseline differences in plasma SPM between the groups. The patients with Type 2 diabetes mellitus in this study had elevated baseline hsCRP levels compared with healthy volunteers indicative of low grade inflammation. Thus it is possible that the elevated levels of SPMs in patients with Type 2 diabetes mellitus is a homeostatic response to inflammation

and that the addition of alcohol was not a sufficient stimulus to further increase plasma SPM levels.

This study has shown that moderate alcohol consumption consumed as red wine does not alter plasma SPM in patients with Type 2 diabetes mellitus. The observation that a number of plasma SPM are elevated in patients with Type 2 diabetes mellitus compared with healthy volunteers needs to be replicated in a larger sample size which can control for major confounders.

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Disclosures

None of the authors has a conflict of interest to declare.

Table 1 Comparison of baseline characteristics of healthy volunteers and patients with Type 2 diabetes mellitus.

	Healthy Volunteers (n=22)	Type 2 diabetes mellitus (n=24)	P Values
Age (y)	54.1 ± 1.4	59.3 ± 1.1	P = 0.006
Gender (males/females)	22/0	19/5	
BMI (kg/m^2)	27.5 ± 0.6	29.3 ± 1.0	
Blood pressure (mmHg)	128 ± 2 / 79 ± 2	130± 2 / 78 ± 1	
*Alcohol consumption (g/wk)	343 (287, 409)	260 (220, 309)	P = 0.03
Glucose (mmol/L)	4.8 ± 0.4	7.5 ± 0.4	P < 0.001
HbA1c (%)	Not measured	6.65 ± 0.27	
Cholesterol (mmol/L)	5.3 ± 0.2	5.1 ± 0.2	
*γGT U/L	28.0 (21.5, 36.6)	33.1 (25.4,42.3)	
*Triglycerides (mmol/L)	1.1 (0.7, 1.4)	1.6 (1.3, 1.9)	P = 0.017
HDL Cholesterol (mmol/L)	1.3 ± 0.1	1.3 ± 0.1	
LDL Cholesterol (mmol/L)	3.5 ± 0.2	3.1 ± 0.2	

Values are Mean ± SEM or *geometric mean and 95% CI

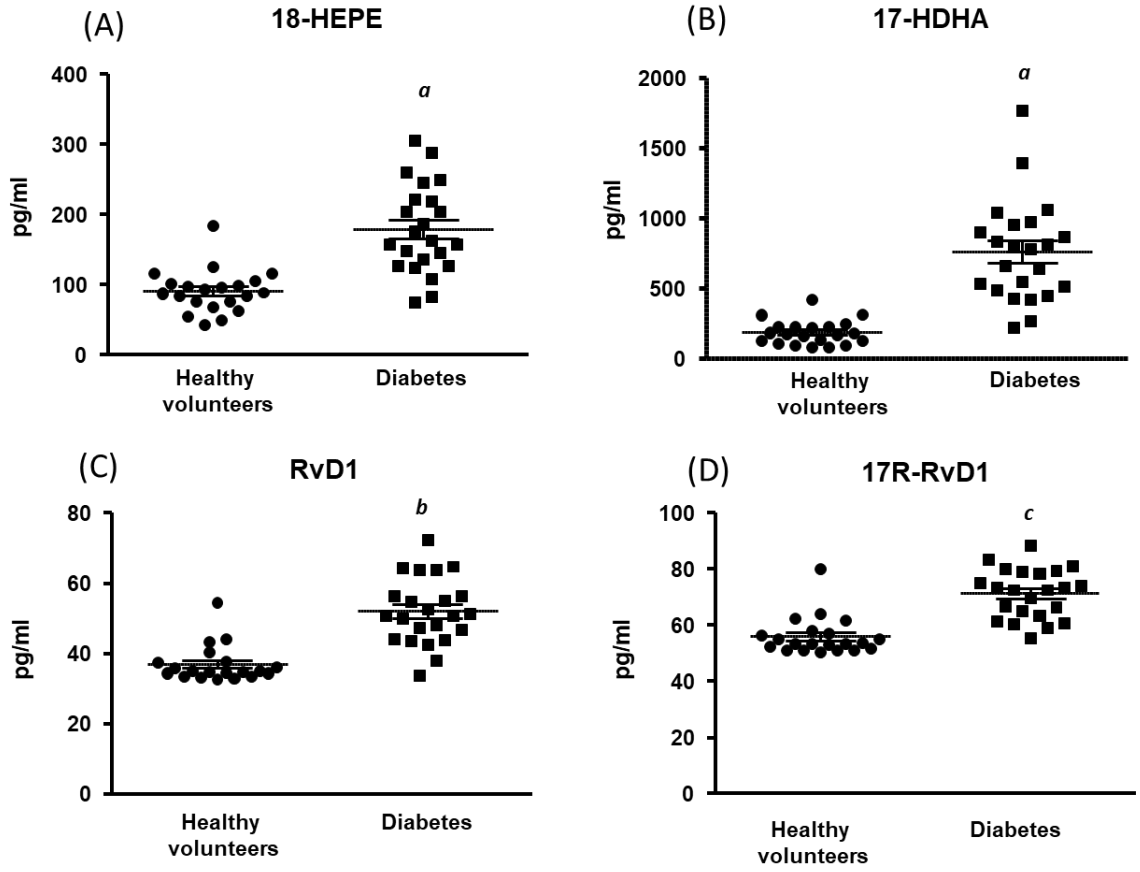
Table 2. Plasma SPM (18-HEPE, E series resolvins, 17-HDHA, D-series resolvins, PD1, 14-HDHA and MaR1) at baseline and after 4 weeks of drinking either water, dealcoholized red wine (DRW) or red wine in patients with Type 2 diabetes mellitus

