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Beneficial effects of designed dietary fatty acid compositions on lipids in triacylglycerol-rich lipoproteins among Chinese patients with type 2 diabetes mellitus☆

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Abstract

Elevated levels of postprandial triacylglycerol-rich lipoproteins (ppTRLs) are atherogenic. Patients with type 2 diabetes mellitus (T2DM) have exaggerated postprandial lipemia associated with elevation or prolonged residence of ppTRL remnants. We examined whether dietary fatty acid compositions (DFACs) decrease atherogenic lipid profiles in ppTRL subfractions in T2DM Chinese patients. A single-blind randomized controlled trial was conducted among 28 T2DM patients. Patients consumed 1 of 3 standardized DFAC-specific fat meals: equidominant (1:1:1), polyunsaturated fatty acid (PUFA)–dominant (PUFA-D, 1:1.7:2.3), or monounsaturated fatty acid (MUFA)–dominant (MUFA-D, 1:1.7:1.2) meals. Numbers in parenthesis, respectively, represent the ratio of saturated fatty acids, MUFA, and PUFA to saturated fatty acids. The MUFA-D meal was the control. Triacylglycerol and cholesterol levels were measured in Svedberg flotation rate (S_f) greater than 400, S_f 60 to 400, S_f 20 to 60, and S_f 12 to 20 ppTRL subfractions at fasting (0 hour) and 2, 4, and 6 hours after the consumption of the fat meals. Effects of DFACs on mean concentrations of triacylglycerols and cholesterol averaged over 0, 2, 4, and 6 hours in ppTRL subfractions were assessed using linear mixed models. Stability and robustness were validated with 1000 bootstrap replicates. Contrasted to the control, equidominant meal reduced 6-hour average triacylglycerol levels in S_f greater than 400 ($P = .002$, bootstrap $P < .05$) and S_f 20 to 60 ($P = .02$, bootstrap $P < .05$) subfractions, and decreased average S_f 20 to 60 cholesterol ($P = .04$, bootstrap $P < .05$); PUFA-D decreased S_f greater than 400 average triacylglycerol levels ($P = .09$, bootstrap $P < .05$). Bootstrap samples suggested that PUFA-D decreased average S_f 20 to 60 cholesterol levels (bootstrap $P < .05$). Therefore, modifying DFACs attenuates the atherogenic lipid profile of ppTRLs in T2DM patients; but increasing PUFA ratio may be more feasible.

Trial registration: This study was registered as a randomized clinical trial on www.ClinicalTrials.gov (ID: NCT00467168).

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1. Introduction

The incidence and prevalence of type 2 diabetes mellitus (T2DM) are increasing dramatically in developing countries because of thriving economics and changing lifestyles. Type 2 diabetes mellitus is becoming a life-threatening disease worldwide. Atherosclerosis is the leading cause of death in T2DM patients. Abnormal metabolism of triacylglycerol-rich lipoproteins (TRLs) contributes to the higher mortality of cardiovascular disease [1]. The longer residence time and higher remnant concentrations of chylomicron and very low-density lipoprotein (VLDL) in blood are significant predictors of coronary heart disease [2]. Patients with T2DM characteristically have fasting hypertriglyceridemia and exaggerated postprandial lipemia. The latter occurs even when fasting triacylglycerol concentrations are normal, accounted for by decreased catabolism of chylomicron remnants and VLDL remnants and prolonged residence of these remnants in blood [3]. Therefore, a diet reducing postprandial TRL (ppTRL) concentrations should be useful in preventing atherosclerosis associated with T2DM [2,4].

