ORIGINAL ARTICLE

Unsaturated fatty acid composition in serum phospholipids in patients in the acute phase of myocardial infarction

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KEY WORDS
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ABSTRACT

BACKGROUND Recent improvements in optimal cardiovascular therapy have questioned the beneficial effects of polyunsaturated fatty acids (PUFAs) observed in previous studies.

AIMS We investigated the fatty acid (FA) composition in serum phospholipids in patients with an established acute phase of myocardial infarction (MI) and in high-risk patients with stable atherosclerotic cardiovascular disease (CVD).

METHODS We studied 83 patients hospitalized within 12 hours from the onset of the first clinical symptoms of MI. As a control group, we assessed 74 patients at high cardiovascular risk with an established stable atherosclerotic CVD treated at an outpatient cardiology clinic. Gas chromatography was used to evaluate the FA composition in serum phospholipids in both groups.

RESULTS The final analysis included 52 patients with acute MI and 74 controls. In both groups, saturated FAs constituted the largest fraction of serum phospholipid FAs (median, 1574.67 µmol/l), followed by n-6 PUFAs (median, 1106.99 µmol/l). The levels of total saturated FAs, monounsaturated FAs, n-6 PUFAs, as well as the ratio of n-6 to n-3 PUFAs significantly differed between groups. Palmitic acid constituted the largest fraction of serum phospholipids both in patients and controls (31.9% and 31.16%, respectively). In a multivariate logistic regression analysis, body mass index, low-density lipoprotein cholesterol, aspartate aminotransferase, high-sensitivity C-reactive protein, and palmitoleic and eicosadienoic acids were independently associated with MI.

CONCLUSIONS We showed major differences in the FA composition of serum phospholipids between patients with acute MI and high-risk individuals with stable atherosclerotic CVD. Eicosadienoic and palmitoleic acids, apart from typical cardiovascular risk factors, were independently associated with MI.

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INTRODUCTION Previous landmark studies revealed the beneficial effect of n-3 polyunsaturated fatty acids (PUFAs) in patients with stable atherosclerotic cardiovascular disease (CVD).[1-4] Serum concentrations of n-3 PUFAs were shown to be inversely correlated with all-cause mortality, independently of cardiovascular risk factors.[4] However, with the improvement in optimal cardiovascular therapy, the results of subsequent randomized controlled trials have questioned the beneficial impact revealed by previous observational and interventional studies.[1-3] It was shown that type 2 diabetes (T2D) could reduce this positive effect, at least in patients with established atherosclerotic CVD.[5-8] Since then, there have been conflicting reports on the association between n-3 PUFAs and cardiovascular events.[4,9-11] A systematic review of randomized

WHAT'S NEW?

The beneficial effects of polyunsaturated fatty acids (PUFAs) observed in previous landmark studies have been questioned by recent improvements in optimal cardiovascular therapy. We investigated the fatty acid (FA) composition in serum phospholipids in patients in the acute phase of myocardial infarction (MI) and patients at high cardiovascular risk with established atherosclerotic cardiovascular disease, using gas chromatography. The levels of total saturated FAs, monounsaturated FAs, n-6 PUFAs, and the ratio of n-6 to n-3 PUFAs were significantly higher in the MI group. Interestingly, there were no significant differences in n-3 PUFA levels. A multivariate analysis demonstrated that palmitoleic and eicosadienoic acids (together with other factors such as body mass index, low-density lipoprotein cholesterol, aspartate aminotransferase, high-sensitivity C-reactive protein) were independently associated with MI, which is a novel finding.

controlled trials did not find any significant effects of n-3 PUFAs on mortality or cardiovascular events.[12] However, a recently published REDUCE-IT study (Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial) brought our attention to the proper dosage of eicosapentaenoic acid (EPA) ethyl ester in high-risk patients with established atherosclerotic CVD or T2D, and demonstrated a significant reduction of cardiovascular mortality.[13,14] Therefore, we sought to investigate the differences in fatty acid (FA) composition in serum phospholipids between patients with an established acute phase of myocardial infarction (MI) and high-risk patients with stable atherosclerotic CVD, and to assess the predictive value of these FAs in the diagnosis of MI.

