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ARTICLE

Erythrocyte *trans*-fatty acids, type 2 diabetes and cardiovascular risk factors in middle-aged and older Chinese individuals

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Abstract
Aims/hypothesis Few data are available about intakes and food sources of *trans*-fatty acids (TFAs) or their associations with cardiometabolic outcomes in Asian people who consume a prudent diet but are experiencing rapid nutritional transitions. We aimed to investigate the relationships between TFA biomarkers and type 2 diabetes and cardiovascular risk factors in Chinese individuals.
Methods Erythrocyte fatty acids were measured by gas chromatography among 3,107 men and women (50–70 years) recruited from urban and rural areas in Beijing and Shanghai, China.
Results Total *trans*-18:1 and two *trans*-18:2 isomers were detected and accounted for 0.37% of the total fatty acids in the erythrocytes. Concentrations of TFAs were higher in women than men, and in urban than rural residents. Of the TFAs, *trans*-18:1, but not *trans*-18:2, showed a modest association with dairy consumption ($\beta=0.27$), but not with other foods. After adjustment for BMI, social-demographic, lifestyle and dietary factors and other TFAs, erythrocyte *trans*-18:1 was shown to be associated with a lower risk of type 2 diabetes (OR comparing extreme [first and fourth] quartiles 0.68, 95% CI 0.48, 0.97, $p_{trend}=0.02$), as well as 20–50% lower odds of central obesity, dyslipidaemia, hyperglycaemia, insulin resistance and chronic inflammation. In contrast, *trans*-18:2 fatty acids were positively associated with high triacylglycerol ($p_{trend}<0.001$) and LDL-cholesterol ($p_{trend}=0.03$) levels, but not with diabetes and other cardiometabolic risk factors.
Conclusions/interpretation Among middle-aged and older Chinese individuals with overall low erythrocyte TFAs levels, *trans*-18:1 might serve as a marker of dairy intake. Higher *trans*-18:1 levels were associated with a lower risk of type 2 diabetes, whereas higher *trans*-18:2 levels were associated with dyslipidaemia.

Keywords Biomarkers · Diet · Dyslipidaemia · *trans*-Fatty acids · Type 2 diabetes

Abbreviations

CHS	Cardiovascular Health Study
FAME	Fatty acid methyl ester
FFQ	Food-frequency questionnaire
GI	Glycaemic index
HOMA-IR	HOMA of insulin resistance
hs-CRP	High-sensitivity C-reactive protein
iTFA	Industrially produced *trans*-fatty acid

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NHS	Nurses' Health Study
PHVO	Partially hydrogenated vegetable oil
rTFA	Ruminant *trans*-fatty acid
TFA	*trans*-Fatty acid
VA	Vaccenic acid

Introduction

In recent decades, many Asian countries including China have been undergoing rapid nutritional and lifestyle transitions. Excessive energy intakes and an increased consumption of animal foods are likely to be the major drivers of the current chronic disease epidemics seen in developing countries [1]. According to the 1992–2002 China Health and Nutrition Surveys, meat and dairy intakes were almost doubled and energy intake from fat and animal foods increased from 22.0% to 29.8% and from 9.3% to 13.7%, respectively, over this period [2]. Consequently, both the quantity and the quality of dietary fats have been concomitantly altered with the shift from a traditional to a Westernised dietary pattern. Meanwhile, metabolic disorders, such as central obesity, dyslipidaemia, insulin resistance and type 2 diabetes, have now become major public health challenges in China. It is thus critical to elucidate the role of dietary factors, including dietary fatty acids, in the development of cardiometabolic outcomes in Chinese individuals.

trans-Fatty acids (TFAs) have been demonstrated to have adverse effects on cardiometabolic diseases [3, 4]. A meta-analysis of 13 clinical trials has shown that iso-energy substitution of TFAs for other fatty acids increased triacylglycerol levels and total to HDL-cholesterol ratios [5]. TFAs, however, have two primary food origins: industrially produced partially hydrogenated vegetable oils (PHVOs), or industrially produced *trans*-fatty acids (iTFAs), and ruminant fats (naturally produced ruminant TFAs [rTFAs]). Most previous studies have focused on total TFAs or iTFAs, the dominant TFAs in the Western diet [3], whereas results with respect to rTFAs among populations with a low overall iTFA consumption are scarce and inconsistent [6, 7].

Food-frequency questionnaires (FFQs) have been commonly used to assess TFA consumption [8, 9]. This method is, however, subject to measurement errors caused by recall bias and inaccurate estimation of food contents for TFAs, which vary by brand, degree of hydrogenation, season and even cow feed [10, 11]. Therefore, especially for regions or countries without comprehensive and accurate food composition data, such as China, objective markers of TFA intake are preferred because diet is the only source of these fatty acids in human tissues [3].

