Associations of plasma very long chain saturated fatty acids and metabolic syndrome in adults

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Abstract

Plasma levels of very long chain saturated fatty acids (VLCSFAs) are associated with metabolic syndrome (MetS). However, the associations may vary by different biological activities of individual VLCSFAs or population characteristics.We aimed to examine the associations of VLCSFAs and MetS risk in Chinese adults. Totally, 2008 Chinese aged 35 to 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 odds ratios (ORs) and 95% confidence intervals (CIs). Cross-sectionally, each VLCSFAs was inversely associated with MetS risk; comparing to the lowest quartile, the multivariate-adjusted ORs (95% CI) for the highest quartile were 0.18 (0.13, 0.25) for C20:0, 0.26 (0.18, 0.35) for C22:0, 0.19 (0.13, 0.26) for C24:0 and 0.16 (0.11, 0.22) for total VLCSFAs (All *P* for trends <0.001). The associations remained significant after further adjusting for C16:0, C18:0, C18:3n3, C22:6n3, n-6 polyunsaturated fatty acids and monounsaturated fatty acids, respectively. Based on follow-up data, C20:0 or C22:0 were also inversely associated with incident MetS risk. Among the 5 individual MetS components, higher levels of VLCSFAs were most strongly inversely associated with elevated triglyceride (≥1.7mmol/L). Plasma levels of VLCSFAs were significantly and inversely associated with MetS risk and individual MetS components, especially triglyceride. Further studies are warranted to confirm the findings and explore underlying mechanisms.

Introduction

Saturated fatty acids (SFAs) have been linked to the development of metabolic disorders, including diabetes[1], hyperlipidemia[2] and hypertension[3]. The underlying mechanisms include activating nuclear factor-κB (NF-κB) to alter gene expression, serving as signal molecules, and modulating membrane fluidity[4, 5]. The effect of straight chain SFAs 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 SFAs (VLCSFAs) with 20-24 carbon atoms may play a favorable role in metabolic disorders. Several epidemiological studies reported that higher circulating levels of VLCSFAs were inversely associated with blood triglyceride[8], insulin resistance[9], incident diabetes[1, 8], arterial fibrillation[10], coronary heart disease[11], and cardioembolic stroke[12] and cardiovascular disease (CVD) mortality[13].

Circulating VLCSFAs 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 beta-oxidation and elongation[14]. VLCSFAs are found at significant quantities in a limited range of foods, including peanuts, lotus nuts, and rapeseed/canola oil[15]. In vivo, VLCSFAs 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 exhibits anti-proliferative and pro-apoptotic effects[20]. Previous epidemiological studies show that VLCSFAs were reversely correlated with plasma phospholipid palmitic acid[8,10].

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

Methods

Study Population

The study population was from an investigation conducted by Chinese Center 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 one year, had no plan to move within one 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 Centers for Disease Control) and other lifestyles. Height, weight, blood pressure, and waist circumference were measured twice. Fasting venous blood was obtained for biochemical testing, including total cholesterol, high-density lipoprotein cholesterol, triglyceride, and plasma glucose. Plasma glucose was tested within 3 hours and plasma lipids on the day of sampling. All biochemical testing was performed with an automatic biochemical analyzer (Brand, manufacturer, city, province/state). Plasma was separated at 1500g/min centrifugation for 15min and stored at -80°C. The above information and bio-samples were collected a second time after two years.

Among the 1915 participants with available baseline fatty acid data, we excluded 185 participants with coronary heart diseases, stroke, chronic respiratory diseases, and malignant tumor 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 two-year follow-up data.

The study protocol was approved by the Ethical Review Committee of the National Center for Chronic and Non-communicable Disease Control and Prevention, Chinese Center 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 metabolic syndrome

MetS was defined according to the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian Americans[21]. Participants with any 3 of the 5 following items were identified as having MetS: 1) waist circumferences ≥ 90cm in men or ≥ 80cm in women, 2) triglycerides ≥ 1.7mmol/L, 3) HDL cholesterol ≤ 1.03mmol/L in men or ≤ 1.30mmol/L in women, 4) blood pressure ≥ 130/85mmHg or current use of antihypertensive medications and 5) fasting plasma glucose ≥ 5.6mmol/L or use of oral anti-diabetic agents or insulin.

Plasma fatty acids measurement

Gas chromatography/flame ionization detector (GC/FID) was used to measure plasma fatty acids profile[22, 23]. Briefly, 100ul plasma with 1,2-dihenarachidoyl-sn-glycero-3-phosphocholine as internal standard was extracted in dichloromethane/methanol and the extracted lipids was reacted with methanol and sulfuric acid to yield fatty acid methyl ester (FAMEs). After methylation, FAMEs were extracted with n-hexane and re-dissolved in isooctane. Analysis was performed using

an Agilent 6890 GC/FID equipped with a Supelco SP-2560 capillary column (100m*0.25mm inside diameter *0.20um thickness). Data were expressed as weight percentage of total fatty acids. Samples were organized in batches of up to 20, which included two samples from a standard pool for quality control (QC). The CVs 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 chi-square test for categorical variables were used to examine differences of basic characteristics between groups with MetS and without MetS. Spearman correlation was used to evaluate correlations of VLCSFAs and other plasma fatty acids. Logistic regression analysis was applied to examine the associations of VLCSFAs and risk of MetS. VLCSFAs were classified into four groups based on the quartiles of VLCSFAs in participants without metabolic syndrome at baseline. Several models were fitted to examine the association of each VLCSFA and risk of 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); 3) models additionally adjusted for the potential mediators based on fully adjusted model, including C16:0, C18:0, C18:3n3, C22:6n3, n-6 polyunsaturated fatty acids (n-6 PUFAs) and monounsaturated fatty acids (MUFAs) (exploration models). These selected fatty acids were significantly correlated with VLCSFAs with the spearman coefficients more than 0.25. As the fully adjusted models didn't change the results, main model was used in the following analyses. The associations of VLCSFAs and risk of incident metabolic syndrome during two year follow-up were conducted only in participants without MetS at baseline. Non-linear odds ratio of MetS was estimated by applying a restricted cubic spline regression model with 3 knots at the 5th, 50th and 95th percentiles[24].Multinomial logistic regression was used to estimate the associations of VLCSFAs and risk of metabolic syndrome according to the number of metabolic syndrome components at baseline. As the sample size in each group was limited, the VLCSFAs levels were classified into high or low group based on the median of VLCSFAs in subjects without metabolic syndrome. Low VLCSFAs levels group were assigned as the reference. When the associations between VLCSFAs and individual components of 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 VLCSFAs 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 versus low levels based on the median levels in controls. The ORs were expressed as the risk of metabolic syndrome for one SD increase of VLCSFAs. 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 1,729 participants were included in this study; 565(32.7%) of these were identified as MetS at baseline. Baseline characteristics of participants with or without MetS were presented in Table 1. The two groups were similar in sex, smoking status, alcohol drinking and physical activity. Compared to participants without MetS, 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, C22:6n3, C18:2n6, C20:4n6, and n-6 PUFA, but had higher C16:0, MUFA, C18:3n3, C20:5n3, and n-3 PUFAs. Women and individuals without diabetes had higher levels VLCSFAs (Supplemental Table S1a, Supplemental Table S1b). In addition, higher VLCSFAs were associated with lower BMI, lower triglycerides, 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 (Supplemental Table S1a). Furthermore, older ages were inversely related to higher C20:0, but directly to higher C24:0 (Supplemental Table S1a, Supplemental Table S1b). Participants with higher C24:0 were more likely to have hypertension (Supplemental Table S1b).

