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Research Article

Determinants of the Fatty Acid Profile in Patients with and without Coronary Heart Disease

Monica G. Giroli[1]*, Federica Laguzzi[2], Manuela Amato[1,3], Patrizia Risé[4], Lorenzo Vigo[1], Alice Bonomi[1], Fabrizio Veglia[1], Jose Pablo Werba[1], and Elena Tremoli[1]

[1]*Centro Cardiologico Monzino, IRCCS, Italy*
[2]*Unit of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Sweden*
[3]*Department of Clinical Medicine and Surgery, Federico II University, Italy*
[4]*Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy*

***Corresponding author**
Monica Gianna Giroli, Centro Cardiologico Monzino,Via Parea 4, Milan, Italy, Tel: 39-02-58002617; Fax: 39-02-58002942; E-mail: monica.giroli@ccfm.it

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Abstract

Objective: Fatty acids (FA) influence lipoprotein levels and take part in metabolic pathways of inflammation and thrombosis, potentially representing a risk marker or an emerging cardiovascular risk factor. The purpose of this study was to search for independent predictors of blood fatty acid distribution in subjects with and without coronary heart disease (CHD).

Methods: In this case-control study, 179 patients with CHD and 155 healthy age- and gender- frequency matched subjects were included. Clinical variables (anthropometric data, traditional risk factors, current drug therapies) and life habits (diet by EPIC food frequency questionnaire, smoke, physical activity) were compared, between CHD patients and controls in univariate analysis. Clinical features that differed significantly were included in a multivariate linear regression analysis to assess their independent association with levels of blood fatty acid types and classes (assessed by gas chromatography).

Results: a) Fish intake was associated positively with n-3 and negatively with n-6 FA; b) coronary disease was associated positively with total saturated FA, C16:0 and C24:0, and negatively with C16:1n-7, C20:3n-6, C20:3n-9, and estimated Δ9 stearoyl-CoA desaturase (16:1n7/16:0 ratio); c) statins use was associated negatively with C18:2n-6 and C18:3n-3, and positively with C18:3n-6, C20:4n-6, the estimated Δ6 desaturase (C18 :3n-6/C18 :2n-6 ratio), C20:4n-6/C18:2n-6, C20:5n-3/C18:3n-3 and C22:6n-3/C18:3n-3 ratios.

Conclusions: The blood fatty acid distribution is associated, besides with diet quality, with the CHD status and with statin utilization, possibly in relation to a distinct fatty acid metabolism in patients prone to CHD and to pleiotropic effects of statins beyond LDL-C lowering.

ABBREVIATIONS

AA: Arachidonic Acid; ALA: α-Linolenic Acid; CABG: Coronary Artery Bypass; CHD: Coronary Heart Disease; CV: Cardiovascular; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; FA: Fatty Acid; FFQ: Food Frequency Questionnaire; HDL-C: High Density Lipoprotein Cholesterol; LA: Linoleic Acid; LDL-C: Low Density Lipoprotein Cholesterol; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Acids; SCD1: Δ^9 Stearoyl-CoA Desaturase; SFA: Saturated Acids.

INTRODUCTION

Cardiovascular (CV) disease is a leading cause of death in most industrialized countries. Traditional risk factors have been widely implicated in determining CV risk, but they explain it only in part [1]. Thus, emerging factors may account for the residual risk. One possible candidate is the quality of fatty acids (FA) in the body. Indeed, FA influence plasma levels of atherogenic lipoproteins [2] and affect several aspects of platelet function and inflammation [3], two processes intimately implicated in atherothrombosis [4]. Among a range of debated dietary recommendations to decrease the risk of CV disease, reducing the intake of saturated fatty acid (SFA) or replace it by monounsaturated or polyunsaturated fatty acid is a priority in cardiovascular prevention guidelines [5,6]. However, the results of two meta-analyses have questioned the link between saturated fat intake and risk [7,8].

Even though the FA profile of individuals is influenced by dietary intake - as is the case for the essential linoleic acid (LA) and linolenic acid (ALA) - it is also modulated endogenously by the activity of specific enzymes [9]. Thus, the fatty acid profile may be determined not only by diet composition but also by derangements of the fatty acid metabolism associated with specific diseases or by exogenous modulators of the fatty acid pathways. Previous studies [10-13] indicate that patients with coronary heart disease (CHD) show, as compared with healthy

subjects, higher blood levels of trans fatty acids and SFA and lower levels of n-3 polyunsaturated fatty acids (PUFA), with controversial data about n-6 PUFA, especially arachidonic acid (AA) [14]. However, the discrete contributions of diet, clinical and life-style features or use of cardiovascular drugs to the FA profile are not well defined. Thus, the present study has been carried out to assess the factors, either dietary or not, that may influence the blood fatty acid profile in patients with and without CHD.

