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Erythrocyte membrane *trans*-fatty acid index is positively associated with a 10-year CHD risk probability

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Abstract

Industry-generated *trans*-fatty acids (TFA) are detrimental to risk of CHD, but ruminant-originated TFA have been reported as neutral or equivocal. Therefore, the total TFA amount should not be the only factor considered when measuring the effects of TFA. In the present study, we addressed whether a version of the TFA index that unifies the effects of different TFA isomers into one equation could be used to reflect CHD risk probability (RP). The present cross-sectional study involved 2713 individuals divided into four groups that represented different pathological severities and potential risks of CHD: acute coronary syndrome (ACS, *n* 581); chronic coronary artery disease (CCAD, *n* 631); high-risk population (HRP, *n* 659); healthy volunteers (HV, *n* 842). A 10-year CHD RP was calculated. Meanwhile, the equation of the TFA index was derived using five TFA isomers (*trans*-16 : 1*n*-7, *trans*-16 : 1*n*-9, *trans*-18 : 1*n*-7, *trans*-18 : 1*n*-9 and *trans*-18 : 2*n*-6*n*-9), which were detected in the whole blood, serum and erythrocyte membranes of each subject. The TFA index and the 10-year CHD RP were compared by linear models. It was shown that only in the erythrocyte membrane, the TFA isomers were significantly different between the groups. In the ACS group, industry-generated TFA (*trans*-16 : 1*n*-9, *trans*-18 : 1*n*-9 and *trans*-18 : 2*n*-6*n*-9) were the highest, whereas ruminant-originated TFA (*trans*-16 : 1*n*-7 and *trans*-18 : 1*n*-7), which manifested an inverse relationship with CHD, were the lowest, and vice versa in the HV group. The TFA index decreased progressively from 7·12 to 5·06, 3·11 and 1·92 in the ACS, CCAD, HRP and HV groups, respectively. The erythrocyte membrane TFA index was positively associated with the 10-year CHD RP (R^2 0·9981) and manifested a strong linear correlation, which might reflect the true pathological severity of CHD.

Key words: ***Trans*-fatty: acid index: CHD: Erythrocyte membranes**

The effect of blood *trans*-fatty acid (TFA) levels on human diseases has recently aroused considerable attention[1–3]. Chavarro *et al.*[4] reported that the whole-blood TFA level was associated with an increased risk of non-aggressive prostate tumour. Chajes *et al.*[5] showed that a high serum level of TFA contributed to the risk of invasive breast cancer in women. Benatar *et al.*[6] proposed that plasma total TFA may be associated with vascular disease and increased C-reactive protein in patients with severe coronary artery disease. Meanwhile, Lemaitre *et al.*[7] found that a high erythrocyte membrane TFA level was correlated with an increased risk of sudden cardiac arrest.

It is well accepted that TFA are highly associated with CHD risk, and different kinds of TFA isomers play different roles in CHD events[8,9]. Industry-originated TFA, such as *trans*-16 : 1*n*-9, *trans*-18 : 1*n*-9 and *trans*-18 : 2*n*-6*n*-9, are considered to have deleterious effects on cardiovascular health[2,10,11], while TFA from ruminant sources are associated with a slightly neutral risk[12], because *trans*-16 : 1*n*-7 and *trans*-18 : 1*n*-7, the dominant TFA isomers in milk, can be biotransformed to conjugated linoleic acid (CLA) such as 9-*cis*, 11-*trans*-18 : 2*n*-6 CLA by Δ9-desaturase, which is conducive to anticancer, anti-diabetic and anti-CHD effects[13]. Therefore, the effects of TFA on CHD vary in differently originated TFA, and it may be irrational to estimate the effects of TFA simply by considering the total amount of TFA levels. However, if there were combined parameters, taking into account both industry-originated TFA and ruminant-sourced TFA, the effects

Abbreviations: ACS, acute coronary syndrome; CCAD, chronic coronary artery disease; CLA, conjugated linoleic acid; HRP, high-risk population; HV, healthy volunteers; RP, risk probability; TFA, *trans*-fatty acids.

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of TFA on CHD could be estimated according to different TFA isomers and their own physico-chemical characteristics.

In the present study, we explored a TFA index that unifies the disparate impacts of different TFA isomers on CHD by discriminating the different effects into one equation. Meanwhile, a 10-year CHD risk probability (RP) was also calculated in the present cross-sectional study to represent different pathological severities and potential risks of CHD. The index may be used to confirm the presence of a true association between TFA and CHD. The aims of the present study were to verify whether the TFA index was associated reliably with the 10-year CHD RP and to identify which study sample (whole blood, serum or erythrocyte membrane) could truly reflect the change in body TFA levels.