Dietary saturated fatty acids (SFA) increase the risk for cardiovascular disease, whereas monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) decrease those risks by modification of fasting lipids [5]. These fatty acids also influence ppTRL metabolism [6-11]. Polyunsaturated fatty acids reduce fasting blood concentrations of triacylglycerol [12] and cholesterol [13] to a greater extent than MUFA, but are much more susceptible to oxidation than MUFA [14], which in turn may increase risk for atherosclerosis [14]. Given that all dietary sources of fat consist of combinations of SFA, MUFA, and PUFA and that human beings consume a whole diet (ie, a combination of various foods, rather than only a single food), there should be an optimal and practical dietary combination of SFA, MUFA, and PUFA that reduces ppTRL concentrations without concerns about harmful effects from oxidation. The best way to characterize the combination of SFA, MUFA, and PUFA is to use ratios of SFA, MUFA, and PUFA to SFA, respectively (SFA:MUFA:PUFA), that is, the fatty acid composition. The need for optimal dietary fatty acid composition (DFAC) becomes imperative among T2DM Chinese patients, as many patients do not consistently adhere to a low-fat diet. Modification of DFAC, therefore, could be a practical alternative approach to prevent atherosclerosis. However, lack of evidences on the influence of DFACs on lipids in different ppTRL subfractions among Chinese T2DM patients limits the ability to intervene in this population through modification of the DFAC.

In this randomized controlled pilot trial, we sought to elucidate the influence of DFACs on ppTRLs during the first 6 postprandial hours after DFAC-specific fat meals. We focused on n-6 fatty acids because they represent most dietary fatty acids. To validate our findings, a bootstrap resampling method was used.

2. Research design and methods

2.1. Study design

This study was a single-blind, 3-arm parallel randomized controlled trial conducted in Guangzhou, Guangdong, China. Outpatients having T2DM were enrolled in Guangzhou, Guangdong, China, between 1995 and 1998. Participants (1) were nonsmokers; (2) had no prior cardiovascular, hepatic, or renal disease, or other diseases influencing fat absorption; (3) had no previous history of gastroenteric surgery; (4) used no medications or vitamins known to affect plasma lipids; (5) had not changed either type or dose of antidiabetic agents during the 4 weeks preceding the trial; (6) were able to withhold hypoglycemic drugs on the day of the study until postprandial tests were completed; (7) ate a diet where fat provided less than 40% of total calorie before the enrollment and were instructed to consume a diet with less than 35% of total caloric intake from fat during the 2 weeks before the trial; (8) were men or postmenopausal women; and (9) were able to provide verbal informed consent to participate. The protocol, which was approved by the Sun Yat-sen University of Medical Sciences, was fully explained to the patients; and all patients gave their oral informed consent before enrollment.

2.2. Randomization

Eligible subjects were stratified by sex and dichotomized body mass index (BMI, <24 or ≥24 kg/m^2) and were randomly assigned to 1 of 3 DFAC-specific fat meals (see below).

2.3. Design of DFAC

The 3 DFACs were 1:1:1, 1:1.7:2.3, and 1:1.7:1.2. Dietary fatty acid composition 1:1:1 is thought to be beneficial in reducing cardiovascular risk [15]. Dietary fatty acid composition 1:1.7:2.3 (PUFA dominant) was designed given that PUFA reduce fasting triacylglycerol levels much more than MUFA [12]; T2DM patients are characterized by hypertriglyceridemia. Dietary fatty acid composition 1:1.7:1.2 (MUFA dominant), chosen as the control meal, was the DFAC among T2DM patients in our previous Guangzhou study [16].

2.4. Oral fat load test

The test meal was a slight modification of that of Karpe et al [17] based on Chinese eating habits. Butter, sunflower oil, olive oil, and lard were used to prepare 3 mixed oils with 1:1:1, 1:1.7:2.3, or 1:1.7:1.2 of DFAC (Table 1). These 3 types of oils had different contents of PUFA rich in n-6 fatty acids, mainly linoleic acid (n-6). Fatty acid distributions were analyzed using gas chromatography. The test meal contained egg-white protein (25 g/m^2 body surface area), dried egg-yolk powder (6.3 g/m^2), carbohydrates (50 g/m^2), mixed oil (50 g/m^2; 1:1:1 for equidominant diet [EQUA-D], 1:1.7:2.3 for PUFA-dominant diet [PUFA-D], or 1:1.7:1.2 for MUFA-dominant diet [MUFA-D]). Carbohydrates were

Table 1
Fatty acids of oils used in the fat meals (grams per 100 g)

