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Serum palmitic acid–oleic acid ratio and the risk of coronary artery disease: a case-control study☆

Ling-bing Sun[a,1], Yan Zhang[a,1], Qiang Wang[b,1], Hao Zhang[a], Wei Xu[a], Jie Zhang[b], Jie Xiang[a], Qing-guo Li[b], Yang Xiang[a,*], Dong-jin Wang[b,*], Chen-yu Zhang[a,*]

[a]Jiangsu Diabetes Center, State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, China
[b]Department of Thoracic Surgery, the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210008, China

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Abstract

Serum free fatty acids are risk factors for future coronary artery disease (CAD). We investigated the association between serum palmitic acid (PA)–oleic acid (OA) ratio and CAD risk in a case-control (n=108/129) study. The PA–OA ratio was associated with future CAD events independently of standard lipid values. The PA–OA ratio was significantly associated with the risk of fatal CAD [odds ratio (OR): 60.4; 95% confidence interval (CI): 11.5–316.9; P<.001] while inversely associated in nonfatal CAD group (OR: 0.11; 95% CI: 0.02–0.53; P<.01), and no distinct modification by sex was found. Receiver-operating characteristic (ROC) analysis found that PA–OA ratio did as well as triglyceride (TG) and apolipoprotein B (apo B)–high-density lipoprotein cholesterol (HDLC) ratio at discriminating fatal CAD (area under ROC, TG, 0.692; apo B–HDLC, 0.683; PA–OA, 0.768, P<.001), and had similar effect with HDLC at discriminating nonfatal CADs (area under ROC, HDLC, 0.649; PA–OA, 0.659, P<.01).These findings suggested that PA–OA ratio did as well as and even better than traditional risk factors and arteriography examination in discriminating fatal and nonfatal CAD events. Serum PA–OA ratio could be a new factor for CAD risk assessment and prediction.

Keywords: Serum free fatty acids; PA–OA ratio; Coronary artery disease; Risk assessment

1. Introduction

Serum free fatty acids (FFAs), mainly mobilized form adipose tissue rather than from diet [1] are elevated in obesity, Type 2 diabetes and other insulin resistance states [2,3]. There is accumulating evidence that an increased concentration of FFAs is a risk factor for coronary artery disease (CAD) [4–6]. The negative clinical outcome associated with elevated FFAs in CAD might be attributed to metabolic alterations, such as suppression of glucose use or mitochondrial dysfunctions [7,8]. In addition, there are also studies suggesting that increased FFAs level may cause endothelial dysfunction by facilitating endothelial transfer of low-density lipoprotein (LDL) and cholesterol-rich remnant particles, reducing the endothelial protective properties of albumin, and impairing ability of endothelial cells to inhibit platelet aggregation [9–12].

The most common monounsaturated fatty acid (FA) and saturated FA in plasma are palmitic acid (PA) and oleic acid (OA) [13,14]. Studies showed that elevated OA [15] could promote vascular smooth muscle cells (VSMCs) from contractile to synthetic type, stimulate VSMC proliferation and migration to subendothelium, and contribute to the formation of organized atherosclerotic plaque [16]. In our previous study, PA could prevent OA-induced VSMC proliferation and migration, thus contributing to prevention of atherosclerosis [17]. Despite epidemiological support for different effects of dietary monounsaturated and saturated FAs in CAD [18–20], there is little controlled clinical trial that are designed to compare the effect of monounsaturated and saturated FFAs ratio on CAD end points, especially the effect of PA and OA ratio.

In this case-control study, we explored whether the ratio of particular FAs PA and OA in the serum could be independent marker of CAD risk, or whether the PA–OA ratio is parallel to other traditional risk factors. This work is part of a larger study investigating association between CAD and FFAs composition in serum.

2. Methods and materials

2.1. Study population

This study enrolled 108 individuals with CAD aged from 25 to 74, and 129 age- (up to 5 years older or younger than the cases), sex-, obesity- and diabetes-matched controls in Nanjing, China from 2006 to 2008. Patients were eligible according to

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* Corresponding authors. Tel.: +86 25 83686234; fax: +86 25 83686234.
E-mail address: cyzhang@nju.edu.cn (C-Y. Zhang).
[1] L-B. Sun, Y. Zhang, Q. Wang contributed equally to this work.

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diagnostic symptoms together with electrocardiogram. The control subjects were recruited from the local hospitals in the course of a routine check-up visit. The inclusion criteria for the control group were: no history or diagnosis of atherosclerosis, vascular disease, chronic heart failure and arrhythmias and no pathological electrocardiogram patterns. Treatment with antihypertensive and oral antidiabetic agents was maintained during the study. None of the patients were using hypolipidemic agents. Diabetes was defined as fasting blood glucose ≥7.0 mmol/L or use of antidiabetic agents; obesity was defined as body mass index (BMI)>25; hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive agents. The local Ethics Committee approved the protocol, and patients gave their written informed consent.