So far, only a few studies have used measured TFA biomarkers to examine the specific effect of individual *trans*-isomers. For instance, in the Cardiovascular Health Study (CHS), *trans*-18:1 isomers were inversely and *trans*-18:2 isomers were positively associated with an increased risk of CHD, although no significant association was detected for total TFA in plasma phospholipids [12]. Moreover, *trans*-16:1n-7, a marker of dairy fat, was associated with a lower incidence of type 2 diabetes and favourable metabolic traits [13]. To date, however, few data have been available regarding TFA status and related health consequences in Chinese individuals, whose TFA consumption is typically low. We therefore measured erythrocyte fatty acids using gas chromatography and determined various TFA isomers in relation to type 2 diabetes and multiple cardiovascular risk factors among 50–70-year-old Chinese men and women.

Methods

Study population A population-based sample was obtained from the Nutrition and Health of Aging Population in China study, which recruited 1,458 men and 1,831 women aged 50–70 years to examine environmental and genetic factors in relation to chronic diseases [14]. Beijing and Shanghai were selected as representative cities in northern and southern China, respectively. For each city, one rural county and two urban districts were sampled to represent low, middle or high socioeconomic level. The detailed study design and inclusion/exclusion criteria have been described elsewhere [14]. After excluding 31 individuals with insufficient erythrocyte samples and 151 individuals with an extreme total energy intake (<3,347 or >16,736 kJ/day for men and <2,092 or >14,644 kJ/day for women), a total of 3,107 eligible participants were included in the current analyses. The study was approved by the institutional review board of the Institute for Nutritional Sciences, and written informed consent was provided by all participants.

Data and sample collection A standardised questionnaire was used during home interviews to collect information on social demographics, health status, smoking (current, former or never), alcohol consumption (yes/no) and physical activity [14]. A family history of chronic diseases was considered positive if a parent or first-degree sibling had CHD, stroke, type 2 diabetes or hypertension. Educational level was classified according to self-reported school years (0–6, 7–9, ≥10 years). Physical activity was categorised as low, moderate and high from the sum of metabolic equivalent minutes expended per week [15].

Food intakes were obtained by a 74-item FFQ modified from the 2002 China Health and Nutrition Surveys [16]. The FFQ used in the national survey has been validated and widely used among studies in China [17, 18]. There were five

questions regarding dairy food consumption (milk, milk powder, yogurt, ice cream and other dairy products). Intakes of energy, macronutrients and fibre were calculated by converting frequencies and serving sizes of specific foods into grams per day and linking these to the Chinese Food Composition Table, except for TFAs, which were not included in this database [19]. The glycaemic indexes (GIs) of all carbohydrate-containing foods were extracted from the International Tables of Glycemic Index and Glycemic Load Values [20], and the weighted average was calculated to obtain dietary GI.

After an overnight fast, trained physicians and staff members gave all participants a physical examination. Measurements of height, weight, waist circumference and blood pressure have been described elsewhere [14]. Blood samples were collected using EDTA as an anticoagulant, centrifuged to separate the plasma, buffy coat and erythrocytes and then stored at −80°C. Measurement of plasma fasting glucose, insulin, triacylglycerol, lipoproteins and high-sensitivity C-reactive protein (hs-CRP) has also previously been described [14, 15].

Outcome assessment Type 2 diabetes was defined as a fasting plasma glucose ≥7.0 mmol/l, prior diagnosed diabetes mellitus or the self-reported use of antidiabetic medications [21]. Other metabolic disorders were defined according to the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian–Americans [22]. Specifically, central obesity was defined as a waist circumference ≥90 cm in men and ≥80 cm in women; hyperglycaemia was defined as a fasting plasma glucose ≥5.6 mmol/l; high blood pressure was defined as blood pressure ≥130/85 mmHg or current use of antihypertensive medication; hypertriacylglycerolaemia was defined as a triacylglycerol value ≥1.7 mmol/l; and low HDL-cholesterol was defined as an HDL-cholesterol level <1.03 mmol/l in men and <1.30 mmol/l in women. The HOMA model of insulin resistance (HOMA-IR) was computed using updated methods (www.dtu.ox.ac.uk). Insulin resistance was defined as a HOMA-IR higher than 75% level in non-diabetic participants (2.08 in this study). Chronic inflammation was defined as an hs-CRP value >3 mg/l, according to the American Heart Association criterion [23].