MATERIALS AND METHODS

Subjects

This case-control study was performed at the Cardiologico Monzino Center, Milan, Italy. Enrolment started in June 2014 and ended in June 2016. Overall, 334 adult residents in north Italy (93% males, mean age 62.3 ± 7.7 years {30-75}) were included in the study. Patients with diagnosis of CHD (n=179) were identified among those admitted to our Institution for programmed coronary artery bypass surgery (CABG) indicated by angiographic documentation of significant stenosis (> 70%) in at least two main coronary arteries. Controls (n=155) were subjects without a history or clinical signs or symptoms of any form of atherosclerotic disease recruited among the hospital staff, relatives or acquaintances, frequency matched with cases for age and gender.

Information about socio-educational factors, smoking habits, physical activity, family history of cardiovascular diseases and personal history of previous or current clinical diseases or surgical interventions, and current drug therapies was recalled using a structured questionnaire by a trained physician, who also obtained anthropometric measures (blood pressure, heart rate, weight, height, and waist circumference) using calibrated and validated tools. Patients were categorised as dyslipidemic if at least one of these conditions was met: hypolipidemic drug therapy, LDL-Cholesterol values higher than the goal recommended for their own risk [15], or triglycerides higher than 150mg/dl. Patients were classified in terms of smoking habits as never smokers, current smokers and former smokers (at least 6 months of cessation). Pack-years (calculated as number of cigarettes smoked per day multiplied by number of years smoked/20) was used as a measure of the lifelong exposure to cigarette smoke. In terms of physical activity, subjects were categorized as sedentary (any kind of at least moderate physical activity at work or at leisure time) or active. Besides, minutes per week of at least moderate physical activity (such as vigorous walking) was estimated as a measure of physical activity volume.

Exclusion criteria were age < 30 or > 75 years, treatment with fibrates, heparin or n-3 supplements.

Nutritional assessment

A semi-quantitative food-frequency questionnaire (FFQ) of European Prospective Investigation into Cancer and Nutrition (namely EPIC, validated for the Italian population), was administered by a trained nutritionist to collect information on food intake over the previous year. The FFQ considers 248 items concerning the frequency of consumption (daily, weekly, monthly or never) and the portion sizes of foods and beverages commonly consumed in Italy. The serving size of most foods was estimated by displaying three different photographs of standard portions. Information on intake frequency and portion size were used to estimate the amount of each food item in grams consumed on average per day. Completed FFQs were processed by the Nutritional Epidemiology Unit of Milan, National Cancer Institute, using specially-developed software [16]. The software calculates frequency and quantity of consumption of foodstuffs and the overall intake of nutrients, using a reference database for food chemical composition. The 248 food items were grouped in 24 food categories for statistical analysis.

Biochemical studies

Peripheral venous blood was obtained at fasting to determine serum levels of total cholesterol, LDL-C, HDL-C, triglycerides and glucose. Capillary blood, obtained at fasting from a fingertip, was collected on a special adsorbent paper embedded with the antioxidant butylhydroxytoluene (Blood Collection kit, Sigma-Aldrich, St. Louis, MO) to perform fatty acid analysis in whole blood. Fatty acid methyl esters, directly prepared by transesterification, were analyzed by gas-liquid chromatography (GC-2010, Shimadzu), equipped with a PTV injector and a FID detector, using a DB-FFAP capillary column (15m x 0.1mm ID x 0.10µm, Agilent); the oven temperature was programmed from 120°C to 235°C [17]. The blood level of each FA was expressed as the relative percentage (%) of the total fatty acid pool. Product to precursor ratios of specific fatty acids were calculated as surrogates of the activity of specific enzymes involved in FA metabolism; in particular, C16:1n-7/C16:0 ratio estimated Δ^9 stearoyl-CoA desaturase-1 (SCD1) and C18:3n-6/ C18:2n-6 ratio estimated Δ^6 desaturase (D6D).

Ethical considerations

The study was approved by the Review Board and the Ethical Committee of the Centro Cardiologico Monzino, IRCCS, Milan, Italy. Each participant provided written informed consent before enrolment in the study.