All the three VLCSFAs 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, Supplemental Table S2). In the fully adjusted model, compared to the lowest quartile, the highest level of VLCSFAs was associated with increased risk of MetS; the multivariate-adjusted ORs (95% CI) were 0.18 (0.13, 0.25) for C20:0, 0.26 (0.18, 0.35) for C22:0, 0.19 (0.13, 0.26) for C24:0, and 0.16 (0.11, 0.22) for total VLCSFAs (Table 2). Odds ratios of MetS were non-linear in C20:0, C22:0, C24:0, and total VLCSFAs (Figure 1, $P_{\text{non-linearity}}$<0.0001 for C20:0 and total VLCSFAs, $P_{\text{non-linearity}}$=0.002 for C22:0, $P_{\text{non-linearity}}$=0.021 for C24:0). The VLCSFAs were significantly correlated with C16:0, C18:0, MUFAs, C18:3n3, C22:6n3 and n-6PUFAs with absolute r_s more than 0.25 (Supplemental Table S2). Separately adjusted for these fatty acids did not affect the associations between VLCSFAs and MetS risk (Supplemental Table S3). In addition, the VLCSFAs levels were inversely associated with the number of MetS components (all the P_{trend}<0.0001, Supplemental Table S4). In the multinomial logistic regression analysis, the associations of VLCSFAs and MetS risk were stronger with the increase of the number of MetS components (Table 3).

Among 899 subjects without MetS at baseline, we identified 212 incident cases of MetS during the two-year follow up. The level of C20:0, C22:0, and total VLCSFAs were significantly associated with decreased incident MetS risk; comparing to the lowest quartile, the multivariate-adjusted ORs

(95% CI) for the highest quartile were 0.21 (0.12, 0.35) for C20:0, 0.20 (0.11, 0.34) for C22:0, 0.75 (0.44, 1.26) for C24:0, 0.28 (0.17, 0.46) for total VLCSFAs (Table 4).

VLCSFA levels and single MetS component associations were analyzed. Among the five MetS components, the associations were strongest for VLCSFAs and elevated triglyceride (Q4 vs Q1: ORs (95% CI) were 0.11 (0.07, 0.17) for C20:0, 0.10 (0.06, 0.16) for C22:0, 0.07 (0.04, 0.12) for C24:0, 0.04 (0.02, 0.07) for total VLCSFAs, P_{trend}<0.0001, Figure 2, Supplemental Table S5).

We also explored effect modification on the associations of VLCSFAs 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 (Figure 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) (Figure 3).

Discussion

In a population of 1729 Chinese adults, we observed that plasma VLCSFAs levels were significantly and inversely associated with prevalence and incidence of MetS. Further adjustment for each of C16:0, C18:0, C18:3n3, C22:6n3, n-6 polyunsaturated fatty acids (n-6 PUFAs), or monounsaturated fatty acids (MUFAs) did not negate the associations. The associations of VLCSFAs and MetS risk became stronger with the increase of the number of MetS components. Among the five individual MetS components, higher plasma VLCSFAs were strongly associated with plasma triglycerides.

Our observations are generally in line with several previous studies that have reported the relationship between blood VLCSFAs and various metabolic conditions in other populations[1, 8, 10-13, 25]. Three prospective studies published recently showed that higher levels of three VLCSFAs (C20:0, C22:0, and C24:0) were associated with lower risk of incident diabetes[1, 8, 27]. A study with 3179 participants from the Cardiovascular Health Study (CHS) reported that higher plasma phospholipid VLCSFAs were cross-sectionally associated with lower blood triglyceride and waist circumference (WC)[8]. In the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS), plasma VLCSFAs were positively correlated with high density lipoprotein (HDL)[11]. Diabetes, WC, circulating triglycerides and HDL are components of MetS. Consistently, we found inverse associations between plasma VLCSFAs and MetS risk; triglycerides showed the strongest associations with VLCSFAs. 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 triglycerides 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, VLCSFAs were associated with lower risk of diabetes, but if blood triglyceride was adjusted in the model, the associations were not significant[8]. This suggested that the associations of VLCSFAs with blood glucose might be mediated by lower triglycerides. Mechanistic studies are needed to confirm these results. Other recently published studies reported inverse associations of circulating levels of VLCSFAs with atrial fibrillation[10], cardioembolic stroke[12], coronary heart diseases (CHDs)[11], and total mortality[13]. MetS was a major intermediate risk factor of these diseases and may explain these associations[26-28].

The biological functions of VLCSFAs in vivo remain unclear. VLCSFAs are major components of ceramides and sphingomyelins which are key intermediates linking saturated fats and inflammatory cytokines to regulate cell function. MetS is associated with insulin resistance. C16-ceramide has been suggested to induce β-cell apoptosis, modulate insulin signaling and reduce insulin synthesis, which causes insulin resistance[29]. In contrast, little is known about the role of VLCSFAs-ceramide in insulin resistance. C16-ceramide may damage the heart, pancreas, and vasculature, and thus may induce diabetes, cardiovascular diseases, and atherosclerosis[30]. However, very long chain ceramides are likely to have opposite biological activities. For example, in cell culture and animal studies, C16 ceramide promoted hepatocellular hyperplasia whereas C24 ceramide did not[16]. In Langmuir–Blodgett monolayer experiments, C16 ceramide mixed well with cholesterol whereas C24 ceramide did not[31], suggesting that lipid raft functionality with higher level C24 ceramides may be impaired and apoptosis reduced subsequently[32]. 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[33].

The sources of circulating VLCSFAs include both exogenous diet and endogenous synthesis[14]. The VLCSFAs are found in limited foods, including peanuts, lotus nuts, and rapeseed/canola 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 favorable plasma lipid profile, including lower LDL cholesterol, and total cholesterol in the Nurses' Health Study[34]. VLCSFAs can be synthesized from C18:0 by elongases, especially the ELOVL1, which selectively elongates SFAs with more than 18 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→20:0 and 20:0→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 the ceramide synthase CERS2, a key enzyme for C24 sphingolipid synthesis[35]. This regulation may ensure that the production of C24-CoA by elongation is coordinated with its utilization. How the dietary intake and endogenous metabolism contribute to circulating levels of VLCSFAs and subsequently affect the metabolic disorders requires further study.