Methods

Ethical statement

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Institutional Ethics Committee, the First and Second Affiliated Hospital, Nanchang University, China. Written informed consent was obtained from all subjects/patients.

Patients

In the present study, we divided CHD into two relatively independent clinical pathological forms: a severely harmful acute coronary syndrome (ACS), which is manifested by myocardial infarction and unstable angina, and a chronic/stable form known as chronic coronary artery disease (CCAD), which has a phenotype of stable angina and ischaemic cardiomyopathy[14]. The ACS and CCAD patients were selected to represent an acute and a chronic CHD pathology, respectively. A high-risk population (HRP) and healthy volunteers (HV) served as controls for CHD patients in the ACS and CCAD groups. Thus, the four groups represented different pathological severities and potential risks of CHD.

A cross-sectional survey on six large physical populations of 12 380 individuals was conducted at the First and Second Affiliated Hospitals, Nanchang University between 2007 and 2010. Excluding confounding factors and other diseases, 2713 individuals were screened in the ACS (*n* 581), CCAD (*n* 631), HRP (*n* 659) and HV (*n* 842) groups to represent different pathological severities and potential risks of CHD. The ACS was diagnosed on the basis of pre-specified criteria for acute myocardial infarction or unstable angina[15,16]. The CCAD patients were selected on the basis of the objective clinical and diagnostic criteria of the American Heart Association[14,17]. The HRP of CHD was classified on the basis of the International Statistical Classification of Diseases codes and previous studies[18,19]. Exclusion criteria included: (1) inadequate multi-detector computed tomography imaging, due to heavily calcified lesions by visual estimation; (2) a culprit lesion in the left main coronary artery; (3) atrial fibrillation; (4) malignant disease; (5) dialysis; (6) diabetes mellitus; (7) renal insufficiency.

Blood sample preparation

After an overnight fasting of the participants, blood (5 ml) was drawn from the antecubital vein into vacutainer tubes containing ethylene diamine tetraacetic acid. Serum was separated from erythrocytes by centrifugation at 2000 ***g*** at 4°C for 10 min. Erythrocytes were washed three times with ice-cold isotonic saline to remove the buffy coat. Membranes were isolated by a modification of Burton's method[20]. Briefly, packed cells were lysed with cold distilled water, centrifuged at 20 000 ***g*** at 10°C for 20 min, and washed several times to eliminate Hb residues. Whole-blood, serum and erythrocyte membrane samples from each participant were stored at −80°C under liquid N_2 until lipid extraction.

Extraction and analysis of fatty acids

Lipids were extracted by chloroform–methanol (1:1) and methylated by sodium methoxide as described previously[21]. Fatty acid methyl esters were analysed by a gas chromatograph (GC 6890 N; Agilent) equipped with a flame ionisation detector, an autosampler injector (7683-B; Agilent) and a fused silica capillary column (CP-Sil 88, 100 m × 0·25 mm inner diameter, 0·20 μm film thickness; Varian). The temperature was held at 45°C for 4 min, and then ramped to 175°C at a flow rate of 13°C/min, held for 27 min, and, finally, increased to 215°C at a flow rate of 4°C/min, held for 35 min.

The levels of five TFA (*trans*-16 : 1*n*-7, *trans*-16 : 1*n*-9, *trans*-18 : 1*n*-7, *trans*-18 : 1*n*-9 and *trans*-18 : 2*n*-6*n*-9) were calculated from the GC results using normalisation and internal standard methods, as described previously[22]. The results were compared with standard fatty acid methyl esters (GLC-463; Nu-Chek Prep, Inc.), with 21 : 0 fatty acid methyl esters added. Fatty acid profiles were expressed as the percentage of TFA. The TFA index was defined by the following equation:

$$\text{TFA index} = \exp\left[\sum \frac{\textit{trans}\text{-16:1}n\text{-9}+\textit{trans}\text{-18:1}n\text{-9}+\textit{trans}\text{-18:2}n\text{-6}n\text{-9}}{\textit{trans}\text{-16:1}n\text{-7}+\textit{trans}\text{-18:1}n\text{-7}}\right]. \quad (1)$$

In this equation, the numerator adds the levels of industrial TFA (*trans*-16 : 1*n*-9, *trans*-18 : 1*n*-9 and *trans*-18 : 2*n*-6*n*-9) together, and the denominator adds the levels of ruminant TFA (*trans*-16 : 1*n*-7 and *trans*-18 : 1*n*-7) together. An exponential function was used to make the index stronger and more obvious as the TFA index.

