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IS PALM MID FRACTION A HEALTHIER CHOICE AS A COCOA BUTTER EQUIVALENT?

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ABSTRACT

Palm mid fraction (PMF) is a fraction of palm oil rich in 1, 3-dipalmitoyl-2- oleoylglycerol (POP) triacylglycerol (TAG) that is obtained through re-fractionation of either palm olein or palm stearin. POP-, 1(3)-1, 3 distearoyl-2-oleoylglycerol (SOS)- and triolein (OOO)- type of fats have different melting characteristics that may affect postprandial lipid and glucose metabolism. We aimed to study the effects of palmitic, stearic or oleic acid situated at the sn-*1 and* sn-*3 positions of edible fats on postprandial lipemia, glucose and insulin responses. A randomised, double-blind crossover (3 x 3 arms) orthogonal Latin-square design was used. A total of 36 healthy adults received three different test muffins, each containing 53 g of test fat from palm mid fraction (PMF as POP-rich fat), shea stearin (SS as SOS-rich fat) or high-oleic sunflower oil (HOSF as OOO-rich fat) plus a low-fat milkshake in random order separated by two weeks. No significant differences (P>0.05) were observed between the three test meals for postprandial responses in plasma total cholesterol, Lp(a), glucose and insulin levels. However, plasma TAG levels were found significantly higher (P<0.05) in PMF- and HOSF- subjects compared with SS- subjects after 90 min. Plasma C-peptide levels were found lower (P<0.05) in the SS-subjects compared to the PMF- and HOSF- subjects. The results suggested that dietary fats containing palmitic (PMF) and oleic acid (HOSF) at the* sn-*1, 3 positions of the TAG backbone exert similar postprandial lipid and glucose responses compared with that of a stearic acid-rich* sn-*1,3 dietary fat (SS). In the food industry, there is demand for edible fats with different forms of TAG which can serve as a cocoa butter equivalent (CBE)* i.e. *as an important alternative for chocolates and other confectionary products.*

Keywords: fatty acids, glucose, insulin, postprandial lipemia, *sn*-1, *sn*-3.

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INTRODUCTION

The effects of edible fats on cholesterolemia have been well-documented (Hu *et al*., 2001). Different types of saturated fatty acids (SFA) pose different effects on blood lipid. In fact, equations arising from human feeding trials have been formulated to predict blood cholesterol/lipid levels brought about by changes in dietary fat (Hegsted *et al*., 1965; Mensink *et al*., 2003).

Much of our knowledge on the impact of dietary fats on cardiovascular heart diseases is based on measurements of plasma lipid and lipoprotein profile in the fasting state, with the lowest low density lipoprotein cholesterol (LDL-c), LDL-c/high density lipoprotein cholesterol (HDL-c) and triacylglycerol (TAG) being considered ideal. Lopez-Miranda and Marin (2010) highlighted the importance of dietary and physiological impacts on postprandial lipid metabolism. In this connection,

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postprandial hypertriglyceridemia is reported to increase the risk of type 2 diabetes mellitus, obesity, atherosclerosis, cardiovascular heart disease and stroke (Ortega *et al*., 2012).

The effects of stereospecific positioning of fatty acids in TAG molecules of dietary fats have been studied. Previous studies reported that palm oil has 41% palmitic acid (C16:0) with the majority of the fatty acid located at the *sn*-1 and *sn*-3 positions, but with only 3%-5% of its C16:0 at the *sn*-2 position (Karupaiah and Sundram, 2007; Kritchevsky, 2000). Chemical interesterification of palm oil is able to increase the amount of C16:0 at the *sn*-2 position to 13.6% which results in a significant increase in atherogenicity of the interesterified fat (Karupaiah and Sundram, 2007; Kritchevsky, 2000).

In animal models, Storlien *et al*. (1991; 1993) found that diets high in saturated, monounsaturated (ω-9) and polyunsaturated (ω-6) fatty acids induced severe insulin resistance, whereas long-chain omega (ω-3) fatty acids normalised the insulin action. However, published studies on the effects of different dietary fats on postprandial lipemia and insulin response in humans have not been consistent. Tholstrup *et al*. (2001) reported that the intake of long-chained saturated acids (palmitic acid and stearic acid) by 16 healthy men resulted in a relatively lower lipemic response than did intake of the unsaturated fatty acids. In a randomised, crossover study with 10 healthy men, Teng *et al*. (2011) found that palm olein (POP-rich fat) and olive oil (OOO-rich fat) caused a higher increase in postprandial lipemia than lard (SPO-rich fat). In contrast, Zampelas *et al.* postprandial study (Zampelas *et al*., 1994) and Galgani *et al.* (2008) extensive review reported that the positional distribution of fatty acids on dietary TAG or dietary fat quality does not influence postprandial lipemia nor insulin response.

