See corresponding editorial on page 1392.

Diets high in palmitic acid (16:0), lauric and myristic acids (12:0 + 14:0), or oleic acid (18:1) do not alter postprandial or fasting plasma homocysteine and inflammatory markers in healthy Malaysian adults[1–3]

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ABSTRACT

Background: Dietary fat type is known to modulate the plasma lipid profile, but its effects on plasma homocysteine and inflammatory markers are unclear.

Objective: We investigated the effects of high-protein Malaysian diets prepared with palm olein, coconut oil (CO), or virgin olive oil on plasma homocysteine and selected markers of inflammation and cardiovascular disease (CVD) in healthy adults.

Design: A randomized-crossover intervention with 3 dietary sequences of 5 wk each was conducted in 45 healthy subjects. The 3 test fats, namely palmitic acid (16:0)–rich palm olein (PO), lauric and myristic acid (12:0 + 14:0)–rich CO, and oleic acid (18:1)–rich virgin olive oil (OO), were incorporated at two-thirds of 30% fat calories into high-protein Malaysian diets.

Results: No significant differences were observed in the effects of the 3 diets on plasma total homocysteine (tHcy) and the inflammatory markers TNF-α, IL-1β, IL-6, and IL-8, high-sensitivity C-reactive protein, and interferon-γ. Diets prepared with PO and OO had comparable nonhypercholesterolemic effects; the postprandial total cholesterol for both diets and all fasting lipid indexes for the OO diet were significantly lower ($P < 0.05$) than for the CO diet. Unlike the PO and OO diets, the CO diet was shown to decrease postprandial lipoprotein(a).

Conclusion: Diets that were rich in saturated fatty acids prepared with either PO or CO, and an OO diet that was high in oleic acid, did not alter postprandial or fasting plasma concentrations of tHcy and selected inflammatory markers. This trial was registered at clinicaltrials.gov as NCT00941837. *Am J Clin Nutr* 2011;94: 1451–7.

INTRODUCTION

Dietary factors have been shown to influence the pathogenesis of CVD[4] through multiple biological pathways. Intermediary biological mechanisms and markers, such as lipid concentrations, blood pressure, thrombotic tendency, cardiac arrhythmia, endothelial dysfunction, systemic inflammation, insulin sensitivity, oxidative stress, and homocysteine concentration, have been implicated in risk of CVD (1).

Plasma tHcy concentrations have been reported to increase risk of coronary artery disease events by ~20% for each increase by 5 μmol/L plasma tHcy (2). Elsewhere, the Hordaland Homocysteine Study (3) reported that a 12–15 g increase in the intake of saturated fat was associated with a 6% rise in the plasma tHcy concentration. To our knowledge, the Hordaland Homocysteine Study did not conduct feeding trials that involved palm oil–rich and CO-rich diets; in addition, the mechanism of the action of SFAs on plasma tHcy was not clearly understood.

The biochemical link between homocysteine and phospholipid metabolism is supported by a research finding that suggested that an increase of the saturation of fatty acids in the diet increased the synthesis of phosphatidylcholine via the phosphatidylethanolamine methyltransferase pathway (4). Vasculopathic effects of homocysteine are mediated via a decreased expression of apo A-I, which results in a decrease in HDL antiinflammatory activity (5). This activity might explain why hyperhomocysteinemia is a risk factor for atherosclerosis and thrombotic and fibrotic vascular disease, as originally noted by McCully and Wilson (6).

Dietary fat type that affect plasma lipid factors (7) and nonlipid risk factors, such as the proinflamatory cytokines IL-1β, -6, and -8 and TNF-α, have been shown to affect lipid metabolism in the development of atherosclerotic lesions (8–10). These cytokines modulate the acute-phase reactant, hsCRP secretion, and the expression of cell adhesion molecules (11). hsCRP is currently known as a more novel risk marker of CVD that shows up in the development of atherosclerotic lesions (12, 13) and may be affected by dietary intake (14). However, hsCRP secretion was shown to respond to IL-6 (15). It was reported in a review by Galli and Calder (16) that there is an inverse relation between dietary long-chain PUFAs and inflammation. Although the intake and potential adverse health effects of SFAs in Western countries have received considerable attention, little is known about the effects of the intake of PO and CO, which are rich sources of SFAs, on the Southeast Asian populations. In addition, the involvement of SFAs

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[4] Abbreviations used: apo, apolipoprotein; CO, coconut oil; CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein; IFN-γ, interferon-γ; Lp(a), lipoprotein(a); OO, olive oil; PO, palm olein; TC, total cholesterol; tHcy, total homocysteine.

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and MUFAs in the inflammatory processes has not been extensively studied in humans.

In this study, we tested the hypothesis that SFA-rich fats incorporated into diets that comprise common Malaysian foods will have an influence on nonlipid (tHcy, hsCRP, and cytokines) and lipid risk factors [total, HDL, LDL, apo A-100, apo B-I, and Lp(a)] in human volunteers.