METHODS

Study population

Myocardial infarction group We assessed 83 patients hospitalized within 12 hours from the onset of the first clinical symptoms of MI. We included both patients with ST-segment elevation MI (STEMI) and those with non–ST-segment elevation MI (NSTEMI). The inclusion criteria were the diagnosis of STEMI or NSTEMI and age of 45 years or older. Both STEMI and NSTEMI diagnoses were established according to the Third Universal Definition of Myocardial Infarction.[15-23] The exclusion criteria were as follows: prior n-3 PUFA treatment, known sensitivity or allergy to fish or PUFA supplements (or other conditions resulting in the lack of dietary intake of n-3 or n-6 PUFAs), pregnancy, active bleeding, acute infection, serum creatinine levels higher than 177 µmol/l (2 mg/dl), liver injury (alanine transaminase levels >1.5 times above the upper limit of the reference range), alcohol or drug abuse, history of malignancy (unless disease free for >10 years or nonmelanoma skin carcinoma), life expectancy of less than 12 months due to concomitant diseases, abnormal laboratory or imaging findings that would interfere with the interpretation of the results, and any life-threatening condition during the study.

Control group The control group included 74 patients with established stable atherosclerotic CVD and T2D treated at an outpatient cardiology clinic. The exclusion criteria were as follows: acute coronary syndrome (within the previous 3 months), percutaneous coronary intervention or coronary artery bypass grafting (within the previous month). Other exclusion criteria were identical to those in the MI group.

The study was approved by the local ethics committee (no., KBET 122.6120.271.2015 and 122.6120.92.2015). Each patient provided written informed consent before enrollment to the study.

Laboratory investigations

Blood samples were obtained on admission to the emergency department (MI group), or between 8 AM and 10 AM on admission to the department after overnight fasting (control group). The samples were processed 30 to 60 minutes after blood collection and stored at –70°C until further analysis. Blood was taken from the antecubital vein with minimal stasis. Routine blood tests, including the measurement of complete blood count, lipid profile (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], and triglycerides), and the levels of aspartate aminotransferase, alanine transaminase, and serum creatinine, were done by automated laboratory techniques. Glycated hemoglobin A_{1c} levels were measured using a turbidimetric inhibition immunoassay.

Analysis of fatty acid composition in serum phospholipids

The analytical procedure consisted of a few separate steps: 1) extraction of serum total lipids; 2) separation of the lipid fraction on Sep-PakNH2 columns (Waters, Milford, Massachusetts, United States); 3) methylation and separation of the FA from the phospholipid fraction by gas chromatography (6890N Network GC Systems, Agilent Technologies, Wilmington, Delaware, United States) equipped with an Agilent J&W HP-88 capillary column (100 m, 0.250 mm, 0.20 µm) (Agilent Technologies). As an internal standard, 1,2-dipentadecanoil-sn-glicero-3-phosphocholine was used (Sigma-Aldrich, Steinheim, Germany). The method was calibrated using a calibration mixture (all FAs, Sigma-Aldrich). The serum levels of saturated FAs (SFAs) (lauric, C12:0; myristic, C14:0; palmitic, C16:0; stearic, C18:0; and lignoceric, C24:0) and unsaturated acids (n-7 [palmitoleic (POA), C16:1], n-9 [oleic, C18:1], n-3 [α-linolenic (ALA), C18:3; EPA, C20:5; and DHA, C22:6], and n-6 [linoleic (LA), C18:2; eicosadienoic (EDA), C20:2; and arachidonic (AA), C20:4] of the phospholipid fraction were quantitatively measured by gas chromatography. The serum concentration of FAs of the phospholipid

fraction was expressed as µmol/l. All laboratory tests were performed by investigators blinded to the sample origin.

Statistical analysis Categorical variables were presented as numbers and percentages. Continuous variables were expressed as mean (SD) or median and interquartile range (IQR). Differences between groups were compared using the Student or Welch *t* test, depending on the equality of variances for normally distributed variables. The Mann–Whitney test was used for nonnormally distributed continuous variables. Normality was assessed by the Shapiro–Wilk test. The equality of variances was assessed using the Levene test. Categorical variables were compared by the Fisher exact test for 2×2 tables or by the Pearson χ^2 test for other tables. The Pearson correlation coefficient was computed to measure the linear dependence between 2 normally distributed variables. The Spearman rank correlation coefficient was calculated to measure monotonic trends between 2 variables if the distribution of the variables was nonnormal.