Erythrocyte fatty acid measurement Fatty acids were measured following the protocol used in the Nurses' Health Study (NHS), with minor modification [24]. Briefly, erythrocytes (400 μl) were mixed with isopropanol, hexane and internal standard (1 μg 1,2-dihenarachidoyl-sn-glycero-3-phosphocholine dissolved in 1 ml chloroform), followed by methylation (methanol and sulphuric acid). After 2.5 h of incubation, fatty acid methyl esters (FAMEs) were twice extracted by hexane, evaporated under nitrogen and suspended in isooctane.

FAMEs were analysed by gas chromatography (Agilent 6890 GC with flame ionisation detector; SP-2560 capillary column: 100 m×0.25 mm i.d. ×0.2 μm film; Supelco, Bellefonte, PA, USA; carrier gas nitrogen). Esters were identified by electron impact mass spectrometry, and methane was used as a reagent gas for positive chemical ionisation (Agilent 6890N-5975B; Supelco). Quantification was calibrated by the internal standard and an equal-weight FAMEs mixture (GLC-569B; Nu-Chek Prep, Elysian, MN, USA). Chromatography conditions have been previously reported [25]. The relative amount of each fatty acid (% of total fatty acids) was quantified. Within-run coefficients of variations were 14.5% for *trans*-18:1 and 21.4% for *trans*-18:2 fatty acids.

Statistical analysis Multiple linear regressions were used to evaluate associations between erythrocyte TFAs and major sources of dietary *trans*-fats in traditional Chinese diets (e.g. dairy products, ruminant meats, fried foods and cooking oil). Multivariable logistic regressions were applied to estimate the risks of metabolic disorders, adjusting for potential confounders, including age (continuous), sex, northern or southern geographical region, urban or rural residence, family history of chronic diseases, educational attainment, physical activity level, smoking, alcohol consumption, daily energy intake (continuous), consumption of carbohydrate and protein (% of energy) and BMI (continuous). Dietary factors including GI, red meat, vegetables, fruit and dietary fibre were further controlled for in the models (continuous and energy-adjusted by residual method) [26]. Values of p for trend were determined by assuming median values of quartiles as continuous variables. Stratified analyses were conducted according to sex, age, region, physical activity, waist circumference and BMI. Interactions were calculated using the likelihood ratio test. All statistical analyses were performed using Stata 9.2 (College Station, TX, USA) with a two-tailed $p<0.05$.

Results

Erythrocyte TFA concentrations and related food sources A total of three TFA isomers were quantified in erythrocytes. The mean content as a percentage of total fatty acids was 0.20 for total *trans*-18:1 (isomers with a different double-bond location could not be further separated because of low levels), and the median levels were 0.093 for *trans*-18:2n-6 9c12t and 0.062 for *trans*-18:2n-6 9t12c (Table 1). Female and northern residents had slightly but significantly higher total TFAs and *trans*-18:1 than their male and southern counterparts, whereas urban participants had significantly higher levels of all three detected TFAs than rural participants.

Table 1 Erythrocyte TFA content and distribution[a]

Variable	Total TFAs	*trans*-18:1	18:2n-6 9c12t	18:2n-6 9t12c
Total participants	0.37±0.11	0.20±0.06	0.093 (0.092, 0.094)	0.062 (0.061, 0.063)
Female (n=1,778)	0.38±0.12[b]	0.21±0.06[b]	0.095 (0.093, 0.096)	0.063 (0.062, 0.064)
Male (n=1,329)	0.36±0.11[b]	0.20±0.06[b]	0.091 (0.090, 0.093)	0.060 (0.059, 0.062)
Northern China (n=1,515)	0.38±0.10[b]	0.21±0.06[b]	0.095 (0.094, 0.097)	0.064 (0.063, 0.065)
Southern China (n=1,592)	0.36±0.12[b]	0.20±0.07[b]	0.091 (0.090, 0.093)	0.060 (0.059, 0.061)
Urban (n=1,574)	0.41±0.12[b]	0.22±0.06[b]	0.103 (0.101, 0.104)[b]	0.071 (0.069, 0.072)[b]
Rural (n=1,533)	0.33±0.09[b]	0.18±0.06[b]	0.085 (0.083, 0.086)[b]	0.054 (0.053, 0.055)[b]

[a]Data (% of total fatty acids) are means±SD or geometric mean (95% CI)

[b]p significant for comparison by sex or region

To determine potential food sources for erythrocyte TFAs, multiple linear regressions were applied with adjustment for age, sex, north/south region, urban/rural location, education, smoking and drinking habits, physical activity, total energy intake and BMI. Levels of *trans*-18:1 were significantly associated with dairy food consumption: each SD of dairy intake was associated with a 0.27 SD higher concentration of *trans*-18:1 (p<0.001), whereas the associations with other foods, including ruminant meats, fried foods and cooking oil, were not significant. In addition, no associations were detected between *trans*-18:2 isomers and dairy consumption.