SUBJECTS AND METHODS

Subjects

Ethical approval for this study was obtained from the Research and Ethics Committee of the International Medical University, Kuala Lumpur, Malaysia, and the study was registered at clinicaltrials.gov as NCT00941837. Before selection, informed consent was obtained from potential volunteers, after which a health-history and lifestyle questionnaire, abbreviated physical examination, and biochemical tests were used to assess the health status of all the volunteers. Biochemical screening that were used were liver-function tests (ie, for serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and total bilirubin, tests for serum creatinine, fasting plasma glucose, LDL, TC, hsCRP, and hemoglobin, as well as a full blood count, were used).

Pregnancy tests for premenopausal female subjects were also conducted during screening. Anthropometric measurements of height and weights of volunteers were measured to calculate BMI (in kg/m^2). Finally, 45 apparently healthy, adult volunteers (36 women and 9 men; average age: 30 y) from the staff of the Malaysian Palm Oil Board, Bangi, Malaysia, were recruited for the study.

We included apparently healthy subjects with BMI that ranged from 18.0 to 29.9. Exclusion criteria used for this study were BMI $\geq$30 or $<$18.0; having any one of the following chronic diseases: diabetes mellitus, coronary artery disease, cancer, liver diseases, renal diseases, blood dyscrasia, and hypertension; and having a plasma TC $>$7.8 mmol/L. Subjects who were alcoholics, chronic smokers, or pregnant or who had difficulty following the study guidelines were also excluded. In addition, we stratified women according to *1*) menstrual cycles, *2*) TC, and *3*) tHcy concentrations to minimize effects of hormonal changes to some endpoints.

Study design

A crossover 3 $\times$ 3 Latin-square design was used. The primary outcome measured was a change in tHcy, and the secondary outcomes were changes in other lipid and nonlipid CVD risk markers, including inflammatory markers and lipid profiles. A power calculation was based on the 42 subjects, which gave an 80% power to detect a difference in means of 10% for tHcy. With the allowance for a dropout of 3 subjects, the final sample size was 45 subjects per treatment group.

A 3-wk standardization diet that comprised 30% of kilocalories from fat, 15% of kilocalories from protein, and 55% of kilocalories from carbohydrate was provided to subjects as the baseline diet. At the end of the standardization period, the 45 subjects were randomly stratified to each dietary treatment by using a computer-based procedure to balance the assignment by tHcy, sex, and fasting serum TC.

Test fats and diets

The test fats (PO, OO, and CO), each which contributed two-thirds of the total dietary fat, were incorporated into common Malaysian diets that contained 30% of kilocalories from fat, 20% of kilocalories from protein, and 50% of kilocalories from carbohydrate. PO was obtained from Unitata, CO was obtained from Wilmar, and extra virgin OO was imported from Spain via a local supermarket. Detailed instructions on the menu plan, portion size, and procedures for incorporation of test fats into diets were given to a cook or caterer responsible for the preparation of experimental diets. A uniform 5-d rotation menu was used for all 3 diet periods, which differed only in the type of test fat incorporated. An additional 5-d back-up menu was also prepared in case subjects complained of monotony that resulted from the consumption of the same menu week after week. Three meals a day (ie, namely, breakfast, lunch, and dinner), which provided a total of 2000 kcal, were served in each of the 5-wk dietary periods. Volunteers consumed breakfast and lunch under the supervision of a nutritionist and a study investigator from Monday to Friday. Dinner was individually packaged for home consumption by each subject. The test fat concerned was also provided as cooking oil to the households of subjects for each specific dietary period, especially for use during weekends, with appropriate dietary guidelines. The menu serving for the 5 weekdays was fixed according to the meal plan, which comprised a bowl of rice or noodles, 2 meats (chicken and/or fish), and one type of vegetable for each meal (*see* supplemental Table 2 under "Supplemental data" in the online issue). Macronutrients and fatty acid content of sample experimental meals were collected and analyzed by chemical analyses in triplicate. Body weights of subjects were recorded fortnightly to ensure that their weights did not change significantly during the course of the study. For practical reasons, the investigator was not blind to the treatment allocation.

Sample collection

Twelve-hour fasting blood samples (15 mL each) were collected from subjects at week 2 of the standardization period and at the end of the fifth week of each dietary period. Two-hour postprandial samples after the consumption of breakfast were also collected from subjects at the end of fourth week of each dietary period. Each subject was bled a total of 7 times throughout the study. The 15 mL blood collected each time was dispensed into evacuated tubes for the determination of biochemical indexes as previously mentioned.