2.2. Serum lipids measurements

Peripheral venous blood samples were collected after a 12-hour overnight fast in heparin-treated tubes, and centrifuged at 3000 rpm for 10 min at room temperature to get the serum. The serum separated was immediately frozen at −70°C for later measurement. Serum total cholesterol (TC) [Determiner L TC II (Kyowa Medex, Tokyo, Japan), normal reference <5.18 mmol/L, coefficients of variation (CV) ≤3%], high-density lipoprotein (HDL, Determiner L HDL-C (Kyowa Medex, Tokyo, Japan), normal reference ≥1.04 mmol/L, CV≤4%), and LDL [Determiner L LDL-C (Kyowa Medex, Tokyo, Japan), normal reference <3.37 mmol/L, CV≤4%] were measured on an automatic analyzer (HITACHI 7600-020, Tokyo, Japan). Serum triglyceride (TG) was measured enzymatically (Daiichi Pure Chemicals, Tokyo, Japan, normal reference <1.70 mmol/L, CV ≤5%). Reference levels were established essentially according to the guidelines of the dyslipidemia prevention and treatment of China adult. Serum apolipoprotein AI (apo AI) and apolipoprotein B (apo B) were determined by immunonnephelometry (Ningbo Medical System Biotechnology, Zhejiang, China; CV ≤5%) with calibration traceable to the International Federation of Clinical Chemistry primary standards [21].

2.3. Ratio of PA–OA measurement [22–25]

Blood samples were collected and the serum was separated following the means mentioned above. Followed by incubation at 56°C with sulphuric acid and methanol, the methyl-etherficated FFAs were extracted by a chloroform/water extraction and dried with anhydrous sodium sulphate. Then the extract was evaporated to dryness under nitrogen and re-diluted with appropriate chloroform.

The ratio of PA–OA was measured using a gas chromatograph (HP-6890 Series GC System, Hewlett-Packard, Palo Alto, CA, USA). Separation was performed on a HP-INNOWAX column (30 m×0.25 mm injected dose, 0.25 μm). Operation conditions were as follows: the injection volume of the sample was 1 μl. The split-splitless injector was used with a split ratio of 50:1. The injector and detector temperature were kept at 300°C. The initial column temperature was 160°C, increased at 4°C/min to 250°C and held at this temperature for 5 min (total run time: 27.5 min). Nitrogen was used both as the carrier gas and as the make-up gas. Data acquisition and processing were performed with a HP-Chemstation software for GC systems. The identity peaks of PA–OA were determined by comparison of their relative retention times with those of PA–OA standard sample (the ratio of PA–OA was 1:1, diluted in methanol with a final concentration of 10 μmol/ml). The relative amount of PA–OA was quantified by integrating the area under the peak and dividing the respective area by the total area for all methyl-etherifications. The results were expressed in relative amount (the ratio of PA–OA peak area).

2.4. Coronary arteriography

Selective coronary arteriography of the right and left coronary arteries was performed using the Judkins technique [26] on a Digital Cardiovascular X-ray Imaging System (Innova 2000, GE Healthcare, WI, USA). Coronary artery images were visually assessed independently by two angiographers unaware of other clinical or image data, and the percent diameter stenosis of lesions and their location noted. Clinically significant coronary artery disease (fatal CAD) was diagnosed when there was a narrowing of 70% or more of the diameter of the lumen in the left anterior descending, left circumflex, right coronary artery, or narrowing of 50% or more of the diameter of the lumen in the left main coronary artery. And a narrowing of less than 50% of the diameter of major epicardial vessels was diagnosed as nonfatal CAD (Fig. 1A). In case of a disagreement in the interpretation of a given coronary arteriogram, it was read by a third cardiologist to determine the correct category.

2.5. Statistical analysis

Statistical analyses were performed using SPSS software, version 12.0.1 (SPSS, Chicago, Illinois, USA). A *P* value less than .05 was considered statistically significant. Before being used as continuous variables in the analyses, triglyceride and apo B–apo AI ratio were log-transformed to approach a normal distribution. Baseline characteristics were compared between cases and controls, taking into account the matching. A mixed-effects model was used for continuous variables, and conditional logistic regression was used for categorical variables.

In the analysis, population distributions were computed for each biomarker and Spearman correlation coefficients were used to discern interrelationships between the various lipid fractions and each other, as well as PA–OA ratio. To evaluate the association between a risk factor and occurrence of CAD, odds ratio and 95% confidence interval (CI) were calculated by using conditional logistic regression analysis, taking into account the matching [27,28]. First, we calculated odds ratios of HDL cholesterol (HDLC), apo AI, apo B, PA–OA, TC–HDLC, LDL cholesterol (LDLC)–HDLC, apo B–apo AI and apo B–HDLC by single-variable models analysis in fatal CAD, nonfatal CAD and total subjects. Then, each risk factor in fatal and nonfatal CAD was entered a forward logistic-regression selection model, and odds ratio was calculated respectively.