We also explored the modifying effects by several *a priori* factors on the relation of VLCSFAs 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 MetS[36], and may neutralize the beneficial effects of VLCSFAs on MetS. We also observed moderate interaction effects of sex on the association of C20:0 with 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 VLCSFAs 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 two-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 VLCSFAs with MetS risk. Third, we analyzed statistically plasma fatty acids in units of weight% 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 concentrations (e.g. mg/ml plasma) is more appropriate to assess whether the mass of a specific circulating fatty acid is outside of a range needed to support the metabolism under study. In addition, the fatty acids in various lipid classes (for instance, triglyceride, sphingomyelins) were not measured and would provide more specific insight into VLCSFAs' biological function. Fourth, no information on the lipid-lowering medication use was available. However, the hyperlipidemia awareness rates (8.3%), treatment rates (7.0%), and detection rates (26.2%) were very low in China, especially in rural area[37], which suggests a possible mild lipid-lowering medication influence. 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 VLCSFAs.

In summary, we confirmed that higher levels of plasma VLCSFAs are significantly associated

with lower risk of MetS in Chinese adults. The association of VLCSFAs with blood triglycerides was stronger than other four MetS components. Further studies are warranted to explore the underlying mechanisms.

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Conflict of Interest

None

Authorship

Ying Gao and Jing Zhao formulated the research questions and designed the study. Jing Zhao, Xiaofan Li, Xiang Li, Qianqian Chu, Yunhua Zhou and Zi Li carried it out. Jing Zhao, Xiaofan Li and Hong Zhang analyzed the data. Jing Zhao, Thomas J Brenna, Yiqing Song and Ying Gao wrote the article

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Table 1.Basic characteristics of subjects with and without metabolic syndrome at baseline

Characteristics*	With MetS (n=565)	Without MetS (n=1164)	P value†
Age	50.5 (5.95)	48.5 (6.28)	<0.0001
Sex			0.52
Male	185 (33%)	401(34%)	
Female	380 (67%)	763 (66%)	
Education			0.43
illiteracy	18 (3%)	25 (2%)	
Primary School	73 (13%)	126 (11%)	
Middle School	353 (62%)	746 (64%)	
High School	115 (20%)	251 (22%)	
College	6 (1%)	16 (1%)	
Health education	262(46%)	598(51%)	0.06
Current smoking status	116 (21%)	265 (23%)	0.32
Current alcohol drinking	146 (26%)	304 (26%)	0.95
Agricultural work	243 (43%)	612 (53%)	0.0002
Physical activity	72 (13%)	150 (13%)	0.99
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	308 (55%)	218 (19%)	< 0.0001
Hypertension	398 (70%)	415 (36%)	< 0.0001
Diabetes	112 (20%)	33 (3%)	< 0.0001
TG	2.33 (1.81)	1.07 (1.06)	< 0.0001
TC	5.44 (1.05)	5.17 (0.88)	< 0.0001
HDL-c	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 VLCSFAs	1.30 (0.32)	1.53 (0.32)	< 0.0001
MUFAs[‡]	20.6 (3.11)	18.0 (2.46)	< 0.0001
C18:3n3	0.93 (0.34)	0.74 (0.26)	< 0.0001
C20:5n3	0.48 (0.20)	0.48 (0.28)	< 0.0001
C22:6n3c	1.87 (0.57)	1.95 (0.60)	0.002
n3PUFAs[§]	3.77 (0.73)	3.65 (0.82)	< 0.0001
C18:2n6	33.9 (3.80)	36.1 (3.80)	< 0.0001
C20:4n6	7.32 (1.79)	8.55 (1.73)	< 0.0001
n6PUFAs[‖]	44.2 (3.99)	47.6 (3.06)	< 0.0001

[*]Continuous variables were expressed as mean (SD) and categorical variables were expressed as frequency(percentage among cases or controls).

[†]*P*-value were calculated from Wilcoxon rank sum test for continuous variables and $\chi 2$-test for categorical variables

[‡]MUFAs: monounsaturated fatty acids, which includes C14:1n5, C16:1n7, C16:1n9, C18:1n7, C18:1n9, C20:1n9, C22:1n9 and C24:1n9 in this study

[§]n-3PUFAs: n-3 polyunsaturated fatty acids, which includes C18:3n3, C20:3n3, C20:5n3, C22:3n3, C22:5n3 and C22:6n3

[‖]n-6PUFAs: n-6 polyunsaturated fatty acids, which includes C18:2n6, C18:3n6, C20:2n6, C20:3n6, C20:4n6, C22:2n6, C22:4n6 and C22:5n6 in this study

Table 2. The associations of VLCSFAs and prevalence of metabolic syndrome at baseline

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
C20:0					
$N_{MetS/non-MetS}$	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					
$N_{MetS/non-MetS}$	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					
$N_{MetS/non-MetS}$	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 VLCSFAs					
$N_{MetS/non-MetS}$	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 ORs and CIs. VLCSFAs were classified into four group based on the quartiles of VLCSFAs in subjects without 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 VLCSFAs.

Model 1: adjusted for sex, age, agricultural work.

Model 2: additionally adjusted for education, smoking, alcohol drinking, physical activity.

Table 3. The associations of VLCSFAs and risk of metabolic syndrome according to the number of metabolic syndrome components at baseline

	MS=0	MS=3	MS=4	MS=5
20:0				
$N_{high/low}$	582/582	80/251	44/138	14/38
ORs	1	0.33 (0.25, 0.44)	0.33 (0.23, 0.47)	0.39 (0.21, 0.72)
22:0				
$N_{high/low}$	582/ 582	81/ 250	69/ 113	14/ 38
ORs	1	0.32 (0.24, 0.43)	0.61 (0.44, 0.84)	0.36(0.19, 0.67)
24:0				
$N_{high/low}$	582/ 582	134/ 197	8/ 174	3/49
ORs	1	0.63 (0.49, 0.81)	0.04 (0.02, 0.09)	0.05 (0.02, 0.18)
Total VLCSFAs				
$N_{high/low}$	582/ 582	97/234	24/158	2/50
ORs	1	0.40 (0.31, 0.53)	0.15 (0.09, 0.23)	0.04 (0.01, 0.16)

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

Table 4. The associations of VLCSFAs and risk of incident metabolic syndrome after two years

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
C20:0					
$N_{MetS/non-MetS}$	89 /172	62/171	43/172	18/172	
	1	0.67 (0.45, 1.00)	0.47 (0.30, 0.72)	0.21 (0.12, 0.35)	<0.001
C22:0					
$N_{MetS/non-MetS}$	89 /172	65/171	38/172	20 /172	
	1	0.64 (0.43, 0.95)	0.40 (0.25, 0.62)	0.20 (0.11, 0.34)	<0.001
C24:0					
$N_{MetS/non-MetS}$	38 /172	87/171	52/172	35/172	
	1	1.94 (1.25, 3.06)	1.15 (0.71, 1.87)	0.75 (0.44, 1.26)	0.11
Total VLCSFAs					
$N_{MetS/non-MetS}$	80 /172	62 /171	44 /172	26/172	
	1	0.71 (0.47,1.07)	0.51(0.33, 0.78)	0.28 (0.17, 0.46)	<0.001