Fatty acids	EQUA-D	PUFA-D	MUFA-D
10:0	1.4	0.3	0.4
12:0	1.4	0.3	0.5
14:0	3.6	1.0	1.5
15:0	0.3	0.1	0.1
16:0	17.4	12.2	15.7
16:1	1.6	1.0	1.6
17:0	0.3	0.1	0.1
18:0	7.7	5.5	6.6
18:1	30.8	32.3	41.2
18:2	32.0	44.6	29.5
18:3	0.9	1.2	1.1
18:4	…[a]	…	…
20:0	0.5	0.1	0.1
20:1	0.5	0.5	0.5
21:0	0.1	0.1	0.2
22:0	0.2	0.3	0.2
22:1	…	…	…

[a] Undetectable.

primarily provided using *Mantou* (Chinese food) made from high-protein wheat flours without fat. Other meal components were mixed with 200 mL of water to create an emulsion. The test meal provided 792 kcal/m^2 energy, 60% from fat, 27% from carbohydrates, and 13% from protein [18], corresponding to 53 g fat, 52 g carbohydrates, and 26 g protein per square meter of body surface area. Patients were allowed to be ambulatory during the study and were given 20 minutes to consume a single-blind test meal between 8:00 AM and 9:00 AM after fasting overnight for 14 hours. Water but not food was allowed during the first 6 postprandial hours. All subjects tolerated the protocol well.

2.5. Anthropometric measurements

Height and weight were measured to calculate sex-specific body surface area using the formula specific for the Chinese population [19] and BMI. Waist and hip circumferences were measured to calculate waist-to-hip ratio.

2.6. Blood collection

Before the test meal (fasting, 0 hour) and 2, 4, and 6 hours after the fat load, blood samples were taken from the antecubital vein into precooled sterile tubes containing Na_2EDTA (1.4 mg/mL) and instantly put into ice water. Plasma was separated within 30 minutes by low-temperature centrifugation (1750*g*, 20 minutes, +1°C) and processed according to established methods [17].

2.7. Triacylglycerol-rich lipoprotein fractions

As described previously by Karpe et al [17], ppTRLs were subfractionated to the Svedberg flotation rate (S_f) greater than 400 (chylomicrons), S_f 60 to 400 ($VLDL_1$, large VLDL), S_f 20 to 60 ($VLDL_2$, small VLDL), and S_f 12 to 20 (intermediate-density lipoproteins) subfractions by cumulative density gradient ultracentrifugation [17].

2.8. Biochemical assay

Triacylglycerol and total cholesterol concentrations in plasma and ppTRLs were assayed with standardized enzymatic method by using commercial kits (TRACE Scientific, Melbourne, Australia). Total plasma apolipoprotein (apo) A-I concentrations were determined with immunoturbidimetry by using commercial kits (bioMerieux Vitek, Marcy-L'Etoile, France). Plasma high-density lipoprotein (HDL) cholesterol concentrations were determined by using commercial kits (Daiichi Pure Chemical, Tokyo, Japan). The intra- and interseries coefficients of variation were less than 5%. All these biochemical analyses were conducted by using a clinical automatic analyzer (Hitachi 720A; Hitachi, Tokyo, Japan).

2.9. Statistical analyses

Triacylglycerol and cholesterol concentrations in 4 subfractions of ppTRLs were transformed with the square root for normal distributions. Effects of DFACs on lipid levels were evaluated using random coefficient models (containing fix and random effects) accounting for correlations of lipid concentrations due to repeated measures. In the initial model, sex and dichotomized BMI (<24, ≥ 24 kg/m^2) were forced into models as fixed effects because of the study design and less than perfect randomization due to the small sample size; other fix effect covariates included age (continuous), weight (continuous), and fasting plasma biochemical measures (continuous) (glucose, total triacylglycerols, total cholesterol, HDL cholesterol, and apo A-I). They were selected a priori based on clinical and physiologic rationale and after elimination of *potentially collinear covariates* defined as those with pairwise correlations greater than 0.7 [20]. As fasting plasma glucose concentrations were highly correlated with hemoglobin A_{1c} (HbA_{1c}) concentrations, we selected fasting plasma glucose concentrations because it was more related to ppTRLs. Random effects in initial models included intercept and time variable. The parsimonious model for each of 8 outcomes was selected using backward elimination [21,22]. The ppTRL lipid levels estimated from the parsimonious model were adjusted means of lipid concentrations averaged over 0, 2, 4, and 6 hours [23] (Fig. 1 unadjusted concentrations), which were referred to as *average concentration*.