STATISTICAL ANALYSIS

In univariate analysis, numerical variables were compared between CHD patients and controls by the Wilcoxon rank-sum test and categorical variables by chi-square test. A multivariable linear regression analysis was run by using as dependent variables the relative amount of each blood FA and as independent variables the clinical features, intake of food or nutrients and drug therapies that differed significantly ($p<0.05$) between CHD patients and controls in univariable analysis (pack-years, BMI, LDL- and HDL-cholesterol, blood glucose, heart rate, systolic and diastolic blood pressure, dyslipidemia, type 2 diabetes, hypertension, cardiovascular family history, intake of vegetable fat, monounsaturated fat, oleic acid, sodium, riboflavin, folic acid, retinol and vitamin D, and treatment with statins, antihypertensive, platelet aggregation inhibitors, insulin, diuretics) with further adjustment for age, sex and energy intake.

Variables with a skewed distribution were log-transformed before analysis. In multivariable analysis, due to the large number of tests performed, only p values <0.001 were considered as significant. All analyses were performed using SAS v. 9.4 statistical package.

RESULTS

The general characteristics of CHD patients and controls are shown in Table 1. As a result of matching, patients and controls were comparable in terms of age and sex. As expected, the CHD group had a higher prevalence of cardiovascular risk factors and a higher consumption of CV drugs. Most participants were males and there were more previous smokers and less current smokers among patients with CHD than among controls. Life-long cigarette smoke exposure was higher in the CHD group. Body mass index, waist circumference and glucose levels were higher whereas blood pressure, heart rate, total cholesterol, LDL-C and HDL-C levels were lower in CHD patients than in controls. The prevalence of physically active subjects and the time spent in physical activity was similar in both groups.

Among 24 food categories evaluated with the FFQ, CHD patients reported a significantly lower consumption of fish, nuts, red meat, eggs, pastry, chocolate and alcoholic beverages and a higher consumption of pasta and rice, butter and cold cuts than controls (Table 2). In terms of macro and micro nutrients, CHD patients had a lower intake of vegetable fats (mostly represented by the monounsaturated oleic acid) and vitamin D and a higher intake of sodium and some other minerals and vitamins than controls (Table 3). No differences between groups in the intake of total calories, total fat, saturated FA and PUFA were observed.

No significant differences in food intake or FA distribution were observed between patients with a long history of CHD events or interventions before enrolment (n=97) and patients referred to CABG after a *new* diagnosis of CHD (n=82), with the exception of a higher intake of red wine in the former (p= 0.005) (data not shown).

Several components of the blood fatty acid profile differed between cases and controls (Table 4). CHD patients had significantly higher total saturated FA (in particular C16:0; C22:0 and C24:0). Total monounsaturated fatty acid (MUFA) and C18:1 were similar in both groups, whereas C16:1; C20:1; C22:1 and C24:1 were higher in CHD patients. CHD also had lower total PUFA, C20:3n-9, C18:2n-6, C20:3n-6, C18:3n-3 and C20:5n-3, and higher C20:4n-6 and C22:4n-6 than controls. The table also shows differences between groups in FA ratios.

A multivariable linear regression analysis was run to investigate twenty-two candidate predictors of blood FA (Table 5). The variables showing significant independent associations with the distribution of blood FA were consumption of fish, CHD status, use of statins and levels of LDL-C. Consumption of fish correlated positively with total n-3 FA (specifically with C20:5 and C22:6), EPA/AA and DHA/AA ratios, and negatively with n-6 FA (C22:4 and C22:5) and n-6/n-3 ratio. Intake of nuts correlated positively with C18:3n-3.

CHD status correlated positively with total saturated FA, C16:0 and C18:1n-7/C16:1n-7 ratio and negatively with the

Table 1: Characteristics of control subjects and CHD patients.

