Associations of plasma very-long-chain SFA and the metabolic syndrome in adults[‡]

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Abstract

Plasma levels of very-long-chain SFA (VLCSFA) are associated with the metabolic syndrome (MetS). However, the associations may vary by different biological activities of individual VLCSFA or population characteristics. We aimed to examine the associations of VLCSFA and MetS risk in Chinese adults. Totally, 2008 Chinese population aged 35–59 years were recruited and followed up from 2010 to 2012. Baseline MetS status and plasma fatty acids data were available for 1729 individuals without serious diseases. Among 899 initially metabolically healthy individuals, we identified 212 incident MetS during the follow-up. Logistic regression analysis was used to estimate OR and 95 % CI. Cross-sectionally, each VLCSFA was inversely associated with MetS risk; comparing with the lowest quartile, the multivariate-adjusted OR for the highest quartile were 0·18 (95 % CI 0·13, 0·25) for C20 : 0, 0·26 (95 % CI 0·18, 0·35) for C22 : 0, 0·19 (95 % CI 0·13, 0·26) for C24 : 0 and 0·16 (0·11, 0·22) for total VLCSFA (all $P_{for\ trend} < 0.001$). The associations remained significant after further adjusting for C16 : 0, C18 : 0, C18 : 3*n*-3, C22 : 6*n*-3, *n*-6 PUFA and MUFA, respectively. Based on follow-up data, C20 : 0 or C22 : 0 was also inversely associated with incident MetS risk. Among the five individual MetS components, higher levels of VLCSFA were most strongly inversely associated with elevated TAG (≥1·7 mmol/l). Plasma levels of VLCSFA were significantly and inversely associated with MetS risk and individual MetS components, especially TAG. Further studies are warranted to confirm the findings and explore underlying mechanisms.

Key words: Cross-sectional studies: Hypertriacylglycerolaemia: Metabolic syndrome: SFA: Follow-up studies

SFA have been linked to the development of metabolic disorders, including diabetes[1], hyperlipidaemia[2] and hypertension[3]. The underlying mechanisms include activating NF-*κ*B to alter gene expression, serving as signal molecules and modulating membrane fluidity[4,5]. The effect of straight chain SFA on metabolic process depends on the carbon chain length. Excess dietary palmitic acid (C16 : 0) impairs insulin sensitivity and increases blood pressure and lipids[6,7]. In contrast, very-long-chain SFA (VLCSFA) with 20–24 carbon atoms may play a favourable role in metabolic disorders. Several epidemiological studies reported that higher circulating levels of VLCSFA were inversely associated with blood TAG[8], insulin resistance[9], incident diabetes[1,8], arterial fibrillation[10], CHD[11], cardioembolic stroke[12] and CVD mortality[13].

Circulating VLCSFA are from minor dietary constituents and are also derived by endogenous synthesis from C16 : 0 or C18 : 0 as well as inter conversions among one another mediated by *β*-oxidation and elongation[14]. VLCSFA are found at significant quantities in a limited range of foods, including peanuts, lotus nuts and rapeseed/rapeseed oil[15]. *In vivo*, VLCSFA are major elements of ceramides and sphingomyelins, and the lower level of ceramides have been suggested to increase hepatocyte apoptosis and proliferation[16], obesity risk[17] and inflammatory response[17] among many other structural roles in, for instance, the brain and skin[18,19]. Further studies have reported that some functions of ceramides are chain-length dependent[20]. For example, based on cell culture experiments, C24 : 0 promotes proliferation, whereas C16 : 0 often has antiproliferative

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and pro-apoptotic effect[20]. Previous epidemiological studies showed that VLCSFA were inversely correlated with phospholipid C16 : 0 levels[8,10], which suggested that VLCSFA and C16 : 0 might have competitive effect in some situation with opposite biological functions.

To our knowledge, no study to date has assessed the relation of VLCSFA to the metabolic syndrome (MetS) in a Chinese population. We aimed to explore the associations of plasma VLCSFA with prevalence of the MetS at baseline and risk of incident MetS of all initially metabolically healthy participants in a 2-year follow-up study. Further, we additionally evaluated the associations between VLCSFA with five MetS components.

Methods

Study population

The study population was from an investigation conducted by Chinese Centre for Disease Control and Prevention from April 2010 to December 2012 in Shunyi district of Beijing. Eligible participants had to live in the area for more than 1 year and had no plan to move within 1 year, no serious diseases of heart, lung, liver and kidney and no pregnancy. A total of 2008 participants aged between 35 and 59 years were recruited in Shunyi. All participants were interviewed in-person to collect information on demographic characteristics, history of diseases, medical conditions, current cigarette smoking status (at least one cigarette a day), alcohol drinking status (at least once a week during the past year), physical activity status (at least once a week, excluding walking and riding), agricultural working (typical agricultural workers), health education (conducted by local Centres for Disease Control) and other lifestyles. Height, weight, blood pressure and waist circumference (WC) were measured twice. Fasting venous blood was obtained for biochemical testing, including total cholesterol, HDL-cholesterol, TAG and plasma glucose. Plasma glucose was tested within 3 h and plasma lipids on the day of sampling. All biochemical testing was performed with an automatic biochemical analyser at Centre for Disease Control. Plasma was separated at 1500 **g**/min centrifugation for 15 min and stored at −80°C. The above information and biosamples were collected a second time after 2 years.