Model selection procedures such as backward elimination are widely used to develop parsimonious statistical models, but may produce biased estimation particularly for studies with small sample sizes [24]. Statisticians have long advocated the use of resampling methods as preferable [25]; these approaches may be especially useful in dietary and metabolism studies where the number of candidate predictors is relatively large [26]. In this study, we used the bootstrap approach to validate the stability and robustness of the importance of DFACs in explaining outcome variations. Bootstrapping randomly samples individual subjects with replacement from the original complete data set to form a new data set of equal size [27-29]. Because bootstrapped samples are generated from the actual data and there are virtually no

assumptions about the parameters of the parent population [27-29], the large sample size assumption, which is required for most traditional statistical methods, is not needed in the bootstrap approach. Moreover, model selection algorithms can be repeated for each bootstrapped sample, allowing for more accurate estimation of confidence intervals and statistical significance [27-29] and validation of the choice of covariates based on their frequencies of selection [30]. We generated 1000 resampled data sets using the nonparametric bootstrap method [31,32]. The initial model was fitted repeatedly to these bootstrap samples. If DFAC was important in explaining outcome variations and such an importance was not entirely dependent on results from only a few participants, DFAC groups should frequently appear as a statistically significant predictor at $\alpha = .05$ in the initial model. Such an appearance in at least 50% of models fitted to the bootstrap replicates was considered an indication of a robust result [31]. Finally, we compared findings from bootstrap data sets with those from the parsimonious models.

We defined P less than .05 as a significant difference and $.05 < P < .1$ as a marginally significant difference for the DFAC group comparison. Data on continuous nontransformed variables were presented as mean ± standard error (SEM) unless otherwise indicated. Analyses were performed with SAS software package version 9.1 (SAS Institute, Cary, NC).

3. Results

3.1. General characteristics

Thirty subjects met the inclusion criteria; 2 subjects were excluded because of their inability to attend scheduled experiments. Our analyses were thus based on 13 men and 15 postmenopausal women: 9 for EQUA-D, 10 for PUFA-D, and 9 for MUFA-D. No statistically significant difference was found across groups for any of the characteristics of subjects at baseline (Table 2). Of all patients, 86% were on sulfonylureas and 14% were on biguanides; but none of them was on insulin therapy.

3.2. Effects of DFACs on lipids in large ppTRLs

The equidominant diet significantly decreased the average S_f greater than 400 triacylglycerol concentrations over 6 hours by 10% ($P = .008$), whereas PUFA-D only marginally significantly reduced the ppTRL average triacylglycerol levels by 7% ($P = .056$). The equidominant diet also decreased the average S_f 60 to 400 cholesterol by 6% ($P = .04$) in comparison with MUFA-D (Table 3 adjusted values).

3.3. Effects of DFACs on lipids in small ppTRLs

In contrast to MUFA-D, EQUA-D and PUFA-D reduced the average S_f 20 to 60 triacylglycerol concentrations over 6 hours by 7% ($P = .02$) and 7% ($P = .06$), respectively; and EQUA-D decreased the average S_f 20 to 60 cholesterol concentrations by 6% ($P = .04$) (Table 3 adjusted values).

No significant differences in the average S_f 12 to 20 triacylglycerol concentration were found between EQUA-D and MUFA-D or between PUFA-D and MUFA-D (Table 3). No significant differences in the average S_f 12 to 20 cholesterol concentrations over 6 hours were found between EQUA-D and MUFA-D or between PUFA-D and MUFA-D (Table 3 adjusted values).