Only subjects without MetS at baseline were included in the analysis. VLCSFAs were classified into four group based on the quartiles of VLCSFA in subjects without 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 and1.72 for total VLCSFAs. Models were adjusted for sex, age, agricultural work

Figure Legend

Figure 1 Odds ratios of MetS by VLCSFAs levels at baseline. Lines represent odds ratios (95% CI) based on restricted cubic splines for VLCSFAs levels with knots at the 5th, 50th and 95th percentiles. Odds ratios were estimated using a logistic regression model after adjustment for age, sex, agricultural work; Bars represent the numbers of participants. (A) C20:0 ($P_{non\text{-}linearity} < 0.001$), (B) C22:0 ($P_{non\text{-}linearity} = 0.002$), (C) C24:0 ($P_{non\text{-}linearity} = 0.021$), (D) total VLCSFAs ($P_{non\text{-}linearity} < 0.0001$)

Figure 2 The associations of VLCSFAs and risk of metabolic syndrome components at baseline. Logistic regression was used to estimate the ORs and CIs. All the ORs was 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 metabolic syndrome definition. BP: blood pressure, FBG: fasting blood glucose, HDL: high density lipoprotein, TG: triglyceride, WC: waist circumference

Figure 3 Stratified analysis of the associations of VLCSFAs and risk of metabolic syndrome at baseline. All the ORs were adjusted for age, sex, and agricultural work. The ORs meant the risk of metabolic syndrome for one SD increase of VLCSFAs.

Supplemental Table 1a. Characteristics of participants according to plasma C20:0 and C22:0

Characteristics*	C20:0†					C22:0†				
	Q1	Q2	Q3	Q4	P_{trend}	Q1	Q2	Q3	Q4	P_{trend}
Age	50(6)	50(6)	49(6)	48 (6)	<0.0001	49 (6)	49 (6)	49(6)	49(6)	0.14
Sex					<0.0001					<0.0001
Male	189(44%)	144(33%)	131(30%)	122(28%)		202(47%)	138(32%)	119(27%)	128(30%)	
Female	243(56%)	288(67%)	302 (70%)	311(72%)		230 (53%)	294 (68%)	314 (73%)	305(70%)	
Education					0.32					0.15
illiteracy	10(2%)	9(2%)	11(3%)	13 (3%)		11(3%)	10(2%)	9(2%)	13(3%)	
Primary School	52(12%)	54(12%)	56 (13%)	37(9%)		50 (12%)	60(14%)	46(11%)	43(10%)	
Middle School	276(64%)	269(62%)	277(64%)	78(64%)		280(65%)	275(64%)	272(63%)	273(63%)	
High School	86(20%)	97(22%)	85 (20%)	98(23%)		83(19%)	84(19%)	99(23%)	100(23%)	
College	8(2%)	3(1%	4 (1%)	7(2%)		8(2%)	3(1%)	7 (2%)	4(1%)	
Current smoking status	123(28%)	86(20%)	84(19%)	88(20%)	0.01	141(33%)	76(18%)	74(17%)	91(21%)	0.0002
Current alcohol drinking	133(31%)	107(25%)	106(24%)	104(24%)	0.03	146(34%)	98(23%)	103(24%)	104(24%)	0.004
Agricultural work	190(44%)	211(49%)	220(50%)	234(54%)	0.003	207(48%)	217(50%)	206(48%)	226(52%)	0.3
Physical activity	52(12%)	54(12%)	61(14%)	55(13%)	0.67	53(12%)	72(17%)	52(12%)	46(11%)	0.17
BMI(kg/m^2)	27.1(3.3)	26.7(3.8)	26.0(3.5)	25.4(3.7)	<0.0001	26.3(3.6)	26.5(3.5)	26.4(3.6)	25.4(3.7)	<0.0001
WC(cm)	88.9(9.7)	87.0(10.2)	84.9(10.1)	82.7(10.6)	<0.0001	88.3(9.6)	86.6(10.1)	85.8(10.3)	82.8(10.8)	<0.0001
Obesity	169(39%)	147(34%)	116(27%)	95 (22%)	<0.0001	164 (38%)	141 (33%)	133 (31%)	88 (20%)	<0.0001
Hypertension	245(57%)	222(51%)	202(47%)	145(33%)	<0.0001	250 (58%)	212 (49%)	200 (46%)	151 (35%)	<0.0001
Diabetes	57(13%)	40(9%)	28(6%)	20(5%)	<0.0001	43(10%)	44 (10%)	34(8%)	24(6%)	0.01
TG	2.27(1.91)	1.43(0.98)	1.19(0.98)	1.04(1.5)	<0.0001	2.39(2.41)	1.4 0(0.79)	1.2(0.84)	0.94(0.61)	<0.0001
TC	5.48(1.02)	5.34(0.90)	5.17(0.87)	5.03(0.9)	<0.0001	5.41(1.06)	5.23(0.92)	5.26(0.90)	5.12(0.88)	<0.0001
HDL-c	1.34(0.31)	1.44(0.32)	1.50(0.34)	1.53(0.35)	<0.0001	1.35(0.34)	1.42 (0.32)	1.48(0.32)	1.54(0.34)	<0.0001

*Continuous variables were expressed as mean (SD) and categorical variables were expressed as frequency (percentage of case).

†VLCSFAs were classified into four group based on the quartiles of VLCSFAs in all subjects: 0.17, 0.20 and 0.22 for C20:0; 0.57, 0.68 and 0.81 for C22:0.

Supplemental Table 1b. Characteristics of participants according to plasma C24:0 and total VLCSFAs

