Serum fatty acids profile and association with early-onset coronary artery disease

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Abstract

Background: Fatty acids (FAs) play crucial roles in modulating and preventing diseases in humans, including early-onset coronary artery disease (EOCAD). In this study, we aimed to provide a profile of FAs in the serum of EOCAD patients and identify potential EOCAD-associated FAs.

Methods: In the first stage, we analyzed the FAs profiles in pooled samples of patients with EOCAD using gas chromatography-mass spectrometry. In the second stage, the serum levels of the candidate FAs were validated in EOCAD patients.

Results: A total of 128 EOCAD patients and 64 controls were included in the study. Forty-nine serum FAs were quantified in pooled samples; three ω-3 FAs were identified to be associated with EOCAD. Moreover, results from the validation stage indicated that serum levels of docosahexaenoic acid (DHA) were significantly lower in EOCAD patients ($55.43 \pm 33.86\,\mu g/$ml) and myocardial infarction (MI) patients ($47.49 \pm 28.44\,\mu g/ml$) than those in the controls ($70.65 \pm 43.56\,\mu g/ml$). Multivariate regression analysis revealed that elevated serum DHA level was an independent protective factor for EOCAD [odds ratio (OR) = 0.8917, 95% confidence interval (CI): 0.879–0.957] and MI (OR = 0.835, 95% CI: 0.799–0.862). Decreased serum levels of docosapentaenoic acid (DPA) and eicosapentaenoic acid (EPA) were observed in the early-onset MI group.

Conclusion: The study provided the serum FAs profile of EOCAD and confirmed that the decrease in serum levels of DHA, DPA, and EPA was associated with EOCAD. These findings might contribute to understanding the cardiovascular effects of FAs, particularly the protective effects of ω-3 polyunsaturated FAs.

Keywords: early-onset coronary artery disease, fatty acids, docosahexaenoic acid, docosapentaenoic acid, eicosapentaenoic acid

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Introduction

Cardiovascular disease (CVD) continues to be one of the major contributors to morbidity and mortality worldwide. The complex etiology of CVD encompasses a complicated interplay between genetic predisposition and environmental factors. The association between fatty acids (FAs) and CVD, particularly coronary artery disease (CAD), has been well documented.[1–3]

FAs, the building blocks of lipids, are carboxylic acids with long aliphatic chains and they represent the predominant source of cellular energy. Depending on the length of the aliphatic chain, FAs are classified as short-chain FAs (aliphatic tails of up to seven carbons), medium-chain FAs (aliphatic tails of 6–12 carbons), long-chain FAs (aliphatic tails of 13–21 carbons), and very long-chain FAs (aliphatic tails of 22 carbons or more; >C22). As well, based on the presence of the carbon–carbon double bonds (–CH=CH–) in the hydrocarbon chain, FAs can be either saturated FAs (SFAs; having only single bonds) or unsaturated FAs (having double bonds). FAs are essential for diverse biological functions, including lipid metabolism, modulation of the immune

system, endothelial function, platelet aggregation, and redox status. An alteration in these functions can result in several lipid dysregulation-related diseases, including CAD.[4] FA plays a crucial dual role in the occurrence of CAD. Generally, SFA is known to contribute to the risk of CAD, whereas polyunsaturated FA (PUFA) protects against CVD and significantly reduces the risk of CAD. Recently, increasing epidemiological evidence also indicates that trans-fatty acid, unsaturated FAs with at least one non-conjugated double bond in the trans configuration, can significantly increase the risk of cardiovascular events.[5] Among PUFAs, omega-3 FAs (ω-3 FAs) have gained increasing attention owing to their potential in preventing the development and progression of many disorders, including neurodegenerative, neuropsychiatric, and inflammatory diseases. Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are the most represented ω-3 FAs. However, the association between these ω-3 FAs and CVD remains controversial.[6]

CAD predominantly affects the elderly; however, the prevalence of CAD in the young is increasing with changing lifestyles. CAD that occurs in individuals less than 50 years of age is defined as early-onset coronary artery disease (EOCAD). Several etiological factors, including family history, lipid metabolism, gender, and environmental risk factors, have been associated with EOCAD.[7–10] Furthermore, the long-term prognosis of patients with EOCAD remains poor.[11] Thus, it is imperative to recognize risk factors for the early diagnosis and screening of EOCAD. In this study, we quantitatively identified 49 FAs in the serum of patients with EOCAD and in normal healthy controls using gas chromatography-mass spectrometry (GC-MS). Furthermore, the serum levels of the potential candidate FAs were validated in patients with EOCAD.

Material and methods

Subjects
In this hospital-based retrospective case–control study, all patients attending the CAD clinic at the Affiliated Hospital of Qingdao University between January 2019 and May 2020 were recruited. A total of 128 patients with CAD were included in the study. Eligible patients fulfilled the following criteria: (1) were less than 50 years of age; (2) met the diagnostic criteria for CAD after being clinically examined using coronary angiography; (3) clinical diagnosis of CAD was based on the presence of at least 50% stenosis in one or more of the major coronary arteries in coronary angiography, combined with the clinical symptoms of angina, changes in cardiac troponin levels, or/and electrocardiographic changes. Exclusion criteria were patients with liver diseases, kidney diseases, tumors, and other serious illnesses that may interfere with the study results. All patients were divided into two groups depending on the presence or absence of myocardial infarction (MI): CAD without MI group ($n = 64$) and MI group ($n = 64$). Moreover, 64 age- and sex-matched individuals with no history of CVD and no signs or symptoms of cardiovascular events, determined using electrocardiography, were included as healthy controls. The control subjects were volunteers recruited from the Health Management Center with a similar geographical background. The study was conducted in accordance with the ethical standards laid down in the 1975 Declaration of Helsinki. The study protocols were approved by the research ethics committee of the Affiliated Hospital of Qingdao University (QYFYWZLL26377). Written informed consent was obtained from all participants.

Sample preparation
Blood samples were collected after overnight fasting of at least 8 h on the morning after admission. Blood was drawn into BD® anticoagulant-free vacuum blood collection tubes. The samples were then allowed to stand at 4°C for 0.5 h and centrifuged at 3500 rev/min for 10 min to obtain serum samples. All samples were frozen at −80°C until further analysis.