Analytic methods

tHcy concentrations were measured by using an enzyme-linked immunosorbent assay (Bio-Rad Laboratories); IL-6, -1β, and -8, IFN-γ, and TNF-α were also measured by using an enzyme-linked immunosorbent assay (R&D Systems). Serum TC, HDL cholesterol, LDL cholesterol, triglyceride, apo A-100, apo BI, Lp(a), and hsCRP were measured by enzymatic colorimetric assays with a Hitachi 902 autoanalyzer (Roche Diagnostics GmbH) by using reagents supplied by the manufacturer. The fatty acid composition of test fats extracted from test diets

was measured by using gas-liquid chromatography on an SP-2560 column (100 m × 0.23 mm × 0.2 mm; Agilent Technologies) with a flame ionization detector on an autosystem (Perkin Elmer). The carrier gas (helium) was set at 40 psi, whereas the injector temperature was set at 250°C. The oven temperature was set at 240°C for 58 min without ramping. Fatty acids were measured in the form of methyl esters as previously described (17). A 37-fatty acid methyl ester mixture (Sigma-Aldrich) was used as an external standard. Results were expressed in percentage of energy (**Table 1**).

Statistical analyses

Data were analyzed by using repeated-measures ANOVA by using Bonferroni post hoc analysis with PASW Statistics 18 software (SPSS Inc) to detect any significant difference between diets. Shapiro-Wilk's normality test was used to check for the normal distribution of data, and logarithmic transformations were used when appropriate. Data were expressed as means ± SDs and adjusted to geometric mean values when necessary. Baseline values were included as covariates for all variables.

RESULTS

Dietary intakes of the 45 participants in a 3 × 5-wk intervention are shown in Table 1. All diets were maintained at ~20% of kilocalories from protein, 30% of kilocalories from fat, and 50% of kilocalories from carbohydrates. Only protein and fat contents were analyzed in food samples. The PO diet was rich in palmitic acid (16:0) and oleic acid (18:1), which contributed 9.7 ± 0.5% and 12.3 ± 1.0% of kilocalories, respectively. The OO diet mainly consisted of 18:1 (19.1 ± 1.0% of kcal). The CO diet was the richest in total SFA, which contributed 20.6 ± 2.4% of kilocalories. Sources of SFAs were mainly lauric acid (12:0; 8.5 ± 1.8% of kcal), myristic acid (14:0; 3.5 ± 0.7% of kcal), 16:0 (5.0 ± 0.6% of kcal), and others. Dietary PUFAs were slightly lower (2.9 ± 0.7% of kcal) for the CO diet than for the PO and OO diets at 4.9 ± 0.6% and 4.4 ± 0.4% % of kilocalories, respectively.

TABLE 1
Dietary intake of 45 healthy subjects who participated in a 3 × 5-wk intervention[1]

	Palm olein	Olive oil	Coconut oil
Energy (kcal)	2171.9 ± 194.0	2190.8 ± 224.1	2165.4 ± 201.6
Percentage of energy			
Protein	22.7 ± 1.8	23.1 ± 1.3	23.1 ± 1.4
Fat	30.6 ± 2.3	31.0 ± 2.8	30.6 ± 2.0
SFA	12.5 ± 1.6	6.6 ± 0.7	20.6 ± 2.4
MUFA	13.0 ± 1.0	20.1 ± 1.1	6.9 ± 1.8
PUFA	4.9 ± 0.6	4.4 ± 0.4	2.9 ± 0.7
12:0	0.4 ± 0.4	0.1 ± 0.1	8.5 ± 1.8
14:0	0.6 ± 0.2	0.3 ± 0.1	3.5 ± 0.7
16:0	9.7 ± 0.5	4.8 ± 0.6	5.0 ± 0.6
18:0	1.4 ± 0.2	1.4 ± 0.4	1.1 ± 0.1
18:1	12.3 ± 1.0	19.1 ± 1.0	6.3 ± 1.8
18:2	4.0 ± 0.6	3.5 ± 0.2	2.3 ± 0.7
18:3	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
Others	2.0 ± 0.0	1.5 ± 0.0	3.7 ± 0.0

[1] All values are means ± SDs.

The CONSORT diagram for recruitment is shown in **Figure 1.** A total of 86 subjects were screened, and 45 subjects were recruited and participated in and completed the study. Data from all 45 subjects were analyzed. Baseline characteristics of the study subjects are shown in **Table 2.** The ethnic mix of subjects was typical of the Malaysian population and was predominantly native Malay with ~10% of subjects from ethnic minorities (Chinese and Indian). Subjects were, on average, 30 y old with BMI <30. The average TC and tHcy concentrations were 4.71 ± 0.66 mmol/L and 7.95 ± 2.81 μmol/L, respectively, which were in the normal range. None of the subjects had any metabolic syndrome or a medical history of coronary artery disease, which had >20% risk of developing CVD for the next 10 y, as assessed by the Framingham risk score (18).