All baseline characteristics, as well as laboratory investigations and fatty acid concentrations, were assessed as potential predictors of MI using a univariate logistic regression analysis. If a *P* value from simple logistic regression for a specific variable was 0.15 or lower, the variable was included in a multiple logistic regression model. Two-sided *P* values of less than 0.05 were considered significant. All calculations were done with JMP, version 14.0.0 (SAS Institute Inc., Vienna, Austria, 2018).

In cases of possibility of separation problems in logistic regression, the Firth bias-adjusted method was used. This maximum likelihood–based method has been shown to produce better estimates and tests than maximum likelihood–based models that do not use bias correction. In addition, bias correction ameliorates separation problems that tend to occur in logistic-type models.

RESULTS

Baseline characteristics The final analysis included 52 patients in the MI group and 74 controls. The baseline characteristics of the study patients, including comorbidities and medication use, are shown in TABLE 1. We did not observe any differences in age, sex, current smoking status, prior MI, hypertension, and chronic kidney disease between groups. However, the percentage of patients with obesity, T2D, and use of antidiabetic medication was higher in the MI than in the control group (TABLE 1). In addition, as expected, there were significant differences between groups in high-sensitivity C-reactive protein (hs-CRP) levels, lipid profile (TABLE 2), left ventricular ejection fraction, as well as the use of some of the drugs, for example, clopidogrel or acetylsalicylic acid (TABLE 1).

Fatty acid composition in serum phospholipids Saturated FAs constituted the largest fraction of serum phospholipid FAs both in the MI and control groups (median, 1574.67 µmol/l [IQR, 1402.43–1855.81 µmol/l] and 1441.56 µmol/l [IQR, 1282.93–1599.76 µmol/l], respectively), followed by n-6 PUFAs as the second (median, 1106.99 µmol/l [IQR, 986.84–1264.19 µmol/l] and median, 1004.72 µmol/l [IQR, 877.78–1118.32 µmol/l], respectively). Interestingly, in the MI group, the third major fraction of serum phospholipids were monosaturated FAs (MUFAs) (median, 348.48 µmol/l [IQR, 299.82–443.54 µmol/l]), while in the control group, n-3 PUFAs (median, 359.11 µmol/l [IQR, 280.13–425.70 µmol/l]).

Significant differences between the MI and control groups were found for every type of the FA fraction of serum phospholipids, including SFAs, MUFAs, n-3 PUFAs, and n-6 PUFAs (TABLE 3). Importantly, total SFAs, MUFAs, n-6 PUFA, as well as the ratio of n-6 to n-3 PUFAs significantly differed between groups (FIGURE 1A–1G), while there were no significant differences in DHA and EPA concentrations.

Considering single FAs, palmitic acid constituted the largest fraction of serum phospholipids: 31.9% and 31.16% in the MI and control groups, respectively. In the MI group, LA and AA were the second and third largest fractions of serum phospholipids (16.4% and 15.8%, respectively). In the control group, AA was the second largest fraction, while LA constituted the third largest fraction (17.6% and 14.4%, respectively) (TABLE 3).

Univariate and multivariate logistic regression analyses Significant predictors of MI in the study population are presented in TABLE 4. The multivariate logistic regression analysis demonstrated that body mass index, LDL-C, aspartate aminotransferase, hs-CRP, POA (C16:1), and EDA (C20:2) were independently associated with MI (TABLE 4). The predictive model showed a high degree of cross-validated calibration and discrimination, with an area under the curve of 0.99.

DISCUSSION

In our study, we demonstrated that patients with MI had significantly increased concentrations of total n-6 PUFAs, total SFAs, total MUFAs, and an increased ratio of n-6 to n-3 PUFAs when compared with patients with stable atherosclerotic CVD. Furthermore, to the best of our knowledge, this is the first study to show that the concentrations of POA (MUFA) and EDA (n-6 PUFA) are independent predictors of MI. We found that higher concentrations of both FAs, together with known cardiovascular risk factors such as LDL-C and hs-CRP levels, were significantly associated with MI.