Variable	Controls (n=155)	CHD patients (n=179)	*p* value
Subjects[b] (Male)	142 (91.6%)	168 (93.3%)	0.55
Age (years)	62.16 (58.13-67.56)	64.65 (57.3-69.06)	0.10
Current Smoker[b]	34 (21.9%)	24 (13.3%)	0.04
Former smoker[b]	68 (53.1%)	110 (66.3%)	0.02
Pack years	15.00 (6.9-34.5)	26.63 (11.25-43.75)	0.003
Active subjects[b]	94 (60.7%)	93 (51.7%)	0.10
Physical activity (minute/week)	180 (120 - 350)	210 (120 - 360)	0.62
BMI (Kg/m^2)[a]	26.31 ± 3.22	27.4 ± 3.59	0.005
Waist circumference[a] (cm)	97.22 ± 9.44	99.67 ± 9.76	0.02
Total-cholesterol[a] (mg/dl)	209.49 ± 38.41	188.89 ± 44.81	<0.0001
LDL-cholesterol[a] (mg/dl)	132.94 ± 38.01	119.14 ± 39.55	0.004
HDL-cholesterol[a] (mg/dl)	52.08 ± 14.87	44.74 ± 11.02	<0.0001
Triglycerides (mg/dl)	109 (80-150)	110 (82-147)	0.60
Glucose (mg/dl)	97.85 (91-105)	110.5 (98-140)	<0.0001
Heart rate (bpm)	70 (61-73)	64 (60-70)	0.005
Systolic blood pressure[a] (mmHg)	135.69 ± 15.05	130.75 ± 18.6	0.002
Diastolic blood pressure[a] (mmHg)	80 (79-90)	80 (70-80)	<0.0001
Dyslipidemia[b]	42 (27.1%)	103 (57.9%)	<0.0001
Type 2 diabetes[b]	9 (5.8%)	57 (32.0%)	<0.0001
Hypertension[b]	53 (34.2%)	110 (61.8%)	<0.0001
Statins[b]	18 (11.6%)	111 (61.7%)	<0.0001
Antihypertensive[b-c]	61 (39.4%)	167 (92.8%)	<0.0001
Platelet aggregation inhibitors[b]	14 (9.0%)	125 (69.4%)	<0.0001
Insulin therapy[b]	1 (0.7%)	20 (11.1%)	<0.0001
Diuretics[b]	19 (12.3%)	43 (23.9%)	0.006
Antiarrhythmic[b]	5 (3.2%)	9 (5.0%)	0.42
Cardiovascular family history[b]	60 (38.7%)	99 (55.6%)	0.002
Menopause[b]	12 (92.3%)	11 (84.6%)	1.00

Table 2: Food intake in controls and CHD patients.

Food variable (g/day)	Controls (n=155)	CHD patients (n=179)	*p* value
Pasta/Rice	61.3 (55.8 - 67.3)	70.2 (64.4 - 76.6)	0.04
Bread	117.9 (108.2 - 128.4)	116.8 (107.8 - 126.5)	0.87
Red meat	44.1 (38.9 - 49.9)	33.1 (29.5 - 37.2)	0.001
White meat	27.0 (23.2 - 31.4)	26.9 (23.4 - 31.0)	0.97
Fish products	35.1 (31.0 - 39.9)	28.3 (25.1 - 31.8)	0.01
Dairy products	47.0 (33.8 - 65.4)	56.4 (41.4 - 76.6)	0.43
Cheeses	42.9 (38.0 - 48.4)	47.9 (42.8 - 53.6)	0.19
Cold cut	18.1 (16.0 - 20.4)	21.7 (19.4 - 24.2)	0.03
Eggs	12.4 (10.8 - 14.2)	9.0 (7.9 - 10.2)	0.001
Legumes	19.6 (16.3 - 23.5)	16.4 (13.8 - 19.5)	0.17
Potatoes	19.4 (16.5 - 22.8)	17.0 (14.6 - 19.7)	0.24
Raw vegetables	84.8 (73.9 - 97.4)	85.1 (74.8 - 96.8)	0.97
Cooked vegetables	35.6 (31.4 - 40.4)	30.1 (26.8 - 33.9)	0.06
Fruit	250.9 (228.2 - 275.8)	273.1 (250.0 - 298.2)	0.20
Dry fruit and nuts	2.31 (2.04 - 2.61)	1.81 (1.62 - 2.03)	0.005
Butter	1.46 (1.32 - 1.61)	1.77 (1.61 - 1.93)	0.005
Margarines	1.06 (1.02 - 1.10)	1.09 (1.05 - 1.13)	0.37
Olive oil	22.1 (20.0 - 24.5)	21.1 (19.2 - 23.2)	0.45
Seed oil	1.14 (1.03 - 1.25)	1.17 (1.07 - 1.28)	0.65
Chocolate	3.95 (3.36 - 4.64)	2.55 (2.20 - 2.97)	0.0001
Pastry	61.6 (54.4 - 69.7)	47.8 (42.6 - 53.7)	0.004
Sugar	5.38 (4.30 - 6.74)	4.65 (3.77 - 5.74)	0.35
Soft drinks	16.4 (11.9 - 22.7)	17.2 (12.8 - 23.2)	0.84

Median (Interquartile range 25-75). *p* calculated with Chi-square test adjusted for Energy intake (KJ)

Table 3: Nutrient intake in controls and CHD patients.