To evaluate the ability of the PA–OA ratio to predict CAD (i.e., to discriminate between patients who will and will not develop a future CAD event), we constructed receiver-operating characteristic (ROC) curves [29,30] and calculated the areas under the curves (AUCs) from regression models that included PA–OA ratio, apo B–HDLC ratio, TG in fatal CAD, PA–OA ratio and HDLC in nonfatal CAD.

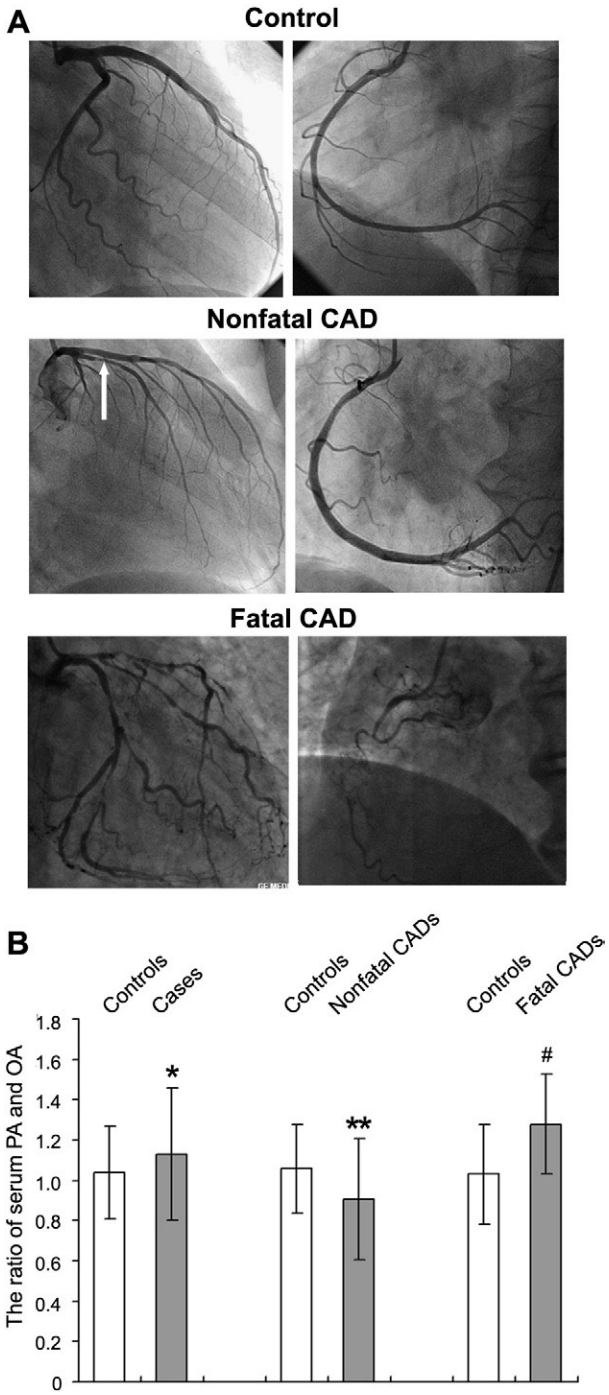


Fig. 1. The ratio of PA and OA differed in CAD subjects. (A) A typical comparison of control, nonfatal and fatal CAD with coronary arteriography of the right and left coronary arteries. (B) Comparisons of serum PA–OA ratio between all cases, fatal cases, nonfatal cases and control groups. $^{*}P<.05$. $^{**}P<.01$. $^{\#}P<.001$ for comparison.

3. Results

3.1. Characteristics of study population

Of the patients, 45 persons (41.3%) had nonfatal events and 63 (58.7%) had fatal events. We were able to match two controls to 21 cases each and one control to 87 cases each by age (within 5 years), sex, obesity (BMI>25) and diabetes (fasting blood ≥7.0 mmol/L or use of antidiabetic agents) (Table 1). Cases were more likely than controls to have hypertension. Systolic blood pressure, diastolic blood pressure, TG and apo B values were statistically significantly higher in cases than in controls, whereas HDLC and apo AI values were significantly lower. Cases had higher TC and LDLC levels, though not statistically significant. The ratios of TC to HDLC, LDLC to HDLC, apo B to apo AI, and apo B to HDLC also was statistically significantly higher in cases. The patterns for these differences were similar when cases were divided into fatal and nonfatal CAD and when men and women were analyzed separately (data not shown).

3.2. The ratio of PA and OA increased in fatal CADs while decreased in nonfatal CADs

In our study, we separated the CAD subjects into fatal and nonfatal events according to diagnostic symptoms together with the arteriography results. Fig. 1A show a typical comparison of control, nonfatal and fatal CAD with coronary arteriography of the right and left coronary arteries. Nonfatal patient only showed a coronary artery stenosis (arrow) in major epicardial vessels and the coronary lesion estimated as less than 50% diameter stenosis by visual assessment, who received medical conservative treatment. Compared with that, fatal CAD showed a narrowing of 70% or more of the diameter of major epicardial vessels, who needed an urgent coronary artery bypass surgery.