It was previously shown that the FA composition of serum phospholipids is dependent on

TABLE 1 Characteristics of patients with myocardial infarction, controls with stable atherosclerotic cardiovascular disease, and the whole study population

Variable	MI group (n = 52)	Control group (n = 74)	Total population (n = 126)	*P* value
Age, y, mean (SD)	67.98 (11.67)	65.58 (6.84)	66.57 (9.18)	0.19
Male sex	36 (69.23)	48 (64.86)	84 (66.67)	0.61
Weight, kg	81.5 (70–90)	90.20 (81.05–100)	87 (75.1–98)	0.001
Waist circumference, cm, mean (SD)	99.76 (14.64)	106 (9.66)	103.48 (12.26)	0.01
BMI, kg/m^2	27.40 (25.19–31)	31.00 (27.83–33.30)	29.90 (26.55–32.85)	0.001
Previous MI	12 (23.08)	9 (25)	21 (23.86)	0.84
Obesity	24 (46.15)	43 (58.11)	67 (53.17)	0.02
Current smoking	17 (32.69)	4 (21.05)	21 (29.58)	0.34
Type 2 diabetes	19 (36.54)	74 (100)	93 (73.81)	0.001
Hypertension	47 (90.38)	62 (83.78)	109 (86.51)	0.29
Hypercholesterolemia	50 (96.15)	50 (67.57)	100 (79.37)	0.001
Hypertriglyceridemia	6 (11.54)	27 (36.49)	33 (26.19)	0.002
Heart failure	31 (62)	6 (31.58)	37 (53.62)	0.02
Chronic kidney disease	11 (21.15)	9 (12.16)	20 (15.87)	0.17
Left ventricular ejection fraction, %	48 (40–55)	60 (50–62.8)	50 (45–60)	0.001
Systolic BP, mm Hg	142.5 (128.75–163)	140 (130–150)	140 (130–155.25)	0.39
Diastolic BP, mm Hg	86 (78.25–94.75)	80 (75–86.25)	84.5 (75–90)	0.046
Medication				
β-Blocker	42 (80.77)	59 (79.73)	101 (80.16)	0.89
ACEI	43 (82.69)	50 (67.57)	93 (73.81)	0.06
ARB	4 (7.69)	16 (21.62)	20 (15.87)	0.04
Clopidogrel	46 (88.46)	33 (44.59)	79 (62.70)	0.001
Ticagrelor	1 (1.92)	0 (0)	1 (0.79)	0.23
ASA	51 (98.08)	65 (87.84)	116 (92.06)	0.04
Nitrate	4 (7.69)	11 (14.86)	15 (11.9)	0.22
CCB	8 (15.38)	31 (41.89)	39 (30.95)	0.002
Fibrate	0 (0)	1 (1.35)	1 (0.79)	0.4
Loop diuretic	16 (30.77)	9 (12.16)	25 (19.84)	0.02
Diuretic	5 (9.62)	21 (28.38)	26 (20.63)	0.01
MRA	18 (34.62)	9 (12.16)	27 (21.43)	0.003
Statin	49 (94.23)	64 (86.49)	113 (89.68)	0.16
Metformin	9 (17.31)	48 (64.86)	57 (45.24)	0.001
Insulin	7 (13.46)	32 (43.24)	39 (30.95)	0.001
Acarbose	0 (0)	3 (4.05)	3 (2.38)	0.14
DPP-4 inhibitor	0 (0)	1 (1.35)	1 (0.80)	0.41
Sulphonylurea	4 (7.69)	31 (41.89)	35 (27.78)	0.001

Data are presented as number (percentage) or median (interquartile range) unless otherwise indicated.

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid; BMI, body mass index; BP, blood pressure; CCB, calcium channel blocker; DPP-4, dipeptidyl peptidase 4; MI, myocardial infarction; MRA, mineralocorticoid receptor antagonist