ORs of metabolic disorders according to trans-18:1 levels Participants with higher quartiles of erythrocyte *trans*-18:1 were more likely to be female, northern or urban residents, have a positive family history of chronic diseases and have higher levels of educational attainment and physical activity, but were less likely to be current smokers (see electronic supplementary material [ESM] Table 1). Participants mostly ate a traditional Chinese diet, characterised as high carbohydrate and low fat intakes (mean±SD [% of energy]: 59.3±9.5% and 28.6±8.2%, respectively) as well as high vegetable and fruit, but low red meat, consumption (547±308 g/day and 45.9±45.0 g/day, respectively). However, individuals with higher *trans*-18:1 levels tended to consume more protein, fat, red meat, dietary fibre, fruits and all kinds of dairy products. Meanwhile, they were more likely to have a lower energy and carbohydrate intake and a lower dietary GI.

From the lowest to highest quartile of *trans*-18:1, the ORs and 95% CIs were 1.00 (reference), 0.75 (0.55, 1.02), 0.59 (0.43, 0.81) and 0.61 (0.44, 0.85) for type 2 diabetes (Model 2; p_{trend}=0.002) (Table 2). This inverse association was independent of social-demographic and lifestyle variables, daily energy and macronutrients intakes and BMI. Further adjustment for dietary GI, red meat, vegetable, fruit and fibre intake and erythrocyte *trans*-18:2 levels did not change the results (Model 3, OR [95% CI] 0.68 [0.48, 0.97] comparing extreme (first and fourth) quartiles; p_{trend}=0.02).

Similarly, participants in the highest *trans*-18:1 quartile had significantly lower odds of central obesity (OR [95% CI] 0.66 [0.46, 0.94]), elevated triacylglycerol (0.50 [0.38, 0.65]) and fasting glucose (0.80 [0.62, 1.02]) levels, and reduced HDL-cholesterol (0.56 [0.44, 0.72]), insulin resistance (0.62 [0.47, 0.81]) and chronic inflammation (0.69 [0.49, 0.97]) compared with those in the lowest quartile (Model 3; all p_{trend}<0.05). However, the significant associations between erythrocyte *trans*-18:1 levels and elevated blood pressure were attenuated after adjusting for BMI and lifestyle and/or dietary covariates (p_{trend}<0.001 in Model 1; p_{trend}=0.23 in Model 3, respectively).

ORs of metabolic disorders according to trans-18:2 levels In contrast to *trans*-18:1, increasing quartiles of *trans*-18:2 (the sum of *trans*-18:2n-6 9c12t and *trans*-18:2n-6 9t12c) were not significantly associated with risk of type 2 diabetes, central obesity, hyperglycaemia, high blood pressure, insulin resistance, chronic inflammation or low HDL-cholesterol levels, but were positively associated with hypertriacylglycerolaemia and elevated total cholesterol and LDL-cholesterol levels (Table 3, Model 2). Comparing the highest with the lowest quartiles, ORs (95% CI) were 1.91 (1.43, 2.54), 1.32 (1.02, 1.72) and 1.33 (1.05, 1.68), respectively. Further adjustment for erythrocyte saturated fatty acids did not substantially alter these associations (Model 3).

Secondary analyses In sensitivity analyses, after we had excluded participants with previously diagnosed diabetes (n=245) or any chronic diseases such as hypertension, high cholesterol levels and diabetes (n=1,634), *trans*-18:1 levels remained inversely associated with newly diagnosed diabetes; the OR (95% CI) across extreme quartiles was 0.35 (0.15, 0.79) (p_{trend}=0.01). Meanwhile, further adjustment for dairy intake or plasma 25-hydroxyvitamin D levels did not abolish the inverse associations between *trans*-18:1 and diabetes (ORs [95% CI] across extreme quartiles 0.60 [0.42, 0.86], p_{trend}=0.003 and 0.69 [0.48, 0.98], p_{trend}=0.03, respectively). However, additionally controlling for waist circumference slightly attenuated the inverse association: ORs (95% CI) across *trans*-18:1 quartiles were 1.00 (reference), 0.74 (0.54, 1.02), 0.59 (0.43, 0.83) and 0.72 (0.51, 1.03) (p_{trend}=0.05), respectively.