Characteristic data

Serum lipids (TAG, total cholesterol and HDL), apoA (mainly included apoAI), apoB (included both apoB100 and apoB48) and lipoprotein α were measured in the hospital clinical laboratory with an automatic biochemistry analyser (Beckman

Table 1. Characteristics of study participants (*n* 2713)

(Mean values and standard deviations; number of patients and percentages)

	ACS (*n* 581)				CCAD (*n* 631)				HRP (*n* 659)				HV (*n* 842)				
	Mean	SD	*n*	%	Mean	SD	*n*	%	Mean	SD	*n*	%	Mean	SD	*n*	%	*P*
Age (years)	56·78[c]	19·78			48·21[a,b]	8·23			52·99[b]	12·11			46·93[a]	9·34			<0·05
Male			371	63·86[a]			412	65·29[b]			420	63·73[b]			509	60·45[c]	<0·05
Tobacco use																	
Current (%)				20[a]				23[a]				40[b]				20[a]	<0·001
Ex (%)				69[c]				31[a,b]				37[b]				28[a]	<0·001
BMI (kg/m^2)	26·83[c]	2·11			25·30[b,c]	1·98			23·01[b]	0·95			21·81[a]	1·09			<0·05
Weight (kg)	80·30	2·98			75·08	3·31			68·87	4·20			69·79	9·18			0·084
Waist circumference (cm)																	
Male	90·12	0·92			89·21	0·32			80·23	1·21			78·60	0·19			0·201
Female	82·87	1·23			76·30	0·98			75·90	1·63			72·59	1·87			0·098
College education			120	20·65			210	33·28			298	45·22			321	38·12	0·121
Blood lipids*																	
TAG (mmol/l)	2·19[b]	0·92			1·85[b]	1·03			1·82[b]	0·91			1·12[a]	1·09			<0·05
TC (mmol/l)	6·61[c]	1·57			5·45[b]	2·31			6·14[c]	1·22			4·24[a]	1·22			<0·05
HDL (mmol/l)	0·90	0·19			1·01	1·01			1·19	0·91			1·08	0·53			0·059
LDL (mmol/l)	3·58[b]	2·11			3·51[b]	1·33			3·62[b]	0·89			2·35[a]	0·86			<0·001
ApoA (g/l)	1·87	0·58			1·76	0·19			1·29	0·21			1·30	0·28			0·098
ApoB (g/l)	1·26	0·02			1·01	0·71			1·29	0·23			0·99	0·07			0·061
Lp-α (mg/l)	178·89	9·81			150·18	8·12			134·19	7·31			140·31	31·23			1·981
Blood pressure (mmHg)																	
Systolic	129·21	3·21			139·12	4·22			131·11	2·11			113·98	1·01			0·061
Diastolic	86·31	5·31			82·21	1·09			80·98	1·19			81·56	2·11			0·059
Glucose (mmol/l)	6·21	0·31			5·91	0·11			6·10	0·92			5·27	0·78			0·073

ACS, acute coronary syndrome; CCAD, chronic coronary artery disease; HRP, high-risk population; HV, healthy volunteers; TC, total cholesterol; Lp-α, lipoprotein α.

[a,b,c] Values within a row with unlike superscript letters were significantly different ($P<0·05$ or $P<0·001$).

* Blood lipids tested include: TAG; TC; HDL-cholesterol (HDL); LDL-cholesterol (LDL); apoA (mainly including apoAI); apoB (including both apoB100 and apoB48); Lp-α.

CX9). Levels of LDL were calculated by the Friedewald equation. Other standard risk factors, including age, sex, BMI, tobacco use, duration of diabetes mellitus, systolic/diastolic blood pressure, waist circumference and history of diagnosed hypertension, were collected as variables (Table 1).

Statistical analysis

The 10-year RP of CHD was calculated on the basis of the Cox proportional hazards multivariate model formulation and the Framingham risk equation[23]. The risk score was defined as

$$f_{(\text{group})}(X,M) = \beta_1(X_1 - M_1) + \beta_2(X_2 - M_2) + \beta_3(X_3 - M_3) + \cdots + \beta_i(X_i - M_i). \quad (2)$$

Furthermore, the 10-year CHD RP was calculated from the following equation:

$$\text{RP}_{(\text{group})} = 1 - S_0(t)^{\exp(f_{(\text{group})}[X,M])}, \quad (3)$$

where $\text{RP}_{(\text{group})}$ is the CHD probability within the next 10 years of each group (ACS, CCAD, HRP and HV); $S_0(t)$ is the hazard function at time t; $X_1, X_2, X_3, \ldots, X_i$ are independent risk factor variables for each individual; $M_1, M_2, M_3, \ldots, M_i$ are the average levels of risk factors in each group; $\beta_1, \beta_2, \beta_3, \ldots, \beta_i$ are the partial regression coefficients of different risk factors.