The serum lipid profile for the 3 dietary interventions is shown in **Table 3**. Compared with the OO diet, the PO diet demonstrated no difference in serum TC, HDL, and LDL during fasting and nonfasting states. The CO diet demonstrated a higher fasting serum TC concentration at 4.95 ± 0.69 mmol/L, HDL concentration at 1.37 ± 0.30 mmol/L, and LDL concentration to 3.30 ± 0.75 mmol/L than did the OO diet. A comparison between CO and OO diets showed significant differences between meals ($P < 0.05$). No difference was observed when PO and CO groups were compared for fasting serum TC, HDL, and LDL. However, the CO diet had a significantly higher nonfasting serum TC concentration at 4.85 ± 0.68 mmol/L than did the PO and OO diets. In addition, the CO diet was also shown to have higher nonfasting serum HDL and LDL at 1.29 ± 0.31 and 3.11 ± 0.67 mmol/L, respectively, compared with those of the OO diet. However, no significant differences were observed when PO to CO diets were compared for nonfasting HDL and LDL. The 3 diets caused no differences in serum TC:HDL cholesterol and triglyceride concentrations. There was also no significant statistical difference between the effects of the 3 diets on apo A-100 and apo B-I in both fasting and nonfasting states. Differences were observed in nonfasting Lp(a) for which CO was shown to have significantly lower Lp(a) concentration at 1.31 ± 1.11 mmol/L compared with that of the PO and OO diets. However, PO and OO exerted no different effects on this biomarker.

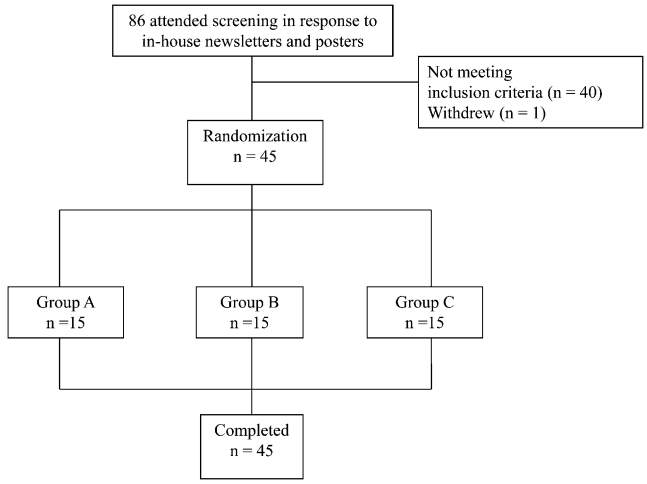

FIGURE 1. CONSORT diagram of subject flow throughout the study of a 3 × 5-wk intervention. A total of 45 subjects completed the study, and all data were analyzed.

TABLE 2
Baseline characteristics of study subjects who participated in a 3 × 5-wk intervention[1]

Characteristic	Values
n	45
Men	9
Women	36
Race or ethnic group (*n*)	
Native Malay	40
Chinese	3
Indian	2
Age (y)	30.1 ± 8.3[2]
BMI (kg/m^2)	23.1 ± 3.7
Waist-to-hip ratio	0.81 ± 0.07
Systolic BP (mm Hg)	114 ± 11
Diastolic BP (mm Hg)	72 ± 9
Fasting glucose (mmol/L)	5.18 ± 0.32
TC (mmol/L)	4.71 ± 0.66
TAG (mmol/L)	0.96 ± 0.44
HDL cholesterol (mmol/L)	1.23 ± 0.28
LDL cholesterol (mmol/L)	3.06 ± 0.58
TC:HDL	4.02 ± 1.03
Apolipoprotein A-100 (μmol/L)	44.70 ± 11.01
Apolipoprotein B-I (μmol/L)	2.56 ± 0.72
Lp(a) (mmol/L)	1.22 ± 1.69
tHcy (μmol/L)	7.95 ± 2.81
TNF-α (pg/mL)	117.38 ± 216.29
IL-1β (pg/mL)	28.90 ± 66.00
IL-6 (pg/mL)	10.59 ± 39.81
hsCRP (mg/L)	1.81 ± 1.82
IFN-γ (pg/mL)	67.61 ± 151.49
IL-8 (pg/mL)	176.00 ± 323.02

[1] BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; IFN-γ, interferon-γ; Lp(a), lipoprotein(a); TAG, triglycerides; TC, total cholesterol; tHcy, total homocysteine.
[2] Mean ± SD (all such values).

The fasting and nonfasting plasma tHcy and serum inflammatory marker concentrations after test diets are shown in **Table 4**. The current results demonstrated that there was no difference in tHcy concentration across the 3 diets. However, compared with the standardization diet, the fasting tHcy concentration was shown to be significantly higher from a mean of 7.95 ± 2.81 μmol/L to 8.88 ± 3.05 μmol/L in the PO diet, to 8.76 ± 2.96 μmol/L in the OO diet, and to 9.13 ± 3.17 μmol/L in the CO diet. In addition, no difference was observed for fasting and nonfasting tHcy; TNF-α, IL-1β, -6, and -8; hsCRP; and IFN-γ when comparisons were made across the 3 test diets.

