Alcohol consumption and n–3 polyunsaturated fatty acids in healthy men and women from 3 European populations[1–4]

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ABSTRACT

Background: Because high dietary and blood n–3 (omega-3) fatty acids (FAs) are protective against coronary heart disease and sudden cardiac death, the alcohol-associated increase in blood n–3 FAs could be considered an original mechanism of alcohol's cardioprotective effect.

Objective: Our objective was to assess whether alcohol consumption is associated with concentrations of very-long-chain "marine" (eg, fish oil) n–3 FAs both in plasma and in red blood cell membranes.

Design: In the framework of the IMMIDIET (Dietary Habit Profile in European Communities with Different Risk of Myocardial Infarction: the Impact of Migration as a Model of Gene-Environment Interaction) Project, 1604 subjects (802 women-men pairs), aged 26–65 y, were enrolled in Italy, Belgium, and England. A 1-y-recall food-frequency questionnaire was used to evaluate dietary intake.

Results: In fully adjusted multivariate analyses, alcohol intake was positively associated with plasma eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and EPA + DHA concentrations ($P < 0.0001$, $P = 0.036$, and $P = 0.002$, respectively) in women and with EPA and the EPA + DHA index in red blood cells ($P < 0.0001$ and $P = 0.037$, respectively). In men, only plasma and red blood cell EPA concentrations were associated with alcohol intake ($P = 0.003$ and $P = 0.004$, respectively). Stratified analyses showed an association between alcohol and both plasma and red cell EPA ($P = 0.008$ and $P = 0.002$, respectively), DHA ($P = 0.014$ and $P = 0.008$, respectively), and the EPA + DHA index ($P = 0.010$ and $P = 0.006$, respectively) in wine drinkers, whereas no association was found in those who drink beer and spirits.

Conclusions: Alcohol intake was associated with higher plasma and red blood cell concentrations of marine n–3 FAs. Components of wine other than alcohol (polyphenols) might exert these effects. Part of the alcohol-induced cardioprotection may be mediated through increased marine n–3 FAs. *Am J Clin Nutr* 2009;89:354–62.

INTRODUCTION

Several observational studies and meta-analyses have consistently shown that moderate alcohol consumption, including wine consumption, is associated with protection against coronary artery disease (CAD) and ischemic stroke (1–3). A number of pathways were implicated in the protective effect of alcohol, although the mechanisms are not completely defined. They include increased concentrations of HDL cholesterol and fibrinolysis, decreased platelet aggregation and coagulation factors, and beneficial effects on endothelium function and inflammation (4, 5). Alcohol intake might influence the metabolism of essential polyunsaturated fatty acids (PUFAs) (6, 7). In particular, low doses of alcohol may increase PUFA concentration through stimulation of fatty acid (FA) anabolism (6, 8); in contrast, at higher alcohol doses PUFA concentration decreases because of increased FA catabolism (6). Moderate alcohol consumption has been associated with increased concentrations of very-long-chain (marine) n–3 FAs both in plasma and in blood cell membranes, both in humans and rats (7, 9). Because high concentrations of dietary and plasma n–3 FAs are protective against CAD and sudden cardiac death (10, 11), the alcohol-induced increase in marine n–3 FAs could be considered an original mechanism of the protective effect of alcohol.

The Lyon Diet Heart Study included only French male patients with cardiovascular disease and measurements of marine plasma n–3 FA concentrations (7). Whether such findings would be the same in other (healthy) populations is not known. In the present

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study we aimed to extend the investigation on the association between alcohol intake and n–3 FAs to healthy male-female pairs from 3 European regions: Abruzzo (Italy), Limburg (Belgium), and Southwest London (United Kingdom); these regions were considered to have populations with different dietary habits and risk of CAD [the IMMIDIET Project (Dietary Habit Profile in European Communities with Different Risk of Myocardial Infarction: the Impact of Migration as a Model of Gene-Environment Interaction)] (12, 13). Moreover, we examined whether different alcoholic beverages may have any specific effect. Indeed, red and white wine, in addition to alcohol, contains polyphenols (although in higher concentration in red wine), with antioxidant activity that could counteract PUFA oxidation (14).

SUBJECTS AND METHODS

The IMMIDIET Project and the recruitment of subjects were previously described (12, 13, 15). Briefly, the IMMIDIET Project is a cross-sectional study comparing individual members from healthy pairs living in Italy, Belgium, and the United Kingdom to evaluate the risk components in the 3 communities with different dietary habits and CAD risk. Pairs were apparently healthy male-female spouses or partners living together and were recruited through local general practice offices. To protect against selection bias, eligible pairs were randomly selected in each country: local general practice networks recruited 270 pairs in the Abruzzo region of Italy, 268 in the Flemish territory of Limburg in Belgium, and 263 in Southwest London in the United Kingdom. A computerized list of all eligible pairs in each practice was generated in advance, and an invitation was made by letter or phone call. The recruitment strategies were carefully defined and standardized across the 3 recruiting centers. Subjects were examined in general practice offices by trained research personnel, who examined the subjects according to good clinical practices. Between October 2001 and October 2003, 1604 subjects (802 women-men pairs), aged 26–65 y (mean ± SD age: women, 44 ± 7.6 y; men, 47 ± 7.7 y) were enrolled in the study. The participation rate ranged between 70% and 90% in the different centers. Exclusion criteria for all groups were as follows: history of cardiovascular disease, diabetes mellitus, familial hypercholesterolemia, or malignancies; chronic diseases such as heart, liver, or renal failure; hypothyroidism or hyperthyroidism; and epilepsy. After exclusion of 142 subjects receiving treatment with cholesterol-lowering drugs or with a caloric intake <800 and >6000 kcal, 1457 healthy volunteers (747 women and 710 men) were studied to assess whether there were any relations between alcohol intake and n–3 FA concentrations.