Blood samples were collected and the serum was separated. The ratios of serum PA and OA were detected by gas-chromatogram. Data showed that PA–OA ratio was statistically significantly higher in cases than in controls (1.13±0.33 versus 1.04±0.23, $P<.05$ versus controls, Fig. 1B, Table 1). The patterns for these differences were similar when men and women were analyzed separately (data not shown). When cases were divided into fatal and nonfatal CAD, fatal CAD patients also had much significantly higher levels of PA–OA ratio than the controls (1.28±0.25 versus 1.03±0.25, $P<.001$ versus controls, Fig. 1B), however, nonfatal CAD patients had significantly lower levels of PA–OA ratio than control subjects (0.91±0.30 versus 1.06±0.22, $P<.01$ versus controls, Fig. 1B).

Table 1
Characteristics analysis between controls and cases

Characteristic[a]	Controls (n=129)	Cases (n=108)	p value
Men, n (%)	82 (63.6)	74 (68.5)	Matched
Mean age (S.D.), y	61.02 (10.61)	62.83 (10.62)	Matched
Mean body mass index (S.D.), kg/m^2	23.37 (3.42)	24.13 (3.53)	Matched
Persons with diabetes, n (%)	19 (14.7)	19 (17.6)	Matched
Persons with hypertension, n (%)	17 (13.2)	39 (36.1)	<.01
Mean systolic blood pressure (S.D.), mm Hg	126.35 (11.4)	132.47 (17.01)	<.01
Mean diastolic blood pressure (S.D.), mm Hg	75.24 (10.57)	78.43 (11.32)	<.05
Median TG level (IQR)[b], mmol/L	1.19 (0.83–1.61)	1.46 (1.10–2.10)	<.001
Mean TC level (S.D.), mmol/L	4.07 (0.84)	4.18 (1.21)	.45
Mean HDLC level (S.D.), mmol/L	1.00 (0.24)	0.86 (0.23)	<.001
Mean LDLC level (S.D.), mmol/L	2.21 (0.48)	2.26 (0.83)	.57
Mean apo B level (S.D.), g/L	0.71 (0.21)	0.81 (0.25)	<.01
Mean apo AI level (S.D.), g/L	1.19 (0.31)	1.06 (0.29)	<.01
Median TC–HDLC ratio (IQR)[b]	4.10 (3.46–4.85)	4.94 (3.69–5.9)	<.001
Mean LDLC–HDLC ratio (S.D.)	2.31 (0.69)	2.70 (1.05)	<.01
Median apo B–apo AI ratio (IQR)[b]	0.57 (0.45–0.77)	0.79 (0.57–0.97)	<.001
Median apo B–HDLC ratio (S.D.)	0.75 (0.27)	0.99 (0.35)	<.001

IQR, interquartile range.
[a] Case-patients and age-, sex-, obesity- and diabetes-matched controls were compared by using conditional logistic regression for categorical variables and a mixed-effects model for continuous variables.
[b] TG and apo B–apo AI ratios were log-transformed before analysis.

3.3. Analysis of Spearman correlation coefficients between measured study variables

Table 2 presents the Spearman correlation coefficients between each lipid parameter. As expected, strong correlations were observed between TC and LDLC (r=0.902), TC and apo AI (r=0.856), LDLC and apo AI (r=0.851), HDLC and apo B (r=0.667), TG and apo AI (r=0.481) and most importantly between LDLC and TC (r=0.91). We also found significant correlations among TG, TC, HDLC, LDLC and apo B (Table 2), ranging from 0.20 to 0.39. HDLC was inversely associated with TG (r=0.218). The correlation coefficients between PA–OA ratio and the measured lipid parameters were r=0.139 for TG, r=−0.001 for TC, r=−0.053 for HDLC, r=−0.012 for LDLC, r=−0.036 for apo B and r=0.079 for apo AI. These data showed that PA–OA ratio was only weakly associated with the other markers (Table 2). We also analyzed the correlations between PA–OA, TC-HDLC, LDLC-HDLC, apo B-apo AI, and apo B-HDLC ratio. Results showed strong correlations among TC-HDLC ratio, LDLC-HDLC ratio, apo B-apo AI ratio, and apo B-HDLC ratio, ranging from 0.71 to 0.93 (data not shown), but PA–OA ratio was still weakly associated with these lipid ratios (data not shown). The patterns for all these correlations were similar when cases were divided into fatal and nonfatal CAD and when men and women were analyzed separately (data not shown).