The positional distribution of fatty acids in the TAG molecule varies greatly among fats and oils of different origins. In the food industry, there is a demand for edible fats with different forms of TAG which can serve as a CBE *i.e.* as an important alternative for chocolates and other confectionary products. Palm oil, illipe and shea are listed in the European Chocolate Directive 2000/36EEC as CBE (EU Commission, 2000). Besides, vegetable oils such as PMF, olive oil, mango fats, kokum and teaseed oil have also been used to prepare CBE. PMF is a fraction of palm oil rich in POP TAG that is obtained through re-fractionation of either palm olein or palm stearin. Therefore, it is important to find out if a CBE such as PMF has similar effects as SS on postprandial lipoprotein metabolism, glycemia and insulinemic response. This forms the research question as well as the justification for the present study reported.

SUBJECTS AND METHODS

Subjects

Thirty-six healthy adults (18 males and 18 females, aged between 25 to 50 years) were recruited to participate in this study. All volunteers underwent a health screening which included: a) an abbreviated medical/physical examination [blood pressure, weight, height, body mass index (BMI)], b) serum lipid profiling, c) plasma glucose determination, d) liver function tests [serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT)] and e) kidney function test (serum creatinine). The inclusion criteria for this study were BMI 18.5-25.0 kg m^{-2}, systolic pressure <140 mmHg, diastolic pressure <90 mmHg, fasting total cholesterol (TC) <6.2 mM $litre^{-1}$ (<240 mg dL^{-1}), fasting TAG <1.70 mmol $litre^{-1}$ (<150 mg dL^{-1}), fasting glucose 4.0-7.0 mmol $litre^{-1}$. Exclusion criteria for this study were individuals on cholesterol or blood glucose medication, substance abuse (*e.g*. alcohol, cigarette smoking), going overseas during period of study, having blood clotting problem and pregnant or lactating for women.

This study was approved by the IMU Joint-Committee of Research and the Ethics Committee, International Medical University, Kuala Lumpur, Malaysia and this trial was registered at clinicaltrials.gov.my as *NCT01428960*.

Study Design

A randomised, double-blind crossover (3 x 3 arms) orthogonal Latin-square design was used. Blood collections were conducted according to the study protocol at every 30 min interval. Each subject received three experimental test meals in a random order, two weeks apart, over a six-week period. Subjects were randomly allocated to one of following six treatment sequences: ABC, BCA, CAB, ACB, CBA, or BAC, with PMF as A, SS as B and HOSF as C. Each set of test meal contained a muffin and a glass milkshake. The test meal provided 875.6 kcal, 16 g protein, 83 g carbohydrate and 53 g test fat.

Sample Size Calculation

Sample size was calculated based on 90% power at $P<0.017$ for cross-over within group comparison to detect a 0.5-SD unit change in the area under the curve (AUC) for plasma TAG concentrations, that provided a sample size of 36 subjects after allowing for 10% dropout. Secondary outcomes were changes in TC, Lp(a), glucose, insulin and C-peptide. The characteristics of the study participants are shown in *Table 1*.

Test Fats

The three test fats were PMF (iodine value = 34.9) and SS (iodine value = 34.1) which were obtained from Wilmar PGEO Edible Oils Sdn. Bhd. while HOSF was obtained from Intercontinental Specialty Fats Sdn. Bhd., Selangor, Malaysia. PMF contained a similar proportion of SFA (62.7% *vs*. 62.6%) and oleic acid (33.7% *vs*. 33.0%) when compared to SS but contained more palmitic acid (57.1% *vs*. 1.8%) and less stearic acid (5.0% *vs*. 60.8%). PMF and SS were blended with a small amount of sunflower oil (Mazola, Switzerland) so that linoleic acid (C18:2,w-6) was standardised across the diets at 7% kcal. The fatty acid composition of the test fats is shown in *Table 2*. The test fats were incorporated into muffins, labelled with a code, and stored frozen until being consumed.

The TAG composition of the test fats is illustrated in *Table 3*. PMF consists mainly of the TAG species 1, 3-dipalmitoyl-2- oleoylglycerol (POP) (67.6%), while the majority of the TAG species in SS is 1(3)- 1, 3 distearoyl-2-oleoylglycerol (SOS) (74.2%) with oleic acid in the *sn*-2 positions. The main TAG species of HOSF is triolein (OOO) (66.5%).

The three test fats consist of approximately equal oleic acid (C18:1) at the *sn*-2 position [(PMF = 72%, SS = 80%, HOSF = 88%)]. Differential scanning calorimetry showed that PMF shows a single sharp melting peak at approximately 31.3°C, whereas SS shows a higher melting point at approximately 38.0°C. HOSF shows a melting point at approximately 0.14°C.