The estimated partial regression coefficients, hazard ratio and their corresponding 95 % CI are shown in Table 2; the descriptive characteristics are shown in Table 1. These parameters and data were substituted into equation 2 and the risk score for the ACS group was calculated as $f_{(\text{ACS})}(X,M)$, which, in turn, was substituted into equation 3 and the average 10-year $\text{RP}_{(\text{ACS})}$ was calculated as $1 - 0{\cdot}9876^{\exp(f_{(\text{ACS})}[X,M])}$. In the same way, the average 10-year RP_{CCAD} of the CCAD group was calculated as $1 - 0{\cdot}9821^{\exp(f_{(\text{CCAD})}[X,M])}$, the average 10-year $\text{RP}_{(\text{HRP})}$ of the HRP group as $1 - 0{\cdot}9781^{\exp(f_{(\text{HRP})}[X,M])}$, the average 10-year $\text{RP}_{(\text{HV})}$ of the HV group as $1 - 0{\cdot}9753^{\exp(f_{(\text{HV})}[X,M])}$ (For the equation calculation process, see the Supplementary material, available online).

The assumption of proportional hazards and the calibration were adjusted for confounding factors such as baseline age, sex, smoking and LDL, and verified by the Hosmer and Lemeshow test[24,25]. The proportional hazards assumption was considered to be valid when the difference in the P value was $<0{\cdot}05$.

SPSS for Windows version 18.0 was used to calculate the regression equations and correlation coefficients between the TFA index and the 10-year CHD-RP for the different groups. Bonferroni correction was performed for ANOVA and correlation analysis to verify statistically significant differences with a P value $<0{\cdot}05$.

Results

Trans-fatty acid profile and index

The erythrocyte membrane, serum and whole-blood TFA profiles are listed in Table 3. Only in erythrocyte membranes were

Table 2. Partial regression coefficients of the hazard score in the Cox model analysis
(Mean values and standard deviations; hazard ratios (HR) and 95 % confidence intervals)

	ACS (*n* 581)					CCAD (*n* 631)					HRP (*n* 659)					HV (*n* 842)				
	RC					RC					RC					RC				
	Mean	SD	HR	95 % CI	*P*	Mean	SD	HR	95 % CI	*P*	Mean	SD	HR	95 % CI	*P*	Mean	SD	HR	95 % CI	*P*
Age (years)	0·0329	0·0021	1·02	1·01, 1·04	0·0003	0·0172	0·0001	1·19	1·10, 1·21	0·0002	0·0119	0·0001	1·12	1·09, 1·18	0·0003	0·0108	0·0092	1·31	1·21, 1·40	0·0030
Sex*	0·6386	0·0101	0·98	0·91, 0·99	0·0002	0·2198	0·0101	0·81	0·79, 0·90	0·0001	0·2352	0·0012	0·92	0·90, 0·99	0·0008	0·1035	0·0009	0·79	0·69, 0·86	0·0051
Tobacco use†	0·2681	0·0211	1·22	1·19, 1·28	0·0001	0·3621	0·0129	1·31	1·28, 1·34	0·0039	0·2094	0·0002	1·17	1·12, 1·28	0·0005	0·1653	0·0021	1·41	1·38, 1·47	0·0015
Weight (kg)	0·4821	0·0129	1·38	1·29, 1·46	0·0019	0·1346	0·0028	1·17	1·09, 1·23	0·0009	0·0986	0·0091	1·24	1·19, 1·30	0·0023	0·0982	0·0028	1·37	1·21, 1·42	0·0027
TC (mmol/l)	0·3122	0·0812	1·09	1·03, 1·17	0·0043	0·2153	0·0091	1·13	1·06, 1·32	0·0056	0·1093	0·0031	1·33	1·23, 1·38	0·0001	0·1754	0·0016	1·24	1·18, 1·34	0·006
TAG (mmol/l)	0·5148	0·0123	1·16	1·01, 1·23	0·0069	0·1428	0·0008	1·25	1·20, 1·32	0·0012	0·1291	0·0072	1·51	1·50, 1·61	0·0003	0·2532	0·0814	1·49	1·37, 1·50	0·0011
LDL-C (mmol/l)	0·3221	0·1921	1·28	1·22, 1·34	0·0103	0·1392	0·0172	1·30	1·29, 1·42	0·0001	0·1481	0·0109	1·09	1·01, 1·20	0·0029	0·1987	0·0103	1·39	1·27, 1·41	0·0016

ACS, acute coronary syndrome; CCAD, chronic coronary artery disease; HRP, high-risk population; HV, healthy volunteers; RC, regression coefficient; TC, total cholesterol; LDL-C, LDL-cholesterol.
* Sex= 1 if woman, 0 if man.
† Tobacco use = 1 if yes, 0 otherwise.