Biochemical measurements
Serum fasting blood glucose (FBG), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), serum creatinine (SCr), and lipoprotein(a) [Lp(a)] levels were determined using an automatic biochemistry analyzer (Hitachi HCP-7600, Hitachi, Japan).

Metabolites extraction
Serum samples (50 μl) were transferred into a 1.5-ml tube and extracted with 430 μl of extraction

solution ($V_{Isopropanol}$:$V_{n-hexane}$ = 2:3); 20 µl of internal standard solution (1 mg/l in n-hexane) were added to the sample, vortexed (30 s), and then sonicated (5 min). Tubes were centrifuged (12,000*g*, 15 min) and the supernatant was collected into a fresh 1.5-ml tube and blow-dried with nitrogen. Next, 200 µl methanol and 100 µl (trimethylsilyl) diazomethane was added to the pellet, vortexed (10 s), and allowed to stand at room temperature for 15 min and blow-dried with nitrogen. Subsequently, the pellet was redissolved in 160 µl of n-hexane, centrifuged (12,000*g*, 5 min) at 4°C, and the supernatant was collected into a fresh vial for GC-MS analysis.

GC-MS

GC-MS was performed using an Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent 5977B mass spectrometer. The system utilized a DB-FastFAME capillary column. The analyte sample (1 µl) was injected in the split mode (5:1). Helium was used as a carrier gas with a front inlet purge flow rate of 3 ml/min and gas flow rate through the column of 46 psi with constant pressure. After 1 min at 75°C, the oven was heated to 200°C at a rate of 50°C/min (hold time 15 min), then ramped up by 2°C/min to 210°C (hold time 1 min); last, the temperature was raised by 10°C/min to 230°C (hold time 16.5 min). The injection, transfer line, quad, and ion source temperatures were 240°C, 240°C, 230°C, and 150°C, respectively. The electron energy was set to −70 eV in electron impact mode. After a solvent delay of 7 min, data were acquired from the m/z range of 33–400 in the Scan/SIM mode.

Limit of detection and limit of quantitation

The calibration standard solution was prepared by stepwise dilution of 2-fold and analyzed using GC-MS. The lower limits of detection were determined based on a signal-to-noise (S/N) ratio of 3, whereas the lower limits of quantitation were established based on an S/N ratio of 10, according to the US Food and Drug Administration guidelines for bioanalytical method validation.

Experiment protocol

The study was divided into two stages. In the first stage, 200 µl of serum from each sample was taken and mixed into a sample pool for every eight samples. There were eight sample pools in the CAD (without MI) group, eight sample pools in the MI group, and 16 sample pools in the controls. We performed GC-MS to analyze the levels of 49 serum FAs in different groups of sample pools and to screen for potential differences. In the second stage, we used GC-MS to verify the screened differential FAs in each sample and investigated their association with CAD.

Statistical analysis

Statistical analyses were conducted using Statistical Package for Social Sciences SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism Software 8 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to test the null hypothesis that a set of data comes from a normal distribution. The comparisons of categorical variables were performed using the chi-square test or Fisher exact test. Independent samples *t*-test or one-way analysis of variance (ANOVA) was used to assess differences between the groups, followed by a *post hoc* least significant difference test for multiple comparisons. Logistic regression was performed to assess the association between variables. A *p*-value of < 0.05 was considered statistically significant. Power calculations were used to determine the number of subjects required with 0.90 power at an alpha level of 0.05. Estimates were calculated using power analysis and PASS software (version 15.0, NCSS, LLC).

Results

Characteristics of participants

We included 64 CAD patients (mean age 43.27 ± 4.26 years) and 128 healthy controls (mean age 43.56 ± 4.78 years) in this study. No significant differences were observed in gender, age, body mass index (BMI), hypertension, diabetes, alcohol consumption, TC, HDL-C, LDL-C, and SCr between EOCAD patients and healthy controls. However, FBG, TG, and Lp(a) levels were found to be significantly elevated in patients with EOCAD compared with the controls. As well, patients with EOCAD had a higher smoking rate compared with controls. In the group of patients with EOCAD, 64 were diagnosed with MI. The clinical characteristics of all study subjects are summarized in Table 1.

Table 1. Demographic and clinical characteristics of patients with EOCAD and controls.

Variable	EOCAD *n* = 128	Control *n* = 64	*p*-value
Gender, male, *n* (%)#	128 (98.44)	64 (98.44)	1.000
Age, years*	43.27 ± 4.26	43.56 ± 4.78	0.670
BMI, kg/m²*	26.68 ± 5.43	25.98 ± 5.98	0.417
Hypertension, *n* (%)#	32 (25.00)	26 (20.31)	0.456
Diabetes, *n* (%)#	26 (20.31)	18 (14.06)	0.246
Smoking, *n* (%)#	68 (53.13)	42 (32.81)	0.002
Alcohol consumption, *n* (%)#	70 (54.69)	62 (48.44)	0.381
FBG, mmol/l*	6.08 ± 3.22	5.02 ± 2.59	0.023
TG, mmol/l*	2.20 ± 1.92	1.65 ± 1.46	0.045
TC, mmol/l*	5.02 ± 1.59	4.84 ± 1.45	0.448
HDL-C, mmol/l*	1.09 ± 0.38	1.18 ± 0.45	0.148
LDL-C, mmol/l*	2.77 ± 1.23	2.55 ± 1.13	0.232
Lp(a), mmol/l*	299.27 ± 287.65	215.60 ± 218.56	0.042
SCr, μmol/l*	74.56 ± 20.22	75.28 ± 22.17	0.822
Myocardial infarction, *n* (%)	64 (50.00)	–	–

#Categorical variables are expressed as percentages. The *p*-value of the categorical variables was calculated using the χ^2 test.
*Continuous variables are expressed as mean ± SD. The *p*-value of the continuous variables was calculated using the unpaired *t*-test.
BMI, body mass index; EOCAD, early-onset coronary artery disease; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); SCr, serum creatinine; TC, total cholesterol; TG, triglyceride.

Profiling of FAs

In the first stage, 49 FAs were quantified using GC-MS. The details of the targeted FAs are presented in Table 2. Among the 49 FAs, significant differences in serum levels of DHA ($p = 0.007$), docosapentaenoic acid (DPA; $p = 0.030$), and EPA ($p = 0.037$) were observed between EOCAD patients and control groups (one-way ANOVA).