TABLE 2 Baseline laboratory test results of the study groups

Variable	MI group (n = 52)	Control group (n = 74)	Total population (n = 126)	*P* value
Creatinine, µmol/l	87 (73.25–107)	84 (77–93.25)	84 (75.5–94.25)	0.28
hs-TnT, µg/l	1.39 (0.25–5.76)	–	–	–
HbA_{1c}, %	–	7 (6.6–7.5)	–	–
Glucose, mmol/l	6.9 (5.73–10.8)	–	–	–
TC, mmol/l	4.96 (4.33–5.79)	3.77 (3.22–4.34)	4.21 (3.44–5.1)	0.001
LDL-C, mmol/l	3.44 (2.71–4)	1.92 (1.52–2.65)	2.55 (1.74–3.39)	0.001
HDL-C, mmol/l	1.27 (1.08–1.65)	1.23 (0.98–1.45)	1.24 (1.02–1.56)	0.13
TG, mmol/l	1.06 (0.74–1.56)	1.38 (1.12–1.92)	1.26 (0.95–1.84)	0.005
AST, U/l	49 (29.50–91)	19 (16–23)	23 (18–32)	0.001
ALT, U/l	29.5 (24.75–40.25)	22 (14–28)	25 (17–33.75)	0.001
hs-CRP, mg/l	4.46 (1.90–12.85)	1.6 (0.74–2.77)	2.12 (1.13–5.59)	0.001
eGFR, ml/min	75 (56.25–89.25)	78 (69.25–89.18)	78 (63.75–89.18)	0.21

Data are presented as median (interquartile range).

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease formula); HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; others, see TABLE 1

TABLE 3 Fatty acid composition of serum phospholipids in the study groups

Variables		MI group (n = 52)	Control group (n = 74)	*P* value
SFAs, µmol/l	C12:0 (lauric)	2.23 (1.01–4.03)	2.06 (1.31–3.22)	0.74
	C14:0 (myristic)	20.16 (13.17–26.56)	16.64 (14.65–19.52)	0.05
	C16:0 (palmitic)	1080.92 (954.22–1294.80)	949.92 (857.51–1073.60)	0.001
	C18:0 (stearic)	477.65 (407.48–554.74)	418.61 (349.10–489.05)	0.02
	C24:0 (lignoceric)	29.52 (23.40–35.09)	24.48 (20.29–29.42)	0.003
n-7 MUFAs	C16:1 (palmitoleic)	20.33 (14.02–30.38)	14.93 (12.11–21.08)	0.006
n-9 MUFAs	C18:1 (oleic)	329.58 (279.81–415.52)	276.71 (244.13–334.06)	0.001
n-3 PUFAs, µmol/l	C18:3 (α-linolenic)	8.85 (5.20–11.88)	6.45 (4.84–8.86)	0.01
	C20:5 (EPA)	46.17 (31.72–82.03)	62.17 (44.57–81.29)	0.10
	C22:6 (DHA)	261.52 (222.06–353.39)	285.51 (236.01–337.17)	0.41
n-6 PUFAs, µmol/l	C18:2 (linoleic)	556.93 (465.59–653.24)	438.84 (373.24–534.49)	0.001
	C20:2 (EDA)	20.03 (15.40–24.89)	16.82 (13.54–20.89)	0.01
	C20:4 (arachidonic)	533.52 (437.18–612.34)	535.12 (460.67–621.50)	0.46

Data are presented as median (interquartile range).

Abbreviations: DHA, docosahexaenoic acid; EDA, eicosadienoic acid; EPA, eicosapentaenoic acid; MUFAs, monounsaturated acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; others, see TABLE 1

the dietary intake of FAs during the previous weeks. However, in addition to diet, the FA concentration is determined by endogenous FA metabolism including FA synthesis, desaturation, elongation, retroconversion, and oxidation.[6,24,25]

Saturated fatty acids The current European Society of Cardiology guidelines on cardiovascular disease prevention in clinical practice recommend a reduction of SFA intake to less than 10% of total energy, through replacement by PUFAs.[26] It was calculated that the risk of coronary artery disease (CAD) is reduced by 2% to 3% when 1% of energy intake from SFAs is replaced by PUFAs.[26,27] It was also shown that even isocaloric replacement of SFAs with PUFAs resulted in a reduction of LDL-C levels and the ratio of TC to HDL-C levels, which are established risk factors for CAD.[28] A recent

TABLE 4 Univariate and multivariate logistic regression analyses of predictors of myocardial infarction