DISCUSSION

In the current study, the effects of 3 cooking oils (ie, saturated PO and CO and monounsaturated OO) on CVD risk factors were compared. The test fats each contributed ~20% of energy in experimental diets that consisted of 30% of kilocalories from fat, 20% of kilocalories from protein, and ~50% of kilocalories from carbohydrates. The linoleic acid content was not standardized across the 3 diets in the study. The CO diet contained the lowest linoleic acid content (2.9% of energy) and was meant to serve as a positive control with respect to serum lipid endpoints that were measured. This purpose was achieved, to some extent, because the postprandial and fasting TC values obtained with the CO diet were significantly higher ($P < 0.05$) than those observed for the OO diet.

The finding that the serum lipid profile obtained with the PO and OO diets was comparable suggested 2 things. First, the 16:0-rich PO and 18:1-rich OO had comparable cholesterolemic effects on the serum lipid profile of the healthy adult subjects. The noncholesterolemic effects of the PO diet were in agreement with similar findings previously reported by Ng et al (19) and Choudhury et al (20) in healthy adult subjects. Second, the 16:0 in the PO had a weaker cholesterol-raising potential than did the 12:0 + 14:0 in the CO. In a study in nonhuman primates, Hayes et al (21) reported that 16:0 from palm oil was essentially neutral. It was previously suggested that the unique distribution of 16:0 predominantly in the sn-1 and sn-3 positions of the palm-fat molecule, as well as the microcomponents in PO, confer noncholesterol-raising properties to palm fat (22–24). However, it could not be ascertained to what degree these properties of palm fat beneficially influenced the lipid results measured in this study. In addition, a sufficient amount of linoleic acid (18:2) intake from

TABLE 3
Fasting and nonfasting serum lipid profile in 45 healthy subjects who participated in a 3 × 5-wk intervention[1]

	Fasting			Nonfasting		
Serum variable	PO	OO	CO	PO	OO	CO
TC (mmol/L)[2]	4.81 ± 0.74^{a,b}	4.65 ± 0.71^{a}	4.95 ± 0.69^{b}	4.65 ± 0.73^{a}	4.62 ± 0.67^{a}	4.85 ± 0.68^{b}
TAG (mmol/L)[2]	0.85 ± 0.31	0.84 ± 0.37	0.90 ± 0.39	1.22 ± 0.50	1.28 ± 0.47	1.20 ± 0.56
HDL cholesterol (mmol/L)[2]	1.31 ± 0.26^{a,b}	1.28 ± 0.23^{a}	1.37 ± 0.30^{b}	1.24 ± 0.25^{a,b}	1.21 ± 0.24^{a}	1.29 ± 0.31^{b}
LDL cholesterol (mmol/L)[2]	3.20 ± 0.71^{a,b}	3.06 ± 0.64^{a}	3.30 ± 0.75^{b}	2.93 ± 0.608^{a,b}	2.89 ± 0.62^{a}	3.11 ± 0.67^{b}
TC:HDL cholesterol[2]	3.69 ± 0.90	3.63 ± 0.93	3.65 ± 0.95	3.86 ± 0.88	3.99 ± 1.09	3.95 ± 1.03
apo A-100 (μmol/L)[2]	46.95 ± 8.68	46.09 ± 9.03	47.64 ± 9.71	46.31 ± 9.85	44.33 ± 8.55	46.13 ± 8.67
apo B-1 (μmol/L)[2]	2.79 ± 0.69	2.67 ± 0.73	2.79 ± 0.88	2.65 ± 0.72	2.55 ± 0.69	2.60 ± 0.60
Lp(a) (mmol/L)[3]	0.93 ± 1.86	0.95 ± 1.79	0.92 ± 1.91	1.42 ± 1.01^{a}	1.41 ± 0.89^{a}	1.31 ± 1.11^{b}

[1] Different superscript letters in the same row indicate that values showed statistically significant differences ($P < 0.05$, Bonferroni multiple comparison test) between corresponding columns. Baseline values were included as covariates for all variables. apo, apolipoprotein; CO, coconut oil; Lp(a), lipoprotein(a); OO, olive oil; PO, palm olein; TAG, triglycerides; TC, total cholesterol.
[2] All values are means ± SDs.
[3] All values are geometric means ± SDs.