Characteristics*	**C24:0†**					**Total VLCSFAs†**				
	Q1	Q2	Q3	Q4	P_{trend}	Q1	Q2	Q3	Q4	P_{trend}
Age	48 (7)	49 (6)	50(6)	50 (6)	<0.0001	49(6)	49(6)	49(6)	49(6)	0.24
Sex					0.04					0.005
Male	140(32%)	141(33%)	131(30%)	174(40%)		181(42%)	132(31%)	136(31%)	138(32%)	
Female	292(68%)	291(67%)	302(70%)	259(60%)		251(58%)	300(69%)	297(69%)	295(68%)	
Education					0.89					0.75
illiteracy	9 (2%)	12 (3%)	12 (3%)	10 (2%)		6 (1%)	16 (4%)	9 (2%)	12 (3%)	
Primary School	44 (10%)	61 (14%)	49 (11%)	45 (11%)		47 (11%)	59 (14%)	48 (11%)	45 (10%)	
Middle School	276(64%)	273 (63%)	268(62%)	282(65%)		283 (66%)	267 (62%)	271 (63%)	279 (64%)	
High School	99 (23%)	81 (19%)	97 (22%)	90 (21%)		90 (21%)	86 (20%)	98 (23%)	92 (21%)	
College	4 (1%)	5 (1%)	7 (2%)	6 (1%)		6 (1%)	4 (1%)	7 (2%)	5 (1%)	
Current smoking status	100(23%)	87 (20%)	81(19%)	113(26%)	0.47	116(27%)	87 (20%)	84 (19%)	95(22%)	0.01
Current alcohol drinking	112(26%)	103(24%)	98(23%)	137(32%)	0.11	131(30%)	98(23%)	112(26%)	110(25%)	0.21
Agricultural work	205(47%)	207(48%)	212(49%)	232(54%)	0.08	204(47%)	208(48%)	208(48%)	236(55%)	0.04
Physical activity	53(12%)	67.0(16%)	59 (14%)	43 (10%)	0.28	53 (12%)	60 (14%)	64 (15%)	45 (10%)	0.44
BMI(kg/m^2)	26.4(3.8)	26.8 (3.8)	26.2 (3.5)	25.8 (3.2)	0.01	27.3(3.4)	26.3(3.8)	25.9(3.7)	25.6(3.3)	<0.0001
WC(cm)	85.9(11.6)	87.0(10.5)	85.7(10.1)	84.8(9.2)	0.07	89.2(9.9)	85.8(10.5)	84.8(10.7)	83.7(9.6)	<0.0001
Obesity	146(34%)	154 (36%)	132 (30%)	94 (22%)	<0.0001	185 (43%)	137 (32%)	116 (27%)	88 (20%)	<0.0001
Hypertension	168(39%)	204 (47%)	224(52%)	218 (50%)	0.0002	237 (55%)	193 (45%)	187 (43%)	197 (45%)	0.01
Diabetes	51 (12%)	38.0(9%)	34 (8%)	22 (5%)	0.0004	54 (12%)	39 (9%)	29 (7%)	23 (5%)	<0.0001
TG	2.11(2.46)	1.63 (0.95)	1.22(0.65)	0.97(0.78)	<0.0001	2.49 (2.39)	1.44(0.83)	1.10(0.5)	0.9 (0.75)	<0.0001
TC	5.23(1.06)	5.30(0.88)	5.3(0.90)	5.19(0.95)	0.64	5.35(1.06)	5.28(0.89)	5.21 (0.89)	5.17(0.93)	0.003

HDL-c	1.35(0.36)	1.42(0.32)	1.50(0.32)	1.53(0.33)	<0.0001	1.3 (0.31)	1.44(0.32)	1.50 (0.31)	1.56(0.35)	<0.0001

*Continuous variables were expressed as mean (SD) and categorical variables were expressed as frequency (percentage of case).

†VLCSFAs were classified into four group based on the quartiles of VLCSFAs in all subjects included in analysis: 0.40, 0.57 and 0.70 for C24:0; 1.23, 1.43 and 1.65 for total VLCSFAs.

Supplemental Table 2. Spearman correlation coefficients for plasma fatty acids

	C20:0	C22:0	C24:0	total VLCSFAs	C16:0	C18:0	MUFAs‡	C18:3n3	C20:5n3	C22:6n3	n3PUFAs§	C18:2n6	C20:4n6	n6PUFAs‖
Percentage of total fatty acids †	0.20 (0.17, 0.22)	0.68 (0.57, 0.81)	0.57 (0.40, 0.70)	1.43 (1.23, 1.65)	20.1 (19.0, 21.2)	8.31 (7.78, 8.84)	18.5 (16.8, 20.3)	0.76 (0.59, 0.96)	0.42 (0.29, 0.63)	1.82 (1.55, 2.16)	3.59 (3.17, 4.13)	35.6 (33.1, 37.9)	8.21 (6.86, 9.44)	46.9 (44.4, 49.0)
C20:0	1.00													
C22:0	0.75**	1.00												
C24:0	0.23**	0.21**	1.00											
total VLCSFAs	0.69**	0.79**	0.72**	1.00										
C16:0	-0.34**	-0.34**	-0.13**	-0.31**	1.00									
C18:0	0.48**	0.36**	0.13**	0.35**	-0.23**	1.00								
MUFAs	-0.48**	-0.49**	-0.22**	-0.47**	0.54**	-0.51**	1.00							
C18:3n3	-0.37**	-0.42**	-0.27**	-0.45**	0.03	-0.25**	0.10**	1.00						
C20:5n3	0.12**	0.26**	-0.50**	-0.11**	0.04	0.16**	-0.15**	0.05*	1.00					
C22:6n3	0.23**	0.27**	-0.15**	0.10**	-0.12**	0.19**	-0.20**	-0.11**	0.41**	1.00				
n3PUFAs	0.05*	0.10**	-0.42**	-0.16**	-0.05*	0.10**	-0.16**	0.34**	0.71**	0.76**	1.00			
C18:2n6	0.23**	0.25**	0.12**	0.22**	-0.68**	0.06*	-0.62**	0.11**	-0.21**	-0.15**	-0.17**	1.00		
C20:4n6	0.28**	0.27**	0.27**	0.36**	-0.29**	0.31**	-0.42**	-0.39**	0.20**	0.29**	0.13**	-0.13**	1.00	
n6PUFAs	0.35**	0.37**	0.24**	0.39**	-0.81**	0.22**	-0.83**	-0.07*	-0.09**	-0..004	-0.08**	0.85**	0.35**	1.00

[†]present as median(interquartile)

[‡]MUFAs: monounsaturated fatty acids, which includes C14:1n5, C16:1n7, C16:1n9, C18:1n7, C18:1n9, C20:1n9, C22:1n9 and C24:1n9 in this study

[§]n3PUFAs: n-3 polyunsaturated fatty acids, which includes C18:3n3, C20:3n3, C20:5n3, C22:3n3, C22:5n3 and C22:6n3

[‖] n6PUFAs: n-6 polyunsaturated fatty acids, which includes C18:2n6, C18:3n6, C20:2n6, C20:3n6, C20:4n6, C22:2n6, C22:4n6 and C22:5n6 in this study

**P*-value < 0.05; ***P*-value < 0.001

Supplemental Table 3. The associations of VLCSFAs and prevalence of metabolic syndrome at baseline