Table 2 ORs for type 2 diabetes and metabolic disorders according to quartiles of *trans*-18:1 fatty acid

trans-18:1	Q1 (median 0.138)	Q2 (median 0.177)	Q3 (median 0.214)	Q4 (median 0.270)	*p* for trend
Type 2 diabetes[a]	116	111	97	101	
Model 1	1	0.81 (0.61, 1.08)	0.65 (0.48, 0.88)	0.65 (0.48, 0.88)	0.003
Model 2	1	0.75 (0.55, 1.02)	0.59 (0.43, 0.81)	0.61 (0.44, 0.85)	0.002
Model 3	1	0.75 (0.55, 1.03)	0.58 (0.42, 0.81)	0.68 (0.48, 0.97)	0.02
Central obesity[a]	381	429	368	322	
Model 1	1	1.11 (0.90, 1.38)	0.72 (0.58, 0.90)	0.47 (0.38, 0.59)	<0.0001
Model 2	1	1.15 (0.83, 1.60)	0.87 (0.62, 1.21)	0.67 (0.47, 0.95)	0.004
Model 3	1	1.14 (0.82, 1.60)	0.85 (0.60, 1.19)	0.66 (0.46, 0.94)	0.004
Hypertriacylglycerolaemia[a]	205	215	182	154	
Model 1	1	0.92 (0.73, 1.16)	0.68 (0.54, 0.87)	0.48 (0.38, 0.62)	<0.0001
Model 2	1	0.93 (0.73, 1.18)	0.75 (0.59, 0.96)	0.58 (0.44, 0.75)	<0.0001
Model 3	1	0.91 (0.72, 1.16)	0.70 (0.55, 0.91)	0.50 (0.38, 0.65)	<0.0001
Low HDL-cholesterol[a]	372	333	321	290	
Model 1	1	0.75 (0.61, 0.92)	0.67 (0.54, 0.83)	0.47 (0.38, 0.59)	<0.0001
Model 2	1	0.73 (0.59, 0.91)	0.74 (0.59, 0.92)	0.57 (0.45, 0.72)	<0.0001
Model 3	1	0.73 (0.58, 0.91)	0.73 (0.58, 0.92)	0.56 (0.44, 0.72)	<0.0001
Hyperglycaemia[a]	307	334	320	295	
Model 1	1	1.00 (0.81, 1.24)	0.86 (0.69, 1.07)	0.75 (0.60, 0.94)	0.004
Model 2	1	0.96 (0.77, 1.20)	0.87 (0.70, 1.09)	0.80 (0.63, 1.00)	0.04
Model 3	1	0.97 (0.78, 1.21)	0.86 (0.68, 1.08)	0.80 (0.62, 1.02)	0.04
High blood pressure[a]	575	575	530	519	
Model 1	1	0.97 (0.77, 1.23)	0.72 (0.57, 0.91)	0.71 (0.56, 0.90)	0.0007
Model 2	1	1.00 (0.78, 1.28)	0.80 (0.63, 1.03)	0.88 (0.68, 1.14)	0.20
Model 3	1	0.99 (0.77, 1.27)	0.79 (0.62, 1.01)	0.89 (0.68, 1.16)	0.23
Insulin resistance[a]	229	244	197	164	
Model 1	1	1.02 (0.82, 1.27)	0.72 (0.58, 0.91)	0.51 (0.40, 0.65)	<0.0001
Model 2	1	1.02 (0.81, 1.29)	0.80 (0.63, 1.03)	0.63 (0.49, 0.82)	<0.0001
Model 3	1	1.00 (0.79, 1.28)	0.79 (0.61, 1.01)	0.62 (0.47, 0.81)	0.0001
Chronic inflammation[a]	109	102	76	81	
Model 1	1	0.83 (0.62, 1.12)	0.57 (0.42, 0.79)	0.59 (0.43, 0.81)	0.0003
Model 2	1	0.82 (0.61, 1.11)	0.61 (0.44, 0.84)	0.67 (0.48, 0.93)	0.008
Model 3	1	0.81 (0.60, 1.10)	0.61 (0.44, 0.84)	0.69 (0.49, 0.97)	0.01

Model 1: adjusted for age, sex and north/south and urban/rural locations

Model 2: further adjusted for family history of chronic diseases, education, physical activity, smoking, alcohol consumption, energy intake, consumption of carbohydrate and protein (% of energy) and BMI

Model 3: further adjusted for energy-adjusted dietary GI, red meat, vegetables, fruits, dietary fibre and erythrocyte *trans*-18:2 fatty acids levels

[a] Number of participants in Q1–Q4 were 777, 777, 776 and 777, respectively. Numbers of cases in each quartile are shown

In stratified analyses, no significant interaction was observed between the *trans*-18:1 tertiles and sex, age group, region, physical activity levels or central obesity status for type 2 diabetes (ESM Fig. 1a–e). However, the association between *trans*-18:1 level and diabetes was more pronounced in overweight/obese participants (OR [95% CI] across extreme [first and third] tertiles 0.56 [0.38, 0.81]; p_{trend}= 0.002) than in their normal-weight counterparts (1.01 [0.60, 1.72]; p_{trend}=0.97) (ESM Fig. 1f, $p_{interaction}$=0.008).