TABLE 4
Fasting and nonfasting plasma tHcy and serum inflammatory marker concentrations in 45 healthy subjects who participated in a 3 × 5-wk intervention[1]

	Fasting			Nonfasting		
	PO	OO	CO	PO	OO	CO
Plasma variable						
tHcy (μmol/L)	8.88 ± 3.05	8.76 ± 2.96	9.13 ± 3.17	8.13 ± 2.42	8.41 ± 2.38	8.74 ± 3.19
Serum variable						
TNF-α (pg/mL)	98.90 ± 221.44	99.67 ± 230.98	95.14 ± 185.55	105.90 ± 238.11	101.78 ± 230.81	95.73 ± 187.44
IL-1β (pg/mL)	23.09 ± 57.93	23.63 ± 57.95	25.93 ± 71.05	24.18 ± 61.35	23.26 ± 55.33	25.05 ± 69.13
IL-6 (pg/mL)	8.52 ± 32.19	8.71 ± 31.15	9.91 ± 44.07	8.86 ± 34.35	8.40 ± 31.14	10.01 ± 44.63
hsCRP (mg/L)	2.15 ± 2.89	2.19 ± 2.36	1.96 ± 2.01	1.59 ± 1.62	2.23 ± 3.52	2.02 ± 2.32
IFN-γ (pg/mL)	17.04 ± 37.78	16.20 ± 36.86	11.53 ± 30.78	25.55 ± 83.99	19.76 ± 48.44	18.48 ± 49.46
IL-8 (pg/mL)	67.15 ± 108.46	71.02 ± 130.10	47.35 ± 85.30	23.75 ± 49.67	36.65 ± 88.99	23.56 ± 49.43

[1] All values are means ± SDs. Baseline values were included as covariates for all variables. CO, coconut oil; hsCRP, high-sensitivity C-reactive protein; IFN-γ, interferon-γ; OO, olive oil; PO, palm olein; tHcy, total homocysteine.

the PO diet (4.9% of energy) might also have counterbalanced the cholesterol-raising effect of 16:0.

Compared with OO, both PO and CO produced no differences in serum TC:HDL. Saturated fats from the experimental diets were able to raise HDL, which balanced the plasma TC:HDL ratio. Our results were in line with our previous study that used high-oleic PO, palm stearin, and partially hydrogenated soybean oil in a 3 × 5-wk intervention (17) in that no differences were shown in serum TA, apo A-100, and apo B-I concentrations. We acknowledge the relatively short 4–5-wk consumption periods before blood collection. However, lipid responses in our study demonstrated the strong compliance of study subjects to the consumption intervention.

The consumption of the CO diet in our study resulted in a decrease in the postprandial Lp(a) concentration compared with that of the OO and PO diets. This observation was in agreement with that of Tholstrup and Samman (25), who reported that long-chain SFAs (eg, 18:0 and 16:0) elevated postprandial Lp(a). The reason for this result was that the CO diet mainly contained 12:0 and 14:0, and thus, the shorter chain-length fatty acids decreased postprandial Lp(a). In other words, the reduction of 16:0 in the CO diet could have been responsible for the decrease. This finding suggested that there was an early phase degradation of Lp (a) in the CO diet compared with in the OO and PO diets. The increase in 18:1 intake from the OO diet did not seem to affect Lp (a) in fasting and nonfasting states.

The 3 different experimental diets did not differ in their effects on the plasma tHcy concentration. However, the fasting plasma tHcy obtained from the 3 experimental diets was shown to be significantly higher than that of standardization diet. This result could have been attributed to the higher protein content in the experimental diets (20% of kcal) compared with that of the standardization diet (15% of kcal). Verhoef et al (26) suggested that a higher-protein diet may raise the postprandial but not fasting plasma tHcy concentration after consumption of a high-protein supplementation that consisted of methionine for 8 d. The longer consumption intervention and more subjects increased the power of our study, and therefore, we could observe a significant higher fasting tHcy compared with that of the standardization diet. We agree with Verhoef et al (26) that the increase in tHcy could be modified by the amino acid composition in high-protein diets. Furthermore, we are also aware that a recent meta-analysis reported that homocysteine-lowering therapies were not beneficial in reducing risk of mortality and other disease risks (27). Homocysteine could be involved in the very early stages of atherosclerosis, which allows vitamin B supplementation to be involved in the prevention stage; however, there are limited trials to support this hypothesis. In addition, most of the published data were derived from Western populations. There is still a lack of data from Asian populations to support this view. Therefore, our findings suggested that high tHcy could be a marker of a high-protein diet, and we do not support the contention that dietary fats influence plasma tHcy concentrations. What this means is that if the plasma tHcy concentration is indeed an independent CVD risk factor, modulation of plasma concentrations are not exerted via dietary fats.

To our knowledge, there are insufficient studies that used SFA-rich diets to alter the immune response. A review by Kennedy et al (28) demonstrated the proinflammatory effects of saturated fats. However, most of the evidence was obtained from cellular and animal studies. Studies have also shown that SFAs induced the expression of cyclooxygenase-2, which is an enzyme that is responsible for inflammation and pain, as well as of inflammatory markers (eg, IL-6 and TNF-α) that are mediated by a Toll-like receptor (29–31). However, changes in plasma concentrations of inflammatory markers were not observed in a study when butter, OO, and walnut oil were compared (32). It was shown that dietary fats with different fatty acid compositions altered plasma IL-6, TNF-α, E-selectin, and hsCRP (17, 33), but no change was observed postprandially (34, 35). Baer et al (33) showed that oleic acid intake was associated with decreased IL-6 and E-selectin concentrations relative to the intake of SFAs.