Table 2
Demographic, anthropometric, and clinical characteristics of T2DM patients (mean values and standard error of means)

Variable	EQUA-D		PUFA-D		MUFA-D		P value[b]
	Mean	SEM	Mean	SEM	Mean	SEM	
n	9		10		9		
Age (y)	64	2.3	62	2.1	64	2.5	0.84
Sex (male/female)	4/5		5/5		4/5		0.38
BMI (kg/m^2)	24.3	0.6	24.9	1.2	23.9	0.6	0.69
Body surface area (m^2)							
Male	1.72	0.06	1.75	0.05	1.77	0.06	0.82
Female	1.60	0.03	1.60	0.05	1.50	0.05	0.25
Waist-to-hip ratio	0.97	0.02	0.95	0.02	0.93	0.01	0.31
Clinical characteristics							
Diabetic duration (mo)	101	27	91	25	97	19	0.95
Fasting concentrations							
Glucose (mmol/L)	7.5	0.51	7.9	0.70	7.3	0.58	0.74
HbA_{1c} (%)	7.6	0.32	7.7	0.53	8.2	0.80	0.69
Triacylglycerols (mmol/L)	2.08	0.45	1.60	0.16	1.50	0.18	0.34
Total cholesterol (mmol/L)[a]	5.68	4.03-6.35	5.78	3.00-7.67	6.15	5.43-7.66	0.14
HDL cholesterol (mmol/L)[a]	1.21	1.07-1.94	1.44	0.78-2.02	1.21	1.12-1.86	0.83
Apo A-I (g/L)	0.98	0.05	1.09	0.08	1.08	0.06	0.63
Biguanides/sulfonylureas (n/n)	1/8		2/8		1/8		1.00

[a] Values are medians (ranges).
[b] Continuous variables were examined using analysis of variance; sex proportion and use of hypoglycemic medication were tested using Fisher exact test.

Table 3
The means of lipid concentrations in ppTRL subfractions averaged over 0 hour and 2, 4, and 6 hours postprandially (means ± SEM)

Outcome	EQUA-D	PUFA-D	MUFA-D	EQUA-D vs MUFA-D *P* value		PUFA-D vs MUFA-D *P* value	
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Model based	Bootstrap based*	Model based	Bootstrap based*
The 6-h average triacylglycerol concentrations (mmol/L)							
Unadjusted means using transformed values estimated from model							
S_f >400	1.28 ± 0.06	1.22 ± 0.06	1.34 ± 0.05	.37	>.05	.09	>.05
S_f 60-400	2.15 ± 0.08	1.96 ± 0.15	2.09 ± 0.16	.7	>.05	.5	>.05
S_f 20-60	1.09 ± 0.05	1.00 ± 0.05	1.06 ± 0.05	.7	>.05	.4	>.05
S_f 12-20	0.92 ± 0.03	0.94 ± 0.02	0.95 ± 0.02	.3	>.05	.7	>.05
Adjusted means using transformed values estimated from model							
S_f >400	1.22 ± 0.03	1.26 ± 0.04	1.36 ± 0.05	.008	<.05	.056	<.05
S_f 60-400	2.10 ± 0.08	2.00 ± 0.11	2.08 ± 0.14	.9	>.05	.6	>.05
S_f 20-60	1.02 ± 0.02	1.02 ± 0.03	1.10 ± 0.03	.02	<.05	.06	>.05
S_f 12-20	0.90 ± 0.02	0.96 ± 0.02	0.93 ± 0.02	.2	>.05	.3	>.05
The 6-h average cholesterol concentrations (mmol/L)							
Unadjusted means using transformed values estimated from model							
S_f >400	0.75 ± 0.02	0.76 ± 0.04	0.74 ± 0.02	.7	>.05	.7	>.05
S_f 60-400	1.06 ± 0.05	1.05 ± 0.05	1.07 ± 0.04	.9	>.05	.7	>.05
S_f 20-60	0.97 ± 0.05	0.91 ± 0.03	0.95 ± 0.02	.8	>.05	.3	>.05
S_f 12-20	1.61 ± 0.002	1.61 ± 0.002	1.61 ± 0.001	.037	>.05	.15	>.05
Adjusted means using transformed values estimated from model							
S_f >400	0.74 ± 0.02	0.76 ± 0.03	0.76 ± 0.02	.3	>.05	.9	>.05
S_f 60-400	1.02 ± 0.02	1.07 ± 0.02	1. 09 ± 0.03	.04	>.05	.6	>.05
S_f 20-60	0.91 ± 0.02	0.95 ± 0.01	0.97 ± 0.01	.04	<.05	.11	<.05
S_f 12-20	1.61 ± 0.001	1.61 ± 0.001	1.61 ± 0.001	.6	>.05	.8	>.05

Values of means are the least square means, lipid concentrations averaged over 0 hour and 2, 4, and 6 hours postprandially, estimated from mixed model accounting for clustering due to repeated measures. Adjusted values were obtained from corresponding parsimonious mixed models including baseline values of characteristic factors. The unbiased effects of DFACs on each outcome were estimated from its parsimonious model using restricted maximum likelihood accounting for the small sample size [33].