Variable	Univariate analysis			Multivariate analysis (AUC, 0.99)	
	OR (95% CI)	*P* value	AUC	OR (95% CI)	*P* value
Weight, kg	0.97 (0.945–0.993)	0.01	0.67	–	–
Waist circumference, cm	0.956 (0.924–0.987)	0.0046	0.66	–	–
BMI, kg/m^2	0.856 (0.773–0.940)	0.0008	0.70	0.24 (0.2–0.56)	<0.0001
Creatinine, μmol/l	1.016 (1.001–1.034)	0.02	0.56	–	–
Heart failure, n (%)	3.535 (1.189–11.559)	0.02	0.65	–	–
Type 2 diabetes, n (%)	0.0039 (0.00003–0.03)[a]	<0.0001	0.82	–	–
LVEF, %	0.883 (0.805–0.949)	0.0001	0.78	–	–
Diastolic BP, mmHg	1.031 (1.004–1.062)	0.03	0.60	–	–
Hypercholesterolemia	12 (3.32–77.158)	<0.0001	0.64	–	–
Hypertriglyceridemia	0.227 (0.079–0.568)	0.001	0.62	–	–
TC, mmol/l	2.766 (1.873–4.334)	0.0001	0.78	–	–
LDL-C, mmol/l	3.759 (2.373–6.438)	0.0001	0.82	2.34 (1.32–10.26)[b]	<0.0001
TG, mmol/l	0.618 (0.36–0.995)	0.048	0.65	–	–
AST, U/l	1.284 (1.164–1.463)	0.0001	0.95	1.92 (1.26–5.03)	<0.0001
ALT, U/l	1.08 (1.041–1.129)	0.0001	0.76	–	–
hs-CRP, mg/l	1.099 (1.035–1.199)	0.0001	0.75	1.35 (1.04–2.40)	0.001
C14:0 (myristic), μmol/l	1.066 (1.016–1.126)	0.009	0.60	–	–
C16:0 (palmitic), μmol/l	1.003 (1.001–1.004)	0.002	0.67	–	–
C18:0 (stearic), μmol/l	1.004 (1.001–1.008)	0.02	0.62	–	–
C24:0 (lignoceric), μmol/l	1.076 (1.027–1.133)	0.002	0.66	–	–
C16:1 (palmitoleic), μmol/l	1.051 (1.017–1.091)	0.003	0.64	1.48 (1.13–2.61)	0.001
C18:1 (oleic), μmol/l	1.008 (1.004–1.014)	0.0002	0.69	–	–
C18:2 (linoleic), μmol/l	1.006 (1.003–1.01)	0.0001	0.73	–	–
C18:3 (α-linolenic), μmol/l	1.091 (1.006–1.194)	0.03	0.63	–	–
C20:2 (EDA), μmol/l	1.078 (1.018–1.145)	0.01	0.63	0.32 (0.05–0.68)	<0.0001
Total SFAs μmol/l	1.002 (1.001–1.003)	0.003	0.66	–	–
Total n-6 PUFAs μmol/l	1.002 (1.000–1.004)	0.02	0.65	–	–

a Values calculated with the Firth bias-adjusted method; **b** Per 0.1-unit increase

Abbreviations: AUC, area under the curve; CI, confidence interval; LVEF, left ventricular ejection fraction; OR, odds ratio; see TABLES 1, 2, and 3

analysis of EPIC-Norfolk and EPIC-Denmark cohorts by Praagman et al[29] suggested that a higher consumption of C4:0–C10:0, C12:0, and C14:0 was associated with a lower MI risk. The conflicting evidence on the association between SFAs and CAD could be due to differences in the types of SFAs, based on their carbon-atom chain lengths.[29] In our study, the majority of SFAs were inversely correlated with MI; however, the levels of lauric acid (C12:0), with the longest carbon-atom chain in our study, were similar between groups.

Monosaturated fatty acids Interventional studies demonstrated that MUFAs could improve cardiovascular risk factors, but prospective data with regard to CAD risk are limited and remain controversial.[30-33] It was found that the total intake of MUFAs is significantly inversely correlated with total mortality, but no association with CAD was observed.[34] Some authors underline the role of MUFA origin for their impact on CVDs.[31] The replacement of SFAs with plant-based MUFAs was associated with a significantly lower risk of CAD when compared with animal-based MUFAs.[31] Some epidemiologic evidence suggests that the length of carbon chains in MUFAs might play a role in the prediction of CVDs.[35] Li et al[35] demonstrated that the higher concentrations of long-chain (14–18 carbon atoms) MUFAs