The study was approved by the ethical committees of all participating institutions. All study participants provided written informed consent.

Trained research personnel in the different recruitment centers performed blood pressure (BP) and anthropometric measurements with the use of methods that had been standardized before and during preliminary meetings at which IMMIDIET consortium partners participated. Interviews were performed using a standardized questionnaire previously adopted in the Olivetti Prospective Heart Study (16).

BP was measured with an automated device (OMRON-HEM-705CP; OMRON Corporation, Amsterdam, Netherlands.) (17). BP values were measured 3 times on the nondominant arm; the average of the last 2 values was recorded as the BP. Measurements were performed in a quiet room with a comfortable temperature and the participants seated for ≥5 min. Body weight and height were measured in subjects without shoes and wearing light clothing with the use of a standard beam balance scale and attached ruler. Body mass index (BMI; in kg/m^2) was calculated. Waist and hip circumferences were measured according to the National Institutes of Health, National Heart, Lung, and Blood Institute guidelines (18), and waist-to-hip ratio was calculated.

Lifestyle assessment

Subjects were classified as nonsmokers (if they had never smoked cigarettes), ex-smokers (if they had smoked cigarettes in the past), and current smokers if they were currently smoking ≥1 cigarettes/d. Physical activity was assessed by using a standardized questionnaire (19). Subjects were grouped into 3 categories of physical activity (low, middle, or high). Socioeconomic status was scored on the basis of 3 variables: education, job, and housing, with higher score representing higher socioeconomic status.

Dietary habit assessment

To evaluate dietary intake, the validated Italian and English European Prospective Investigation into Cancer and Nutrition (EPIC) semiquantitative food-frequency questionnaires (FFQs) (20, 21) were used; the FFQs contain questions on the average consumption of 164 food items over the past year. On the basis of these 2 questionnaires, a Belgian FFQ (unpublished data, 2008) was implemented. The 3 FFQs refer to simple foods (eg, carrot, apple, or cod) and complex foods containing several ingredients (eg meat sauce or vegetable pie) with daily quantities that were specific to each country. Questionnaires had been developed to estimate total energy intake, macronutrients (carbohydrate, protein, lipids, and alcohol), and principal vitamins and micronutrients from the diet. The questionnaire was self-administered and checked by a dietitian for completeness and ambiguous answers.

A computer program, NUTRITION ANALYSIS OF FFQ (NAF) (22), was developed by the Epidemiology Unit of the Istituto Nazionale Tumori of Milan to convert questionnaire dietary data into frequencies of consumption and average daily quantities of foods, energy, and nutrients consumed. For the present project, NAF was linked to the McCance Food Composition Tables (FCTs) (23), the Belgian FCT (24–26), and the Italian FCT for Epidemiologic Studies (27). Marine food intake was defined as the total intake of fish, shellfish, cuttlefish, squid, octopus, shrimp, and crab.

Alcohol intake assessment

For the 3 European populations, total daily alcohol intake was estimated on the basis of the consumption of wine, beer, and spirits during the past year as reported in drinks per day in the FFQ; there was one question each on white wine, red wine, rosé wine, fortified wines (sherry, port, vermouth), beer, and spirits consumption. In particular, one drink of wine (glass) was considered to be equivalent to 120 mL of wine containing 12% alcohol (vol:vol, %); one drink of beer (bottle or can) was equivalent to 200 mL for the Italian population and 250 mL for the Belgian population, whereas it was equivalent to 284 mL for the English population. One drink of spirits (small glass) was equivalent to 40 mL containing 36% alcohol (vol:vol, %). Various categories of alcohol percentage in beer

were indicated in the FFQ as follows: 1–6.5%, 7–9.5%, 10–13.5%, and >13.5% (vol:vol, %). Possible responses for frequency of each beverage were graded in an 8-point scale ranging from never to ≥7/d. The validity of these questions was reported elsewhere for the Italian and English EPIC questionnaires (20, 21, 28). For the Belgian questionnaire, the correlation between diet records and FFQ for total alcohol intake was 0.90 for men ($n = 35$) and 0.83 for women ($n = 35$; $P < 0.001$). The mean alcohol intake was 17 g/d for men and 5 g/d for women when calculated from the FFQ and 20 g/d for men and 6 g/d for women with the use of a repeated 24-h dietary recall.

Daily intake was estimated in grams by multiplying the volume (expressed in mL/d) of each type of alcoholic beverage by the percentage of alcohol, which was corrected for alcohol density (0.79).

Biochemical and anthropometric measurements

Blood samples were obtained between 0700 and 1000 from patients who had been fasting overnight and who had not smoked for ≥6 h. Aliquots were kept on dry ice until biochemical analysis, which was performed in central laboratories.

Total cholesterol, HDL cholesterol, and triglyceride concentrations were assessed by using an automated analyzer (Roche Cobas Mira Plus; Roche Applied Science, Meylan, France). LDL cholesterol was calculated according to the Friedewald formula (29). Plasma and red blood cell n–3 FAs were measured by gas chromatography (7, 9). In brief, lipids were extracted by a monophasic method that uses of hexane to isopropanol (3:2; vol:vol) after adding heptadecanoic acid as an internal standard. Extracted lipids were saponified and methylated with 14% boron trifluoride in alcohol. After extraction, the methylated FAs were quantified by gas chromatography with flame ionization detection (CPG 6850; Agilent Technologies Inc, Santa Clara, CA) on a capillary column (Quadrex 007 cyanopropyl methyl silicone, 30-m length, 0.25-mm internal diameter, film thickness 0.25 μm; Quadrex Corporation, Woodbridge, CT). Hydrogen was the carrier gas. FA peaks were identified and quantified by comparison with known standards, and FA composition is reported as weight percentage of total FAs.