* *P* less than .05 obtained using 1000 bootstrap samples.

3.4. Bootstrap validation on the stability and robustness of the importance of DFACs in explaining outcome variability

Bootstrap samples confirmed findings from the original analyses with respect to the effect of DFACs on average levels of triacylglycerols and cholesterol in large ppTRLs over 6 hours. The bootstrap approach, however, did not completely support the observed impact of DFACs on the lipids in small ppTRLs (Table 3).

Similar results were obtained when we replaced the fasting plasma glucose concentration with the HbA_{1c} concentration and thus were not shown.

4. Discussion

This study demonstrated for the first time the overall effects of DFACs on 4 different TRL subfractions isolated from postprandial plasma of T2DM Chinese patients. The test meals were unique for the high ratio of MUFA and PUFA to SFA, respectively. Our study showed that EQUA-D and PUFA-D decreased the adjusted average concentration of S_f greater than 400 ppTRLs triacylglycerol and that EQUA-D also reduced the adjusted average triacylglycerols and cholesterol in S_f 20 to 60 ppTRLs in comparison with MUFA-D.

Although previous studies have demonstrated an influence of SFA, PUFA, and MUFA with emphasis on n-6 fatty acids on ppTRL metabolism [6-11], to date, none have reported ppTRL subfractions as detailed as in our study. These studies were also performed using edible oils as a surrogate for a single type of fatty acids, SFA, MUFA, or PUFA, unlike ours, where the coexistence of SFA, MUFA, and PUFA as in the whole diet was the focus. Finally, the impact of DFACs, characteristic of diets in China, has not been studied before. For example, the Chinese DFAC was 1:1.5:1 in the mainland of China [34] and 1:1.7:1.2 in T2DM patients in Guangzhou, a city in southern China close to the sea [16]. Comparing with 1:1.7:0.4 of the Greek DFAC [35] and 1:1.0:0.5 of the Americans [36], directly obtained or derived from published reports [35,36], the Chinese DFAC was MUFA predominant and PUFA enriched.

Our results indicated that EQUA-D decreased chylomicron triacylglycerol concentrations compared with MUFA-D, consistent with some [6,7] but not all [8,9] previous studies. Our data agreed with some but not all [7] studies comparing safflower oil [10] or soybean oil [11] with olive oil, in that PUFA-D reduced chylomicron triacylglycerol levels compared with MUFA-D. Our results were consistent with a previous study comparing edible oils [10] where DFACs affected chylomicron cholesterol levels.