TNF system activity, IL-6, C-reactive protein, E-selectin, soluble intracellular and vascular cell adhesion molecules (sICAM-1 and sVCAM) were shown to be increased with the consumption of diets rich in *trans* fatty acids (33, 36–38). IL-6 and TNF-α were also shown to increase in peripheral blood mononuclear cells in overweight subjects fed a *trans* fatty acid diet compared with a high-PUFA diet (15). However, no difference in concentrations was observed for IL-6, -1β, and -8, IFN-γ, TNF-α, and hsCRP in the 3 test diets in our study. Our findings were in agreement with a recent study from our group (17) that showed that a palmitic acid–rich diet did not differ from an oleic acid–rich diet in their effects on serum concentrations of hsCRP; IL-6, -8, and -1β; TNF-α; and IFN-γ. No

significant difference in effects was observed between SFA-rich meals (PO and CO diets) and the MUFA-rich meal (OO diet) in our study. However, compared with a palmitic acid–rich diet, the oleic acid–rich diet in our previous study decreased IL-8 (17). Palm stearin, a solid fraction from palm oil, contributed 16.4 ± 0.1% of energy from SFAs and 3.6 ± 0.1% of energy from 18:2 in the previous study. The oleic acid–rich diet contained 10.8 ± 0.1% of energy from SFAs and 5.8± 0.0% of energy from 18:2. The high PUFA content of the oleic acid–rich diet might have caused the decrease in IL-8 in the previous study. The small changes in our study may have been due to the PUFA content, which did not vary critically across diets. However, our diet formulations were in the range of the recent dietary recommendation for PUFAs with 2.5–3.5% of energy from 18:2 intakes to prevent deficiency symptoms. Furthermore, our study subjects were healthy young adults and not at high CVD risk to give a significant immune response in a short period of time. A 5-wk intervention might not have been long enough to give significant effects in the chosen markers, although high-SFA diets were used in the study. Besides, between-subject variations in inflammatory results might also have contributed to the null effects in this study.

Our study was designed to investigate the effects of common commercial fats for normal household cooking in a sample of Malaysian volunteers but not to distinguish between the effects of individual fatty acids. Therefore, the 18:2 content was not standardized across diets. Another limitation was that most of the subjects were young and women with a low-risk profile. Therefore, we did not see much difference in terms of nonlipid risk markers in response to dietary fatty acids. We intended to recruit older adults, but younger adults came for the initial screening for subject recruitment.

In conclusion, the results of this study indicated that it may be premature to judge SFA-rich diets as contributing to CVD risk solely on the basis of their SFA content. The source of the SFA and linoleic acid contents in the diet may play different roles in lipid and inflammatory responses. The length of SFAs and the fatty acid distribution in the triacylglycerol moiety should be taken into consideration when a recommendation for an intake of a particular dietary fat type is made. Our study provided preliminary data on the effect of dietary fat type (saturated compared with monounsaturated) on nonlipid risk factors for nonfasting and fasting states in a sample of Malaysian volunteers, although the commonly used dietary fats did not cause a change in the nonlipid indexes measured.