Statistical methods

Alcohol intake was expressed as the percentage of total energy intake and categorized into quartiles on the basis of sex-specific distribution (with the lowest value representing the abstainers). Nutrient covariate data were energy-adjusted according to the residual method (30). Multivariable linear regression analysis was used to assess the relation between quartiles of alcohol intake treated as a linear term and n–3 separately for men and women. The basic model was adjusted for age and country. Additional adjustment was performed with all variables that were associated with plasma or red cell n–3 FAs and alcohol intake, with a significance level of at least $P < 0.1$. The final regression models included age; country; social status (categorical); smoking habits (never, past, or current); physical activity (in tertiles); fatty fish intake; intake of saturated and monounsaturated FAs, PUFAs, and total energy; HDL-cholesterol concentration; and BMI. Additional analyses were performed by dividing the whole population into subjects drinking only wine or only beer or spirits (with further adjustment for total alcohol intake). Plasma n–3 FA concentrations that showed a positive skewness were transformed into natural logarithms. Data were reported as geometric means and 95% CI for skewed variables and as means ± SEM for continuous non-skewed variables. Two-sided 95% CIs and P values were calculated; $P < 0.05$ was chosen as the level of significance. The analyses were performed with SAS 9.1.3 for Windows (SAS Institute, Cary, NC).

RESULTS

The characteristics of the population according to quartiles of alcohol intake are shown in **Tables 1** and **2**. In women, alcohol intake accounted for 2.4% of total energy intake (76% of which was derived from wine alcohol), whereas in men it accounted for 5.3% (53% from wine alcohol) of total energy intake. In women, there were only 3 heavy drinkers (1 with an alcohol intake > 20% of total energy intake and 2 with an alcohol intake > 25%); in men the number of heavy drinkers was 15 (11 with alcohol intake > 20% of total energy intake, and 4 with alcohol intake > 25%).

Alcohol intake was associated in women with age, social status, HDL cholesterol, LDL cholesterol, oxidized LDL, BMI, systolic blood pressure, energy intake, and intake of total lipid and saturated and polyunsaturated FAs (Table 1). In men, alcohol consumption was associated with social status, smoking habits, total cholesterol, HDL cholesterol, systolic blood pressure, and intake of total lipid, saturated, and monounsaturated FAs and dietary cholesterol (Table 2). After multivariate analysis, alcohol intake remained significantly associated with age, HDL cholesterol, and intake of saturated and polyunsaturated FAs in women (Table 1) and with social status, HDL cholesterol, smoking habits, and intake of total energy, saturated FAs, and dietary cholesterol in men (Table 2).

Plasma and red cell n–3 FA percentages were positively associated with quartiles of alcohol intake both in women and in men, after adjustment for age and country (**Tables 3** and **4**). In particular, in women, higher alcohol intake was significantly associated with higher plasma and red blood cell eicosapentaenoic acid (EPA, 20:5n–3; $P < 0.0001$ for both), docosahexaenoic acid (DHA, 22:6n–3; $P < 0.0001$ and $P = 0.007$), and EPA + DHA concentrations ($P < 0.0001$ and $P = 0.0004$) (Table 3). In fully adjusted multivariate analyses (Table 3), all associations remained significant in plasma (EPA: $P < 0.0001$; DHA: $P = 0.036$; and EPA + DHA: $P = 0.0002$), whereas only EPA ($P < 0.0002$) and EPA + DHA ($P = 0.037$) were associated with alcohol intake in red blood cells.

In men, higher alcohol intake was significantly associated with lower α-linolenic acid (ALA, 18:3n–3) concentrations in plasma and red blood cells ($P = 0.014$ and $P = 0.037$, respectively), with higher EPA concentrations in both plasma and red blood cells ($P < 0.0001$) and with higher EPA + DHA concentrations ($P = 0.048$) in plasma (Table 4). In fully adjusted multivariate analyses (Table 4), EPA remained significantly associated with alcohol intake in plasma and red blood cells ($P = 0.0003$ and $P = 0.004$), and ALA remained significantly associated with alcohol intake ($P = 0.043$) in plasma.

To evaluate whether the observed association was completely or partially dependent on the alcohol content of different beverages,

TABLE 1
General characteristics and main dietary habits of women in the IMMIDIET population according to quartile (Q) of alcohol intake[1]