Postprandial Protocol

Subjects were asked to avoid consuming foods high in fat as well as not to participate in strenous exercise the day preceding each test meal and to fast overnight beginning at 2200 hr. A low-fat meal (500-700 kcal containing, 10 g fat) was provided to the subjects as their evening meal. The subjects were requested to consume the low-fat meal before 2200 hr and fast overnight.

On the morning of the postprandial test, participants attended the Nutrition Group Clinic between 0800 hr and 1000 hr when a 22G" Vasofix® Brannule (Cat No. 426 8091B, B. Braun, Germany) was inserted into the antecubital vein of the forearm and held in place with a Connecta (Cat No. 394601, Becton – Dickinson, Sweden). Blood was collected by syringe and dispensed into appropriate uncapped blood collection containers. Samples for TAG, TC, Lp(a), insulin and C-peptide analysis were collected using serum vacutainer tubes. The samples collected were allowed to clot at room temperature before centrifugation. Samples for plasma glucose was collected using fluoride containing tube. The serum and plasma were aliquoted accordingly and stored in -80°C freezer prior to analyses.

The subjects were given 10 min to consume a set of test meal. Venous blood samples were collected every 30 min for 4 hr after the test meal. Blood collection was performed by registered staff nurse supervised by medical officer or family physician using antiseptic technique. Drinking water was provided to the subjects over 4 hr. At the end of 4 hr, the subjects were provided with an *ad-libitum* lunch.

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY PARTICIPANTS

Variable	Participants		
	Women (n=18)	Men (n=18)	All (n=36)
Age (yr)	23.0 ± 1.1	23.0 ± 1.9	23.3 ± 1.5
Weight (kg)	52.0 ± 5.6	63.1 ± 7.6	57.5 ± 8.7
Height (cm)	160.0 ± 4.2	172.0 ± 5.7	166.0 ± 8.0
BMI (kg m^{-2})	20.3 ± 1.6	21.3 ± 2.0	20.8 ± 1.8
Systolic blood pressure (mmHg)	111.0 ± 6.1	122.0 ± 10.0	116.0 ± 9.9
Diastolic blood pressure (mmHg)	73.0 ± 5.0	75.0 ± 7.6	74.0 ± 6.4
Total cholesterol (mmol $litre^{-1}$)	4.9 ± 0.7	4.4 ± 0.8	4.7 ± 0.8
HDL-c (mmol $litre^{-1}$)	1.7 ± 0.3	1.4 ± 0.3	1.5 ± 0.3
LDL-c (mmol $litre^{-1}$)	2.9 ± 0.6	2.7 ± 0.7	2.8 ± 0.6
TAG (mmol $litre^{-1}$)	0.7 ± 0.3	0.7 ± 0.4	0.7 ± 0.3
Fasting glucose (mmol $litre^{-1}$)	4.6 ± 0.6	4.7 ± 0.3	4.6 ± 0.3

Note: All values are mean ± SD.
BMI - body mass index; HDL-c - high-density lipoprotein cholesterol; LDL-c - low-density lipoprotein cholesterol; TAG - triacylglycerol; PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil.

TABLE 2. FATTY ACID COMPOSITIONS OF THE TEST FATS

Fatty acids	Mol %		
	PMF	SS	HOSF
C14:0	0.61	ND	ND
C16:0	52.85	2.40	4.44
C18:0	4.87	57.20	2.41
C18:1	33.73	32.29	85.58
C18:2	7.93	7.63	7.56
Others	0.01	0.48	0.01
SFA	58.33	59.60	6.85
MUFA	33.73	32.29	85.58
PUFA	7.93	7.63	7.56

Note: ND - not detected; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil.

TABLE 3. TAG COMPOSITION OF THE TEST FATS

TAG	Mol %		
	PMF	SS	HOSF
PLL	0.1	ND	ND
MLP	0.2	ND	ND
OLO	0.2	ND	6.3
PLO	1	ND	1.1
PLP	8.7	ND	0.2
OOO	0.9	0.3	66.5
POO	3.1	0.6	9
POP	67.6	1	0.5
PPP	2.5	3.2	ND
SOO	0.3	7.1	5.9
POS	13.3	7.4	0.4
PPS	0.4	0.2	ND
SOS	1.5	74.2	ND
Others	ND	5.9	10.1
Slip melting point (°C)	31.1	37.9	<1.0

Note: ND - not detected; TAG - triacylglycerol; L - linoleic acid (18:2n–6); M - myristic acid (14:0); O - oleic acid (18:1n–9); P - palmitic acid (16:0); S - stearic acid (18:0); PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil.