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REFERENCES

1. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA 2002;288:2569–78.
2. Humphrey LL, Fu R, Rogers K, Freeman M, Helfand M. Homocysteine level and coronary heart disease incidence: a systematic review and meta-analysis. Mayo Clin Proc 2008;83:1203–12.
3. Berstad P, Konstantinova SV, Refsum H, Nurk E, Vollset SE, Tell GS, Ueland PM, Drevon CA, Ursin G. Dietary fat and plasma total homocysteine concentrations in 2 adult age groups: the Hordaland Homocysteine Study. Am J Clin Nutr 2007;85:1598–605.
4. Hargreaves KM, Pehowich DJ, Clandinin MT. Effect of dietary lipid composition on rat liver microsomal phosphatidylcholine synthesis. J Nutr 1989;119:344–8.
5. Devlin AM, Lentz SR. ApoA-I: a missing link between homocysteine and lipid metabolism? Circ Res 2006;98:431–3.
6. McCully KS, Wilson RB. Homocysteine theory of arteriosclerosis. Atherosclerosis 1975;22:215–27.
7. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 2003;77:1146–55.
8. Kaul D. Molecular link between cholesterol, cytokines and atherosclerosis. Mol Cell Biochem 2001;219:65–71.
9. Klein RL, Ascencao JL, Mironova M, Huang Y, Lopes-Virella MF. Effect of inflammatory cytokines on the metabolism of low-density lipoproteins by human vascular endothelial cells. Metabolism 2001;50:99–106.
10. Niemann-Jönsson A, Dimayuga P, Jovinge S, Calara F, Ares MP, Fredrikson GN, Nilsson J. Accumulation of LDL in rat arteries is associated with activation of tumor necrosis factor-alpha expression. Arterioscler Thromb Vasc Biol 2000;20:2205–11.
11. Verma S, Szmitko PE, Ridker PM. C-reactive protein comes of age. Nat Clin Pract Cardiovasc Med 2005;2:29–36, quiz 58.
12. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.
13. Hansson GK, Robertson AK, Soderberg-Naucler C. Inflammation and atherosclerosis. Annu Rev Pathol 2006;1:297–329.
14. Clifton PM. Diet and C-reactive protein. Curr Atheroscler Rep 2003;5:431–6.
15. Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ, Meydani SN. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. J Lipid Res 2002;43:445–52.
16. Galli C, Calder PC. Effects of fat and fatty acid intake on inflammatory and immune response: a critical review. Ann Nutr Metab 2009;55:123–39.
17. Teng KT, Voon PT, Cheng HM, Nesaretnam K. Effects of partially hydrogenated, semi-saturated, and high oleate vegetable oils on inflammatory markers and lipids. Lipids 2010;45:385–92.
18. Anderson KM, Odell PM, Wilson PW, Kannel WB. Cardiovascular disease risk profiles. Am Heart J 1991;121:293–8.
19. Ng TK, Hayes KC, DeWitt GF, Jegathesan M, Satgunasingam N, Ong AS, Tan D. Dietary palmitic and oleic acids exert similar effects on serum cholesterol and lipoprotein profiles in normocholesterolemic men and women. J Am Coll Nutr 1992;11:383–90.
20. Choudhury N, Tan L, Truswell AS. Comparison of palmolein and olive oil: effects on plasma lipids and vitamin E in young adults. Am J Clin Nutr 1995;61:1043–51.
21. Hayes KC, Pronczuk A, Lindsey S, Diersen-Schade D. Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in nonhuman primates. Am J Clin Nutr 1991;53:491–8.
22. Khosla P. Palm oil: a nutritional overview. AgroFOOD industry hi-tech. 2006;17:21–3.
23. Kritchevsky D. Fatty acids, triglyceride structure, and lipid metabolism. J Nutr Biochem. 1995;6:172–8.
24. Hayes KC. Synthetic and modified glycerides: effects on plasma lipids. Curr Opin Lipidol 2001;12:55–60.
25. Tholstrup T, Samman S. Postprandial lipoprotein(a) is affected differently by specific individual dietary fatty acids in healthy young men. J Nutr 2004;134:2550–5.

26. Verhoef P, van Vliet T, Olthof MR, Katan MB. A high-protein diet increases postprandial but not fasting plasma total homocysteine concentrations: a dietary controlled, crossover trial in healthy volunteers. Am J Clin Nutr 2005;82:553–8.
27. Clarke R, Halsey J, Bennett D, Lewington S. Homocysteine and vascular disease: review of published results of the homocysteine-lowering trials. J Inherit Metab Dis 2011;34:83–91.
28. Kennedy A, Martinez K, Chuang CC, LaPoint K, McIntosh M. Saturated fatty acid mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. J Nutr 2009;139:1–4.
29. Laine PS, Schwartz EA, Wang Y, Zhang WY. Karnik SK, Musi N, Reaven PD. Palmitic acid induces IP-10 expression in human macrophages via NF-kappaB activation. Biochem Biophys Res Commun 2007;358:150–5.
30. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006;116:3015–25.
31. Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2mediated through Toll-like receptor 4. J Biol Chem 2001;276:16683–9.
32. Jiménez-Gómez Y, Lopez-Miranda J, Blanco-Colio LM, Marin C, Perez-Martinez P, Ruano J, Paniagua JA, Rodriguez F, Egido J, Perez-Jimenez F. Olive oil and walnut breakfasts reduce the postprandial inflammatory response in mononuclear cells compared with a butter breakfast in healthy men. Atherosclerosis 2009;204:e70–6.
33. Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. Am J Clin Nutr 2004;79:969–73.
34. Teng KT, Nagapan G, Cheng HM, Nesaretnam K. Palm olein and olive oil cause a higher increase in postprandial lipemia compared with lard but had no effect on plasma glucose, insulin and adipocytokines. Lipids 2011;46:381–8.
35. Tholstrup T, Teng KT, Raff M. Dietary cocoa butter or refined olive oil does not alter postprandial hsCRP and IL-6 concentrations in healthy women. Lipids 2011;46:365–70.
36. Mozaffarian D. Trans fatty acids – effects on systemic inflammation and endothelial function. Atheroscler Suppl 2006;7:29–32.
37. Mozaffarian D, Rimm EB, King IB, Lawler RL, McDonald GB, Levy WC. Trans fatty acids and systemic inflammation in heart failure. Am J Clin Nutr 2004;80:1521–5.
38. Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, Willett WC, Hu FB. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. J Nutr 2005;135:562–6.