Table 3. Erythrocyte membrane, serum and whole-blood *trans*-fatty acid (TFA) profiles in each group*
(Mean values and standard deviations)

	Trans-16 : 1*n*-7		*Trans*-16 : 1*n*-9		*Trans*-18 : 1*n*-7		*Trans*-18 : 1*n*-9		*Trans*-18 : 2*n*-6*n*-9		Total TFA		TFA index	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Erythrocyte membrane														
ACS	0·19^{a}	0·01	0·21^{b}	0·09	0·35^{a}	0·04	0·43^{c}	0·11	0·42^{c}	0·12	1·6^{b}	0·57	7·12^{d}	1·23
CCAD	0·17^{a}	0·06	0·18^{a,b}	0·04	0·41^{a,b}	0·08	0·38^{c}	0·12	0·38^{b,c}	0·11	1·52^{a,b}	0·09	5·06^{c}	1·03
HRP	0·16^{a}	0·03	0·22^{b}	0·08	0·51^{b}	0·19	0·25^{a,b}	0·08	0·29^{b}	0·03	1·43^{a}	1·03	3·11^{b}	0·89
HV	0·22^{b}	0·11	0·14^{a}	0·01	0·59^{c}	0·21	0·21^{a}	0·10	0·18^{a}	0·09	1·34^{a}	0·98	1·92^{a}	0·66
P	<0·05		<0·05		<0·001		<0·05		<0·05		<0·05		<0·001	
Serum														
ACS	0·03	0·01	0·21	0·09	0·49	0·01	0·12	0·01	0·13^{b}	0·11	0·98	0·11	2·42	1·09
CCAD	0·09	0·03	0·11	0·02	0·31	0·02	0·08	0·01	0·12^{a,b}	0·01	0·71	0·12	2·17	0·89
HRP	0·02	0·01	0·22	0·09	0·51	0·02	0·08	0·04	0·09^{a}	0·01	0·92	0·08	2·09	0·73
HV	0·04	0·01	0·17	0·07	0·38	0·03	0·05	0·02	0·08^{a}	0·02	0·72	0·02	2·04	0·12
P	0·081		0·093		0·098		0·051		<0·05		0·323		0·052	
Whole blood														
ACS	0·05	0·01	0·21	0·01	0·31	0·08	0·21^{b}	0·03	0·18^{b}	0·01	0·86	0·21	5·29	0·01
CCAD	0·09	0·01	0·26	0·06	0·22	0·01	0·18^{b}	0·01	0·13^{a,b}	0·02	0·88	0·09	6·29	1·21
HRP	0·07	0·02	0·13	0·01	0·19	0·02	0·13^{a,b}	0·07	0·11^{a}	0·01	0·63	0·14	4·15	0·12
HV	0·08	0·01	0·19	0·02	0·38	0·11	0·09^{a}	0·02	0·09^{a}	0·01	0·83	0·05	2·24	0·78
P	0·093		0·121		0·113		<0·05		<0·05		0·063		0·102	

ACS, acute coronary syndrome; CCAD, chronic coronary artery disease; HRP, high-risk population; HV, healthy volunteers.
a,b,c,d Mean values within a column with unlike superscript letters were significantly different ($P<0·05$ or $P<0·001$).
* Values are given as the percentages of individual fatty acids relative to the total chromatogram area (FA%).

there significant differences in the levels of all TFA isomers, as well as the TFA index among the different groups. The levels of industry-generated isomers (*trans*-16 : 1*n*-9, *trans*-18 : 1*n*-9 and *trans*-18 : 2*n*-6*n*-9) were the highest in the ACS group, whereas the levels of ruminant-originated isomers (*trans*-16 : 1*n*-7 and *trans*-18 : 1*n*-7) were the highest in the HV group, and the TFA index showed a significant progressive decrease from the ACS to the HV group. In serum, only the levels of *trans*-18 : 2*n*-6*n*-9 were significantly different between these groups; although the TFA index was high in the ACS group, there was still no significant difference. In whole blood, the levels of both *trans*-18 : 1*n*-9 and *trans*-18 : 2*n*-6*n*-9 were significantly higher in the ACS group than those in the other groups, but no difference existed in the other groups. In addition, the total industrial and ruminant TFA levels showed no significant differences between the groups.