3.4. Odds ratios analysis for CAD associated with PA–OA ratio and other risk factors by single-variable models analysis

After adjustment for age, history of CAD in first-degree relatives, body mass index, and physical activity, all variables were introduced into single-variable models, and the significance is listed in Table 3. The odds ratio (OR) for future CAD increased on PA–OA ratio, triglyceride level, and apo AI level, and it decreased on HDLC and apo B level (Table 3, $P<.01$ for all) in all subjects. Estimate of risk was highest for apo B [OR,4.47 [95% CI, 0.95–21.1]; $P<.05$] and lowest for HDLC [OR, 0.09 (95% CI, 0.03–0.29); $P<.001$]. Similar patterns were observed when fatal and nonfatal cases were analyzed separately, except the PA–OA ratio (Table 3). The odds ratio for CAD associated with PA–OA ratio markedly increased in fatal CAD (OR, 60.4 [95% CI, 11.5–316.9]; $P<.001$) but decreased in nonfatal CAD [OR, 0.11 (95% CI, 0.02–0.53); $P<.01$]. On the other side, by combining information of two lipid risk factors into a single clinical variable, all these lipid ratios provided stronger evidence of association than any single lipid factor. Comparative data on risk associated with the ratios of TC to HDLC, LDLC to HDLC, apo B to apo AI and apo B to HDLC appears in Table 3. Data showed that odds ratio for future CAD increased of all these lipid risks. Estimate of risk was highest for apo B-HDLC ratio [OR, 11.56 (95% CI, 3.35–39.9); $P<.001$; in all subjects]. Separate analyses for fatal CAD and nonfatal CAD yielded similar patterns (Table 3).

3.5. OR analysis for CAD associated with PA–OA ratio and other risk factors by multiple logistic-regression

All lipid variables listed in Table 2 and PA–OA ratio were introduced into the forward, stepwise, multiple logistic-regression model. By forward selection, we obtained a final logistic- regression model with three independent variables for fatal and nonfatal CAD (Table 4A and C). The most important one is PA–OA ratio, which was

Table 2
Spearman correlation coefficients between measured study variables[a]

	PA–OA ratio	Triglyceride	Cholesterol			Apolipoprotein	
			Total	HDL	LDL	B	AI
PA–OA ratio	1.0	0.139[a]*,**	−0.001	−0.053	−0.012	−0.036	0.079
Triglyceride		1.0	0.388**	−0.218**	0.306**	0.02	0.481**
Cholesterol							
Total			1.0	0.351**	0.902**	0.380**	0.856**
HDL				1.0	0.198**	0.667**	0.068
LDL					1.0	0.229**	0.851**
Apolipoprotein							
B						1.0	0.109
AI							1.0

[a] Correlation is expressed as Spearman correlation coefficient.
* $P<.05$ for comparison.
** $P<.01$ for comparison.

into the regression model in the first step and maintained the significance until the final step in both fatal and nonfatal CAD. PA–OA ratio for fatal CAD had the highest chi-square value (17.94, $P<.001$), followed by HDLC (6.04, $P<.05$) and TG (5.43, $P<.05$). For nonfatal CAD, apo B had the highest chi-square value (10.1, $P<.01$), followed by PA–OA ratio (8.03, $P<.01$) and LDLC (5.85, $P<.05$). Furthermore, PA–OA ratio and other ratios of 2 lipid risk factors were also introduced into the multiple logistic-regression models. Table 4B showed that PA–OA ratio for fatal CAD had the highest chi-square value (17.99, $P<.001$), followed by apo B–HDLC (13.02, $P<.001$) and LDLC–HDLC ratio (7.26, $P<.001$). In nonfatal patients, PA–OA ratio still had significantly high chi-square value (6.30, $P<.05$), following apo B-HDLC ratio (10.11, $P<.01$, Table 4D). It is worth to mention that the odds ratio of PA–OA ratio was over 1.0 in fatal CAD [Table 4A, OR, 41.90 (95% CI, 7.44–235.9); Table 4B, OR, 50.63 (95% CI, 8.26–310.4); $P<.001$] but under 1.0 in nonfatal CAD [Table 4C, OR, 0.078 (95% CI, 0.013–0.455); $P<.01$; Table 4D, OR, 0.12 (95% CI, 0.021–0.624); $P<.05$], which was similar with results in single-variable models analysis. All these results suggested that PA–OA ratio was statistically significant in CAD risk assessment compared with other lipid risks, and may contribute to the risk assessment of both nonfatal and fatal CAD.

3.6. Discriminative ability of PA–OA ratio by ROC curve analysis

The capacity of PA–OA ratio to differentiate the presence from the absence of fatal and nonfatal CAD was assessed with a ROC curve analysis (Fig. 2). The area under the ROC curve (AUC) when PA–OA ratio was used to differentiate fatal CAD was 0.768 (95% CI, 0.689–0.848; $P<.001$). The performance of PA–OA ratio was as good as that of TG (AUC, 0.692; 95% CI, 0.603–0.781; $P<.001$) and apo B-HDLC ratio (AUC, 0.683; 95% CI, 0.592–0.775; $P<.001$) (Fig. 2A). When PA–OA ratio was used to discriminate nonfatal CAD, the odds ratio for nonfatal CAD was contrary with that of fatal CAD, and the AUC (AUC, 0.659; 95% CI, 0.546–0.773; $P<.01$) was as good as that of HDLC (AUC, 0.649; 95% CI, 0.541–0.758; $P<.05$) (Fig. 2B).