Analytical Methods

Serum TAG, TC, Lp(a) and plasma glucose were analysed using enzymatic colourimetric assays with a Hitachi 902 autoanalyser (Roche Diagnostics GmbH). The fatty acid composition of test fats was measured using gas-liquid chromatography on an SP-2560 column (100 m x 0.23 mm x 0.2 mm; Agilent Technologies) with a flame ionisation detector on an autosystem (Perkin Elmer). The carrier gas (helium) pressure and injector temperature were set to 40 psi and 250°C, respectively. The oven temperature was set isothermal at 240°C for 42 min. Hydrogen and compressed air were used for ignition. A fatty acid methyl esters mixture (Sigma-Aldrich, Australia) was used as the external standard.

TAG composition of the test fats was determined by reversed-phase High Performance Liquid Chromatography (HPLC) system. The method of analysis was modified from the AOCS Official Method Ce 5c-93, slip-melting point was determined according to MPOB Test Method (MPOB, 2004).

Meanwhile, both C-peptide and insulin samples were analysed using (Elecsys module) immunoassay analysers. Although the 'Homeostatic Model Assessment' (HOMA= fasting insulin concentration × fasting glucose concentration /22.5) (Matthews *et al.*, 1985) and the quantitative insulin sensitivity check index [QUICKI= the inverse of the sum of the logarithms of the fasting insulin and fasting glucose, *i.e.* [1 / (log (fasting insulin) + log (fasting glucose)] (Katz *et al.*, 2000) are normally used for fasting blood samples, these indices of insulin resistance were calculated for the 4 hr time-point following the three test meals, which may be considered as the early fasting phase in the study.

Statistical Analyses

Repeated measure analysis of variance (ANOVA) with *Bonferroni post hoc* analysis was conducted using GraphPad Prism version 5 (GraphPad Software, La Jolla, CA 9203, USA) and PASW Statistics 18 to assess the significant difference between diets. The normal distribution of data was assessed using Shapiro-Wilk's normality test and logarithmic transformations were used when necessary. Data are expressed as mean with 95% CI.

RESULTS

Table 4 and *Figure 1* show the postprandial changes in serum TAG and following the three test meals. The meal × time interaction (P=0.000) for the change in postprandial TAG indicated differences between test meals; plasma TAG increased more rapidly following PMF and HOSF compared with that of a SS test meal.

There were significant differences in serum TAG levels between the test diets at 120 min and 180 min. Postprandial changes in TAG between the HOSF and SS showed a significant meal × time interaction (P=0.0041) at 120 min; the difference was 0.24 (95% CI, 0.064, 0.41). Meanwhile, comparison of PMF and HOSF with the SS meal showed a significant meal × time interaction (P=0.0075) at 180 min in which the differences between HOSF and PMF with the SS meal in the change of TAG were 0.26 (95% CI, 0.052, 0.47) and -0.21 (95% CI, -0.42, -0.00076) respectively.

The differences between HOSF and PMF with the SS meal in the change of TAG at 150 min were 0.25 (95% CI, 0.12, 0.37) and -0.22 (95% CI, -0.35, -0.094) respectively, at 180 min 0.23 (95% CI, 0.096, 0.36) and -0.17 (95% CI, -0.30, -0.040) and at 210 min 0.22 (95% CI, 0.084, 0.36) and -0.20 (95% CI, -0.34, -0.060). Moreover, the release of C-peptide following PMF test meals was significantly different (P=0.0028) from SS at 240 min time-point *i.e.* -0.29 (95% CI, -0.48, -0.090).

TAG responses did not differ significantly between the three types of test meal given at 60 min and 240 min. Moreover, there were no significant differences (P>0.05) between meals and times in TC (*Table 5*), Lp(a) (*Table 6*), glucose and insulin.

Figure 2 illustrates the postprandial changes in serum C-peptide following the test meals. The meal x time interaction (P=0.000) for the change in postprandial C-peptide indicated differences between test meals. The total amount of C-peptide released by pancreatic β-cells following PMF and HOSF test meals were higher as compared to the SS test meal. Comparison of the postprandial changes between the HOSF and SS showed a significant difference at 120 min (P<0.048); the difference was 0.15 (95% CI, 0.0021, 0.31). Meanwhile, comparison of PMF and HOSF with the SS meal showed a significant meal × time interaction difference at 150 min (P<0.0001), at 180 min (P=0.0002) and at 210 min (P=0.003).

TABLE 4. POSTPRANDIAL CHANGES IN TAG FOLLOWING THE THREE TEST MEALS

Time (min)	Changes in TAG of test meals (mmol litre^{-1})		
	HOSF	SS	PMF
0	0.77 (0.66, 0.89)	0.86 (0.75, 0.97)	0.82 (0.69, 0.94)
60	0.83 (0.69, 0.95)	0.83 (0.72, 0.94)	0.84 (0.70, 0.97)
120	1.19 (0.99, 1.38)^{a}	0.95 (0.81, 1.1)^{b}	1.12 (0.95, 1.28)^{a}
180	1.47 (1.22, 1.68)^{a}	1.2 (1.01, 1.39)^{b}	1.41 (1.19, 1.62)^{a}
240	1.46 (1.21, 1.68)	1.37 (1.16, 1.56)	1.49 (1.26, 1.69)

Note: Values are geometric means; 95% CIs in parentheses; (n = 36; 18 men, 18 women) for all treatments. Values were log-transformed, analysed by repeated-measures analysis of variance (ANOVA) and showed a diet × time interaction (P=0.000); the meal × gender, time × gender and meal × gender × time interaction were not significant. Different superscript letters in the same row indicate that values showed statistically significant differences (P<0.05, Bonferroni multiple comparison test) between corresponding columns.
TAG - triacylglycerol; PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil.