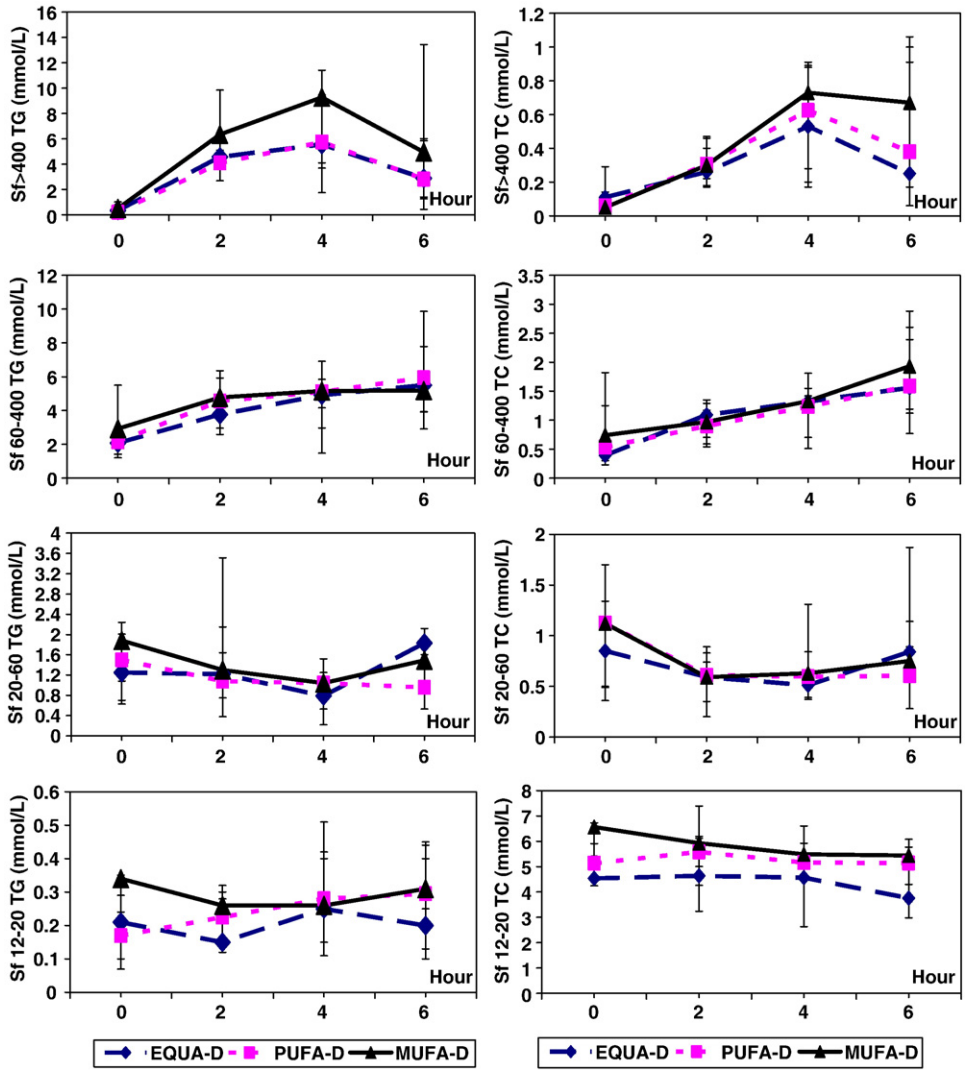

Fig. 1. Unadjusted nontransformed lipid concentrations (median ± interquartile) in 4 subfractions of ppTRLs at 0, 2, 4, and 6 hours after the fat meals with different DFACs.

We did not find significant differences in triacylglycerol and cholesterol concentrations in $VLDL_1$ comparing EQUA-D with MUFA-D or comparing PUFA-D with MUFA-D, as reported by the previous study comparing a mixture of palm oil and cocoa butter or safflower oil with olive oil [10]. We found that EQUA-D led to lower concentrations of triacylglycerols and cholesterol in $VLDL_2$ than MUFA-D, in disagreement with a study comparing SFA-rich oil with MUFA-rich oil [10]. To our knowledge, this is the first report that triacylglycerol and cholesterol concentrations in intermediate-density lipoproteins are not significantly different between EQUA-D and MUFA-D or between PUFA-D and MUFA-D.

Inconsistency of findings across trials in ppTRL lipids may be attributable to variations in laboratory techniques and populations, including the harvest of chylomicrons in terms of S_f value [8], amount of fat ingestion [37], and fatty acid compositions in both the fatty meal and habitual diets of subjects. Our findings provide evidence that a higher proportion of SFA or PUFA in the DFAC has differential effects on lipids in the subfractions of ppTRLs compared with a higher proportion of MUFA in the DFAC.

The underlying mechanisms through which the MUFA-D meal increases chylomicron triacylglycerol concentrations remain unclear. An increased number [10] of small chylomicron particles [38] and the lower affinity of MUFA ppTRLs for lipoprotein lipase hydrolysis [39] may account for the accumulation of triacylglycerols in ppTRLs after MUFA-D.