Serum DHA levels in the control sample pool ($66.40 \pm 15.48\,\mu g/ml$, $n = 8$) group were significantly higher than those in the CAD (without MI) samples pool group ($52.50 \pm 12.21\,\mu g/ml$, $n = 8$, $p = 0.028$) and MI samples pool group [$45.85 \pm 5.39\,\mu g/ml$, $n = 8$, $p = 0.002$; Figure 1(a)]. Serum DPA levels in the MI samples pool group ($8.56 \pm 1.27\,\mu g/ml$, $n = 8$) were significantly lower than that of the control samples pool group [$12.09 \pm 3.53\,\mu g/ml$, $n = 8$, $p = 0.010$; Figure 1(b)]. Similarly, serum EPA levels in the MI samples pools group ($7.72 \pm 1.20\,\mu g/ml$, $n = 8$) were also significantly lower than those in the control samples pool group [$11.73 \pm 4.02\,\mu g/ml$, $n = 8$, $p = 0.011$; Figure 1(c)]. DHA, DPA, and EPA were subsequently selected to be candidate FAs for validation in the next stage.

Validation of the candidate FAs

In the validation stage, the candidate FAs were validated and GC-MS was used to quantify the serum levels of DHA, DPA, and EPA in patients

Table 2. Serum quantitative analysis of 49 fatty acids.

Fatty acids	Control (µg/ml)	CAD (without MI) (µg/ml)	MI (µg/ml)	*p**
Octanoic acid	0.14 ± 0.05	0.21 ± 0.13	0.19 ± 0.09	0.262
Decanoic acid	0.47 ± 0.16	0.42 ± 0.17	0.41 ± 0.08	0.706
Undecanoic acid	0.09 ± 0.02	0.08 ± 0.01	0.10 ± 0.02	0.331
Lauric acid	0.75 ± 0.22	0.79 ± 0.26	0.53 ± 0.20	0.081
Tridecylic acid	0.16 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.800
Myristic acid	15.74 ± 5.85	16.10 ± 4.07	11.99 ± 3.02	0.150
trans-9-tetradecenoic acid	0.12 ± 0.07	0.08 ± 0.04	0.11 ± 0.04	0.370
cis-9-myristoleic acid	0.4 ± 0.22	0.57 ± 0.19	0.34 ± 0.19	0.089
Pentadecanoic acid	2.63 ± 0.52	2.63 ± 0.52	2.25 ± 0.38	0.209
trans-10-pentadecenoic acid	0.05 ± 0.02	0.07 ± 0.01	0.06 ± 0.02	0.087
cis-10-Pentadecenoic acid	0.42 ± 0.12	0.40 ± 0.10	0.36 ± 0.09	0.486
Palmitic acid	510.00 ± 105.99	510.12 ± 123.88	477.67 ± 57.49	0.758
trans-9-palmitelaidic acid	5.69 ± 5.85	6.51 ± 5.61	8.85 ± 1.32	0.402
cis-9-palmitoleic acid	29.57 ± 11.07	32.95 ± 9.22	26.24 ± 4.33	0.323
Heptadecanoic acid	5.76 ± 1.20	5.48 ± 1.19	4.81 ± 0.55	0.188
trans-10-heptadecenoic acid	0.92 ± 0.27	0.58 ± 0.36	0.89 ± 0.27	0.068
cis-10-heptadecenoic acid	2.26 ± 0.69	2.28 ± 0.51	1.94 ± 0.28	0.373
Stearic acid	199.17 ± 42.05	177.69 ± 51.92	160.70 ± 15.10	0.174
trans-6-octadecenoic acid	1.66 ± 0.50	1.62 ± 0.28	1.25 ± 0.43	0.112
trans-9-elaidic acid	0.95 ± 0.55	0.79 ± 0.26	0.85 ± 0.22	0.694
trans-11-octadecenoic acid	0.71 ± 0.30	0.73 ± 0.25	0.91 ± 0.61	0.589
cis-6-octadecenoic acid	0.72 ± 0.26	0.63 ± 0.15	0.61 ± 0.14	0.492
cis-9-octadecenoic acid	728.35 ± 149.98	781.22 ± 217.97	707.36 ± 91.66	0.648
cis-11-octadecenoic acid	38.85 ± 10.32	38.04 ± 10.71	36.57 ± 4.24	0.875
trans,trans-9,12-linolelaidic acid	0.53 ± 0.18	0.41 ± 0.04	0.41 ± 0.05	0.072
cis,cis-9,12-linoleic acid	605.90 ± 97.61	605.29 ± 187.53	530.76 ± 41.32	0.397
trans-nonadecenoic acid	3.15 ± 1.84	4.11 ± 2.32	2.29 ± 1.01	0.157
cis,cis,cis-6,9,12-linolenic acid	5.68 ± 1.6	6.54 ± 1.97	5.45 ± 1.30	0.393

(Continued)

Table 2. (Continued)