TABLE 5. POSTPRANDIAL CHANGES IN TC FOLLOWING THE THREE TEST MEAL

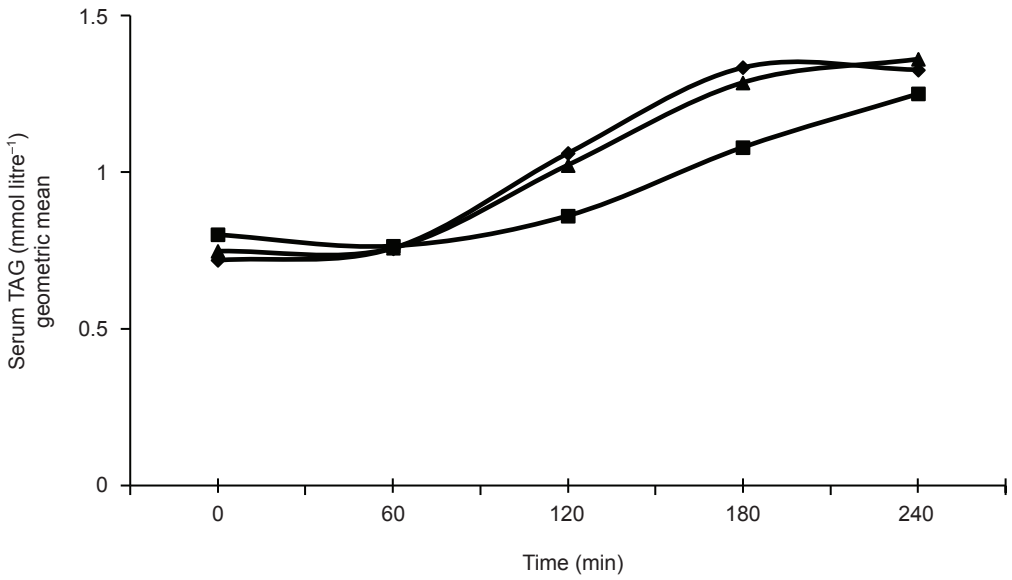
Time (min)	Changes in TC of test meals (mmol litre^{-1})		
	HOSF	SS	HOSF
0	5.00 (4.72, 5.28)	4.90 (4.64, 5.17)	4.97 (4.73, 5.21)
60	4.88 (4.59, 5.17)	4.69 (4.42, 4.95)	4.72 (4.50, 4.94)
120	4.86 (4.58, 5.14)	4.75 (4.46, 5.04)	4.74 (4.53, 4.96)
180	4.85 (4.58, 5.12)	4.74 (4.47, 5.00)	4.74 (4.52, 4.96)
240	4.92 (4.65, 5.19)	4.77 (4.52, 5.03)	4.79 (4.58, 5.01)

Note: Values are means; 95% CIs in parentheses; (n = 36; 18 men, 18 women) for all treatments. Values were analysed by repeated-measures analysis of variance (ANOVA) and diet x time, the meal x gender, time x gender and meal x gender x time interaction were not significant (P>0.05).
TC - total cholesterol; PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil.