Characteristics	Alcohol intake				P^2	P^3
	Q1 (n = 224)	Q2 (n = 176)	Q3 (n = 172)	Q4 (n = 175)		
Alcohol intake[4]	0	0.6 (0.3–1.3)[5]	2.3 (1.6–3.3)	5.8 (3.9–12.2)	—	—
Age (y)	44 ± 0.5[6]	43 ± 0.6	45 ± 0.6	46 ± 0.6	0.012	0.006
BMI (kg/m^2)	26.9 ± 0.3	26.0 ± 0.4	25.0 ± 0.4	25.3 ± 0.4	0.002	0.085
Waist-to-hip ratio	0.83 ± 0.004	0.82 ± 0.005	0.82 ± 0.005	0.81 ± 0.005	0.218	—
Systolic blood pressure (mm Hg)	117 ± 1.1	118 ± 1.2	114 ± 1.2	115 ± 1.2	0.028	0.429
Diastolic blood pressure (mm Hg)	75 ± 0.6	76 ± 0.7	73 ± 0.7	74 ± 0.7	0.065	0.873
Blood glucose (mg/dL)	79 ± 1.0	80 ± 1.1	77 ± 1.1	79 ± 1.1	0.209	—
Total cholesterol (mg/dL)	213 ± 2.4	216 ± 2.6	209 ± 2.7	212 ± 2.7	0.428	—
HDL cholesterol (mg/dL)	55 ± 0.9	55 ± 1.0	58 ± 1.0	62 ± 1.0	<0.0001	0.002
LDL cholesterol (mg/dL)	138 ± 2.3	141 ± 2.5	132 ± 2.6	133 ± 2.6	0.042	0.383
LDL oxidized (mg/dL)	53.6 ± 1.0	53.9 ± 1.0	50.7 ± 1.1	49.9 ± 1.1	0.015	0.993
Triglycerides (mg/dL)	97 ± 3.7	96 ± 4.0	94 ± 4.1	88 ± 4.1	0.346	—
High social status (%)	22	27	33	35	0.031	0.159
Current smoker (%)	18	23	18	22	0.557	—
Wine consumption[4]	0	0.54 ± 0.18	1.68 ± 0.19	5.65 ± 0.19	<0.0001	—
Beer consumption[4]	0	0.06 ± 0.07	0.28 ± 0.07	0.81 ± 0.07	<0.0001	—
Spirits consumption[4]	0	0.09 ± 0.05	0.25 ± 0.06	0.71 ± 0.06	<0.0001	—
Total energy intake (kcal/d)	2170 ± 45	2416 ± 49	2250 ± 49	2112 ± 50	<0.0001	0.073
Total lipids[4]	37.2 ± 0.4	36.5 ± 0.4	36.0 ± 0.4	34.7 ± 0.4	0.0001	0.405
Saturated fat[4]	13.6 ± 0.2	13.4 ± 0.2	12.8 ± 0.2	12.2 ± 0.2	<0.0001	0.006
Monounsaturated fat[4]	14.2 ± 0.2	14.2 ± 0.2	14.0 ± 0.2	14.2 ± 0.2	0.806	—
Polyunsaturated fat[4]	6.4 ± 0.1	6.2 ± 0.2	6.4 ± 0.2	5.6 ± 0.2	0.0006	0.003
Dietary cholesterol (mg/d)	297.6 ± 8.5	305.7 ± 9.2	297.2 ± 9.3	286.1 ± 9.4	0.534	—
Total fish (g/d)	34.2 ± 1.7	31.8 ± 1.8	36.1 ± 1.8	34.8 ± 1.8	0.408	—
Fatty fish (g/d)[7]	24.1 ± 1.3	22.9 ± 1.4	24.9 ± 1.4	24.6 ± 1.5	0.761	—
Crustaceans, mollusks (g/d)	10.0 ± 0.7	8.9 ± 0.8	11.2 ± 0.8	10.1 ± 0.8	0.249	—

[1] IMMIDIET, Dietary Habit Profile in European Communities with Different Risk of Myocardial Infarction: the Impact of Migration as a Model of Gene-Environment Interaction.
[2] Determined by linear regression analysis adjusted for age and country.
[3] Determined by multivariate linear regression analysis adjusted for all the variables associated with quartiles of alcohol intake at the level of $P < 0.10$ in the model adjusted for age and country.
[4] Expressed as a percentage of total energy intake.
[5] Median; interquartile range in parentheses (all such values).
[6] Mean ± SEM (all such values).
[7] Includes salmon, anchovy, sardine, herring, mackerel, trout, swordfish, tuna, flatfish, and cod.

the volume of alcoholic beverage consumption was used instead of total alcohol intake, and our analyses were further adjusted for alcohol content. After the latter adjustment, the association between alcoholic beverages and EPA was slightly attenuated (from $P < 0.0001$ to $P = 0.002$, both in plasma and red blood cells), whereas the associations with DHA (from $P = 0.589$ to $P = 0.038$ in plasma and from $P = 0.943$ to $P = 0.040$ in red blood cells) and EPA + DHA (from $P = 0.231$ to $P = 0.026$ in red blood cells) became significant. These results suggest that the association with plasma and red blood cell n–3 FAs was only partially dependent on the alcohol content of the beverages.

The interaction term for type of alcoholic beverage was statistically significant ($P < 0.0001$ for plasma and red cell n–3 FAs). Therefore, we conducted separate analyses for subjects who drank wine and subjects who only drank beer or spirits. After multivariate analysis, in wine drinkers there was a significant association with EPA and EPA + DHA index both in plasma and red blood cells (**Table 5**), whereas EPA was associated in the group drinking only beer and spirits (**Table 6**). After further adjustment for alcohol intake, both in plasma and red blood cells, the association between wine consumption and EPA and EPA + DHA was, respectively, only slightly or not attenuated, whereas the association with DHA was significant (Table 5). In contrast, none of the associations remained statistically significant after adjustment for alcohol intake in subjects who consumed exclusively beer or spirits (Table 6). In these last analyses, we did not separate men and women to avoid excessive sample size reduction.

DISCUSSION

Moderate alcohol drinking is associated with reduced cardiovascular mortality in humans (3). The mechanisms of this protection are as yet not fully understood (4, 5).