Nutrient Variable	Controls (n=155)	CHD patients (n=179)	*p* value
Energy (KJ)	9212 (8802 - 9640)	8907 (8539 - 9292)	0.30
Protein (g)	84.4 (82.5 - 86.3)	86.9 (85.1 - 88.7)	0.06
Animal protein (g)	56.0 (53.8 - 58.3)	57.5 (55.4 - 59.7)	0.34
Vegetable protein (g)	28.2 (27.4 - 29.0)	29.0 (28.2 - 29.7)	0.18
Fat (g)	80.8 (78.5 - 83.1)	79.3 (77.2 - 81.4)	0.33
Animal fat (g)	42.7 (40.8 - 44.8)	44.8 (42.9 - 46.8)	0.14
Vegetable fat (g)	36.5 (34.6 - 38.4)	33.4 (31.8 - 35.0)	0.02
Cholesterol (mg)	308.6 (295.6 - 322.3)	297.6 (285.9 - 309.8)	0.23
Saturated fat (g)	28.1 (27.1 - 29.2)	28.8 (27.8 - 29.8)	0.38
Monounsaturated fat (g)	39.9 (38.6 - 41.3)	37.9 (36.7 - 39.0)	0.02
Oleic acid (g)	37.6 (36.3 - 38.9)	35.4 (34.3 - 36.6)	0.02
Polyunsaturated (g)	9.77 (9.48 - 10.07)	9.49 (9.23 - 9.77)	0.18
Linoleic acid (g)	7.85 (7.61 - 8.10)	7.64 (7.42 - 7.86)	0.2
Alfa-linolenic acid (g)	2.14 (2.11 - 2.18)	2.17 (2.13 - 2.20)	0.39
Other polyunsaturated (g)	1.72 (1.66 - 1.78)	1.65 (1.60 - 1.70)	0.07
Carbohydrate (g)	251.9 (245.2 - 258.8)	258.3 (251.9 - 264.8)	0.18
Soluble carbohydrate (g)	94.6 (89.9 - 99.6)	93.0 (88.6 - 97.5)	0.62
Starch (g)	151.6 (145.4 - 158.1)	159.5 (153.5 - 165.8)	0.08
Fiber (g)	19.3 (18.6 - 20.1)	19.5 (18.8 - 20.2)	0.77
Ethanol (g)	11.91 (9.71 - 14.61)	9.08 (7.51 - 10.99)	0.06
Sodium (mg)	2163.2 (2076.7 - 2253.3)	2381.1 (2292.4 - 2473.3)	0.001
Potassium (mg)	3098.3 (3016.3 - 3182.5)	3185.4 (3106.9 - 3265.8)	0.14
Iron (mg)	14.5 (14.2 - 14.8)	14.7 (14.5 - 15.0)	0.25
Calcium (mg)	910.6 (869.8 - 953.3)	961.5 (921.4 - 1003.4)	0.09
Phosphorus (mg)	1343.1 (1313.7 - 1373.1)	1384.0 (1355.8 - 1412.8)	0.05
Zinc (mg)	12.4 (12.1 - 12.7)	12.8 (12.5 - 13.0)	0.06
Tiamin (mg)	1.92 (1.90 - 1.95)	1.94 (1.92 - 1.97)	0.26

Riboflavin (mg)	2.43 (2.38 - 2.48)	2.53 (2.48 - 2.57)	0.007
Niacin (mg)	18.25 (17.7 - 18.7)	18.7 (18.2 - 19.2)	0.16
Piridoxine (mg)	2.80 (2.75 - 2.85)	2.85 (2.80 - 2.90)	0.14
Folic acid (µg)	250.4 (242.7 - 258.3)	262.4 (254.9 - 270.1)	0.03
Beta-carotene (µg)	2672.5 (2481.0 - 2878.8)	2562.4 (2391.2 - 2745.9)	0.42
Retinol (µg)	330.5 (299.4 - 364.9)	391.5 (357.1 - 429.2)	0.02
Retinol equivalent (µg)	837.5 (785.2 - 893.3)	889.1 (837.4 - 944.0)	0.19
Vitamin C (mg)	115.8 (108.8 - 123.3)	121.1 (114.3 - 128.3)	0.31
Vitamin D (µg)	3.67 (3.49 - 3.86)	3.36 (3.21 - 3.52)	0.01
Vitamin E (mg)	8.41 (8.12 - 8.71)	8.1594 (7.90 - 8.43)	0.23

Median (Interquartile range 25-75). *p* calculated with Chi-square test adjusted for Energy intake except Energy (KJ)

Table 4: Blood fatty acids (%) and fatty acids ratios in controls and CHD patients.