TABLE 6. POSTPRANDIAL CHANGES IN LP(A) FOLLOWING THE THREE TEST MEALS

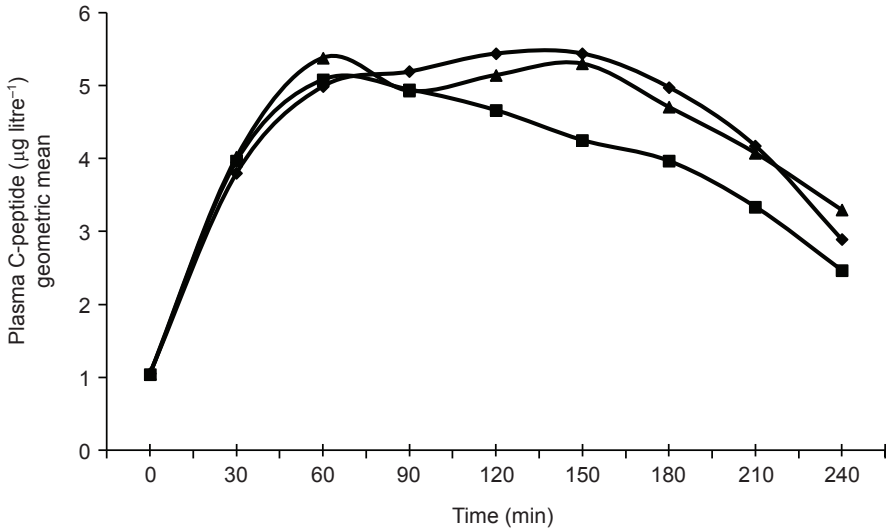
Time (min)	Changes in Lp(a) of test meals (mg dL^{-1})		
	HOSF	SS	HOSF
0	31.05 (25.46, 37.87)	29.99 (24.15, 37.25)	28.15 (22.67, 34.96)
60	29.95 (24.35, 36.83)	28.56 (23.01, 35.47)	29.23 (23.76, 35.96)
120	33.69 (27.78, 40.86)	31.71 (25.61, 39.27)	33.41 (27.50, 40.60)
180	36.44 (30.28, 43.84)	35.24 (28.67, 43.32)	36.56 (30.10, 44.42)
240	37.65 (31.56, 44.91)	38.70 (31.65, 47.32)	38.15 (31.56, 46.11)

Note: Values are geometric means; 95% CIs in parentheses; (n = 36; 18 men, 18 women) for all treatments. Values were log-transformed, analysed by repeated-measures analysis of variance (ANOVA) and diet x time, the meal x gender, time x gender and meal x gender x time interaction were not significant (P>0.05).
Lp(a) - lipoprotein(a); PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil.



Note: PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil; TAG - triacylglycerol.

Figure 1. Postprandial concentrations of serum TAG geometric mean (n = 36; 18 men, 18 women) following three test meals containing 53 g test fat from HOSF (♦), SS (■), and PMF (▲). Deviations from postprandial values were analysed by repeated measures analysis of variance (ANOVA); meal × time interaction P=0.000. Comparisons between meals were adjusted by using a Bonferroni multiple-comparison test.



Note: PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil.

Figure 2. Postprandial concentrations of plasma C-peptide geometric mean (n = 36; 18 men, 18 women) following three test meals containing 53 g test fat from HOSF (♦), SS (■), and PMF (▲). Deviations from postprandial values were analysed by repeated measures analysis of variance (ANOVA); meal × time interaction P=0.000. Comparisons between meals were adjusted by using a Bonferroni multiple-comparison test.

DISCUSSION

PMF and SS are widely used as CBE due to their desirable mouth-feel effects arising from their respective melting point of 31.3°C and 38.0°C (Lipp and Anklam, 1998). PMF displays a steep melting profile, with a narrow melting interval around 35.0°C which makes it an important component of confectionary fats, being the source of palmitic acid-rich TAG in the formulation of CBE (Salas *et al.*, 2009).

To date, there are lack of human studies comparing both PMF and SS as test fats in diets. However, there are several reports of SS being used in postprandial test meals (Berry and Sanders, 2005; Sanders and Berry, 2005). In the present study, the amounts of oleic acid at *sn*-2 position in all the three types of TAG molecules of PMF, SS and HOSF were approximately equal, linoleic acid content was standardised across diets, and the major difference between the test fats are the fatty acid type in the *sn*-1 and *sn*-3 positions, namely palmitic acid in the case of PMF (POP), oleic acid for HOSF (OOO) and stearic acid in the case of SS (SOS). In this study, the PMF and HOSF test fats exerted a similar postprandial TAG profile, which not only suggests that palmitic acid and oleic acid have comparable postprandial triglyceridemic effects but also the two types of fatty acids were similarly absorbed. The SS- (SOS) meal however, resulted in a lower postprandial TAG response compared to HOSF- (OOO) and PMF- (POP) meal.

The lower postprandial level of lipemia following SS in the present study is in agreement with results reported by Sanders and Berry (2005) that non-randomised SS resulted in decreased lipemia as compared to a test meal containing 50 g HOSF. Sanders *et al.* (2000) suggested that the lower postprandial lipemic effect of a stearic acid-rich SS meal was due to a slower absorption rate and led to the formation of smaller lipoprotein particles compared to an oleic acid-rich HOSF meal with a faster absorption rate and larger chylomicrons formation. However, the mechanism involved warrant further investigations. Evidently, differences in the physical characteristics of fats and stereospecific structure of TAG molecules influence the metabolism of lipids and the level of postprandial lipemia. In this connection, Berry *et al.* (2007) proposed that SS contains a higher proportion of solid fat at 37°C compared with HOSF and PMF and this may cause SS to be emulsified less readily and is less absorbed due to its higher melting point, resulting in a slower increase in serum TAG levels. This would suggest that SS might be cleared from the circulation faster than HOSF and PMF and resulted in a more prolonged postprandial lipemia which explains our results obtained.