Among the 1915 participants with available baseline fatty acid data, we excluded 185 participants with CHD, stroke, chronic respiratory diseases and malignant tumour at baseline; one participant with no MetS information was also excluded. Finally, 1729 participants were included in the current analyses. Among them, 81 % had the 2-year follow-up data.

The study protocol was approved by the Ethical Review Committee of the National Centre for Chronic and Non-communicable Disease Control and Prevention, Chinese Centre for Disease Control and Prevention. All participants provided informed written consent. The trial was registered at chictr.org.cn as ChiCTR-EOC-17012759.

Ascertainment of the metabolic syndrome

The MetS was defined according to the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian Americans[21]. Participants with any three of the five following items were identified as having the MetS: (1) WC ≥90 cm in men or ≥80 cm in women, (2) TAG ≥1·7 mmol/ l, (3) HDL-cholesterol ≤1·03 mmol/l in men or ≤1·30 mmol/l in women, (4) blood pressure ≥130/85 mmHg or current use of antihypertensive medications and (5) fasting plasma glucose ≥5·6 mmol/l or use of oral antidiabetic agents or insulin.

Plasma fatty acids measurement

GC/flame ionisation detector (FID) was used to measure plasma fatty acids profile[22,23]. Briefly, 100 µl of plasma with 1,2-dihenarachidoyl-sn-glycero-3-phosphocholine as internal standard was extracted in dichloromethane/methanol and the extracted lipids were reacted with methanol and sulphuric acid to yield fatty acid methyl ester (FAME). After methylation, FAME were extracted with n-hexane and redissolved in isooctane. Analysis was performed using an Agilent 6890 GC/FID equipped with a Supelco SP-2560 capillary column (100 m × 0·25 mm inside diameter × 0·20 µm thickness; Agilent Technologies Inc.). Data were expressed as weight percentage of total fatty acids. Samples were organised in batches of up to 20, which included two samples from a standard pool for quality control (QC). The CV of QC were 10·8 % for C20 : 0 (arachidic acid), 12·4 % for C22 : 0 (behenic acid) and 14·6 % for C24 : 0 (lignoceric acid).

Statistical analysis

Wilcoxon rank-sum test for continuous variables and the χ^2 test for categorical variables were used to examine the differences of basic characteristics between groups with the MetS and without the MetS. Spearman correlation was used to evaluate correlations of VLCSFA and other plasma fatty acids. Logistic regression analysis was applied to examine the associations of VLCSFA and risk of the MetS. VLCSFA were classified into four groups based on the quartiles of VLCSFA in participants without the MetS at baseline. Several models were fitted to examine the association of each VLCSFA and risk of the MetS: (1) model adjusted for age, sex and agricultural work (main model); (2) model adjusted for age, sex, agricultural work, education, smoking, alcohol drinking and physical activity (fully adjusted model) and (3) models additionally adjusted for the potential mediators based on fully adjusted model, including C16 : 0, C18 : 0, C18 : 3n-3, C22 : 6n-3, n-6 PUFA and MUFA (exploration models). These selected fatty acids were significantly correlated with VLCSFA with the Spearman coefficients more than 0·25. As the fully adjusted models did not change the results, main model was used in the following analyses. The associations of VLCSFA and risk of incident MetS during 2-year follow-up were conducted only in participants without the MetS at baseline. Non-linear OR of the MetS was estimated by applying a restricted cubic spline regression model with three knots at the 5th, 50th and 95th percentiles[24]. Multinomial logistic regression was used to estimate the associations of VLCSFA and risk of the MetS according to the number of MetS components at baseline. As the sample size in each group was limited, the VLCSFA levels were classified into high or low group based on the median of VLCSFA in subjects without the MetS. Low

VLCSFA levels group were assigned as the reference. When the associations between VLCSFA and individual components of the MetS were evaluated, each of the other four components was adjusted in the models separately. To explore modification effects of some *a priori* risk factors on the association between VLCSFA and MetS risk, stratified analyses were conducted by age, sex, physical activity, agricultural working, smoking status and current alcohol drinking. Continuous variables were classified into high *v.* low levels based on the median levels in controls. The OR were expressed as the risk of the MetS for 1 SD increase of VLCSFA. Likelihood ratio tests were conducted to examine interactions. All analyses were conducted with R software and SAS 9.3 software. All *P* values were two-sided.