4. Discussion

In this study, we have shown that the ratio of serum nonesterified PA and OA, which is the most common monounsaturated FA and saturated FA in serum, is valuable in CAD risk assessment. The PA–OA ratio was associated with future CAD events independently of standard lipid values. The PA–OA ratio was significantly associated with the risk of fatal CAD (OR: 60.4; 95% CI: 11.5–316.9; $P<.001$) while inversely associated in nonfatal CAD group (OR: 0.11; 95% CI: 0.02–0.53; $P<.01$), and no distinct modification by sex was found.

CAD has a high incidence of mortality among women and men in most industrialized countries [31]. It is the leading cause of death from cardiovascular disease, with about half of the cases occurring in persons without clinically diagnosed heart disease [32]. Although the prevention of CAD in the community remains a challenge, there is growing evidence that the composition of serum free FAs can influence the risk of CAD and other cardiovascular diseases [33–36]. In this study, we showed a simple and noninvasive way to predict CAD by measuring serum PA–OA ratio. The procedure comprises of methylation of serum FFAs and measuring PA–OA ratio by gas chromatographic analysis, which is rapid, is inexpensive and needs a minimum of 0.5 ml serum, suitable for a routine check-up visit. It is worth mentioning that we didn't focus on the optimal procedure of measuring PA–OA ratio, so further work would be needed on optimization in a clinical setting.

The major finding of this case-control study was that the ratio of serum PA–OA was associated with the risk for CAD. As far as we know, no such findings of serum free FA ratio have been published

Table 3
Odds ratios[a] and 95% CI for CAD associated with PA–OA ratio and other risk factors in single-variable model analysis

Risk factors	Fatal CADs		Nonfatal CADs		All subjects	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
PA–OA ratio	60.4 (11.5–316.9)	<.001	0.11 (0.02–0.53)	<.01	2.96 (1.17–7.50)	<.05
Individual lipid variables						
Triglyceride	2.59 (1.47–4.59)	<.01	1.89 (1.08–3.33)	<.05	2.23 (1.50–3.31)	<.001
HDLC	0.09 (0.02–0.39)	<.001	0.08 (0.01–0.67)	<.05	0.09 (0.03–0.29)	<.001
apo AI	0.23 (0.07–0.71)	<.05	0.29 (0.07–1.25)	.1	0.25 (0.10– 0.61)	<.01
apo B	4.47 (0.95–21.1)	<.05	9.61 (1.61–57.3)	<.05	6.63 (2.16–20.42)	<.01
Lipid ratios						
TC–HDLC	1.56 (1.17–2.07)	<.01	1.64 (1.17–2.31)	<.01	1.59 (1.28–1.98)	<.001
LDLC–HDLC	1.53 (1.05–2.24)	<.05	1.89 (1.15–3.13)	<.05	1.64 (1.21–2.23)	<.01
apo B–apo AI	5.57 (1.69–18.4)	<.01	8.92 (1.97–40.3)	<.01	6.71 (2.62–17.15)	<.001
apo B–HDLC	11.56 (3.35–39.9)	<.001	10.2 (2.60–39.7)	<.01	10.9 (4.37–27.3)	<.001

[a] Odds ratios and 95% CI from the single-variable logistic regression model adjusted for age, history of CAD in first-degree relatives, body mass index, and physical activity.

Table 4
Odds ratios[a] and 95% CI for CAD associated with PA–OA ratio and other risk factors by forward logistic-regression selection