Variable	Controls (n=155)	CHD patients (n=179)	*p* value
C16:0 (palmitic acid)	25.09 (24.85-25.33)	26.36 (26.13-26.58)	<0.0001
C18:0 (stearic acid)	11.03 (10.85-11.20)	11.30 (11.09-11.52)	0.07
C20:0 (arachic acid)	0.417 (0.404-0.430)	0.441 (0.430-0.452)	0.20
C22:0	1.18 (1.15-1.21)	1.21 (1.18-1.23)	0.01
C24:0	2.37 (2.30-2.44)	2.40 (2.34-2.46)	<0.0001
C16:1 (palmitoleic acid)	1.718 (1.626-1.810)	1.790 (1.691-1.890)	0.005
C18:1 (oleic acid)	21.67 (21.17-22.16)	21.32 (20.95-21.69)	0.31
C18:1 n-7	1.89 (1.84-1.95)	1.91 (1.85-1.96)	0.71
C20:1	0.209 (0.203-0.215)	0.220 (0.211-0.228)	0.05
C22:1	0.063 (0.056-0.069)	0.069 (0.063-0.074)	0.003
C24:1	2.862 (2.782-2.941)	2.967 (2.878-3.055)	<0.0001
C20:3n-9	0.131 (0.122-0.140)	0.123 (0.115-0.131)	<0.0001
C18:2n-6 (LA)	17.43 (17.05-17.81)	15.62 (15.25-16.00)	<0.0001
C18:3n-6 (GLA)	0.253 (0.240-0.265)	0.256 (0.241-0.270)	0.82
C20:3n-6 (DGLA)	1.36 (1.32-1.41)	1.28 (1.25-1.32)	0.02
C20:4n-6 (AA)	7.674 (7.458-7.890)	8.357 (8.141-8.573)	<0.0001
C22:4n-6	0.898 (0.860-0.936)	0.937 (0.905-0.968)	0.04
C22:5n-6	0.233 (0.217-0.249)	0.295 (0.276-0.314)	0.61
C18:3n-3 (ALA)	0.220 (0.203-0.237)	0.185 (0.174-0.196)	0.02
C20:5n-3 (EPA)	0.491 (0.454-0.529)	0.431 (0.402-0.461)	0.01
C22:5n-3 (DPA)	0.677 (0.652-0.702)	0.634 (0.610-0.658)	0.29
C22:6n-3 (DHA)	1.84 (1.75-1.93)	1.82 (1.72-1.91)	0.35
Saturated Acid	40.09 (39.75-40.44)	41.72 (41.37-42.06)	<0.0001
Monounsaturated Acid	28.42 (27.91-28.93)	28.28 (27.86-28.69)	0.48
Polyunsaturated Acid	31.23 (30.71-31.75)	29.95 (29.49-30.42)	0.001
n-6	27.86 (27.38-28.33)	26.76 (26.34-27.18)	0.001
n-3	3.23 (3.09-3.38)	3.07 (2.93-3.21)	0.69
n-6/n-3	9.135 (8.751-9.518)	9.427 (9.009-9.845)	0.77
D6D (GLA/LA)	0.014 (0.013-0.015)	0.016 (0.015-0.017)	0.003
DGLA/LA	0.079 (0.076-0.082)	0.084 (0.081-0.086)	0.02
AA/LA	0.447 (0.431-0.463)	0.550 (0.530-0.571)	<0.0001
AA/DGLA	5.80 (5.58-6.01)	6.76 (6.48-7.04)	<0.0001
EPA/ALA	2.84 (2.41-3.27)	2.61 (2.38-2.83)	0.37
DHA/ALA	11.13 (9.193-13.08)	11.08 (10.26-11.90)	0.44
ALA/LA	0.012 (0.011-0.013)	0.012 (0.011-0.012)	0.88
EPA/AA	0.066 (0.060-0.072)	0.052 (0.048-0.055)	<0.0001
DHA/AA	0.245 (0.232-0.259)	0.219 (0.208-0.230)	0.15
C18:1n-9/C18:0	2.00 (1.92-2.07)	1.92 (1.86-1.98)	0.09
SCD1 (C16:1/C16:0)	0.068 (0.065-0.072)	0.067 (0.064-0.071)	<0.0001
C18:1n-7/C16:1	1.21 (1.14-1.27)	1.19 (1.12-1.25)	<0.0001