Results

A total of 1729 participants were included in this study; 565 (32·7 %) of these were identified as the MetS at baseline. Baseline characteristics of participants with or without the MetS were presented in Table 1. The two groups were similar in sex, smoking status, alcohol drinking and physical activity. Compared with participants without the MetS, the MetS participants were older and did less agricultural work. The MetS group had lower level of plasma $C18:0$, $C20:0$, $C22:0$, $C24:0$, $C22:6n$-3, $C18:2n$-6, $C20:4n$-6 and n-6 PUFA, but had higher $C16:0$, MUFA, $C18:3n$-3, $C20:5n$-3 and n-3 PUFA. Women and individuals without diabetes had higher levels VLCSFA (online Supplementary Tables S1a and S1b). In addition, higher VLCSFA were associated with lower BMI, lower TAG and higher HDL. Participants with higher levels of $C20:0$ or $C22:0$ tended to be non-smoker and non-alcohol drinker, do more agricultural work, have no hypertension and have lower WC and cholesterol (online Supplementary Table S1a). Furthermore, older ages were inversely related to higher $C20:0$, but directly to higher $C24:0$ (online Supplementary Tables S1a and S1b). Participants with higher $C24:0$ were more likely to have hypertension (online Supplementary Table S1b).

All the three VLCSFA were significantly correlated with each another (r_s 0·75, 0·23, 0·21 for $C20:0$ with $C22:0$, $C24:0$ and $C22:0$ with $C24:0$, online Supplementary Table S2). In the fully adjusted model, compared with the lowest quartile, the highest level of VLCSFA was associated with increased risk of the MetS; the multivariate-adjusted OR were 0·18 (95 % CI 0·13, 0·25) for $C20:0$, 0·26 (95 % CI 0·18, 0·35) for $C22:0$, 0·19 (95 % CI 0·13, 0·26) for $C24:0$ and 0·16 (95 % CI 0·11, 0·22) for total VLCSFA (Table 2). OR of the MetS were non-linear in $C20:0$, $C22:0$, $C24:0$ and total VLCSFA (Fig. 1, $P_{non-linearity} < 0·0001$ for $C20:0$ and total VLCSFA, $P_{non-linearity} = 0·002$ for $C22:0$, $P_{non-linearity} = 0·021$ for $C24:0$). The VLCSFA were significantly correlated with $C16:0$, $C18:0$, MUFA, $C18:3n$-3, $C22:6n$-3 and n-6 PUFA with absolute r_s more than 0·25 (online Supplementary Table S2). Separately adjusted for these fatty acids did not affect the associations between VLCSFA and MetS risk (online Supplementary Table S3). In addition, the VLCSFA levels were inversely associated with the number of the MetS components (all the $P_{trend} < 0·0001$, online Supplementary Table S4). In the multinomial logistic regression analysis, the

Table 1. Basic characteristics of subjects with and without the metabolic syndrome (MetS) at baseline
(Mean values and standard deviations; frequencies and percentages)

Characteristics*	With the MetS (n 565) Mean	SD	Without the MetS (n 1164) Mean	SD	P†
Age (years)	50·5	5·95	48·5	6·28	<0·0001
Sex					0·52
Male					
Frequency	185		401		
%	33		34		
Female					
Frequency	380		763		
%	67		66		
Education					0·43
Illiteracy					
Frequency	18		25		
%	3		2		
Primary school					
Frequency	73		126		
%	13		11		
Middle school					
Frequency	353		746		
%	62		64		
High school					
Frequency	115		251		
%	20		22		
College					
Frequency	6		16		
%	1		1		
Health education					0·06
Frequency	262		598		
%	46		51		
Current smoking status					0·32
Frequency	116		265		
%	21		23		
Current alcohol drinking					0·95
Frequency	146		304		
%	26		26		
Agricultural work					0·0002
Frequency	243		612		
%	43		53		
Physical activity					0·99
Frequency	72		150		
%	13		13		
BMI (kg/m²)	28·5	3·06	25·2	3·36	<0·0001
WC (cm)	92·9	8·54	82·4	9·42	<0·0001
Obesity					<0·0001
Frequency	308		218		
%	55		19		
Hypertension					<0·0001
Frequency	398		415		
%	70		36		
Diabetes					<0·0001
Frequency	112		33		
%	20		3		
TAG	2·33	1·81	1·07	1·06	<0·0001
TC	5·44	1·05	5·17	0·88	<0·0001
HDL-cholesterol	1·23	0·28	1·55	0·31	<0·0001
C16:0	21·1	1·81	19·8	1·45	<0·0001
C18:0	7·95	0·84	8·52	0·81	<0·0001
C20:0	0·18	0·04	0·21	0·04	<0·0001
C22:0	0·63	0·19	0·73	0·20	<0·0001
C24:0	0·49	0·19	0·60	0·21	<0·0001
Total VLCSFA	1·30	0·32	1·53	0·32	<0·0001
MUFA‡	20·6	3·11	18·0	2·46	<0·0001
C18:3n-3	0·93	0·34	0·74	0·26	<0·0001
C20:5n-3	0·48	0·20	0·48	0·28	<0·0001
C22:6n-3c	1·87	0·57	1·95	0·60	0·002
n-3 PUFA§	3·77	0·73	3·65	0·82	<0·0001