The chylomicron remnant triacylglycerol-raising effect of MUFA-D meal is atherogenic. There had been a controversy on the atherogenicity of chylomicron remnants and triacylglycerol. In the past, chylomicron remnants were thought of as being nonatherogenic particles because they were too large to enter the artery wall [40]. However, recent data show that chylomicron remnants not only penetrate the artery wall, but also are retained in the subendothelial space [40].

Recently, experiments' evidence has shown that chylomicron remnant particles are taken up by macrophages and cause extensive triacylglycerol and cholesterol accumulation in the artery wall, thus inducing foam cell formation, the early hallmark of atherosclerosis [41]. The high extracellular levels of triacylglycerol in chylomicron remnants increase the triacylglycerol accumulation in macrophages [41] without requirement for prior lipid oxidation [40]. This evidence suggests that, although MUFA is less susceptible to oxidation than PUFA, MUFA-D may be detrimental among Chinese T2DM patients.

The mechanisms underlying the atherogenicity of chylomicron remnants include dysfunction of endothelium [42], retention in the subendothelial space [43], formation of foam cells [44] via the apo B-48 receptor [45], activation of vascular smooth muscle cells via inducing Egr-1 expression [46], and inhibition of the lipoprotein lipase–mediated catabolism of atherogenic VLDL [47].

There were some limitations in this trial. The sample size was small; and thus, residual confounding is possible. However, our stratified randomization ensured appropriate control for potential confounding factors within sex and BMI groups despite the small trial size (sample size <100) [48,49]. We also controlled for potential confounders in the analysis. Thus, it is less likely that the residual confounding is a problem. A second limitation is the use of a parallel rather than crossover design. Our Cantonese in China expressed reluctance to undergo repeated blood draws even if a large interval were to be allowed in between sampling. A crossover design would have required tripling the amount of blood to be drawn from each subject, therefore greatly increasing the likelihood of noncompliance with the protocol. Therefore, we chose a parallel design to increase feasibility and compliance. Another potential limitation is that the temperature to isolate ppTRLs may affect lipid concentrations in these remnants. This is, however, unlikely to be a problem in our study because we used a conventional published ultracentrifugation protocol to isolate ppTRLs formed after the consumption of the fat meals with the appropriate content of SFA and its ratio to unsaturated fatty acids [50]. Finally, withholding hypoglycemic medications only on the day of the study may not be long enough to prevent potential effects of these drugs on the study outcomes. Withholding diabetes medications for a longer time would have been ethically unacceptable. In addition, only a small proportion of our subjects were treated with biguanides. We believe that our approach yielded a sample more consistent with diabetic patients in actual clinical practice; and thus, our results should be more clinically applicable.

An advantage of using a Chinese population for this study was the feasibility of implementation of designed DFACs through changing cooking oils, the major source of dietary fat in China. Our study is also strengthened by using the bootstrap technique to minimize potential biased results due to the small sample size. Because our bootstrap samples generated from the original data approximated the results achieved by repeating the same study design 1000 times, our findings that PUFA-D decreased average concentrations of S_f 20 to 60 cholesterol in comparison with MUFA-D in bootstrap samples, although not in the original data, appear to be robust. These findings propose a hypothesis for future larger studies. Effects seen in the original data and supported by the bootstrap samples are more persuasive than those observed solely in the original study data or solely in the bootstrap replicates, as they are not dependent on data from only a few participants.

Given that our study population's usual diet is MUFA-D and traditional food sources in this population, it may be more feasible to change their diet from MUFA-D to PUFA-D. This change can be accomplished by increasing the use of PUFA-rich foods such as soybean oil or sunflower oil instead of peanut oil, palm oil, and lard. On the other hand, the alternative approach of changing the diet from MUFA-D to SFA-D would mean increasing the SFA intake by eating more SFA-rich foods not common in the dietary culture of the study population, such as beef, butter, or lamb. This dietary change would also result in an increase in the least desirable type of fatty acids for the prevention of atherosclerosis. Therefore, the DFAC with a PUFA/SFA ratio at or greater than 1.2 is more feasible in the prevention of cardiovascular disease for T2DM Chinese patients. A long-term study using a larger population is needed to determine the efficacy and safety of this dietary approach.

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