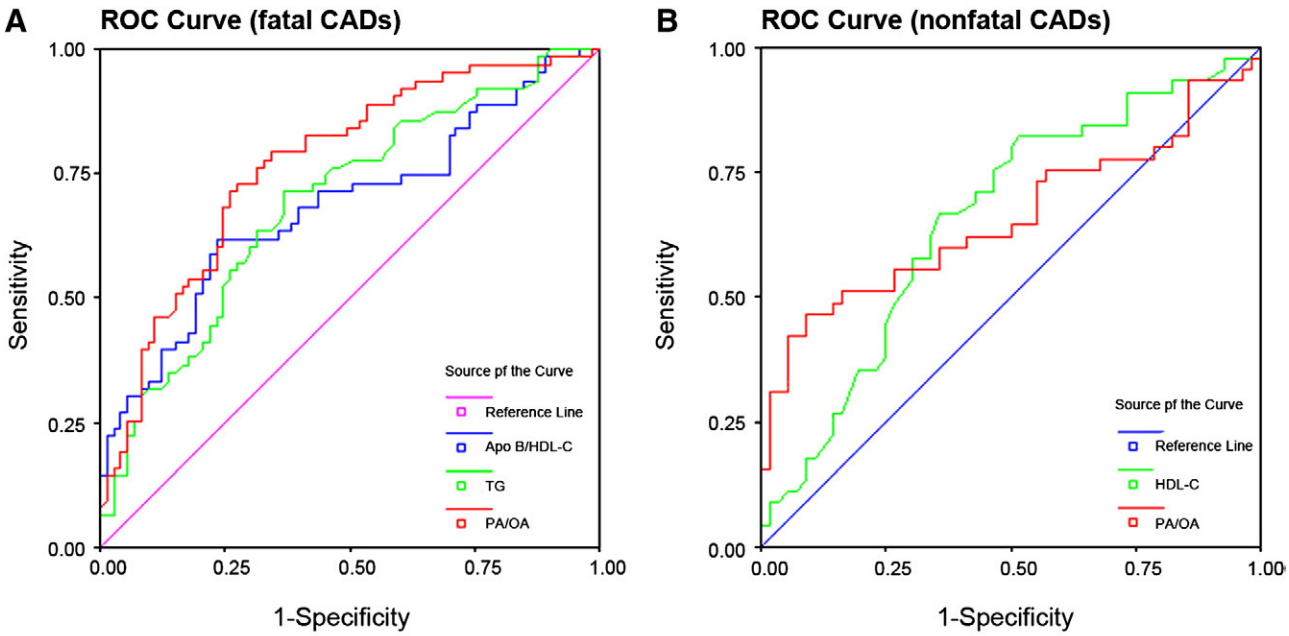
		B	S.E.	Wald	*df*	Sig.	OR	95.0%C.I. for OR	
								Lower	Upper
A. OR and 95% CI for fatal CAD associated with PA–OA *ratio and other lipid factors*									
Step 1	PA–OA	4.100	.846	23.494	1	.000	60.370	11.501	316.896
	Constant	−4.889	1.005	23.655	1	.000	.008		
Step 2	PA–OA	4.024	.874	21.200	1	.000	55.952	10.088	310.322
	HDLC	−2.377	.875	7.374	1	.007	.093	.017	.516
	Constant	−2.620	1.249	4.398	1	.036	.073		
Step 3	PA–OA	3.735	.882	17.945	1	.000	41.898	7.441	235.912
	TG	.744	.319	5.431	1	.020	2.105	1.126	3.936
	HDLC	−2.216	.902	6.035	1	.014	.109	.019	.639
	Constant	−3.567	1.353	6.953	1	.008	.028		
B. OR and 95% CI for fatal CAD associated with PA–OA *ratio and ratios of other two risk lipids*									
Step 1	PA–OA	4.100	.846	23.494	1	.000	60.370	11.501	316.896
	Constant	−4.889	1.005	23.655	1	.000	.008		
Step 2	PA–OA	3.798	.876	18.808	1	.000	44.629	8.019	248.391
	apo B–HDLC	2.128	.698	9.293	1	.002	8.401	2.138	33.008
	Constant	−6.389	1.207	28.029	1	.000	.002		
Step 3	PA–OA	3.924	.925	17.988	1	.000	50.625	8.256	310.439
	LDLC–HDLC	−1.326	.492	7.263	1	.007	.265	.101	.696
	apo B–HDLC	5.717	1.584	13.024	1	.000	303.863	13.625	6776.647
	Constant	−6.331	−1.258	25.320	1	.000	.002		
C. OR and 95% CI for nonfatal CAD associated with PA–OA *ratio and other lipid factors*									
Step 1	PA–OA	−2.228	.816	7.464	1	.006	.108	.022	.533
	Constant	1.983	.827	5.757	1	.016	7.267		
Step 2	PA–OA	−2.233	.844	7.008	1	.008	.107	.021	.560
	apo B	2.249	.938	5.751	1	.016	9.480	1.508	59.588
	Constant	.289	1.082	.072	1	.789	1.336		
Step 3	PA–OA	−2.556	.902	8.027	1	.005	.078	.013	.455
	LDLC	−1.842	.762	5.849	1	.016	.158	.036	.705
	apo B	6.020	1.894	10.098	1	.001	411.529	10.043	16863.462
	Constant	1.961	1.341	2.138	1	.144	7.107		
D. OR and 95% CI for nonfatal CAD associated with PA–OA *ratio and ratios of other two risk lipids*									
Step 1	apo B–HDLC	2.319	.695	11.132	1	.001	10.162	2.603	39.675
	Constant	−2.180	.624	12.192	1	.000	.113		
Step 2	PA–OA	−2.126	.859	6.299	1	.012	.116	.021	.624
	apo B–HDLC	2.301	.723	10.114	1	.001	9.983	2.418	41.217
	Constant	−.014	1.035	.000	1	.989	.986		

[a] Odds ratios and 95% CI from a forward, stepwise, logistic-regression model adjusted for age, history of CAD in first-degree relatives, body mass index and physical activity.

before. A series of studies have confirmed a strong relation between dietary FA composition and a population's coronary heart disease (CHD) rate [37–39]. Populations that consumed high amounts of saturated FAs, including PA, had a high risk for CAD [18,19,40], consistent with the findings that PA in the serum cholesterol ester and phospholipids composition is directly associated with CHD risk [35,41], whilst monounsaturated FA intake, including OA, had received increased attention as being potentially beneficial for risk reduction of CAD [18–20]. However, little controlled clinical trial is designed to elucidate the effect on CAD of FFAs composition reflecting FA synthesis and metabolism rather than FA composition of serum lipid esters mirroring the dietary FA pattern [42]. Our study on ratio of nonesterified PA and OA, which are the most abundant monounsaturated FA and saturated FA in plasma, respectively, fills up the lack of data.