Table 1. *Continued*

Characteristics*	With the MetS (n 565) Mean	SD	Without the MetS (n 1164) Mean	SD	P†
C18:2n-6	33·9	3·80	36·1	3·80	<0·0001
C20:4n-6	7·32	1·79	8·55	1·73	<0·0001
n-6 PUFA‖	44·2	3·99	47·6	3·06	<0·0001

WC, waist circumference; TC, total cholesterol; VLCSFA, very-long-chain SFA.
* Continuous variables were expressed as means and standard deviations and categorical variables were expressed as frequency (percentage among cases or controls).
† P values were calculated from Wilcoxon rank sum test for continuous variables and χ^2 test for categorical variables.
‡ Includes C14:1n-5, C16:1n-7, C16:1n-9, C18:1n-7, C18:1n-9, C20:1n-9, C22:1n-9 and C24:1n-9 in this study.
§ Includes C18:3n-3, C20:3n-3, C20:5n-3, C22:3n-3, C22:5n-3 and C22:6n-3.
‖ Includes C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6, C22:4n-6 and C22:5n-6 in this study.

associations of VLCSFA and MetS risk were stronger with the increase of the number of the MetS components (Table 3).

Among 899 subjects without the MetS at baseline, we identified 212 incident cases of the MetS during the 2-year follow-up. The level of C20:0, C22:0 and total VLCSFA was significantly associated with decreased incident MetS risk; comparing with the lowest quartile, the multivariate-adjusted OR for the highest quartile were 0·21 (95% CI 0·12, 0·35) for C20:0, 0·20 (95% CI 0·11, 0·34) for C22:0, 0·75 (95% CI 0·44, 1·26) for C24:0 and 0·28 (95% CI 0·17, 0·46) for total VLCSFA (Table 4).

The associations between VLCSFA levels and each of five individual MetS components were analysed. Among the five MetS components, the associations were strongest for VLCSFA and elevated TAG (Q4 *v.* Q1: OR was 0·11 (95% CI 0·07, 0·17) for C20:0, 0·10 (95% CI 0·06, 0·16) for C22:0, 0·07 (95% CI

0·04, 0·12) for C24:0 and 0·04 (95% CI 0·02, 0·07) for total VLCSFA, $P_{\text{trend}} < 0.0001$, Fig. 2, online Supplementary Table S5).

We also explored effect modification on the associations of VLCSFA and MetS risk by several *a priori* risk factors, including age, sex, smoking status (yes and no), current alcohol drinking (yes and no), physical activity and agricultural working (Fig. 3). The inverse associations between C20:0 and MetS risk were stronger in women than in men (OR = 0·37 (95% CI 0·31, 0·44) for women and OR = 0·57 (95% CI 0·46, 0·69) for men; P for interaction = 0·002) and in non-alcohol drinkers than in current alcohol drinkers (OR = 0·38 (95% CI 0·32, 0·45) for non-alcohol drinkers and OR = 0·61 (95% CI 0·49, 0·75) for current alcohol drinkers; P for interaction = 0·0008) (Fig. 3).

Discussion

In a population of 1729 Chinese adults, we observed that plasma VLCSFA levels were significantly and inversely associated with prevalence and incidence of the MetS. Further adjustment for each of C16:0, C18:0, C18:3n-3, C22:6n-3, n-6 PUFA or MUFA did not negate the associations. The associations of VLCSFA and MetS risk became stronger with the increase of the number of the MetS components. Among the five individual MetS components, higher plasma VLCSFA were strongly associated with plasma TAG.

Our observations are generally in line with several previous studies that have reported the relationship between blood VLCSFA and various metabolic conditions in other populations[1,8,10–13,25]. Three prospective studies published recently showed that higher levels of three VLCSFA (C20:0, C22:0 and C24:0) were associated with lower risk of incident diabetes[1,8,25]. A study with 3179 participants from the Cardiovascular Health Study (CHS) reported that higher plasma phospholipid VLCSFA

Table 2. The associations of very-long-chain SFA (VLCSFA) and prevalence of the metabolic syndrome (MetS) at baseline*
(Odds ratios and 95% confidence intervals)