	Baseline	Water	DRW	Red wine
18-HEPE (pg/ml)	186.0 (152.6, 226.8)	151.3 (130.8, 174.9)	148.8 (127.3, 174.0)	166.7 (139.8, 198.6)
RvE1 (pg/ml)	50.0 (36.6, 68.2)	57.4 (43.8, 75.2)	40.9 (30.6, 54.6)	45.9 (35.1, 60.0)
RvE2 (pg/ml)	82.3 (58.1, 116.7)	61.1 (43.3, 86.4)	54.1 (39.6, 73.9)	59.7 (41.5, 85.8)
18R-RvE3 (pg/ml)	50.6 (36.4, 70.3)	36.9 (23.4, 58.1)	46.4 (27.0, 79.8)	50.2 (31.1, 80.3)
17-HDHA (pg/ml)	751 (597, 945)	599 (474, 759)	627 (513, 766)	634 (471, 853)
RvD1 (pg/ml)	51.2 (46.0, 56.9)	50.4 (46.3, 55.2)	48.2 (43.9, 52.9)	49.2 (44.9, 53.9)
17R-RvD1 (pg/ml)	71.2 (65.1, 77.8)	69.3 (64.9, 74.0)	68.1 (62.2, 74.6)	68.5 (63.1, 74.4)
RvD2 (pg/ml)	31.6 (28.1, 35.6)	33.8 (29.6, 38.6)	33.6 (29.3, 38.6)	31.4 (27.5, 35.8)
PD1 (pg/ml)	53.5 (46.9, 60.9)	47.6 (42.1, 53.8)	46.7 (41.9, 51.8)	46.7 (42.2, 51.6)
14-HDHA (pg/ml)	1173 (960, 1434)	979 (757, 1266)	1116 (883, 1386)	1086 (762, 1547)
MaR1 (pg/ml)	54.6 (42.6, 69.9)	49.4, (39.4,52.8)	50.0 (39.8, 62.7)	48.4 (40.0, 58.5)

Values are geometric means and 95% confidence intervals. There were no statistical differences in any of the plasma SPM after intake of red wine compared with DRW or water or after DRW compared with water.

Figure Caption

Figure 1. Scattergram of plasma 18-HEPE (Panel A), 17-HDHA (Panel B), RvD1 (Panel C) and 17R-RvD1 (Panel D) in patients with Type 2 diabetes mellitus and healthy volunteers ([a] P<0.0001, [b] P=0.006 and [c] P=0.012 compared with healthy volunteers using general linear model after adjustment for age, gender and alcohol consumption.



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