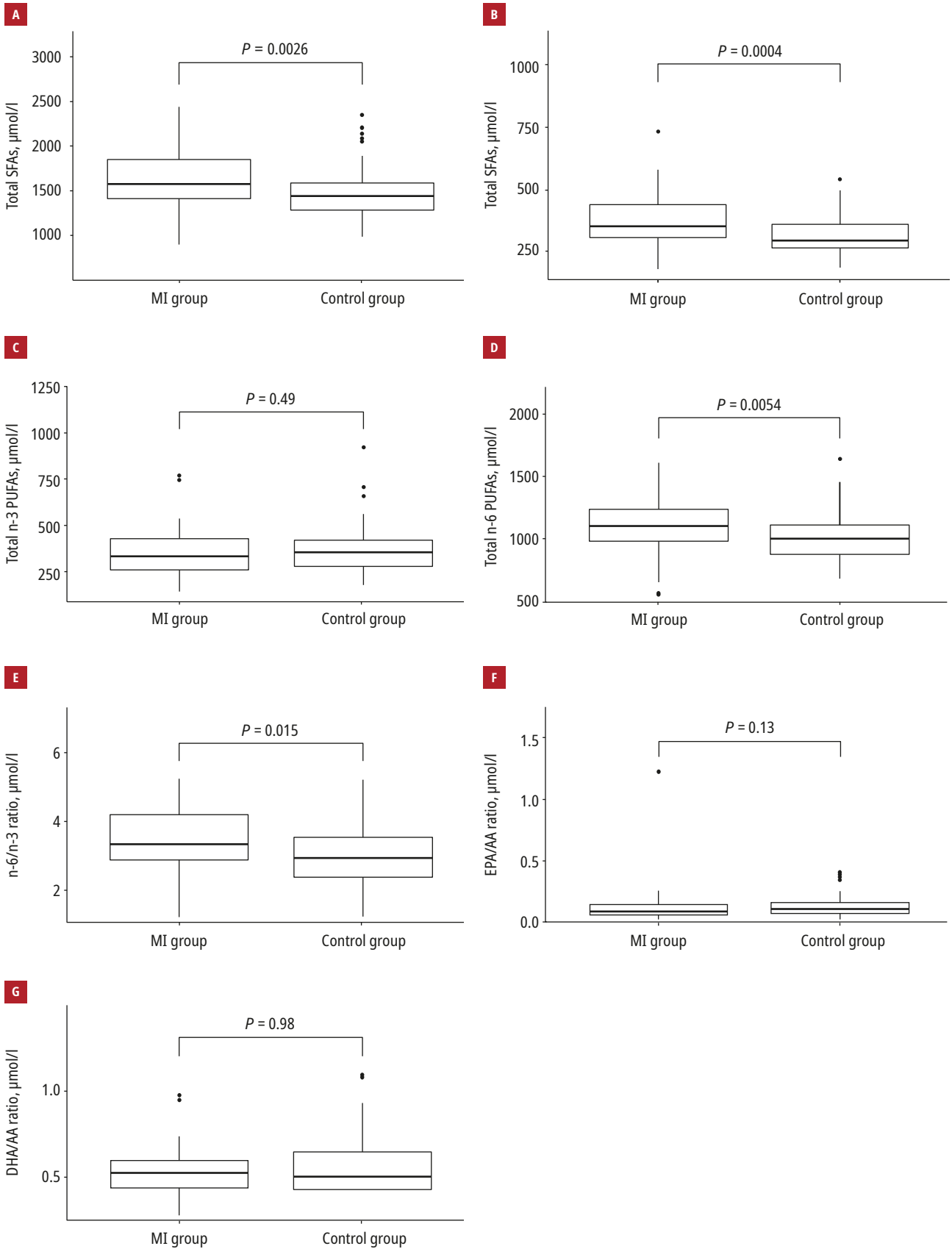

FIGURE 1 Distribution of fatty acid composition in serum phospholipids in patients in the acute phase of myocardial infarction (MI) and in the control group: **A** – total saturated fatty acids (SFAs); **B** – total monosaturated fatty acids (MUFAs); **C** – total n-3 polyunsaturated fatty acids (PUFAs); **D** – total n-6 (PUFAs); **E** – the ratio of n-6 to n-3 PUFAs; **F** – the ratio of eicosadienoic to arachidonic acid (EPA/AA); **G** – the ratio of docosahexaenoic to arachidonic acid (DHA/AA). Boxes represent median and interquartile ranges; whiskers, highest and lowest values; and dots, outliers >1.5 IQR above the upper quartile.