Fatty acids	Control (µg/ml)	CAD (without MI) (µg/ml)	MI (µg/ml)	p^*
cis,cis,cis-9,12,15-linolenic acid	14.88 ± 7.26	19.97 ± 8.80	13.07 ± 1.84	0.125
Arachidic acid	2.00 ± 0.36	1.87 ± 0.67	1.45 ± 0.29	0.074
trans-11-eicosenoic acid	21.69 ± 15.31	18.12 ± 27.57	49.28 ± 38.88	0.085
cis-11-eicosenoic acid	6.62 ± 1.67	6.10 ± 2.95	7.88 ± 2.71	0.362
cis,cis-11,14-eicosadienoic acid	8.69 ± 1.66	7.47 ± 2.06	7.06 ± 0.61	0.119
Heneicosanoic acid	0.18 ± 0.02	0.20 ± 0.06	0.22 ± 0.05	0.339
cis,cis,cis-8,11,14-linolenic acid	27.26 ± 6.74	24.54 ± 6.47	24.10 ± 3.98	0.517
all-cis-5,8,11,14-eicosatetraenoic acid	130.75 ± 31.82	115.12 ± 30.77	102.48 ± 14.81	0.135
all-cis-11,14,17-eicosatrienoic acid	1.33 ± 0.46	1.16 ± 0.37	1.08 ± 0.17	0.366
Behenic acid	0.93 ± 0.27	0.97 ± 0.65	0.69 ± 0.24	0.377
all-cis-5,8,11,14,17-eicosapentaenoic acid	11.73 ± 4.02	10.11 ± 2.75	7.72 ± 1.20	0.037
trans-13-docosenoic acid	0.31 ± 0.16	0.24 ± 0.20	0.46 ± 0.31	0.168
cis-13-erucic acid	0.57 ± 0.18	0.48 ± 0.07	0.45 ± 0.04	0.115
cis,cis-13,16-docosadienoic acid	0.47 ± 0.21	0.42 ± 0.14	0.43 ± 0.26	0.879
Tricosanoic acid	0.36 ± 0.07	0.35 ± 0.12	0.44 ± 0.14	0.259
all-cis-7,10,13,16-docosatetraenoic acid	6.35 ± 1.14	6.37 ± 1.85	6.04 ± 1.16	0.874
all-cis-4,7,10,13,16-docosapentaenoic acid	4.23 ± 1.02	3.68 ± 0.99	3.18 ± 0.59	0.082
Lignoceric acid	0.85 ± 0.19	0.77 ± 0.38	0.64 ± 0.12	0.260
all-cis-4,7,10,13,16,19-docosapentaenoic acid	12.09 ± 3.53	9.8 ± 2.09	8.56 ± 1.27	0.030
cis-15-nervonic acid	0.32 ± 0.17	0.32 ± 0.13	0.29 ± 0.08	0.848
all-cis-7,10,13,16,19-docosahexaenoic acid	66.40 ± 15.48	52.50 ± 12.21	45.85 ± 5.39	0.007

*One-way analysis of variance.
CAD, coronary artery disease; MI, myocardial infarction.

with CAD (without MI), patients with MI, and control samples one by one. The results of multivariate analysis are shown in Table 3.

The serum levels of DHA in CAD (without MI) patients (55.43 ± 33.86 µg/ml, $n = 64$, $p = 0.029$) and in MI patients (47.49 ± 28.44 µg/ml, $n = 64$, $p < 0.001$) were significantly lower than those in healthy controls (70.65 ± 43.56 µg/ml, $n = 64$; Figure 2). After adjusting for age, BMI, hypertension, diabetes, smoking status, alcohol status, FBG, TG, TC, SCr, HDL-C, LDL-C, and Lp(a) in the logistics regression analysis, DHA was found to be significantly associated with the occurrence of CAD without MI [odds ratio (OR) = 0.8917, 95% confidence interval (CI): 0.879–0.957, $p < 0.001$] and MI (OR = 0.835, 95% CI: 0.799–0.862, $p < 0.001$).
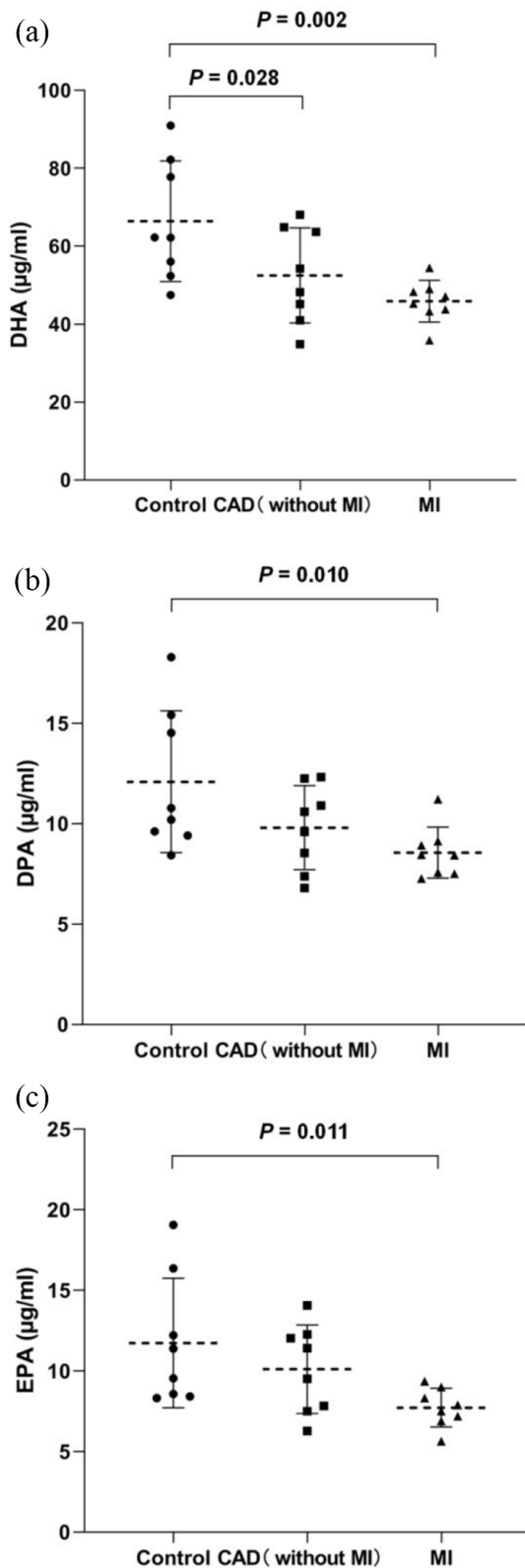
(a)

Figure 1. Serum levels of DHA, DPA, and EPA in different sample pool groups. (a) Compared with the control (66.40 ± 15.48 µg/ml, *n* = 8), serum DHA levels were significantly lower than those in CAD sample pool group (52.50 ± 12.21 µg/ml, *n* = 8, *p* = 0.028) and MI sample pool group (45.85 ± 5.39 µg/ml, *n* = 8, *p* = 0.002). (b) Serum DPA levels in the MI sample pool group (8.56 ± 1.27 µg/ml, *n* = 8) were significantly lower than those in the control samples pool group (12.09 ± 3.53 µg/ml, *n* = 8, *p* = 0.010). (c) Serum EPA levels in the MI sample pool group (7.72 ± 1.20 µg/ml, *n* = 8) were also significantly lower than those in the control samples pool group (11.73 ± 4.02 µg/ml, *n* = 8, *p* = 0.011). CAD, coronary artery disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; MI, myocardial infarction.