Discussion

Among more than 3,000 middle-aged and older Chinese individuals, we observed that TFAs accounted for only 0.37% of the total fatty acids in erythrocytes. *trans*-18:1, the most abundant type of TFA seen in this population, which consumes a traditional Chinese diet, was significantly associated with dairy consumption. Participants with the highest *trans*-18:1 levels had about a 30% lower risk for

Table 3 ORs of type 2 diabetes and dyslipidaemia according to quartiles of *trans*-18:2 fatty acids

trans-18:2	Q1 (median 0.106)	Q2 (median 0.138)	Q3 (median 0.167)	Q4 (median 0.225)	*p* for trend
Type 2 diabetes[a]	112	105	101	107	
Model 1	1	0.83 (0.62, 1.12)	0.73 (0.54, 0.98)	0.78 (0.58, 1.06)	0.12
Model 2	1	0.78 (0.58, 1.05)	0.66 (0.49, 0.90)	0.71 (0.52, 0.98)	0.05
Model 3	1	0.81 (0.60, 1.10)	0.71 (0.52, 0.96)	0.76 (0.55, 1.04)	0.12
Hypertriacylglycerolaemia[a,b,c]	104	135	180	201	
Model 1	1	1.25 (0.94, 1.66)	1.72 (1.31, 2.27)	1.88 (1.43, 2.48)	<0.0001
Model 2	1	1.15 (0.86, 1.55)	1.67 (1.26, 2.22)	1.91 (1.43, 2.54)	<0.0001
Model 3	1	1.27 (0.94, 1.71)	1.98 (1.48, 2.65)	2.34 (1.73, 3.17)	<0.0001
High total cholesterol[a,b,c]	143	177	216	213	
Model 1	1	1.18 (0.91, 1.53)	1.38 (1.07, 1.78)	1.34 (1.03, 1.73)	0.03
Model 2	1	1.15 (0.88, 1.50)	1.34 (1.04, 1.74)	1.32 (1.02, 1.72)	0.04
Model 3	1	1.15 (0.89, 1.51)	1.36 (1.04, 1.76)	1.29 (0.98, 1.70)	0.05
High LDL-cholesterol[a,b,c]	231	277	323	334	
Model 1	1	1.16 (0.92, 1.46)	1.33 (1.06, 1.67)	1.38 (1.09, 1.74)	0.007
Model 2	1	1.12 (0.88, 1.41)	1.27 (1.01, 1.61)	1.33 (1.05, 1.68)	0.02
Model 3	1	1.13 (0.89, 1.43)	1.29 (1.02, 1.64)	1.31 (1.02, 1.68)	0.03
Low HDL-cholesterol[a,b]	280	280	290	289	
Model 1	1	1.00 (0.80, 1.25)	0.99 (0.79, 1.24)	1.01 (0.80, 1.27)	0.95
Model 2	1	0.94 (0.74, 1.19)	0.93 (0.73, 1.17)	0.97 (0.76, 1.24)	0.86
Model 3	1	0.98 (0.78, 1.25)	1.00 (0.78, 1.28)	1.12 (0.86, 1.44)	0.34

Model 1: adjusted for age, sex and north/south and urban/rural location

Model 2: further adjusted for family history of chronic diseases, education, smoking, alcohol consumption, physical activity and BMI

Model 3: further adjusted for saturated fatty acids and *trans*-18:1 fatty acids in erythrocytes

[a] Numbers of participants in Q1–Q4 were 694, 694, 693 and 694, respectively. Numbers of cases in each quartile are shown

[b] Participants (n=332) who took fish oil supplements or hypolipidaemic drugs were excluded

[c] Defined by the National Centers for Environmental Prediction/Adult Treatment Panel III criteria for borderline high (1.7 mmol/l for triacylglycerol, 5.17 mmol/l for total cholesterol and 3.4 mmol/l for LDL-cholesterol)

type 2 diabetes after adjustment for social demographics, lifestyle, BMI, multiple dietary factors and other fatty acids. The associations appeared to be stronger in overweight/obese individuals. In contrast, increased *trans*-18:2 levels were associated with a higher risk of dyslipidaemia, independent of conventional risk factors, and erythrocyte saturated and *trans*-18:1 fatty acids.