Meanwhile, there were no significant differences in postprandial serum TC and Lp(a) levels after ingestion of the three test meals. Our present findings for TC levels are in accordance to the results obtained by Forsythe *et al.* (2007) who reported that that a *sn*-1 + *sn*-3 palmitic acid-rich fat such as palm oil is non-cholesterolemic. In contrast, Tholstrup and Samman (2004) reported that postprandial Lp(a) is affected differently by specific individual dietary fatty acids in healthy young men, while Sanders *et al.* (2000) found that shorter-chain fatty acids will clear Lp(a) at a faster rate after a post-meal study with high and normal fat loads.

TABLE 7. PLASMA GLUCOSE, INSULIN, HOMA AND QUICKI VALUES FOLLOWING THE THREE TEST MEALS AT 240 MIN

Plasma index	HOSF	SS	PMF
HOMA	4.43 (2.90, 5.97)	4.68 (2.91, 6.44)	7.27 (4.85, 9.69)
QUICKI	0.34 (0.32, 0.36)	0.34 (0.32, 0.35)	0.32 (0.30, 0.34)
Glucose	5.15 (4.93, 5.37)	5.27 (4.98, 5.57)	5.54 (5.25, 5.84)
Insulin	18.71 (12.34, 25.07)	18.96 (12.03, 25.90)	28.54 (19.07, 38.00)

Note: Values are means; 95% CIs in parentheses (n = 36; 18 men, 18 women) for all treatments. Values were log-transformed, analysed by repeated-measures analysis of variance (ANOVA); HOMA, diet × time interaction (P>0.05); QUICKI, diet × time interaction (P>0.05).
HOMA - Homeostatic Model Assessment; QUICKI - Quantitative Insulin Sensitivity Check Index; PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil.

C-peptide and insulin are initially synthesised in the same polypeptide. The C-peptide component is later cleaved off leaving the A- and B-chains which form insulin. C-peptide concentrations specifically reflect β-cell function because C-peptide is secreted in equimolar amounts with insulin and is not removed in the first pass through the liver (Hills and Brunskill, 2009; Kim *et al.*, 2012). Moreover, C-peptide is a more stable biomarker of insulin secretion than is the direct measurement of plasma insulin because C-peptide has a substantially longer half-life than insulin (Nimptsch *et al.*, 2011). In the present postprandial study, C-peptide responses peaked in the three test-fat groups about 60 min after the test meals were consumed and its serum concentrations decreased significantly (P<0.05) to around 2.8 μg litre^{-1} towards the end of the 4 hr study. There were significant differences in C-peptide responses among the three groups. Overall, postprandial C-peptide concentrations were lower (P<0.05) after a SS-meal compared to the HOSF and PMF-meals. This suggests that C-peptide concentrations following the SS-meal were cleared faster as compared to the HOSF- and PMF-meals.

Postprandial glucose and insulin concentrations did not differ following the meals high in palmitic acid- (POP) and rich in stearic acid- (SOS) compared to HOSF which was rich in oleic acid- (OOO). The comparable postprandial insulin concentration across the three test-fat groups also strengthens our suggestion that C-peptide concentrations were cleared at a faster rate in the SS-meal group. Our results are consistent with the findings of Lopez *et al.* (2011) which showed no significant difference in postprandial glucose response in subjects who consumed monounsaturated and saturated fats. In agreement with the previous study of Teng *et al.* (2011), a single high fat load may not affect the changes in glucose and insulin levels, as the meal x time interaction was not significant indicating that the peak insulin concentration was reached at the same time across meals, which was at 30 min. Our results suggest that POP-, SOS- and OOO- type of fats did not affect postprandial glucose and insulin responses. The HOMA and QUICKI (*Table 7*) indices at the 4 hr time-point (early fasting phase) were also comparable across the three test-fat groups.

CONCLUSION

The preponderance of either palmitic (16:0), stearic (18:0) or oleic acid (18:1) of the three test fats with different physical characteristics used in the present acute study did not induce any significant differences in postprandial Lp(a), glucose or insulin concentrations. Our findings, however, demonstrated that PMF (POP) and HOSF (OOO) fats induced higher TAG and C-peptide postprandial concentrations compared with the SS (SOS) fat.

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REFERENCES

Berry, S E and Sanders, T A (2005). Influence of triacylglycerol structure of stearic acid-rich fats on postprandial lipaemia. *Proc. Nutr. Soc., 64(2)*: 205-212.

Berry, S E; Miller, G J and Sanders, T A (2007). The solid fat content of stearic acid-rich fats determines their postprandial effects. *Am. J. Clin. Nutr., 85(6)*: 1486-1494.

EU Commission (2000). Directive 2000/36/EC of the European Parliament and of the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption. *Official J. European Communities EG 197*: 19.