(b)

(c)

Figure 1. *(Continued)*

The serum DPA level in the control group (14.12 ± 6.88 µg/ml, *n* = 64) was significantly higher than that in the MI group (9.96 ± 4.23 µg/ml, *n* = 64, *p* < 0.001) and in the CAD (without MI) group (11.91 ± 5.36 µg/ml, *n* = 64, *p* = 0.045; Figure 3). After adjusting for all covariables, serum DPA level was identified to be an independent protective factor for MI (OR = 0.828, 95% CI: 0.791–0.878, *p* < 0.001), but not for CAD without MI (OR = 0.958, 95% CI: 0.917–1.098, *p* = 0.350).

Serum EPA levels in the MI group (8.99 ± 1.76 µg/ml, *n* = 64) were significantly lower than those in the control group (12.83 ± 4.51 µg/ml, *n* = 64, *p* < 0.001). However, the difference was not detected between the control group and the CAD (without MI) group (11.52 ± 3.25 µg/ml, *n* = 64, *p* = 0.062; Figure 4). After adjusting for all factors, the increased serum EPA level was found to be an independent protective factor for MI (OR = 0.848, 95% CI: 0.801–0.895, *p* < 0.001).

Discussion

In the present study, we quantified serum levels of 49 FAs in patients with EOCAD and in healthy controls and identified three FAs that were markedly associated with EOCAD. Results from the validation stage indicated that decreased serum levels of DHA, DPA, and EPA were associated with an increased risk of EOCAD and/or early-onset MI and could be considered independent risk factors for EOCAD.

CAD is a complex multifactorial disease that is influenced by both genetic and environmental

Table 3. Associations between serum DHA, DPA, and EPA levels and the presence of CAD.

Adjustment models		DHA				DPA				EPA			
		CAD (without MI)		MI		CAD (without MI)		MI		CAD (without MI)		MI	
		OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Model 1:	Crude	–	0.029	–	<0.001	–	0.045	–	<0.001	–	0.062	–	<0.001
Model 2:	Adjusting for smoking, FBG, TG, and Lp(a)	0.920 (0.882–0.961)	<0.001	0.845 (0.809–0.891)	<0.001	0.964 (0.921–1.012)	0.127	0.833 (0.798–0.882)	<0.001	0.981 (0.946–1.035)	0.403	0.852 (0.814–0.906)	<0.001
Model 3:	Adjusting for all factors	0.917 (0.879–0.957)	<0.001	0.835 (0.799–0.862)	<0.001	0.958 (0.917–1.010)	0.082	0.828 (0.791–0.878)	<0.001	0.977 (0.928–1.026)	0.364	0.848 (0.801–0.895)	<0.001

CAD, coronary artery disease; CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FBG, fasting blood glucose; Lp(a), lipoprotein(a); MI, myocardial infarction; OR, odds ratio; TG, triglyceride.
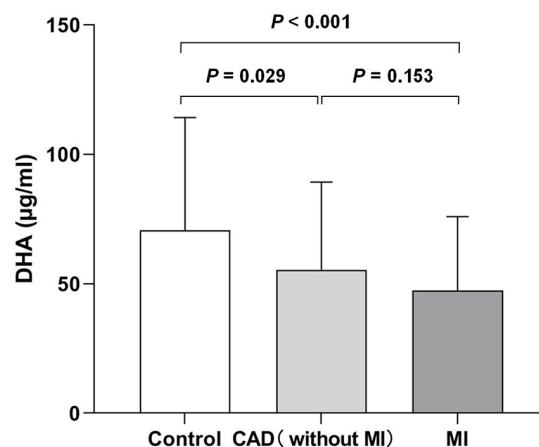

Figure 2. Serum DHA levels in patients with EOCAD and healthy controls. Compared with the controls ($70.65 \pm 43.56\,\mu g/ml$, $n = 64$), serum DHA levels were significantly lower in patients with CAD (without MI) ($55.43 \pm 33.86\,\mu g/ml$, $n = 64$, $p = 0.029$) and early-onset MI ($47.49 \pm 28.44\,\mu g/ml$, $n = 64$, $p < 0.001$). There were no significant differences in serum DHA levels between patients with CAD (without MI) and patients with early-onset MI ($p = 0.153$).
DHA, docosahexaenoic acid; EOCAD, early-onset coronary artery disease; CAD, coronary artery disease; MI, myocardial infarction.
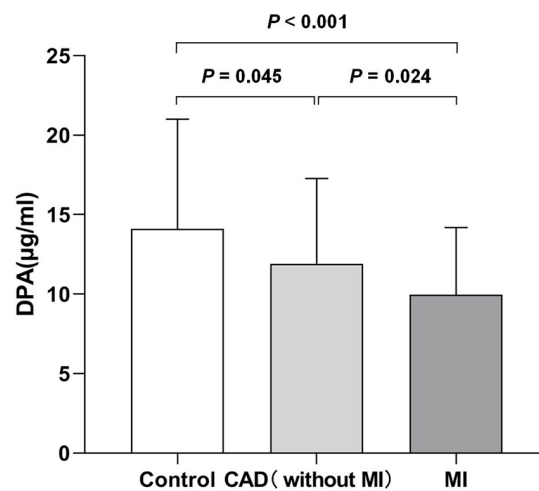