	Quartile 1	Quartile 2 OR	95% CI	Quartile 3 OR	95% CI	Quartile 4 OR	95% CI	P_{trend}
C20:0								
$n_{\text{MetS/non-MetS}}$	326/291	101/291		80/291		58/291		
Model 1†	1	0·30	0·23, 0·40	0·25	0·18, 0·33	0·18	0·13, 0·25	<0·0001
Model 2‡	1	0·30	0·23, 0·40	0·25	0·18, 0·33	0·18	0·13, 0·25	<0·0001
C22:0								
$n_{\text{MetS/non-MetS}}$	248/291	153/291		98/291		66/291		
Model 1†	1	0·58	0·44, 0·75	0·38	0·28, 0·50	0·26	0·18, 0·35	<0·0001
Model 2‡	1	0·58	0·44, 0·75	0·38	0·28, 0·50	0·26	0·18, 0·35	<0·0001
C24:0								
$n_{\text{MetS/non-MetS}}$	260/291	160/291		90/291		55/291		
Model 1†	1	0·55	0·42, 0·72	0·30	0·22, 0·40	0·18	0·13, 0·26	<0·0001
Model 2‡	1	0·55	0·42, 0·71	0·30	0·22, 0·40	0·19	0·13, 0·26	<0·0001
Total VLCSFA								
$n_{\text{MetS/non-MetS}}$	312/291	130/291		70/291		53/291		
Model 1†	1	0·38	0·29, 0·50	0·21	0·15, 0·29	0·16	0·11, 0·22	<0·0001
Model 2‡	1	0·38	0·29, 0·50	0·21	0·15, 0·29	0·16	0·11, 0·22	<0·0001

* Logistic regression was used to estimate the OR and CI. VLCSFA were classified into four group based on the quartiles of VLCSFA in subjects without the metabolic syndrome: 0·18, 0·20 and 0·23 for C20:0; 0·59, 0·70 and 0·84 for C22:0; 0·46, 0·61 and 0·73 for C24:0; 1·32, 1·51 and 1·72 for total VLCSFA.
† Model 1: adjusted for sex, age and agricultural work.
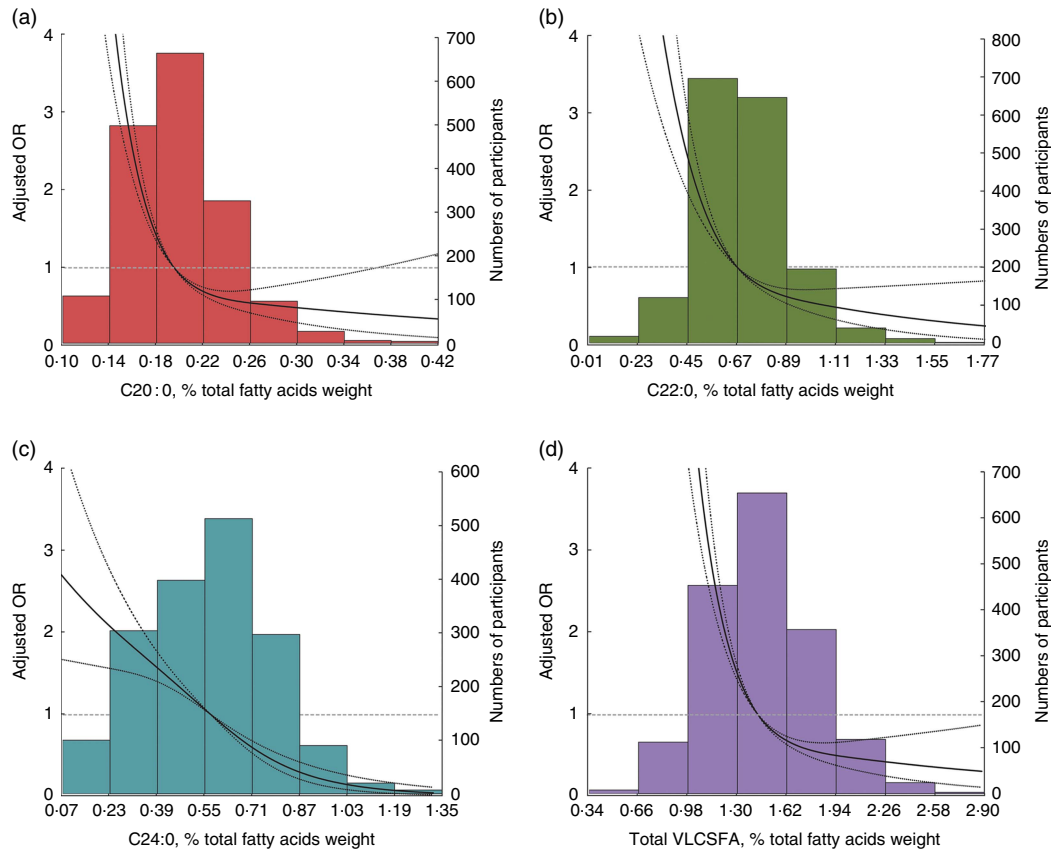‡ Model 2: additionally adjusted for education, smoking, alcohol drinking and physical activity.