	Q1	Q2	Q3	Q4	P_{trend}
C20:0					
$N_{MetS/non-MetS}$	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)	<0.001
Model 2	1	0.43 (0.32, 0.58)	0.40 (0.29, 0.54)	0.28 (0.20, 0.40)	<0.001
Model 3	1	0.39 (0.29, 0.52)	0.35 (0.26, 0.48)	0.26 (0.18, 0.37)	<0.001
Model 4	1	0.45 (0.33, 0.61)	0.44 (0.32, 0.61)	0.38 (0.26, 0.54)	<0.001
Model 5	1	0.41 (0.30, 0.55)	0.36 (0.26, 0.49)	0.32 (0.23, 0.46)	<0.001
Model 6	1	0.37 (0.28, 0.49)	0.33 (0.24, 0.45)	0.28 (0.19, 0.39)	<0.001
Model 7	1	0.30 (0.23, 0.40)	0.25 (0.18, 0.33)	0.18 (0.13, 0.25)	<0.001
C22:0					
$N_{MetS/non-MetS}$	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)	<0.001
Model 2	1	0.88 (0.66, 1.19)	0.69 (0.50, 0.95)	0.51 (0.36, 0.73)	<0.001
Model 3	1	0.72 (0.54, 0.96)	0.54 (0.39, 0.73)	0.41 (0.29, 0.58)	<0.001
Model 4	1	1.00 (0.74, 1.34)	0.82 (0.59, 1.14)	0.73 (0.50, 1.05)	0.06
Model 5	1	0.71 (0.54, 0.94)	0.52 (0.38, 0.70)	0.43 (0.30, 0.60)	<0.001
Model 6	1	0.73 (0.55, 0.96)	0.56 (0.41, 0.76)	0.49 (0.34, 0.69)	<0.001
Model 7	1	0.58 (0.44, 0.75)	0.38 (0.28, 0.50)	0.26 (0.18, 0.36)	<0.001
C24:0					
$N_{MetS/non-MetS}$	260/291	160/291	90/291	55/291	
Model 1	1	0.55 (0.42, 0.71)	0.30 (0.22, 0.40)	0.19 (0.13, 0.26)	<0.001
Model 2	1	0.59 (0.44, 0.79)	0.45 (0.32, 0.62)	0.31 (0.21, 0.45)	<0.001
Model 3	1	0.50 (0.37, 0.66)	0.33 (0.24, 0.46)	0.23 (0.16, 0.33)	<0.001
Model 4	1	0.59 (0.44, 0.78)	0.43 (0.31, 0.59)	0.32 (0.22, 0.47)	<0.001

Model 5	1	0.56 (0.42, 0.74)	0.32 (0.24, 0.44)	0.23 (0.16, 0.32)	<0.001
Model 6	1	0.56 (0.42, 0.73)	0.36 (0.27, 0.50)	0.27 (0.19, 0.38)	<0.001
Model 7	1	0.51 (0.39, 0.66)	0.27 (0.20, 0.37)	0.17 (0.12, 0.24)	<0.001
Total VLCSFAs[a]					
$N_{MetS/non-MetS}$	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)	<0.001
Model 2	1	0.53 (0.40, 0.71)	0.35 (0.25, 0.49)	0.30 (0.21, 0.43)	<0.001
Model 3	1	0.47 (0.35, 0.63)	0.29 (0.21, 0.40)	0.23 (0.16, 0.32)	<0.001
Model 4	1	0.58 (0.43, 0.77)	0.40 (0.28, 0.55)	0.37 (0.25, 0.53)	<0.001
Model 5	1	0.48 (0.36, 0.64)	0.29 (0.21, 0.40)	0.23 (0.16, 0.33)	<0.001
Model 6	1	0.49 (0.37, 0.65)	0.30 (0.21, 0.41)	0.25 (0.17, 0.36)	<0.001
Model 7	1	0.39 (0.30, 0.51)	0.21 (0.15, 0.29)	0.16 (0.11, 0.22)	<0.001

Logistic regression was used to estimate the ORs and CIs. VLCSFAs were classified into four group based on the quartiles of VLCSFAs in subjects without MetS: 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 VLCSFAs.

Model 1: adjusted for sex, age, agricultural work, education, smoking, alcohol drinking, physical activity

Model 2: additionally adjusted for n-6 PUFAs

Model 3: additionally adjusted for C16:0

Model 4: additionally adjusted for MUFAs

Model 5: additionally adjusted for C18:0

Model 6: additionally adjusted for C18:3n3

Model 7: additionally adjusted for C22:6n3

Supplemental Table 4. Levels of VLCSFAs according to the scores of metabolic syndrome at baseline[*]

MetS scores	**≤2**	**3**	**4**	**5**	P_{trend}
C20:0	0.21 (0.04)	0.18 (0.04)	0.18 (0.04)	0.17 (0.04)	<0.0001
C22:0	0.73 (0.20)	0.59 (0.17)	0.69 (0.22)	0.62 (0.18)	<0.0001

C24:0	0.60 (0.21)	0.58 (0.17)	0.36 (0.13)	0.36 (0.14)	<0.0001
Total VLCSFAs	1.53 (0.32)	1.35 (0.34)	1.23 (0.29)	1.15 (0.24)	<0.0001

*Levels of VLCSFAs were expressed as mean (SD)

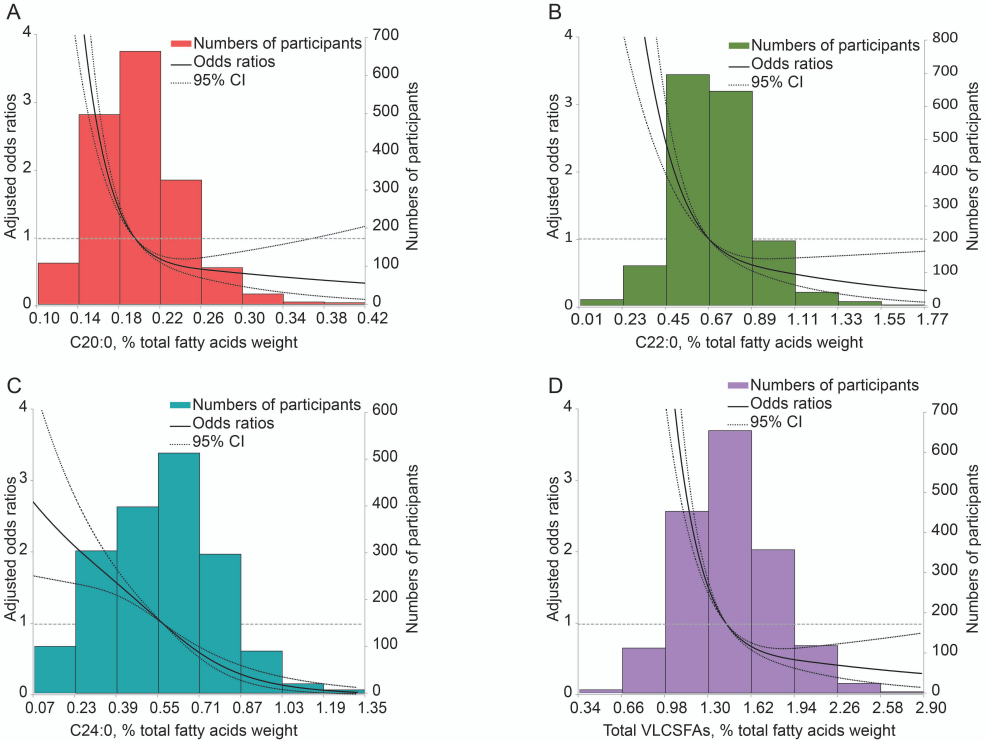
Supplemental Table 5. The associations of VLCSFAs and metabolic syndrome components