In the TFA index equation, the total industrial TFA levels in the numerator exhibited an increasing trend from the HV to the ACS group, while the total ruminant TFA levels in the denominator showed a decreasing trend. To unify the incompatible trend of decreased ruminant TFA and increased industrial TFA from the HV to the ACS group, we divided the total industrial TFA levels by the total ruminant TFA levels. This index value represents a coincidental trend of TFA change. In all the four groups, only the erythrocyte membrane TFA index manifested a significant difference. The TFA index in the erythrocyte membrane fraction progressively decreased from 7·12 (SD 1·23) to 5·06 (SD 1·03), 3·11 (SD 0·89) and 1·92 (SD 0·66) in the ACS, CCAD, HRP and HV groups, respectively ($P<0·001$).

CHD risk probability

The descriptive characteristics of traditional CHD risk factors in each group are described in Table 1. Sex, age, tobacco use and BMI were significantly different between the groups, while waist circumference, weight and education status were not. As for the blood lipids, only total cholesterol, TAG and LDL showed significant differences between the groups. The estimated partial regression coefficients, hazard ratio and their corresponding 95% CI are shown in Table 2. The risk scores in the four groups were determined as follows: ACS, $f_{(ACS)}(X,M) = 31\%$; CCAD, $f_{(CCAD)}(X,M) = 21\%$; HRP, $f_{(HRP)}(X,M) = 13\%$; HV, $f_{(HV)}(X,M) = 4\%$.

Correlation between trans-fatty acid index and 10-year risk probability

The comparison between the erythrocyte membrane TFA index and the 10-year CHD RP showed a strong linear correlation (R^2 0·9981, $P<0·001$; Fig. 1). In contrast, the comparison between the serum or whole-blood TFA index and the 10-year CHD RP showed no significant correlation ($P>0·05$ and $P>0·01$, respectively; Figs. 2 and 3). In each group (HV, HRP, CCAD and ACS), the average erythrocyte membrane TFA index and the 10-year CHD RP coincided with the regression line.

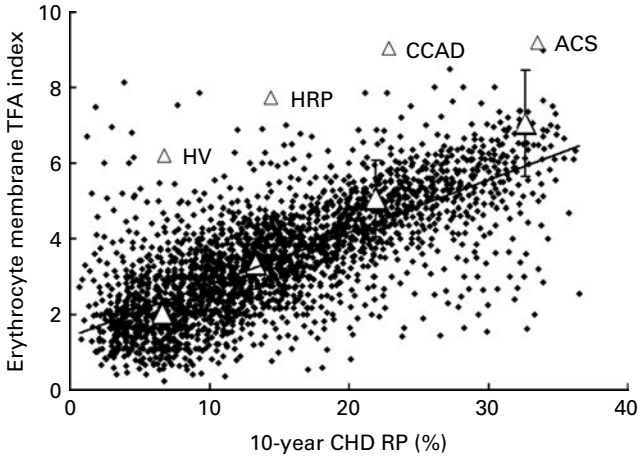

Fig. 1. Linear correlation between the erythrocyte membrane *trans*-fatty acid (TFA) index and the 10-year CHD risk probability (RP) ($y = 17{\cdot}122x + 1{\cdot}1365$ and R^2 0·9981). Values (Δ) are average TFA index and RP in the healthy volunteers (HV, *n* 842), high-risk population (HRP, *n* 659), chronic coronary artery disease (CCAD, *n* 631) and acute coronary syndrome (ACS, *n* 581) groups, respectively, with standard deviations represented by vertical bars.

Discussion

Erythrocyte membrane trans*-fatty acids reflect the change in body* trans*-fatty acids in CHD*

The levels of all of the erythrocyte membrane TFA isomers differed significantly between the groups. In contrast, only *trans*-18 : 1*n*-9 and *trans*-18 : 2*n*-6*n*-9 differed significantly between the whole-blood and serum samples. The probable reasons for this difference were as follows: on the one hand, the serum fatty acid level reflected fat intake over the past few days due to the immediate penetration of dietary fat into the serum[26], and it is hard to get much change in fatty acid profile during a few days, especially TFA; on the other hand, the fatty acid level of erythrocyte membrane reflected chronic lipid storage, as well as the average level of body lipids over the previous 4–12 weeks[27], and there was no fatty acid synthesis, chain elongation or desaturation in the membrane[28]. Therefore, erythrocyte membrane TFA reflected the change in body TFA levels in CHD[8].