Figure 3. Serum DPA levels in patients with EOCAD and healthy controls. Serum DPA levels in patients with early-onset MI ($9.96 \pm 4.23\,\mu g/ml$, $n = 64$) were significantly lower than those in patients with CAD (without MI) ($11.91 \pm 5.36\,\mu g/ml$, $n = 64$, $p = 0.024$) and controls ($14.12 \pm 6.88\,\mu g/ml$, $n = 64$, $p < 0.001$). Serum DPA levels were slightly lower in patients with CAD (without MI) than in healthy controls ($p = 0.045$).
DPA, docosapentaenoic acid; EOCAD, early-onset coronary artery disease; CAD, coronary artery disease; MI, myocardial infarction.
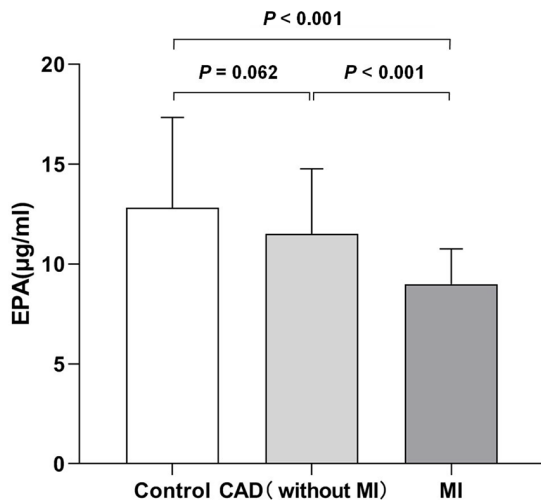
Figure 4. Serum EPA levels in patients with EOCAD and healthy controls. Serum EPA levels in patients with early-onset MI (8.99 ± 1.76 µg/ml, *n* = 64) were significantly lower than those in patients with CAD (without MI) (11.52 ± 3.25 µg/ml, *n* = 64, *p* < 0.001) and controls (12.83 ± 4.51 µg/ml, *n* = 64, *p* < 0.001). There were no significant differences in serum EPA levels between the control and CAD (without MI) patients (*p* = 0.062).
EOCAD, early-onset coronary artery disease; CAD, coronary artery disease; EPA, eicosapentaenoic acid; MI, myocardial infarction.

factors. Although CAD exhibits a wide spectrum of etiologies, atherosclerosis represents the predominant pathogenic process that causes CAD. Atherosclerosis is a progressive chronic inflammatory process characterized by the accumulation of lipids, fibrous elements, and molecules of inflammation in the walls of the large arteries.[12,13] The oxidative modification of LDL is integrally involved in the instability of the atherosclerosis plaque, which triggers a cascade of events leading to the initiation of the atherosclerotic lesion.[14] The early lesions of atherosclerosis comprise accumulations of oxidized low-density lipoprotein (LDL) in macrophages to form foam cells, which serve to perpetuate atherosclerotic plaque formation by releasing chemokines and mitogens that recruit more macrophages, promote the accumulation of lipid-rich necrotic debris, and enhance the migration and proliferation of smooth muscle cells into the intima.[15] Atherosclerotic plaque narrows the coronary arteries and obstructs blood flow, leading to myocardial ischemia, unstable angina, and stroke. The most common acute clinical manifestation is MI, which is caused by the rupture of atherosclerotic plaques, blocking the distal coronary arteries, and ultimately leading to myocardial necrosis. Results of a meta-regression of randomized clinical trials also demonstrate that regression of atherosclerotic coronary plaque volume may represent a surrogate for myocardial infarction and repeat revascularization.[16] The strong association between elevated serum lipid levels and atherosclerosis development has been well-documented.[17] FAs are the important components of lipids and are recognized as essential nutrients for the human body. FAs are biologically active molecules with various cellular processes, including cellular signaling pathways, membrane architecture, cell–cell, cell protein interactions, transcriptional and translational modulation, and energy storage.[18] Depending on the presence of carbon–carbon double bonds, FAs are classified into SFA, monounsaturated fatty acids (MUFAs), and PUFA.[19] SFA does not contain double-bonded carbons. A number of studies in animals have demonstrated that SFAs elevate serum LDL and TC levels by inhibiting the activity of LDL receptors and enhancing apolipoprotein B levels.[20] However, different meta-analyses have provided diverse and conflicting results in humans; thus, the relationship between SFA and CVD risk remains controversial.[21] In this study, no significant differences were detected in serum SFA levels between patients in the EOCAD group and the healthy control group. MUFA has only one double-bonded carbon and is widely present in various foods and can be completely absorbed by the intestines to perform biological functions. Like SFA, the relationship between MUFA and the risk of CVD remains controversial.[22] Yu and co-workers observed that MUFA significantly decreases serum TC and LDL-C and increases serum HDL-C levels.[23] However, Schwingshackl *et al.*[24] conducted a meta-analysis of randomized control trials with a duration of at least 6 months and revealed that high MUFA diet and low MUFA diet had no significant effect on body weight, waist circumference, LDL, HDL, TG, and TC. As well, in several studies where MUFA content exceeds 20% of total energy consumption, no detrimental changes in cardiovascular-related biomarkers have been observed.[25,26] Consistent with these findings, the present study also revealed no associations between serum MUFA levels and EOCAD. PUFA contains more than one unsaturated carbon in its backbone. PUFAs

are divided into two families, ω-3 and ω-6. Among them, the relationship between ω-3 PUFA and the risk of CVD has been extensively studied. There are three main types of ω-3FAs, including EPA, DHA, and ALA. EPA and DHA are commonly found in marine oils, and ALA is found mainly in plant oils. ALA can be obtained only from dietary sources as the enzymes needed to synthesize ALA are lacking in the human body. A study has indicated that approximately 8–20% of ALA can be converted to EPA, and approximately 0.5–9% of ALA is converted to DHA in humans. Observational and retrospective studies have yielded almost the same conclusion, that the intake of ω-3 can significantly reduce the incidence of cardiovascular events.[27] The Diet and Reinfarction Trial (DART) study revealed that eating two to three portions of fatty fish weekly reduced the 2-year all-cause mortality rate among patients with MI by nearly 30%.[28] The GISSI-Prevenzione study also confirmed that the oral administration of ω-3 PUFAs in patients with MI could significantly reduce all-cause mortality and cardiovascular mortality MI.[29] It should be noted that apart from TG, there is still no strong reverse relationship between ω-3 and blood lipids (TC, HDL, and LDL). Therefore, the cardioprotective effect of ω-3 PUFA may not be achieved entirely by regulating blood lipid metabolism. The cardiovascular protective effect of ω-3 PUFAs is due to their actions on the metabolism of lipids, vessels, and platelets, by which they achieve a significant anti-arrhythmic effect, lowered blood pressure, reduced inflammation and improved endothelial dysfunction, increased autonomic vascular tone, and reduced platelet aggregation.[30] In the present study, we also detected a significant decrease in DPA, DHA, and EPA in the serum of patients with EOCAD or early-onset MI.