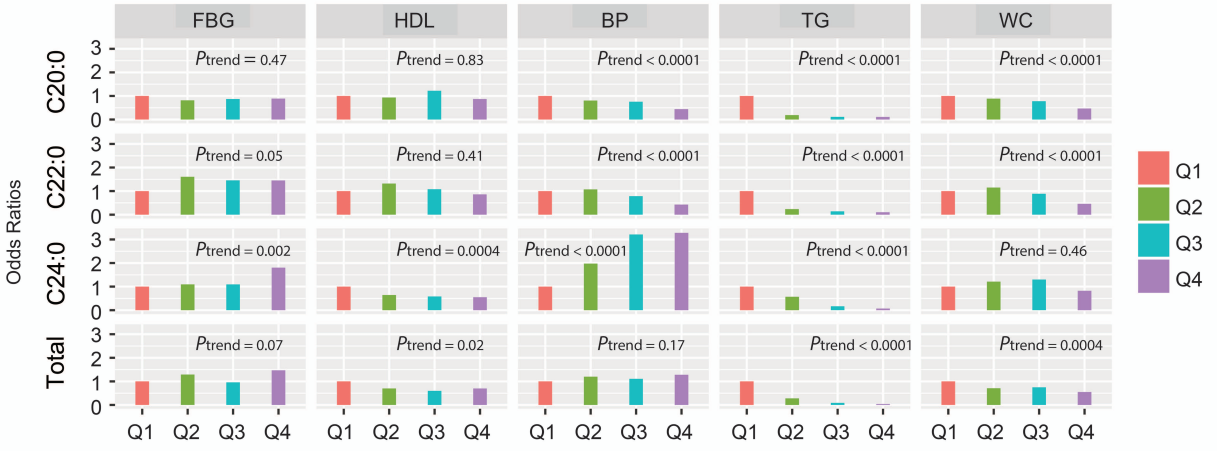
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	P_{trend}
C20:0					
$N_{high\ vs\ low}$	432/ 186	229/ 163	204/ 167	141/ 208	
$WC_{high\ vs\ low}$	1	0.89 (0.66, 1.20)	0.78 (0.57, 1.05)	0.47 (0.34, 0.64)	<0.0001
$N_{high\ vs\ low}$	312/ 306	56/ 336	38/ 333	26/ 323	
$TG_{high\ vs\ low}$	1	0.19 (0.13, 0.26)	0.11 (0.07, 0.17)	0.11 (0.07, 0.17)	<0.0001
$N_{low\ vs\ high}$	196/ 422	76/ 316	81/ 290	52/ 297	
$HDL_{low\ vs\ high}$	1	0.93 (0.66, 1.32)	1.22 (0.86, 1.74)	0.87 (0.58, 1.29)	0.83
$N_{high\ vs\ low}$	447/ 170	251/ 141	224/ 147	159/ 190	
$BP_{high\ vs\ low}$	1	0.80 (0.60, 1.09)	0.76 (0.56, 1.03)	0.44 (0.32, 0.60)	<0.0001
$N_{high\ vs\ low}$	203/ 415	95/ 297	90/ 281	79/ 270	
$FBG_{high\ vs\ low}$	1	0.81 (0.60, 1.10)	0.87 (0.63, 1.19)	0.89 (0.63, 1.24)	0.47
C22:0					
$N_{high\ vs\ low}$	355/ 185	292/ 152	222/ 167	137/ 220	
$WC_{high\ vs\ low}$	1	1.15 (0.85, 1.55)	0.89 (0.65, 1.21)	0.46 (0.33, 0.63)	<0.0001
$N_{high\ vs\ low}$	260/ 280	95/ 349	49/ 340	28/ 329	
$TG_{high\ vs\ low}$	1	0.24 (0.17, 0.32)	0.14 (0.10, 0.21)	0.10 (0.06, 0.16)	<0.0001
$N_{low\ vs\ high}$	154/ 386	120/ 324	80/ 309	51/ 306	
$HDL_{low\ vs\ high}$	1	1.32 (0.95, 1.83)	1.08 (0.75, 1.55)	0.86 (0.57, 1.27)	0.41
$N_{high\ vs\ low}$	379/ 386	309/ 135	234/ 155	159/ 198	
$BP_{high\ vs\ low}$	1	1.07 (0.80, 1.44)	0.79 (0.58, 1.07)	0.43 (0.31, 0.58)	<0.0001

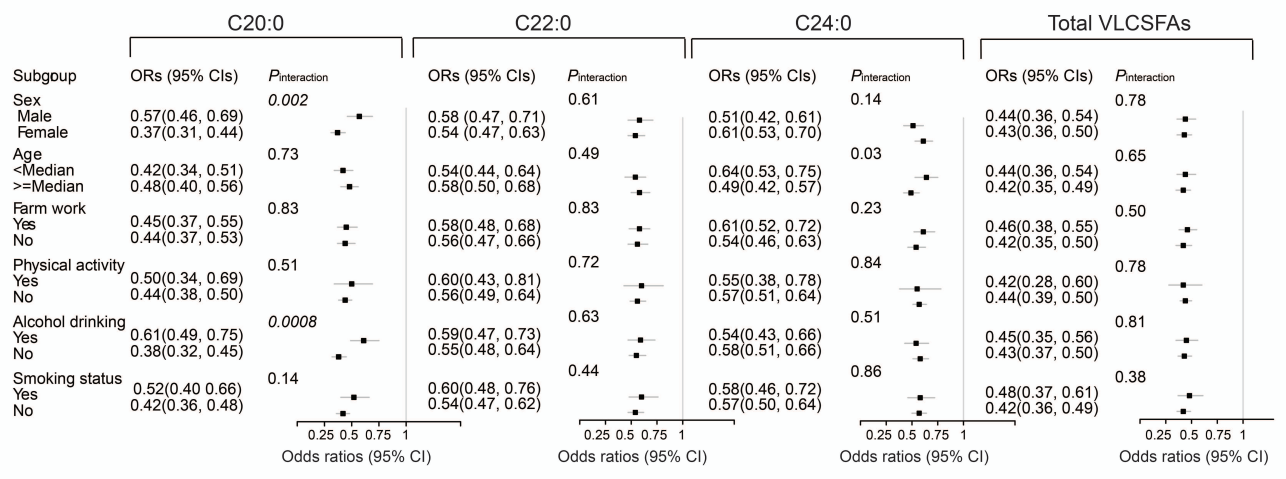
$N_{\text{high vs low}}$	139/ 401	139/ 305	103/ 286	86/ 271	
$FBG_{\text{high vs low}}$	1	1.61 (1.19, 2.17)	1.46 (1.05, 2.01)	1.45 (1.03, 2.05)	0.05
C24:0					
$N_{\text{high vs low}}$	332/ 219	285/ 167	229/ 152	160/ 186	
$WC_{\text{high vs low}}$	1	1.21 (0.90, 1.62)	1.30 (0.95, 1.78)	0.82 (0.60, 1.13)	0.46
$N_{\text{high vs low}}$	235/ 316	136/ 316	44/ 337	17/ 329	
$TG_{\text{high vs low}}$	1	0.57 (0.42, 0.77)	0.17 (0.11, 0.24)	0.07 (0.04, 0.12)	<0.0001
$N_{\text{low vs high}}$	190/ 361	106/ 346	65/ 316	44/ 302	
$HDL_{\text{low vs high}}$	1	0.64 (0.47, 0.88)	0.58 (0.40, 0.83)	0.55 (0.37, 0.82)	0.0004
$N_{\text{high vs low}}$	273/ 278	290/ 161	273/ 108	245/ 101	
$BP_{\text{high vs low}}$	1	1.98 (1.50, 2.62)	3.21 (2.36, 4.40)	3.28 (2.38, 4.55)	<0.0001
$N_{\text{high vs low}}$	142/ 409	120/ 332	93/ 288	112/ 234	
$FBG_{\text{high vs low}}$	1	1.09 (0.81, 1.48)	1.09 (0.78, 1.53)	1.80 (1.29, 2.53)	0.002
Total VLCSFAs					
$N_{\text{high vs low}}$	421/ 183	238/ 183	189/ 172	158/ 186	
$WC_{\text{high vs low}}$	1	0.71 (0.53, 0.96)	0.75 (0.55, 1.02)	0.55 (0.40, 0.76)	0.0004
$N_{\text{high vs low}}$	308/ 296	86/ 335	26/ 335	12/ 332	
$TG_{\text{high vs low}}$	1	0.28 (0.20, 0.38)	0.09 (0.06, 0.14)	0.04 (0.02, 0.07)	<0.0001
$N_{\text{low vs high}}$	214/ 390	87/ 334	53/ 308	51/ 293	
$HDL_{\text{low vs high}}$	1	0.70 (0.50, 0.96)	0.60 (0.41, 0.88)	0.70 (0.47, 1.03)	0.02
$N_{\text{high vs low}}$	386/ 217	266/ 155	214/ 147	215/ 129	
$BP_{\text{high vs low}}$	1	1.20 (0.90, 1.60)	1.11 (0.82, 1.52)	1.28 (0.93, 1.76)	0.17
$N_{\text{high vs low}}$	172/ 432	120/ 301	77/ 284	98/ 246	
$FBG_{\text{high vs low}}$	1	1.29 (0.95, 1.74)	0.96(0.68, 1.35)	1.47 (1.05, 2.07)	0.07