To evaluate the association between PA–OA ratio and CAD events, we compared it with other important risk factors of CAD. We found that the PA–OA ratio was associated with CAD cases independently of standard lipid values and two lipid ratios. In single-variable models, estimate of CAD risk in all subjects was highest for apo B and lowest for HDLC, although the odds ratio for future CAD also increased of PA–OA ratio. When cases were divided into fatal and nonfatal events, the odds ratio for fatal CAD associated with PA–OA ratio markedly increased, which was higher than any other lipid risk factors and lipid ratios. For nonfatal CAD, the odds ratio associated with PA–OA ratio decreased, which was as low as that of HDLC. These analyses suggest that PA–OA ratio had the same importance as traditional lipid risk factors in CAD risk assessment, especially when cases are divided into fatal and nonfatal events. When we further introduced PA–OA ratio into the forward, stepwise, multiple logistic-regression model, PA–OA ratio was into the regression model in the first step and maintained the significance until the final step in both fatal and nonfatal CAD, demonstrating that the most important variable in CAD risk assessment was PA–OA ratio compared with other risk factors. Actually, when we used ROC analysis to evaluate whether and to what extent this apparent advantage of the PA–OA ratio was transformed into CAD risk prediction improvement, we found that it did as well as TG and apo B-HDLC ratio at discriminating fatal CAD, and had similar effect with HDLC at discriminating nonfatal CAD. All these analyses suggest that the PA–OA ratio could be a valuable alternative to traditional lipid-based variables for assessing risk for CAD.

Studies showed that elevated OA [15] could promote vascular smooth muscle cells (VSMCs) from contractile to synthetic type, stimulate VSMC proliferation and migration to subendothelium and contribute to the formation of organized atherosclerotic plaque [16]. In our previous study, we showed that OA could induce VSMC proliferation and migration by inhibiting PPARγ (peroxisome proliferator-activated receptor γ) coactivator-1 alpha (PGC-1α) expression, while PA could stimulate PGC-1α expression, thus attenuate the effect of OA and contribute to prevention of atherosclerosis [17]. Molar ratio of 1:2 OA:PA with a final concentration of 0.4 mmol/L which mimics the high FA under



Area Under the Curve

Test Result Variable(s)	Area
PA/OA	.768
TG	.692
apo B/HDL-C	.683

Area Under the Curve

Test Result Variable(s)	Area
PA/OA ratio	.659
HDL cholesterol	.649

Fig. 2. ROC curves for PA–OA ratio and other risk factors in fatal and nonfatal CAD. (A) Results of models using PA–OA ratio, TG or apo B-HDLC in fatal CAD. (B) Results of models using PA–OA ratio or HDLC in nonfatal CAD.

pathophysiological state stimulated endogenous PGC-1α expression and suppressed VSMC proliferation and migration to a quiescent level in vitro. Hereby, we designed this study to further elucidate the in-vivo effect of PA–OA ratio on cardiovascular system. Fatal and nonfatal CAD are two stages of the CAD development. In clinical treatment, an operation is needed for fatal CAD. To nonfatal cases, it will develop into fatal level without medical care. It is interesting that we also compared the PA/OA ratio between obese subjects (BMI >25) and controls and found a similar pattern as between the nonfatal CAD group and controls (data not shown), suggesting that the obese are at high risk for nonfatal CAD. All these findings imply that when some factor like obesity decreases PA–OA ratio, then relatively high amount of OA could keep inducing VSMC proliferation and migration, contributing to the development of CAD. And if CAD develops into a fatal stage, a feedback response may be involved to elevate PA–OA ratio, and thus, relatively high amount of PA could prevent the promotion effect of OA and pathological process of CAD, although it might be too late. However, the mechanism altering the PA–OA ratio was largely unknown. PGC-1α is originally identified as a transcriptional coactivator of PPARγ, of which the basic biochemical function is regulation of mitochondrial biogenesis. A possible hypothesis of PA and OA affecting VSMC proliferation and migration is that PA stimulates PGC-1α expression, thus increasing PPARγ expression, and negatively regulates ERK MAPK pathway, consequently inhibits OA-induced VSMC proliferation and migration.

Our study has several limitations. First, the relation between PA level, OA level, or PA–OA ratio and future CAD in this subgroup could be different. However, we cannot discriminate the effect of PA and OA separately on CAD risk assessment, as we only detected the relative amount of serum PA and OA. Second, persons with diabetes or the metabolic syndrome are at high risk for CAD. Our investigation contained only few diabetic participants, and information was insufficient to determine the presence of the metabolic syndrome. Therefore, our findings do not apply to these patients.

In conclusion, we observe a modest association of PA–OA ratio with CAD, a strong positive association of PA–OA ratio with fatal CAD, and a strong negative association of PA–OA ratio with nonfatal CAD. These associations need to be confirmed in future studies that distinguish the effect of PA and OA on risk of CAD.

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