Trans*-fatty acid index reflects* trans*-fatty acid hazard on CHD*

The five TFA isomers in the erythrocyte membrane included two *n*-9 TFA (*trans*-16 : 1*n*-9 and *trans*-18 : 1*n*-9), two *n*-7 TFA (*trans*-16 : 1*n*-7 and *trans*-18 : 1*n*-7) and one double-bonded TFA (*trans*-18 : 2*n*-6*n*-9). The two *n*-9 TFA and *trans*-18 : 2*n*-6*n*-9 primarily originate from partially hydrogenated vegetable oils ('industrial' TFA)[29]. The two *n*-7 TFA are generally ruminant-sourced[29], although *trans*-18 : 1*n*-7 may also come from an industrial source. The levels of *n*-9 TFA and *trans*-18 : 2*n*-6*n*-9 were significantly higher in ACS, which is consistent with the proven detrimental impact of industrial TFA in the promotion and induction of CHD events from the Nurses' Health Study in 32 826 participants and 6 years of follow-up[9]. Another study investigating TFA and sudden cardiac death among 86 762 women has shown that the levels of *trans*-18 : 2 and *trans*-16 : 1*n*-9 were positively associated with myocardial infarction ($P < 0{\cdot}001$) after adjusting for established risk factors and other confounders, and that the *trans*-18 : 2 isomer may play a greater role in sudden cardiac death among individuals with clinically manifest atherosclerosis[30].

Remarkably, in contrast, *n*-7 TFA levels were high in the HV group while low in the ACS group, which may result from the ruminant-sourced generation. Ruminant *trans*-16 : 1*n*-7 was associated with *trans*-18 : 1*n*-7 and may be converted into *trans*-18 : 1*n*-7 by the carbochain increase[31]. As a major TFA of ruminant fat, *trans*-18 : 1*n*-7 is produced in the rumen and converted in tissues to 9-*cis*, 11-*trans*-18 : 2*n*-6 CLA by Δ9-desaturase with an average conversion rate of 19 %[32]. 9-*cis*, 11-*trans*-18 : 2*n*-6 CLA could positively modulate HDL-cholesterol metabolism and enhance reverse cholesterol transport, and prevent the progression of atherosclerosis in humans[33]. Hence, the effect of ruminant TFA on CHD may be neutral or somewhat favourable due to the indirect benefit of 9-*cis*, 11-*trans*-18 : 2*n*-6 CLA[34]. However, the industrial TFA are harmful. It has been reported that the consumption of industrial *trans*-18 : 1*n*-9 by LDL receptor-deficient (LDL −/−) mice stimulated atherosclerotic development[35], while consumption of a diet enriched in *trans*-18 : 1*n*-7 reduced cholesterol-induced hyperlipidaemia and atherosclerosis and thus protected against atherosclerotic lesions[36]. Chronic *trans*-18 : 1*n*-7 supplementation also significantly abated dyslipidaemia in both the food-deprived and postprandial states in JCR:LA-cp rats due to reductions in intestinal chylomicrons and hepatic *de novo* lipogenesis pathways[37]. Another study investigating the effects of ruminant-derived TFA on immune function in a model of the metabolic syndrome (JCR:LA-cp rats) has shown that vaccenic acid might protect from CVD due to an anti-inflammatory action[38].

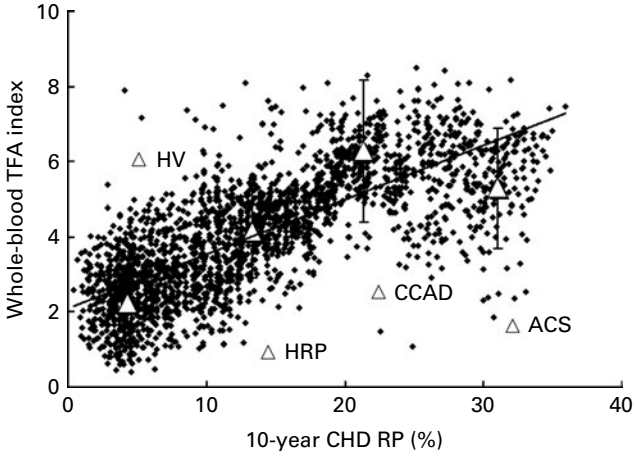

Fig. 2. Linear correlation between the whole-blood *trans*-fatty acid (TFA) index and the 10-year CHD risk probability (RP) ($y = 14{\cdot}365x + 21{\cdot}141$ and R^2 0·5607). Values (Δ) are average TFA index and RP in the healthy volunteers (HV, *n* 842), high-risk population (HRP, *n* 659), chronic coronary artery disease (CCAD, *n* 631) and acute coronary syndrome (ACS, *n* 581) groups, respectively, with standard deviations represented by vertical bars.