Patients with EOCAD were younger than 50 years, and the etiology may have some differences compared with late-onset CAD. In recent years, numerous biomarkers have already been identified for EOCAD, including genes, proteins, and other biomacromolecules. We previously demonstrated that the *MTHFR* and *ALMS1* variants were associated with the risk of EOCAD, and serum levels of asymmetric dimethylarginine, uric acid, and adenosine deaminase were associated with the presence and severity of EOCAD.[31–36] Based on serum differential proteomics, our study confirmed an aberrant metabolism of serum lipoproteins (Apo A-I, Apo A-IV, Apo C-I) in EOCAD.[37]

To the best of our knowledge, only a few studies have analyzed the correlation between EOCAD and serum FAs levels, particularly on FA profiles. Recently, Bittner and co-workers have indicated that low levels of EPA and DHA are associated with early-onset coronary atherosclerosis.[38] As well, a study based on a Japanese population confirmed that decreased EPA/arachidonic acid might be a risk factor for the early onset of acute coronary syndrome.[39] In the first stage of the present study, we quantified the serum concentrations of 49 FAs for the first time in patients with EOCAD and screened out three important FAs associated with disease status. These three FAs are DHA, EPA, and DPA, which are all ω-3 PUFA. In the validation stage, we measured the serum levels of the three FAs in 192 subjects. The results indicated that the serum DHA levels in patients with EOCAD were significantly lower than those in the control group. As well, the serum DPA and EPA levels in patients with early-onset MI were significantly lower than those in the control group. Notably, decreased serum DHA levels were an independent risk factor for EOCAD and early-onset MI. Decreased serum DPA and EPA levels were independent risk factors for early-onset MI. Although this was a retrospective case–control study, the detection of lower levels of DHA, EPA, and DPA in the serum of patients with EOCAD and/or early-onset MI cannot be incidental. DHA has been extensively studied. Although the results remain controversial, some CVD studies have considered it a protective factor.[40,41] As one of the ω-3 PUFAs, DPA is a direct intermediate in converting ALA to DHA and EPA. However, due to the difficulty of purification, the physiological role of DPA is not well understood. Recent reviews have summarized the possible roles of DPA associated with cardiovascular protection, mental health, and cancers. It is generally believed that the protective functions of DHA in the cardiovascular system include anti-inflammatory properties, inhibition of cytokine synthesis, decrease in thrombosis, decrease in plasma lipids, and inhibition of atherosclerosis.[42] In this study, we found that serum DHA levels in control patients were significantly higher than EPA and DPA. Besides, the serum levels of DHA were

significantly decreased in patients with EOCAD (including MI); however, the serum levels of DPA and EPA were significantly decreased in patients with early-onset MI. Together, these findings indicate to a certain extent that DHA may be superior to DPA and EPA in conferring cardioprotective effects. We did not detect significant changes in serum ALA levels in patients with EOCAD and these findings were consistent with those of previous studies.

Our study has some limitations. First, this was a retrospective case–control study, and we could not assess the cause and effect of serum FAs levels on the dynamic progression of EOCAD. Second, most study participants were from coastal areas with seawater fish as a major constituent of the diet. Thus, serum PUFA levels may have been affected by their diet. Third, there were only a few female patients with EOCAD in this study. Although there may be differences in serum FAs levels between the genders, subgroup analysis could not be performed. Thus, studies on larger patient cohorts are warranted to further validate the findings of our study.

Conclusions

To summarize, this study provides a profile of serum FAs in patients with EOCAD. Notably, 49 serum FAs were initially screened and quantified, and three PUFAs were identified to be associated with the risk of EOCAD. The results also indicated that low levels of DHA were associated with EOCAD and early-onset MI, and low levels of DPA and EPA were associated only with early-onset MI. Collectively, these findings provide insights for future studies on the diverse characteristics of lipid metabolism and the protective effects of ω-3 PUFAs in patients with EOCAD.

Author contributions

CX and LML designed the study; HL, QWT, and JJG performed the experiments, CX, HL, and JJG analyzed and interpreted the data; CX wrote the manuscript, GWH reviewed and revised the manuscript; all authors approved the final manuscript.

Availability of supporting data

The datasets used and/or analyzed during this current study are available from the corresponding author on reasonable request.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Consent for publication

Informed consent form for publication was obtained from all authors.

Ethics statement

The study was approved by the ethics committee of The Affiliated Hospital of Qingdao University.

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References

1. Ghosh A, Gao L, Thakur A*, et al*. Role of free fatty acids in endothelial dysfunction. *J Biomed Sci* 2017; 24: 50.

2. Lecerf JM. Fatty acids and cardiovascular disease. *Nutr Rev* 2009; 67: 273–283.

3. Baum SJ, Kris-Etherton PM, Willett WC*, et al*. Fatty acids in cardiovascular health and disease: a comprehensive update. *J Clin Lipidol* 2012; 6: 216–234.

4. Shramko VS, Polonskaya YV, Kashtanova EV, *et al*. The short overview on the relevance of fatty acids for human cardiovascular disorders. *Biomolecules* 2020; 10: 1127.

5. Mazidi M, Cicero AF, Kengne AP*, et al*. Association between plasma trans-fatty acid concentrations and measures of glucose homeostasis and cardiovascular risk factors in adults in NHANES 1999-2000. *Angiology* 2018; 69: 630–637.

6. Aung T, Halsey J, Kromhout D*, et al*. Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: meta-analysis

of 10 trials involving 77 917 individuals. *JAMA Cardiol* 2018; 3: 225–234.

7. Kathiresan S, Voight BF, Purcell S, *et al.* Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat Genet* 2009; 41: 334–341.

8. Khera AV, Chaffin M, Zekavat SM, *et al.* Whole-genome sequencing to characterize monogenic and polygenic contributions in patients hospitalized with early-onset myocardial infarction. *Circulation* 2019; 139: 1593–1602.

9. Pang J, Poulter EB, Bell DA, *et al.* Frequency of familial hypercholesterolemia in patients with early-onset coronary artery disease admitted to a coronary care unit. *J Clin Lipidol* 2015; 9: 703–708.

10. Cipriani V, Mannucci PM, Ardissino D, *et al.* Familial aggregation of early-onset myocardial infarction. *Eur J Intern Med* 2010; 21: 511–515.

11. Cole JH, Miller JI 3rd, Sperling LS, *et al.* Long-term follow-up of coronary artery disease presenting in young adults. *J Am Coll Cardiol* 2003; 41: 521–528.