To our knowledge, this is the first report on TFA biomarkers in relation to type 2 diabetes and cardiometabolic disorders in a large Asian population. Our participants were sampled from the most economically developed regions of China, although the erythrocyte contents of TFAs were 70–80% lower than those seen in Western populations, such as the NHS cohort (total TFAs 1.8%, *trans*-18:1 1.3%) using a similar methodology for fatty acid measurement [27]. Even among urban women, who had the highest TFAs of all the subgroups, the mean levels of total TFAs and *trans*-18:1 were 0.42±0.12% and 0.23±0.06%, respectively, suggesting a low overall intake of TFAs in this Chinese population.

Of note, not only the amount of TFAs, but also the food sources of the TFAs might be different between Chinese and Western populations. Unlike the CHS, in which ten TFAs in plasma phospholipids were detected from 3,330 US adults, only three isomers of erythrocyte TFAs were detected in the current study. Moreover, *trans*-18:1 levels in our study were only significantly associated with dairy products among all foods, whereas all *trans*-18:1 and *trans*-18:2 isomers in the CHS were associated with PHVO-abundant foods, such as fried foods, margarine and bakery foods [28]. Since TFAs cannot be generated from de novo human synthesis, *trans*-isomers in tissues or in the circulation may serve as objective dietary biomarkers [29]. The discrepancies between our investigation and studies conducted in Western populations might be due to a lower consumption of PHVO-rich foods in this 50–70-year-old Chinese population, who mostly cook and eat a traditional Chinese diet at home. In such a diet, dairy products may become the primary source of *trans*-18:1, since dairy fat was reported to contain a substantially higher portion of *trans*-18:1 than *trans*-18:2 fatty acids [30].

The moderate association between *trans*-18:1 and dairy consumption may occur for several reasons. First, the dairy intake in our population (median 40 g/day) was much lower

than that in the USA (median 250 g/day in the US National Health and Nutrition Examination Survey) or in European countries (median 270 g/day in the Netherlands Cohort Study) [31, 32]. Second, consumption of high-fat dairy products, such as cheese and butter, was almost negligible in our study population. A limited variability of dairy intake levels might lead to a relatively weaker association in the current analysis than has been seen in previous studies [24, 33, 34]. Third, FFQ assessments of less frequently consumed foods are prone to measurement errors. Similarly, measurement errors of less abundant erythrocyte fatty acids tend to be higher. Thus, both sources of measurement error tend to weaken the association [35]. Nonetheless, because the errors in FFQ assessment and erythrocyte fatty acid measurement are independent of each other, the significant correlation supports the use of *trans*-18:1 as a biomarker of dairy consumption in this population.

In our study, erythrocyte *trans*-18:1 levels were inversely associated with risk of diabetes and several cardiovascular risk factors. Interestingly, *trans*-18:1 levels were also related to certain healthy lifestyle and dietary factors, but the associations were basically unchanged after carefully controlling for these confounders and other erythrocyte TFAs, saturated and polyunsaturated fatty acids. Similarly, Mozaffarian et al [13] have reported that *trans*-16:1n-7 as a unique marker of ruminant fat only represented 0.2% of total fatty acids in plasma phospholipids and was associated with lower insulin resistance, less dyslipidaemia and a nearly 60% lower risk of incident diabetes. Because of the overall very low amount of TFAs, *trans*-16:1 was undetectable in our population. However, previous studies have shown that markers of dairy fat vary in populations with a different dietary background [29]. Accumulating evidence has shown the beneficial effects of dairy products on metabolic syndrome and diabetes [36, 37]. In a recent meta-analysis, the relative risk of diabetes was 0.85 (0.75, 0.96) comparing the highest with the lowest dairy consumption [36]. Moreover, milk intake was found to be associated with a lower incidence of type 2 diabetes in the Shanghai Women's Health Study, which involved participants who were comparable with ours in terms of dietary pattern and lifestyle practices [38]. The inverse relationship between dairy-related TFA markers and metabolic disorders observed in our study was consistent with these reports.

Meanwhile, the possible beneficial effects of a particular *trans*-18:1 isomer – *trans*-18:1n-7 (vaccenic acid [VA]) – have previously been documented. VA supplementation in rats reduced triacylglycerol levels by 40% compared with a control diet [39]. An antiatherogenic property was also suggested from the administration of VA to LDL-receptor-deficient mice [40]. Because the erythrocyte *trans*-18:1 levels in our study were too low to allow a further distinction between individual *trans*-18:1 fatty acids in terms of the double-bond location, it is unclear whether the inverse association could be largely due to VA, which is also a TFA found in dairy products [10]. On the other hand, dairy products are a rich source of micronutrients such vitamin D, calcium and magnesium, which have shown cardiometabolic benefits in some studies [37]. In our population, plasma 25-hydroxyvitamin D level was positively associated with erythrocyte *trans*-18:1 level ($\beta=0.44$, $p<0.001$). However, adjustment for 25-hydroxyvitamin D did not materially change the inverse associations between *trans*-18:1 levels and diabetes. Certainly, more studies are needed to elucidate the pathophysiological mechanism(s) of specific isomers and also effects of micronutrients from dairy products on cardiometabolic outcomes.