It was recently reported that alcohol drinking is associated with increased blood concentrations of marine n–3 FAs in patients with CAD, a phenomenon referred to as ''fish-like effect of moderate drinking'' and that is independent of dietary n–3 FAs (7). Because n–3 FAs also induce cardioprotection in animals (31, 32) and reduce cardiac mortality in humans (10, 11), part of the alcohol-induced cardioprotection may be mediated through increased marine n–3 FAs. If confirmed, this effect of alcohol drinking might

TABLE 2
General characteristics and main dietary habits of men in the IMMIDIET population according to quartile (Q) of alcohol intake[1]

Characteristics	Alcohol intake				P^2	P^3
	Q1 (n = 50)	Q2 (n = 222)	Q3 (n = 217)	Q4 (n = 221)		
Alcohol intake[4]	0	1.2 (0.4–1.9)[5]	3.9 (2.7–5.8)	10.4 (6.9–18.0)		
Age (y)	47 ± 1.1[6]	46 ± 0.5	48 ± 0.5	48 ± 0.5	0.179	—
BMI (kg/m^2)	27.5 ± 0.5	27.1 ± 0.2	27.1 ± 0.2	27.3 ± 0.2	0.889	—
Waist-to-hip ratio	0.92 ± 0.009	0.93 ± 0.004	0.93 ± 0.004	0.94 ± 0.004	0.330	—
Systolic blood pressure (mm Hg)	130 ± 2.2	127 ± 1.0	127 ± 1.0	132 ± 1.0	0.012	0.125
Diastolic blood pressure (mm Hg)	83 ± 1.4	81 ± 0.6	82 ± 0.7	83 ± 0.6	0.094	0.254
Blood glucose (mg/dL)	83 ± 2.3	83 ± 1.1	85 ± 1.1	83 ± 1.1	0.771	—
Total cholesterol (mg/dL)	214 ± 5.4	216 ± 2.6	226 ± 2.6	227 ± 2.6	0.017	0.473
HDL cholesterol (mg/dL)	45 ± 1.7	46 ± 0.8	49 ± 0.8	51 ± 0.8	<0.0001	0.0001
LDL cholesterol (mg/dL)	147 ± 4.8	144 ± 2.3	148 ± 2.3	147 ± 2.3	0.706	—
LDL oxidized (mg/dL)	59.5 ± 2.2	59.6 ± 1.1	58.8 ± 1.1	60.0 ± 1.1	0.891	—
Triglycerides (mg/dL)	115 ± 12.8	131 ± 6.1	131 ± 6.2	142 ± 6.2	0.232	—
High social status (%)	25	28	43	28	0.0005	0.002
Current smoker (%)	19	25	21	34	0.008	0.007
Wine consumption[4]	0	0.54 ± 0.19	2.40 ± 0.19	6.01 ± 0.19	<0.0001	—
Beer consumption[4]	0	0.51 ± 0.20	1.20 ± 0.21	4.73 ± 0.20	<0.0001	—
Spirits consumption[4]	0	0.18 ± 0.12	0.39 ± 0.12	0.91 ± 0.12	<0.0001	—
Total energy intake (kcal/d)	2610 ± 102	2857 ± 49	2752 ± 49	2582 ± 49	0.0006	0.019
Total lipids[4]	38.0 ± 0.7	36.0 ± 0.3	35.9 ± 0.3	34.0 ± 0.3	<0.0001	0.610
Saturated fat[4]	14.3 ± 0.4	13.3 ± 0.2	12.9 ± 0.2	12.1 ± 0.2	<0.0001	0.015
Monounsaturated fat[4]	14.8 ± 0.4	13.7 ± 0.2	14.1 ± 0.2	13.4 ± 0.2	0.003	0.399
Polyunsaturated fat[4]	6.3 ± 0.3	6.3 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	0.281	—
Dietary cholesterol (mg/d)	347.8 ± 12.4	346.2 ± 5.9	362.3 ± 5.9	349.2 ± 5.9	0.011	0.004
Total fish (g/d)	32.6 ± 3.4	34.5 ± 1.6	38.2 ± 1.6	37.9 ± 1.6	0.304	—
Fatty fish (g/d)[7]	21.7 ± 2.6	23.8 ± 1.2	25.3 ± 1.2	26.0 ± 1.2	0.503	—
Crustaceans, mollusks (g/d)	10.9 ± 1.7	10.7 ± 0.8	12.9 ± 0.8	11.9 ± 0.8	0.324	—

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[5] Median; interquartile range in parentheses (all such values).
[6] Mean ± SEM (all such values).
[7] Includes salmon, anchovy, sardine, herring, mackerel, trout, swordfish, tuna, flatfish, and cod.

have major implications for the prevention of coronary vascular disease. Other studies, in contrast, reported lower concentrations of liver n–3 PUFAs in animals with prolonged heavy alcohol consumption and in humans with alcoholic liver diseases (6).

The best way to confirm such data in humans would be a long-term controlled trial, which is is presently not technically feasible or ethically acceptable (33). Another way is to confirm these data would be to examine whether the association between alcohol and marine n–3 FAs is reproducible in animal experiments in which most potential confounders encountered in human studies can be controlled. This has been done in a recent study in rats (9), in which 7 wk of alcohol drinking resulted in higher plasma marine n–3 FA concentrations (+65% for EPA and +19% for DHA).

The main difference between the studies in patients and rats is that the animals were drinking pure alcohol, whereas French patients were mainly wine drinkers. In addition, the effect on the different series of FAs was different in humans and in rats (7, 9), suggesting that nonalcoholic components of wine, namely polyphenols, could also interact with the metabolism of essential PUFAs.