Fig. 1. Risk of the metabolic syndrome by very-long-chain SFA (VLCSFA) levels at baseline. Lines represent OR (95 % CI) based on restricted cubic splines for VLCSFA levels with knots at the 5th, 50th and 95th percentiles. OR were estimated using a logistic regression model after adjustment for age, sex and agricultural work; bars represent the numbers of participants. (a) C20 : 0 ($P_{\text{non-linearity}} < 0.001$), (b) C22 : 0 ($P_{\text{non-linearity}} = 0.002$), (c) C24 : 0 ($P_{\text{non-linearity}} = 0.021$) and (d) total VLCSFA ($P_{\text{non-linearity}} < 0.0001$).

Table 3. Associations of very-long-chain SFA (VLCSFA) and risk of the metabolic syndrome (MS) according to the number of MS components at baseline*
(Odds ratios and 95 % confidence intervals)

	MS = 0	MS = 3	MS = 4	MS = 5
20 : 0				
$n_{\text{high/low}}$	582/582	80/251	44/138	14/38
OR	1	0·33	0·33	0·39
95 % CI		0·25, 0·44	0·23, 0·47	0·21, 0·72
22 : 0				
$n_{\text{high/low}}$	582/582	81/250	69/113	14/38
OR	1	0·32	0·61	0·36
95 % CI		0·24, 0·43	0·44, 0·84	0·19, 0·67
24 : 0				
$n_{\text{high/low}}$	582/582	134/197	8/174	3/49
OR	1	0·63	0·04	0·05
95 % CI		0·49, 0·81	0·02, 0·09	0·02, 0·18
Total VLCSFA				
$n_{\text{high/low}}$	582/582	97/234	24/158	2/50
OR	1	0·40	0·15	0·04
95 % CI		0·31, 0·53	0·09, 0·23	0·01, 0·16

* Multinomial logistic regression was used to estimate the OR and CI. All the OR was adjusted for sex, age and agricultural work. As the sample size in each case group was limited, VLCSFA were classified into high/low group based on the median of VLCSFA in subjects without the metabolic syndrome. Low group was the reference.

Table 4. Associations of very-long-chain SFA (VLCSFA) and risk of incident metabolic syndrome (MetS) after 2 years*
(Odds ratios and 95 % confidence intervals)

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
C20 : 0					
$n_{\text{MetS/non-MetS}}$	89/172	62/171	43/172	18/172	
OR	1	0·67	0·47	0·21	<0·001
95 % CI		0·45, 1·00	0·30, 0·72	0·12, 0·35	
C22 : 0					
$n_{\text{MetS/non-MetS}}$	89/172	65/171	38/172	20 /172	
OR	1	0·64	0·40	0·20	<0·001
95 % CI		0·43, 0·95	0·25, 0·62	0·11, 0·34	
C24 : 0					
$n_{\text{MetS/non-MetS}}$	38/172	87/171	52/172	35/172	
OR	1	1·94	1·15	0·75	0·11
95 % CI		1·25, 3·06	0·71, 1·87	0·44, 1·26	
Total VLCSFA					
$n_{\text{MetS/non-MetS}}$	80/172	62/171	44 /172	26/172	
OR	1	0·71	0·51	0·28	<0·001
95 % CI		0·47, 1·07	0·33, 0·78	0·17, 0·46	