Consistent with the findings that increased adipose tissue *trans*-18:1 was associated with decreased BMI and waist circumference in 1,785 Costa Rican individuals [41], we observed that erythrocyte *trans*-18:1 was associated with lower odds of central obesity. Motard-Bélanger et al [6] reported that a moderate-rTFA diet (1.5% of energy) was able to reduce the waistline and improve blood lipids compared with a control diet. However, it is still unclear whether a *trans*-configuration could per se modify energy metabolism [42]. Moreover, we observed that the inverse association between *trans*-18:1 level and type 2 diabetes was more evident among overweight/obese individuals in a stratified analysis. However, mechanistic links between *trans*-18:1 and obesity and metabolic diseases need to be illuminated in future studies.

Although *trans*-18:2 isomers in our study only represented less than 0.2% of the total fatty acids in erythrocytes, the positive associations of these TFAs with blood triacylglycerol, total cholesterol and LDL-cholesterol were robust and independent of established risk factors for dyslipidaemia. Previous studies reported that higher *trans*-18:2 but lower *trans*-18:1 levels were associated with greater adiposity [41] and risk of heart disease [12]. However, the influence of unconjugated-*trans*-18:2 (*trans*-18:2n-6 9c12t and 9t12c) on blood lipids and other metabolic disorders needs to be elucidated. In the current study, one of the possible explanations for the opposite associations of the two types of TFA might be that erythrocyte *trans*-18:1 derived from ruminant fats whereas the *trans*-18:2 isomers came from PHVOs or transformed linoleic acid (18:2n-6) during frying [12, 43].

Our study has several strengths. This is the first large-scale population-based study that has investigated the amounts and food sources of measured TFAs and their associations with cardiometabolic diseases in Chinese men and women. The participants were recruited from both urban and rural areas in northern and southern China. Second, rich data on social-demographic, lifestyle and dietary factors as well as other fatty acids were collected, and major established and potential confounders were extensively adjusted to minimise confounding. Finally, TFA exposure was determined by a widely accepted objective measurement of

erythrocytes, which have been shown as excellent biomarkers of long-term *trans*-fat intake [24].

A major limitation of this study is its cross-sectional design, which limits a causal inference. However, because of low levels of health awareness and nutritional education, participants were unlikely to substantially change their diet if they had an elevated BMI or were even diagnosed with hypertension or dyslipidaemia. Nevertheless, in the sensitivity analyses, the overall results were not changed by excluding participants with any previously diagnosed chronic diseases such as hypertension, hypercholesterolaemia or diabetes. Another weakness is that newly developed diabetes was diagnosed by a single measure of fasting plasma glucose level. Misclassification was possible but may have attenuated the association if it were random. In addition, the robust associations with other metabolic biomarkers provide assurance that the observed association with diabetes was less likely to have occurred by chance. Moreover, measurement errors for erythrocyte fatty acids and food intake are inevitable, which may reduce statistical power. In particular, measurement errors for TFAs and dairy consumption may be substantial because of the overall very low levels of these. There might also be other food sources besides dairy products that provide *trans*-18:1, but these might not have been included in our FFQ, or might have been poorly assessed. Finally, regardless of a wide range of potential confounders that were controlled for, residual or unmeasured confounder(s) may still, to some extent, explain the associations.

In conclusion, erythrocyte *trans*-18:1 was associated with dairy intake and showed inverse associations with risk of type 2 diabetes and several cardiovascular risk factors in 50–70-year-old Chinese men and women, whereas higher erythrocyte *trans*-18:2 levels were associated with a higher risk of dyslipidaemia. Our findings may offer novel insights into differential associations between specific TFAs and metabolic disorders in a population with a low consumption of both dairy products and PHVOs. Prospective studies are needed to confirm our findings and clarify the potential underlying mechanisms.

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Contribution statement DXY made substantial contributions to the acquisition and analysis of data and to drafting the article. XWY, AP, HXL, GZ and YHZ made substantial contributions to the acquisition of data and to revising the article critically for important intellectual content. QS and FBH contributed substantially to conception and design, data interpretation and revising the article critically for important intellectual content. XL provided a substantial contribution to the conception and design, to acquisition and interpretation of data and to drafting/revising the article. All authors approved the final version to be published.

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