To extend the observation obtained in male patients or in rats to a large sample of healthy subjects including women and subjects from 3 European populations at different risk of CVD, essential PUFAs were measured both in plasma and red cells, together with a careful evaluation of dietary habits (12, 13). The different dietary and drinking habits of 3 populations offered the opportunity to examine whether different alcoholic beverages may show different associations with n–3 FA concentrations.

In the present study, concentrations of EPA and of DHA and of the EPA + DHA index in women and EPA in men were positively associated with alcohol intake. The association was present in both preliminary (where adjustments were done for age and country only) and multivariate analyses in which the inclusion of other confounders (in particular the intake of fatty fish, which is main source of dietary EPA and DHA PUFAs) did not modify the results. The association was stronger in women than in men; in the latter, indeed, after multivariate analyses only plasma and red blood cell EPA concentrations were associated with alcohol intake. The lack of association between alcohol intake and DHA concentrations in plasma or red blood cells in men might be mainly attributed to the tight regulation of DHA synthesis from ALA and EPA (34–36), whereas female hormones are known to increase the synthesis of EPA and DHA from their precursor ALA (35).

TABLE 3
Multivariate regression analysis of individual plasma and red blood cell n–3 fatty acids according to quartile (Q) of alcohol intake among women[1]

Fatty acids (% of total fatty acids)	Alcohol intake				*P* for trend[2]	*P* for trend[3]
	Q1 (0% of energy intake) (n = 224)	Q2 (0.8 ± 0.2% of energy intake) (n = 176)	Q3 (2.4 ± 0.2% of energy intake) (n = 172)	Q4 (7.3 ± 0.2% of energy intake) (n =175)		
Plasma						
18:3n–3 (ALA)	0.69 (0.66, 0.72)[4]	0.67 (0.64, 0.71)	0.70 (0.66, 0.74)	0.66 (0.63, 0.70)	0.176	0.448
20:5n–3 (EPA)	0.81 (0.76, 0.87)	0.83 (0.78, 0.89)	0.90 (0.84, 0.96)	0.97 (0.90, 1.04)	<0.0001	<0.0001
22:5n–3 (DPA)	0.49 (0.47, 0.52)	0.49 (0.46, 0.51)	0.49 (0.47, 0.52)	0.49 (0.47, 0.52)	0.724	0.886
22:6n–3 (DHA)	2.10 (2.02, 2.19)	2.17 (2.08, 2.26)	2.21 (2.12, 2.31)	2.22 (2.13, 2.32)	<0.0001	0.036
EPA + DHA index	2.98 (2.86, 3.10)	3.05 (2.92, 3.19)	3.16 (3.02, 3.30)	3.26 (3.12, 3.41)	<0.0001	0.002
Red blood cells						
18:3n–3 (ALA)	0.15 (0.15, 0.16)	0.15 (0.14, 0.16)	0.15 (0.14, 0.16)	0.15 (0.14, 0.16)	0.730	0.882
20:5n–3 (EPA)	0.85 (0.81, 0.89)	0.84 (0.80, 0.88)	0.91 (0.87, 0.96)	0.98 (0.93, 1.03)	<0.0001	<0.0001
22:5n–3 (DPA)	2.94 ± 0.05[5]	2.87 ± 0.05	2.89 ± 0.05	2.95 ± 0.05	0.988	0.976
22:6n–3 (DHA)	6.25 ± 0.10	6.28 ± 0.11	6.49 ± 0.11	6.38 ± 0.11	0.007	0.179
EPA + DHA index	7.26 ± 0.12	7.29 ± 0.12	7.56 ± 0.12	7.55 ± 0.12	0.0004	0.037

[1] ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Determined across quartiles of alcohol intake modeled as a continuous variable adjusted for age and country.
[3] Determined from multivariate regression analysis adjusted for age, country, social status (tertiles), smoking habits (never, past, or current), physical activity (tertiles), BMI (continuous), HDL cholesterol (continuous), total energy intake, fish intake, and intake of saturated, monounsaturated, and polyunsaturated fatty acids.
[4] Geometric mean; 95% CI in parentheses (all such values).
[5] Mean ± SEM (all such values).

The association between alcohol intake and marine n–3 FA concentrations could be confounded by varying fish consumption in drinkers or nondrinkers. However, as in our previous study (7), consumption of fatty fish did not substantially vary among quartiles of alcohol intake, and, after controlling for fatty fish consumption, the association was still observed. Alcohol intake was also independently related to other macronutrients, such as consumption of saturated and polyunsaturated FAs in women or saturated FAs and cholesterol in men. Moreover, alcohol intake was associated with lower BMI in women more than in men, an effect that has been previously reported (37). However, adjustment for these variables either did not change or only slightly reduced the association of alcohol intake with marine n–3 FAs.

The question whether the observed association depended on the alcohol content of specific alcoholic beverages or on other components of wine such as polyphenols was also addressed.

TABLE 4
Multivariate regression analysis of individual plasma and red blood cells n–3 fatty acids according to quartile (Q) of alcohol intake among men[1]