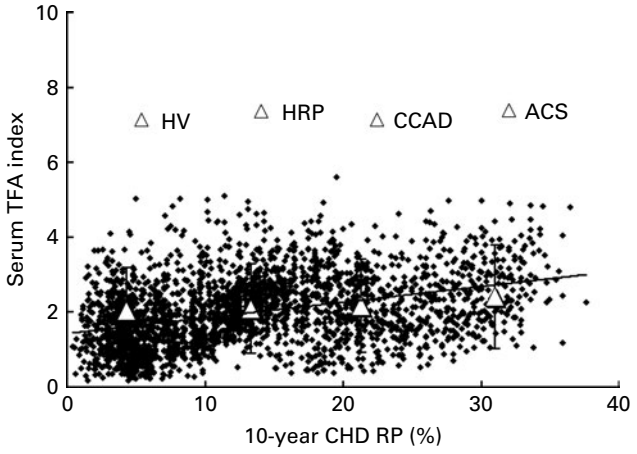

Fig. 3. Linear correlation between the serum *trans*-fatty acid (TFA) index and the 10-year CHD risk probability (RP) ($y = 0{\cdot}104x + 21{\cdot}81$ and R^2 0·012). Values (Δ) are average TFA index and RP in the healthy volunteers (HV, *n* 842), high-risk population (HRP, *n* 659), chronic coronary artery disease (CCAD, *n* 631) and acute coronary syndrome (ACS, *n* 581) groups, respectively, with standard deviations represented by vertical bars.

Although ruminant TFA have been shown to elicit neutral or less detrimental effects, further studies in human subjects are still needed to prove the protective effects.

To unify the inverse trend of decreased ruminant TFA and increased industrial TFA from the HV to the ACS group, we divided the total industrial TFA levels by the total ruminant TFA levels. This index value represents a coincidental trend of TFA change. An exponential function was used to make the index stronger and more obvious as the TFA index. The TFA index could estimate the true objective change in TFA levels comprehensively by unifying the disparate effects of different TFA on the body; accordingly, the hazardous level of all TFA isomers on CHD could be evaluated. In addition, it was interesting to note that none of the five TFA isomers showed any significant differences except the TFA index ($P<0{\cdot}001$) between the ACS and CCAD groups, indicating that the TFA index could discriminate the acute CHD symptoms of the ACS from the chronic CHD symptoms of CCAD.

Trans-fatty acid index is associated positively with 10-year CHD risk probability

The average TFA index in erythrocyte membranes differed between the groups and increased progressively from the HV to the ACS group. The 10-year CHD RP were 4, 13, 21 and 31 % in the HV, HRP, CCAD and ACS groups, respectively. These values generally reflect the potential probability and pathological severity of CHD. A strong linear correlation was seen between the average TFA index and the 10-year CHD RP (R^2 0·9981; Fig. 1), which suggests that as the erythrocyte membrane TFA level goes up, CHD may get worse.

CHD incidence could be influenced directly or indirectly by TFA through TAG accumulation, vasodilation, inflammation, PG translation and/or platelet aggregation[8,39]. *Trans*-fatty acids elicit an unfavourable effect on the lipoprotein profile by stimulating cholesteryl ester transfer protein activity (r 0·58, $P<0{\cdot}005$), increasing the LDL level and decreasing the HDL level (r −0·57, $P<0{\cdot}01$). These changes may contribute to a more atherogenic lipoprotein profile[40–42]. A high intake of TFA could adversely affect endothelial function; this would partially explain why the positive relationship between *trans*-fats and cardiovascular health took precedence over the adverse effects of *trans*-fats on lipids and lipoproteins[43,44]. In addition, a recent study has suggested that *trans*-18 : 1*n*-9 maintains the levels of vascular cell adhesion molecule-I and intercellular cell adhesion molecule-I up-regulated by TNF-α or lipase. This kept the human brain microvascular endothelial function at the stimulated phenotype, which could promote CHD[45].

In summary, the erythrocyte membrane TFA index was proposed as a method to unify the content changes in different TFA isomers and their effects on CHD in one equation. The TFA index manifested a strong and positive linear correlation with the 10-year CHD RP. Although the present study might be limited to the variety of TFA isomers analysed, the results should contribute to further studies on the relationship between TFA and CHD.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114513000196

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