Median (Interquartile range 25-75). *p* calculated with Chi-square test
AA: Arachidonic Acid; ALA: α-Linolenic Acid ; CHD: Coronary Heart Disease; DHA: Docosahexaenoic Acid; DPA: Docosapenaenoic Acid; D6D: Delta-6-desaturase; EPA: Eicosapentaenoic acid; GLA: Gamma Linolenic Acid; DGLA: Dihomo Gamma Linoleic Acid; LA: Linoleic Acid; SCD1: Δ^9 Stearoyl-CoA desaturase.

Table 5: Multivariable analysis: associations between blood fatty acids and clinical features, cardiovascular drugs and food intake.

Variable	BMI	CHD	LDL-C	Statins	Fish	Nuts	Alchoolic drinks
C 16:0 (palmitic acid)	0.162 **(0.004)**	0.371 **(<0.001)**					0.140 **(0.01)**
C24:0		0.269 **(0.003)**					
C 16:1 (palmitoleic acid)		-0.336 **(<0.001)**					0.178 **(0.002)**
C18:1n-7			-0.233 **(<0.001)**				
C20:1			-0.199 **(0.001)**				
C24:1		0.289 **(0.003)**	-0.194 **(0.002)**				
C20:3n-9		-0.367 **(<0.001)**					
C 18:2n-6 (LA)			0.292 **(<0.001)**	-0.256 **(<0.001)**		0.147 **(0.005)**	
C 18:3n-6 (GLA)				0.203 **(0.009)**	-0.176 **(0.004)**		
C 20:3n-6 (DGLA)		-0.253 **(0.01)**			-0.229 **(0.001)**		
C 20:4n-6 (AA)			-0.163 **(0.007)**	0.215 **(0.002)**			
C22:4n-6			-0.227 **(<0.001)**		-0.270 **(<0.001)**		
C 22:5n-6					-0.301 **(<0.001)**		
C 18:3n-3 (ALA)						0.226 **(<0.001)**	
C 20:5n-3 (EPA)	-0.181 **(0.002)**				0.277 **(<0.001)**		
C 22:5n-3 (DPA)	-0.167 **(0.005)**				0.143 **(0.01)**		
C 22:6n-3 (DHA)					0.452 **(<0.001)**		
Saturated acid		0.341 **(<0.001)**					
n-6			0.171 **(0.005)**				
n-3	-0.153 **(0.006)**				0.402 **(<0.001)**		
n-6/n-3					-0.452 **(<0.001)**		
D6D (GLA/LA)				0.302 **(<0.001)**			
DGLA/LA				0.235 **(0.001)**	-0.171 **(0.003)**	-0.147 **(0.01)**	
AA/LA			-0.296 **(<0.001)**	0.319 **(<0.001)**			
AA/DGLA		0.267 **(0.005)**	-0.224 **(<0.001)**		0.148 **(0.01)**		
EPA/ALA				0.205 **(0.005)**			
DHA/ALA				0.245 **(0.001)**			
ALA/LA						0.185 **(0.003)**	
EPA/AA	-0.148 **(0.01)**				0.258 **(<0.001)**		
DHA/AA					0.474 **(<0.001)**		
SCD1 (C16:1/C16:0)		-0.422 **(<0.001)**			-0.134 **(0.01)**		0.159 **(0.006)**
C18:1n-7/C16:1n-7		0.328 **(<0.001)**	-0.260 **(<0.001)**				-0.166 **(0.004)**

Results are presented as standardized β-coefficients (*p*). For clarity, only β-coefficients with p-value below 0.01 were reported. Each independent variable was adjusted for the clinical features, intake of food or nutrients and drug therapies that differed significantly (p<0.05) between CHD patients and controls in univariable analysis (see methods), with further adjustment for age, sex and energy intake.