Fatty acids (% of total fatty acids)	Alcohol intake				*P* for trend[2]	*P* for trend[3]
	Q1 (0 % of energy intake) (n = 50)	Q2 (1.2 ± 0.2% of energy intake) (n = 222)	Q3 (4.1 ± 0.2% of energy intake) (n = 217)	Q4 (11.7 ± 0.2% of energy intake) (n = 221)		
Plasma						
18:3n–3 (ALA)	0.74 (0.68, 0.80)[4]	0.71 (0.68, 0.74)	0.71 (0.68, 0.74)	0.67 (0.65, 0.70)	0.014	0.043
20:5n–3 (EPA)	0.81 (0.72, 0.91)	0.83 (0.78, 0.88)	0.92 (0.87, 0.98)	0.92 (0.87, 0.98)	<0.0001	0.003
22:5n–3 (DPA)	0.54 (0.50, 0.58)	0.54 (0.51, 0.56)	0.55 (0.53, 0.57)	0.55 (0.53, 0.57)	0.545	0.485
22:6n–3 (DHA)	1.90 (1.76, 2.06)	1.86 (1.79, 1.94)	1.90 (1.82, 1.98)	1.81 (1.74, 1.88)	0.855	0.254
EPA + DHA index	2.77 (2.56, 3.01)	2.74 (2.63, 2.86)	2.86 (2.75, 2.99)	2.78 (2.67, 2.90)	0.048	0.569
Red blood cells						
18:3n–3 (ALA)	0.15 (0.13, 0.16)	0.14 (0.13, 0.14)	0.14 (0.13, 0.15)	0.15 (0.14, 0.15)	0.037	0.127
20:5n–3 (EPA)	0.90 (0.83, 0.98)	0.89 (0.85, 0.93)	0.94 (0.90, 0.99)	0.97 (0.93, 1.02)	<0.0001	0.004
22:5n–3 (DPA)	3.16 ± 0.09[5]	3.08 ± 0.04	3.12 ± 0.05	3.19 ± 0.04	0.412	0.174
22:6n–3 (DHA)	6.17 ± 0.18	6.04 ± 0.09	6.00 ± 0.09	5.85 ± 0.09	0.279	0.058
EPA + DHA index	7.21 ± 0.21	7.04 ± 0.10	7.06 ± 0.10	6.95 ± 0.10	0.95	0.337

[1] ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Determined across quartiles of alcohol intake modeled as a continuous variable adjusted for age and country.
[3] Determined from multivariate regression analysis adjusted for age, country, social status (tertiles), smoking habits (never, past, or current), physical activity (tertiles), BMI (continuous), HDL cholesterol (continuous), total energy intake, fish intake, and intake of saturated, monounsaturated, and polyunsaturated fatty acids.
[4] Geometric mean; 95% CI in parentheses (all such values).
[5] Mean ± SEM (all such values).

TABLE 5
Association between main n–3 fatty acid according to quartile (Q) of wine intake in the whole population[1]

	Wine intake					
Fatty acids (% of total fatty acids)	Q1 (0 mL) (n = 274)	Q2 (30 ± 3.9 mL) (n = 341)	Q3 (83 ± 4.1 mL) (n = 317)	Q4 (270 ± 4.2 mL) (n = 303)	P for trend[2]	P for trend[3]
Plasma						
18:3n–3 (ALA)	0.69 (0.66, 0.73)[4]	0.69 (0.66, 0.72)	0.69 (0.66, 0.72)	0.67 (0.64, 0.70)	0.042	0.431
20:5n–3 (EPA)	0.83 (0.78, 0.88)	0.89 (0.84, 0.93)	0.92 (0.87, 0.96)	0.93 (0.88, 0.99)	<0.0001	0.008
22:5n–3 (DPA)	0.51 (0.49, 0.54)	0.53 (0.51, 0.55)	0.51 (0.50, 0.53)	0.51 (0.4, 0.53)	0.880	0.672
22:6n–3 (DHA)	1.95 (1.88, 2.03)	2.01 (1.95, 2.08)	2.05 (1.99, 2.12)	2.09 (2.01, 2.18)	0.246	0.014
EPA + DHA index	2.85 (2.74, 2.97)	2.95 (2.86, 3.06)	3.02 (2.91, 3.12)	3.08 (2.96, 3.21)	0.012	0.010
Red blood cells						
18:3n–3 (ALA)	0.15 (0.14, 0.15)	0.14 (0.14, 0.15)	0.14 (0.14, 0.15)	0.15 (0.14, 0.15)	0.742	0.726
20:5n–3 (EPA)	0.88 (0.84, 0.92)	0.90 (0.86, 0.93)	0.94 (0.90, 0.98)	0.97 (0.93, 1.02)	<0.0001	0.002
22:5n–3 (DPA)	3.06 ± 0.05[5]	3.00 ± 0.04	3.04 ± 0.04	3.06 ± 0.05	0.296	0.833
22:6n–3 (DHA)	6.05 ± 0.09	6.14 ± 0.08	6.23 ± 0.08	6.42 ± 0.09	0.294	0.008
EPA + DHA index	7.09 ± 0.11	7.19 ± 0.09	7.32 ± 0.09	7.58 ± 0.11	0.044	0.006

[1] ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Determined from multivariate regression analysis across quartiles of wine intake modeled as a continuous variable adjusted for age, sex, country, social status (tertiles), smoking habits (never, past, or current), physical activity (tertiles), BMI (continuous), HDL cholesterol (continuous), total energy intake, fish intake, and intake of saturated, monounsaturated, and polyunsaturated fatty acids.
[3] Determined from multivariate regression analysis additionally adjusted for total alcohol intake (in g/d).
[4] Geometric mean; 95% CI in parentheses (all such values).
[5] Mean ± SEM (all such values).