Logistic regression was used to estimate the ORs and CIs. All the ORs was adjusted for sex, age, 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 metabolic syndrome definition.

BP: blood pressure, FBG: fast blood glucose, HDL: high density lipoprotein, TG: triglyceride, WC: waist circumference


A
Numbers of participants
Odds ratios
95% CI
Adjusted odds ratios
Numbers of participants
0 1 2 3 4
0 100 200 300 400 500 600 700
0.10 0.14 0.18 0.22 0.26 0.30 0.34 0.38 0.42
C20:0, % total fatty acids weight
B
Numbers of participants
Odds ratios
95% CI
Adjusted odds ratios
Numbers of participants
0 1 2 3 4
0 100 200 300 400 500 600 700 800
0.01 0.23 0.45 0.67 0.89 1.11 1.33 1.55 1.77
C22:0, % total fatty acids weight
C
Numbers of participants
Odds ratios
95% CI
Adjusted odds ratios
Numbers of participants
0 1 2 3 4
0 100 200 300 400 500 600
0.07 0.23 0.39 0.55 0.71 0.87 1.03 1.19 1.35
C24:0, % total fatty acids weight
D
Numbers of participants
Odds ratios
95% CI
Adjusted odds ratios
Numbers of participants
0 1 2 3 4
0 100 200 300 400 500 600 700
0.34 0.66 0.98 1.30 1.62 1.94 2.26 2.58 2.90
Total VLCSFAs, % total fatty acids weight


FBG
HDL
BP
TG
WC
C20:0
C22:0
C24:0
Total
Odds Ratios
Ptrend = 0.47
Ptrend = 0.83
Ptrend < 0.0001
Ptrend < 0.0001
Ptrend < 0.0001
Ptrend = 0.05
Ptrend = 0.41
Ptrend < 0.0001
Ptrend < 0.0001
Ptrend < 0.0001
Ptrend = 0.002
Ptrend = 0.0004
Ptrend < 0.0001
Ptrend < 0.0001
Ptrend = 0.46
Ptrend = 0.07
Ptrend = 0.02
Ptrend = 0.17
Ptrend < 0.0001
Ptrend = 0.0004
0
1
2
3
Q1
Q2
Q3
Q4


C20:0
C22:0
C24:0
Total VLCSFAs
Subgoup
ORs (95% CIs)
Pinteraction
Sex
Male
Female
0.57(0.46, 0.69)
0.37(0.31, 0.44)
0.002
0.58 (0.47, 0.71)
0.54 (0.47, 0.63)
0.61
0.51(0.42, 0.61)
0.61(0.53, 0.70)
0.14
0.44(0.36, 0.54)
0.43(0.36, 0.50)
0.78
Age
<Median
>=Median
0.42(0.34, 0.51)
0.48(0.40, 0.56)
0.73
0.54(0.44, 0.64)
0.58(0.50, 0.68)
0.49
0.64(0.53, 0.75)
0.49(0.42, 0.57)
0.03
0.44(0.36, 0.54)
0.42(0.35, 0.49)
0.65
Farm work
Yes
No
0.45(0.37, 0.55)
0.44(0.37, 0.53)
0.83
0.58(0.48, 0.68)
0.56(0.47, 0.66)
0.83
0.61(0.52, 0.72)
0.54(0.46, 0.63)
0.23
0.46(0.38, 0.55)
0.42(0.35, 0.50)
0.50
Physical activity
Yes
No
0.50(0.34, 0.69)
0.44(0.38, 0.50)
0.51
0.60(0.43, 0.81)
0.56(0.49, 0.64)
0.72
0.55(0.38, 0.78)
0.57(0.51, 0.64)
0.84
0.42(0.28, 0.60)
0.44(0.39, 0.50)
0.78
Alcohol drinking
Yes
No
0.61(0.49, 0.75)
0.38(0.32, 0.45)
0.0008
0.59(0.47, 0.73)
0.55(0.48, 0.64)
0.63
0.54(0.43, 0.66)
0.58(0.51, 0.66)
0.51
0.45(0.35, 0.56)
0.43(0.37, 0.50)
0.81
Smoking status
Yes
No
0.52(0.40 0.66)
0.42(0.36, 0.48)
0.14
0.60(0.48, 0.76)
0.54(0.47, 0.62)
0.44
0.58(0.46, 0.72)
0.57(0.50, 0.64)
0.86
0.48(0.37, 0.61)
0.42(0.36, 0.49)
0.38
0.25 0.5 0.75 1
Odds ratios (95% CI)