No association was observed between pack years, physical activity, diabetes, hypertension, antihypertensives, diuretics, pasta/rice, cold cuts, butter, pastry, chocolate and blood fatty acids. Age, platelet aggregation inhibitors and red meat exhibited only sparse associations with blood fatty acids and were not presented. Similarly, C18:0, C20:0, C22:0, C18:1 (oleic acid), C22:1, Monounsaturated acid, Polyunsaturated acid, C18:0/C16:0, C18:1n-9/C18:0 showed no association with independent variables.

AA: Arachidonic Acid; ALA: α-Linolenic Acid; DHA: Docosahexaenoic Acid; DGLA: Dihomo Gamma Linoleic Acid; DPA: Docosapenaenoic Acid; D6D: Delta-6-desaturase; EPA: Eicosapentaenoic acid; LA: Linoleic Acid; GLA: Gamma Linolenic Acid; SCD1: Δ^9 Stearoyl-CoA desaturase

MUFA C16:1n-7, C16:1n-7/C16:0 ratio (SCD1) and the PUFA C20:3n-9.

Statins use was correlated negatively with C18:2n-6 and positively with the ratios C18:3n-6/ C18:2n-6 (D6D), C20:4n-6/ C18:2n-6 (AA/LA).

Conversely, LDL-C levels correlated positively with C18:2n-6, and negatively with C18:1n-7, C22:4n-6, the ratios C20:4n-6/ C18:2n-6 (AA/LA) and C20:4n-6/C20:3n-6 (AA/DGLA).

DISCUSSION

Fatty acids have been postulated as emerging and potentially modifiable biomarkers of cardiovascular risk [10]. The present study was aimed to uncover the determinants of the blood fatty acid profile in a population of healthy subjects and patients with CHD. The groups differed significantly in blood levels of many FA and, as expected, in several clinical variables including prevalence of traditional risk factors, specific food intake and consumption of cardiovascular drugs. A multivariate approach was used to assess the independent contribution of each of these clinical variables to the components of the blood FA profile.

As expected, fish intake correlated positively with n-3 at the expense of a reduction in n-6 FA and consumption of nuts was positively linked with levels of the essential ALA [18-20].

The present and former studies show that blood levels of palmitic acid are strongly associated with CHD [21]. In the literature, several authors show a significant reduction in CV risk when SFA intake is replaced by MUFA and/or PUFA in primary and secondary prevention [22-24]. However in our study, the intake of SFA, estimated by the FFQ, was not significantly higher in CHD patients than in controls, in line with other observational studies showing an uncertain relationship between consumption of SFA and risk of CHD [7,8]. In absence of a parallel significant increment in the intake of SFA, the positive independent association between CHD status and blood levels of SFA, in particular palmitic acid, and the negative association between the C16:1n-7/16:0 ratio and CHD status might be related to a reduced SCD1 activity in this condition. Moreover, the blood FA profile may reflect the atherogenic potential of the diet with a greater precision than intakes estimated by FFQs.

Statins use was associated with changes in n-6 PUFA ratios that suggest an increased D6D activity with an augmented conversion from LA to longer and more unsaturated n-6 FA. These results are in line with previous cell culture studies from our group and others, showing that statins induce the activity of specific elongases and desaturases [25-27] and are also compatible with the results of two statin randomized-controlled clinical studies [9,28]. Reasonably, the differences in n-6 PUFA distribution herein observed between CHD patients and controls might be explained by the higher prevalence of statins use in the former (61% Vs 11%, respectively).

This study has some limitations. First, being an observational study, an influence of residual unknown confounding factors on the FA pattern cannot be excluded. Moreover, particular dietary choices of patients long aware of their CHD status impede to understand whether the observed associations between diet, FA and CHD are cause-effect or are due to reverse causality. Yet, food intake and FA distribution were similar in patients with a long history of CHD and those with a new diagnosis at the time of enrolment, which argues in favour of the first possibility. Second, given the large prevalence of males in our study, which is typical of cohorts of patients subjected to CABG, we are unaware whether the present results may apply to a female population. Third, the FA profile was determined using a rapid and convenient analytical method in total blood, which represents a mixed FA pool that may differ from those of plasma, lipoproteins or red blood cells; however, it derives from the balanced proportion of all these pools.

CONCLUSION

Beyond independent influences of diet quality (mainly fish and nuts), LDL-C levels and utilization of statins, the CHD status itself is associated with increased levels of SFA in blood, in particular palmitic acid. Thus, the blood FA profile provides additional information about the atherogenic potential of the diet with respect to FFQs, and appears as a promising novel biomarker for CHD risk.

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