Previous studies showed an association between wine drinking and increased concentration of marine n–3 FAs (7, 38, 39). However, it was not possible to clearly separate the effects of wine from those of beer or spirits. In our study of 3 populations with different dietary habits and different consumption of alcoholic beverage types, we were able to perform interaction and stratification analyses for different types of alcohol. The interaction term for alcoholic beverages was statistically significant for both plasma and red cell marine n–3 FAs, suggesting a different pathway of association according to type of alcoholic beverages (only wine compared with only beer or spirits). Stratification analysis showed that the association between alcohol and marine n–3 FAs was present in both wine drinkers and beer or spirits drinkers. However, adjustment for alcohol content of alcoholic beverages, while completely abolishing the association with n–3 FAs in beer or spirits drinkers, maintained the association with EPA and EPA + DHA and strengthened those associations with DHA in wine drinkers. This suggests that components

TABLE 6
Association between main n–3 fatty acid according to quartile (Q) of beer or spirits intake in the whole population[1]

	Beer or spirits intake					
Fatty acids (% of total fatty acids)	Q1 (0 mL) (n = 274)	Q2 (22 ± 13.2 mL) (n = 267)	Q3 (85 ± 13.3 mL) (n = 262)	Q4 (493 ± 13.5 mL) (n = 255)	P for trend[2]	P for trend[3]
Plasma						
18:3n–3 (ALA)	0.69 (0.66, 0.72)[4]	0.67 (0.64, 0.70)	0.68 (0.66, 0.71)	0.69 (0.65, 0.72)	0.334	0.922
20:5n–3 (EPA)	0.82 (0.77, 0.87)	0.88 (0.84, 0.93)	0.90 (0.85, 0.95)	0.89 (0.83, 0.95)	0.001	0.113
22:5n–3 (DPA)	0.52 (0.50, 0.54)	0.52 (0.50, 0.54)	0.52 (0.50, 0.54)	0.52 (0.50, 0.55)	0.486	0.910
22:6n–3 (DHA)	1.94 (1.86, 2.02)	2.03 (1.96, 2.10)	2.04 (1.97, 2.11)	1.96 (1.88, 2.04)	0.881	0.673
EPA + DHA index	2.83 (2.72, 2.95)	2.96 (2.86, 3.07)	3.00 (2.89, 3.11)	2.90 (2.78, 3.03)	0.188	0.387
Red blood cells						
18:3n–3 (ALA)	0.15 (0.14, 0.16)	0.15 (0.14, 0.15)	0.14 (0.14, 0.15)	0.14 (0.14, 0.15)	0.656	0.270
20:5n–3 (EPA)	0.88 (0.84, 0.92)	0.93 (0.89, 0.97)	0.93 (0.90, 0.97)	0.91 (0.87, 0.95)	0.007	0.350
22:5n–3 (DPA)	3.06 ± 0.05[5]	2.99 ± 0.04	3.06 ± 0.04	2.97 ± 0.05	0.682	0.555
22:6n–3 (DHA)	6.05 ± 0.09	6.24 ± 0.08	6.21 ± 0.08	6.04 ± 0.10	0.209	0.898
EPA + DHA index	7.10 ± 0.11	7.34 ± 0.10	7.29 ± 0.09	7.08 ± 0.11	0.550	0.870

[1] ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Determined from multivariate regression analysis across quartiles of beer or spirits intake modeled as a continuous variable adjusted for age, sex, country, social status (tertiles), smoking habits (never, past, or current), physical activity (tertiles), BMI (continuous), HDL cholesterol (continuous), total energy intake, fish intake, and intake of saturated, monounsaturated, and polyunsaturated fatty acids.
[3] Determined from multivariate regression analysis additionally adjusted for total alcohol intake (in g/d).
[4] Geometric mean; 95% CI in parentheses (all such values).
[5] Mean ± SEM (all such values).

of wine other than alcohol might be associated with marine n–3 FA concentrations.

One potential mechanism by which low-dose alcohol drinking could increase the marine n–3 concentrations in blood and cells is by increasing their synthesis from the precursor ALA through activation of the elongation-desaturation pathway (6, 8). However, the metabolism of alcohol induces production of reactive oxygen species, even after moderate drinking, which may increase PUFA catabolism and utilization. This, in turn, may stimulate the elongation-desaturation processes and increase PUFA synthesis. Antioxidant components of wine may also be involved in that process by preventing alcohol-induced oxidation and delaying the catabolism of PUFAs (14, 40).

Clinical implications

Several mechanisms have been proposed to explain the protective effect of moderate alcohol drinking on CAD and total mortality (4, 5, 7). The increase in blood and tissue marine n–3 FAs might be an additional mechanism. Indeed, n–3 FAs mitigate the risk of CAD death by enriching membrane phospholipids with EPA and DHA (32).

Marine n–3 FA concentrations were shown to be inversely associated with the risk of sudden cardiac death (11, 41), a syndrome that accounts for ≈70% of total cardiac mortality. Moderate alcohol drinking was also shown to decrease the risk of sudden cardiac death (42). Thus, converging data suggest that marine n–3 FAs could be one of the mediators of the protective effect of moderate drinking.

Limitations of this study

Some limitations of our study should be mentioned. First, the cross-sectional design does not enable determination of causality. A second issue regarding the mechanism or mechanisms by which alcohol and nonalcoholic components of wine might influence the metabolism of n–3 FAs can only be speculated. Because this study was not designed to investigate biological mechanisms, further studies are necessary to understand at which level of their respective metabolisms alcohol, polyphenols, and n–3 FAs interact.

Finally, possible errors because of misreporting in subjects with higher alcohol consumption should be acknowledged. The reporting of diet on FFQs is similarly reproducible across different alcohol intakes and BMI (43), although the reproducibility of questionnaires is slightly reduced among heavier drinkers. However, in our study the association between alcohol and n–3 FAs was observed in all alcohol intake categories, and only 3 women and 15 men were classified as heavy drinkers (alcohol intake $>$ 20% of total energy intake).

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APPENDIX A

European Collaborative Group of the IMMIDIET Project

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