12. Davidson J and Rotondo D. Lipid metabolism: inflammatory-immune responses in atherosclerosis. *Curr Opin Lipidol* 2003; 14: 337–339.

13. van Diepen JA, Berbée JF, Havekes LM, *et al.* Interactions between inflammation and lipid metabolism: relevance for efficacy of anti-inflammatory drugs in the treatment of atherosclerosis. *Atherosclerosis* 2013; 228: 306–315.

14. Colles SM, Maxson JM, Carlson SG, *et al.* Oxidized LDL-induced injury and apoptosis in atherosclerosis. Potential roles for oxysterols. *Trends Cardiovasc Med* 2001; 11: 131–138.

15. Maguire EM, Pearce S and Xiao Q. Foam cell formation: a new target for fighting atherosclerosis and cardiovascular disease. *Vascul Pharmacol* 2019; 112: 54–71.

16. Ascenzo F, Agostoni P, Abbate A, *et al.* Atherosclerotic coronary plaque regression and the risk of adverse cardiovascular events: a meta-regression of randomized clinical trials. *Atherosclerosis* 2013; 226: 178–185.

17. Lewis LA, Olmsted F, Page IH, *et al.* Serum lipid levels in normal persons; findings of a cooperative study of lipoproteins and atherosclerosis. *Circulation* 1957; 16: 227–245.

18. Cascio G, Schiera G and Di Liegro I. Dietary fatty acids in metabolic syndrome, diabetes and cardiovascular diseases. *Curr Diabetes Rev* 2012; 8: 2–17.

19. Vissers L, Rijksen J, Boer J, *et al.* Fatty acids from dairy and meat and their association with risk of coronary heart disease. *Eur J Nutr* 2019; 58: 2639–2647.

20. Dietschy JM. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. *J Nutr* 1998; 128(2 Suppl.): 444S–448S.

21. Siri-Tarino PW, Sun Q, Hu FB, *et al.* Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients. *Curr Atheroscler Rep* 2010; 12: 384–390.

22. Schwingshackl L and Hoffmann G. Monounsaturated fatty acids and risk of cardiovascular disease: synopsis of the evidence available from systematic reviews and meta-analyses. *Nutrients* 2012; 4: 1989–2007.

23. Yu S, Derr J, Etherton TD and Kris-Etherton PM. Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. *Am J Clin Nutr* 1995; 61: 1129–1139.

24. Schwingshackl L, Strasser B and Hoffmann G. Effects of monounsaturated fatty acids on cardiovascular risk factors: a systematic review and meta-analysis. *Ann Nutr Metab* 2011; 59: 176–186.

25. Brehm BJ, Lattin BL, Summer SS, *et al.* One-year comparison of a high-monounsaturated fat diet with a high-carbohydrate diet in type 2 diabetes. *Diabetes Care* 2009; 32: 215–220.

26. Due A, Larsen TM, Mu H, *et al.* Comparison of 3 ad libitum diets for weight-loss maintenance, risk of cardiovascular disease, and diabetes: a 6-mo randomized, controlled trial. *Am J Clin Nutr* 2008; 88: 1232–1241.

27. Bilato C. n-3 fatty acids and cardiovascular disease: the story is not over yet. *Aging Clin Exp Res* 2013; 25: 357–363.

28. Burr ML, Fehily AM, Gilbert JF, *et al.* Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet* 1989; 2: 757–761.

29. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999; 354: 447–455.

30. Oppedisano F, Macrì R, Gliozzi M, *et al*. The anti-inflammatory and antioxidant properties of n-3 PUFAs: their role in cardiovascular protection. *Biomedicines* 2020; 8: 306.

31. Zhang SY, Xuan C, Wang Y, *et al*. Association between ALMS 1 variants and early-onset coronary artery disease: a case-control study in Chinese population. *Biosci Rep* 2020; 40: BSR20193637.

32. Xuan C, Liu ZF, Wang Q, *et al*. Increased serum concentrations of asymmetric dimethylarginine (ADMA) in patients with early-onset coronary artery disease. *Clin Chim Acta* 2017; 464: 195–199.

33. Tian TT, Li H, Chen SJ, *et al*. Serum uric acid as an independent risk factor for the presence and severity of early-onset coronary artery disease: a case-control study. *Dis Markers* 2018; 2018: 1236837.

34. Xuan C, Tian QW, Li H, *et al*. Levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, and risk of coronary artery disease: a meta-analysis based on 4713 participants. *Eur J Prev Cardiol* 2016; 23: 502–510.

35. Zhang SY, Xuan C, Zhang XC, *et al*. Association between MTHFR gene common variants, serum homocysteine, and risk of early-onset coronary artery disease: a case-control study. *Biochem Genet* 2020; 58: 245–256.

36. Xuan C, Tian QW, Zhang SY, *et al*. Serum adenosine deaminase activity and coronary artery disease: a retrospective case-control study based on 9929 participants. *Ther Adv Chronic Dis* 2019; 10: 2040622319891539.

37. Xuan C, Li H, Li LL, *et al*. Screening and identification of pregnancy zone protein and leucine-rich alpha-2-glycoprotein as potential serum biomarkers for early-onset myocardial infarction using protein profile analysis. *Proteomics Clin Appl* 2019; 13: e1800079.

38. Bittner DO, Goeller M, Zopf Y, *et al*. Early-onset coronary atherosclerosis in patients with low levels of omega-3 fatty acids. *Eur J Clin Nutr* 2020; 74: 651–656.

39. Yagi S, Aihara K, Fukuda D, *et al*. Reduced ratio of eicosapentaenoic acid and docosahexaenoic acid to arachidonic acid is associated with early onset of acute coronary syndrome. *Nutr J* 2015; 14: 111.

40. Hite AH, Feinman RD, Guzman GE, *et al*. In the face of contradictory evidence: report of the Dietary Guidelines for Americans Committee. *Nutrition* 2010; 26: 915–924.

41. DeSalvo KB, Olson R and Casavale KO. Dietary guidelines for Americans. *JAMA* 2016; 315: 457–458.

42. Drouin G, Rioux V and Legrand P. The n-3 docosapentaenoic acid (DPA): a new player in the n-3 long chain polyunsaturated fatty acid family. *Biochimie* 2019; 159: 36–48.