had a beneficial impact on all-cause and cardiovascular mortality, while very long-chain MUFAs (≥20 carbon atoms) contributed to higher mortality rates.

n-3 Polyunsaturated fatty acids In our study, only the ALA (C18:3) concentration was significantly higher in the MI group. We did not observe any significant differences between other n-3 PUFAs, including DHA and EPA. A recent systematic review of randomized control trials demonstrated no beneficial effects of n-3 PUFAs on mortality or cardiovascular events.[12] In the VITAL study (Vitamin D and Omega-3 Trial; treatment with 1 g/d of n-3 PUFAs) on primary cardiovascular prevention, the authors found a significant 19% reduction of major adverse cardiovascular events, but only in patients with low fish consumption.[36] On the contrary, the REDUCE-IT trial, which investigated the impact of high-dose n-3 PUFAs (4 g/d; pure EPA ethyl ester) on established CVD or T2D and other cardiovascular risk factors, demonstrated significant benefits of an intervention with n-3 PUFAs, including a reduction in major adverse cardiovascular events by 25%.[13] Finally, the membrane composition of FAs (especially EPA and DHA) in the inflammatory cells could alter cell function. Therefore, the anti-inflammatory impact of those acids may contribute to their protective actions against an atherosclerotic process and plaque rupture.[37]

n-6 Polyunsaturated fatty acids (PUFAs) and the ratio of n-6 to n-3 PUFAs A multicenter trial by Nishizaki et al[38] found that a low ratio of EPA to AA levels, but not of DHA to AA levels, was significantly associated with the occurrence of MI. In our study, these ratios were similar in both groups. Nevertheless, we observed that the ratio of n-6 to n-3 PUFAs and total n-6 PUFA levels were much higher in the MI group than in controls.

It was reported that the LA concentration was inversely associated with the occurrence of MI.[39] Moreover, it was shown that low serum levels of n-6 PUFAs could predict a poor long-term prognosis in patients with acute CVD. In particular, decreased dihomo-γ-linolenic acid (DGLA) levels were associated with increased total mortality rates in patients with acute CVD. However, DGLA and AA have the opposite effects, mainly due to their metabolites. The proinflammatory effect of AA comes from the conversion to series-2 prostaglandins and series-4 leukotrienes, which are known to induce platelet aggregation, inflammation, and vasoconstriction.[40,41] On the other hand, DGLA undergoes oxidative metabolism to anti-inflammatory eicosanoids (series-1 prostaglandin and series-3 leukotrienes).[40,42-44]

Palmitoleic and eicosadienoic acids Although previous studies have discussed the impact of POA levels on the risk and progression of atherosclerotic CVD,[45,46] none of them analyzed the effect of POA in patients with MI. Palmitoleic acid was found to act as a lipokine and could be considered as a metabolic modulator.[47] Furthermore, POA was associated with decreased LDL-C and fibrinogen levels as well as increased HDL-C levels.[47-49]

We did not identify any studies regarding the association between EDA levels and the acute phase of MI. Eicosadienoic acid is a naturally occurring n-6 polyunsaturated FA and a relatively minor metabolite of LA. It can be further metabolized to, for example, AA, thereby giving it proinflammatory properties.[50] In our study, we demonstrated a novel association between EDA levels in patients with acute phase MI.

Limitations First, the cross-sectional design of the study did not allow us to infer causality, and there was no follow-up. Second, our patients were provided with dietary advice regarding low-fat and low-carbohydrate meals, but dietary fat intake was not determined. Third, the higher presence of obesity and T2D in the control group could affect the inference between groups. Finally, the multiple logistic regression model for MI per 1-unit increase in EDA showed opposite results to those of the simple logistic regression model for EDA, which was confirmed also after eliminating the observations with the highest influence on the model (Cook's distance). This could be the so called Simpson's paradox resulting from adjustment for known risk factors, such as LDL-C.

Conclusions The major differences in the FA composition of serum phospholipids between patients with MI and high-risk individuals with stable atherosclerotic CVD and T2D were the higher content of SFAs, MUFAs, n-6 PUFAs, as well as the ratio of n-6 to n-3 PUFAs. In the multivariate analysis, apart from known cardiovascular risk factors, EDA and POA levels were independent predictors of MI.

ARTICLE INFORMATION

CONFLICT OF INTEREST None declared.

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