* Only subjects without the MetS at baseline were included in the analysis. VLCSFA were classified into four group based on the quartiles of VLCSFA in subjects without the MetS: 0·18, 0·21 and 0·23 for C20 : 0; 0·60, 0·73 and 0·86 for C22 : 0; 0·46, 0·63 and 0·75 for C24 : 0; 1·32, 1·51 and 1·72 for total VLCSFA. Models were adjusted for sex, age and agricultural work.
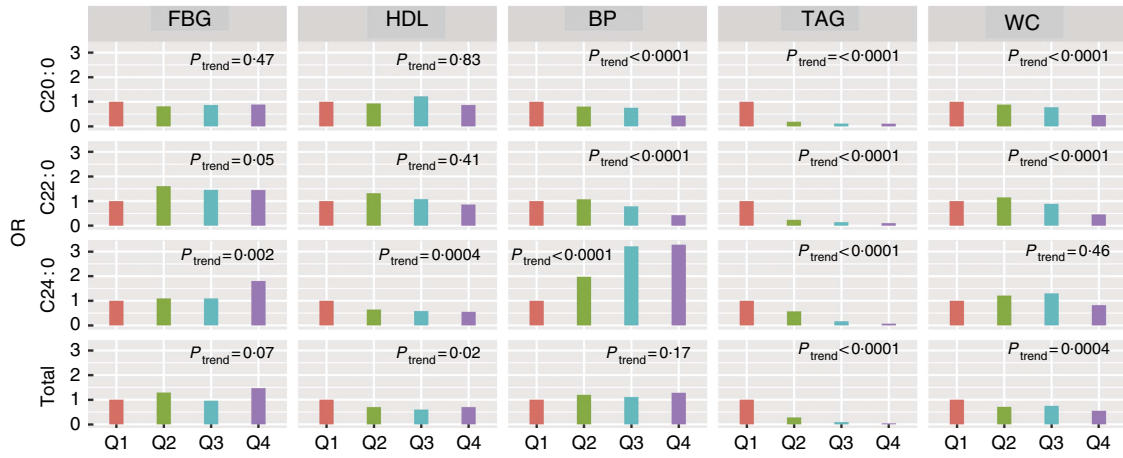
Fig. 2. Associations of very-long-chain SFA and risk of the metabolic syndrome components at baseline. Logistic regression was used to estimate the OR and CI. All the OR were adjusted for age, sex and agricultural work. The five components were adjusted in the models, except for itself. The five components were classified into high/low group according to the cut-off in the metabolic syndrome definition. BP, blood pressure; FBG, fasting blood glucose; WC, waist circumference.
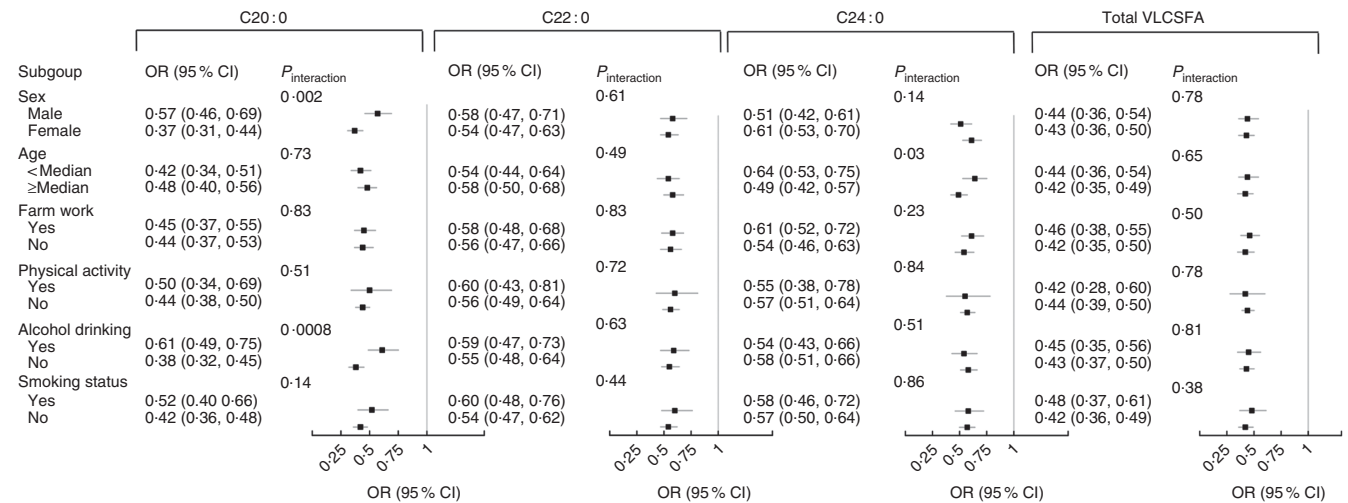

Fig. 3. Stratified analysis of the associations of very-long-chain SFA (VLCSFA) and risk of the metabolic syndrome at baseline. All the OR were adjusted for age, sex and agricultural work. The OR meant the risk of the metabolic syndrome for 1 SD increase of VLCSFA.

were cross-sectionally associated with lower blood TAG and WC[8]. In the Nurses' Health Study and Health Professionals Follow-Up Study, plasma VLCSFA were positively correlated with HDL[11]. Diabetes, WC, circulating TAG and HDL are components of the MetS. Consistently, we found inverse associations between plasma VLCSFA and MetS risk; TAG showed the strongest associations with VLCSFA. In addition, we found significant inverse associations of C20 : 0 and C22 : 0 with WC, and direct associations of C24 : 0 with HDL. However, we observed that C22 : 0 and C24 : 0 were directly associated with fasting blood glucose, which was different from the results of above studies. If the covariate of TAG was excluded from the analyses model in our study, the associations of C22 : 0 and C24 : 0 with fasting blood glucose were reversed. In the CHS study, VLCSFA were associated with lower risk of diabetes, but if blood TAG was adjusted in the model, the associations were not significant[8]. This suggested that the associations of VLCSFA with blood glucose might be mediated by lower TAG. Mechanistic studies are needed to confirm these

results. Other recently published studies reported inverse associations of circulating levels of VLCSFA with atrial fibrillation[10], cardioembolic stroke[12], CHD[11] and total mortality[13]. The MetS was a major intermediate risk factor of these diseases and may explain these associations[26–28].

The biological functions of VLCSFA *in vivo* remain unclear. VLCSFA are major components of ceramides and sphingomyelins, which are key intermediates linking saturated fats and inflammatory cytokines to regulate cell function. Insulin resistance is the key feature of the MetS. VLCSFA are major components of ceramides and sphingomyelins. A large body of evidence from non-human experimental studies has supported a role of ceramides in insulin signalling and β-cell function as well as the development of diabetes[29]. Specifically, C16-ceramide has been suggested to induce β-cell apoptosis, impair insulin signalling and reduce insulin synthesis, which can lead to insulin resistance[30,31]. In contrast, ceramides with VLCSFA appear to be protective. VLCSFA affect liver homoeostasis,

myelin maintenance and anti-inflammatory response through inhibition of endogenous C16-ceramide synthesis[14,16], but the biological pathways have not been fully understood and need to be investigated in future mechanistic studies. In addition, very-long-chain acyl-CoA synthetase may be up-regulated by PPARδ and evidence showed that PPARδ played a role in fat metabolism to prevent obesity[32].

The sources of circulating VLCSFA include both exogenous diet and endogenous synthesis[14]. The VLCSFA are found in limited foods, including peanuts, lotus nuts and rapeseed/rapeseed oil[15]. Several studies have shown that peanut intake increases blood C22:0 and C24:0[8]. Interestingly, peanut butter consumption was significantly associated with a more favourable plasma lipid profile, including lower LDL-cholesterol, and total cholesterol in the Nurses' Health Study[33]. VLCSFA can be synthesised from C18:0 by elongases, especially elongation of very long chain fatty acids protein 1 (ELOVL1), which selectively elongates SFA with more than eighteen carbons[14]. Indeed, our data show that the fatty acids related to each other by a single round of elongation were highly correlated, suggesting that higher dietary intake resulted in higher levels of the elongation product: $18:0 \rightarrow 20:0$ and $20:0 \rightarrow 22:0$ (the Spearman coefficients were 0·48 for C18:0 and C20:0, and 0·75 for C20:0 and C22:0). The *ELOVL1* gene coding for the first committed step in elongation is regulated in concert with ceramide synthase 2 (CERS2), a key enzyme for C24 sphingolipid synthesis[34]. This regulation may ensure that the production of C24-CoA by elongation is coordinated with its utilisation. How the dietary intake and endogenous metabolism contribute to circulating levels of VLCSFA and subsequently affect the metabolic disorders requires further study.

We also explored the modifying effects by several *a priori* factors on the relation of VLCSFA to MetS risk. We found a stronger association between C20:0 and MetS risk in subjects without alcohol drinking than those with current alcohol drinking. This phenomenon was consistent with the finding in the CHS study and our study that C20:0 was inversely correlated with the amount of alcohol drinks and current alcohol drinking status (yes/no)[8]. Heavy alcohol drinking is a risk factor for the MetS[35] and may neutralise the beneficial effects of VLCSFA on the MetS. We also observed moderate interaction effects of sex on the association of C20:0 with the MetS. In the CHS study and our study, women tended to have higher C20:0[8], which may explain our findings that the association of C20:0 and MetS risk was stronger in women than in men.

Our study is the first study to explore the associations of plasma VLCSFA and MetS risk in Chinese population. Our study has some limitations. First, the study design was initially cross-sectional, but we confirmed the cross-sectional results among baseline non-MetS subjects with 2-year follow-up data. Second, the half-life of plasma fatty acids was short and it may not reflect long-term dietary pattern. The fatty acids of erythrocyte or fat adipose tissue were needed to confirm the associations of VLCSFA with MetS risk. Third, we analysed statistically plasma fatty acids in units of weight percentage as is reported in most studies[8,11], which is most useful in assessing whether fatty acid imbalance is related to metabolic status. Specific fatty acid concentration (e.g. mg/ml plasma) is more appropriate to assess whether the mass of a specific circulating fatty acid is outside of a range that is needed to support the metabolism[36]. Best practices for the design, laboratory analysis, and reporting of trials involving fatty acids. *Am J Clin Nutr* in press). In addition, with the advancement of lipidomics technology, a detailed identification and quantification of VLCSFA in different lipid classes or tissues is of crucially helpful in understanding their roles in metabolic disorders. Unfortunately, we did not measure the fatty acids in different lipid classes (TAG, sphingomyelins, etc.). Fourth, no information on the lipid-lowering medication use was available. However, the hyperlipidaemia awareness rates (8·3 %), treatment rates (7·0 %), and detection rates (26·2 %) were very low in China, especially in rural areas[37], which suggests a possible mild lipid-lowering medication influence. Last but not least, compared with other fatty acids such as n-6 PUFA, C16:0 and MUFA, the proportion of VLSCFA was very low. However, after adjusting for each of these fatty acids separately, the association of VLCSFA and risk of the MetS remained statistically significant. Besides, we also adjusted for other potential confounders including lifestyle factors in the statistics model, but residual confounding by non-available or unknown factors is also possible, such as menopausal status of women or dietary VLCSFA.

In summary, we confirmed that higher levels of plasma VLCSFA are significantly associated with lower risk of the MetS in Chinese adults. The association of VLCSFA with blood TAG was stronger than other four MetS components. Further studies are warranted to explore the underlying mechanisms.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0007114518002106

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