Forsythe, C E; French, M A; Goh, Y K and Clandinin, M T (2007). Cholesterolaemic influence of palmitic acid in the *sn*-1, 3 v. the *sn*-2 position with high or low dietary linoleic acid in healthy young men. *Br. J. Nutr., 98(2)*: 337-344.

Galgani, J E; Uauy, R D; Aguirre, C A and Diaz, E O (2008). Effect of the dietary fat quality on insulin sensitivity. *Br. J. Nutr., 100(3)*: 471-479.

Hegsted, D M; McGandy, R B; Myers, M L and Stare, F J (1965). Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr., 17(5)*: 281-295.

Hills, C E and Brunskill, N J (2009). Cellular and physiological effects of C-peptide. *Clin. Sci. (Lond), 116(7)*: 565-574.

Hu, F B; Manson, J E and Willett, W C (2001). Types of dietary fat and risk of coronary heart disease: A critical review. *J. Am. Coll. Nutr., 20(1)*: 5-19.

Karupaiah, T and Sundram, K (2007). Effects of stereospecific positioning of fatty acids in triacylglycerol structures in native and randomized fats: A review of their nutritional implications. *Nut. Metab., 4*: 16.

Katz, A; Nambi, S S; Mather, K; Baron, A D; Follmann, D A; Sullivan, G and Quon, M J (2000). Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. *J. Clin. Endocrinol. Metab., 85(7)*: 2402-2410.

Kim, B Y; Jung, C H; Mok, J O; Kang, S K and Kim, C H (2012). Association between serum C-peptide levels and chronic microvascular complications in Korean type 2 diabetic patients. *Acta Diabetol., 49(1)*: 9-15.

Kritchevsky, D (2000). Overview: Dietary fat and atherosclerosis. *Asia Pac. J. Clin. Nutr., 9(2)*: 141-145.

Lipp, M and Anklam, E (1998). Review of cocoa butter and alternative fats for use in chocolate - Part A. Compositional data. *Food Chem., 62(1)*: 73-97.

Lopez-Miranda, J and Marin, C (2010). Dietary, physiological, and genetic impacts on postprandial lipid metabolism. *Fat detection: taste, texture, and post ingestive effects.* CRC Press/Taylor and Francis.

Lopez, S; Bermudez, B; Ortega, A; Varela, L M; Pacheco, Y M; Villar, J; Abia, R and Muriana, F J (2011). Effects of meals rich in either monounsaturated or saturated fat on lipid concentrations and on insulin secretion and action in subjects with high fasting triglyceride concentrations. *Am. J. Clin. Nutr., 93(3)*: 494-499.

Matthews, D R; Hosker, J P; Rudenski, A S; Naylor, B A; Treacher, D F and Turner, R C (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28(7)*: 412-419.

Mensink, R P; Zock, P L; Kester, A D and Katan, M B (2003). 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., 77(5)*: 1146-1155.

MPOB (2004). *MPOB Test Method* (Aini, K; Siew, W L; Tan, Y A; Nor Aini, I; Tang, T S and Nurul, A I eds.). MPOB, Bangi.

Nimptsch, K; Brand-Miller, J C; Franz, M; Sampson, L; Willett, W C and Giovannucci, E (2011). Dietary insulin index and insulin load in relation to biomarkers of glycemic control, plasma lipids, and inflammation markers. *Am. J. Clin. Nutr., 94(1)*: 182-190.

Ortega, A; Varela, L M; Bermudez, B; Lopez, S; Abia, R and Muriana, F J (2012). Dietary fatty acids linking postprandial metabolic response and chronic diseases. *Food Funct., 3(1)*: 22-27.

Salas, J J; Bootello, M A; Martinez-Force, E and Garces, R (2009). Tropical vegetable fats and butters: Properties and new alternatives. *OCL, 16(4)*: 254-258.

Sanders, T A and Berry, S E (2005). Influence of stearic acid on postprandial lipemia and hemostatic function. *Lipids, 40(12)*: 1221-1227.

Sanders, T A; De Grassi, T; Miller, G J and Morrissey, J H (2000). Influence of fatty acid chain length and cis/trans isomerization on postprandial lipemia and factor VII in healthy subjects (postprandial lipids and factor VII). *Atherosclerosis, 149(2)*: 413-420.

Storlien, L H; Jenkins, A B; Chisholm, D J; Pascoe, W S; Khouri, S and Kraegen, E W (1991). Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle

triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes, 40(2)*: 280-289.

Storlien, L H; Pan, D A; Kriketos, A D and Baur, L A (1993). High fat diet-induced insulin resistance. Lessons and implications from animal studies. *Ann. N Y Acad. Sci., 683*: 82-90.

Teng, K T; Nagapan, G; Cheng, H M and Nesaretnam, K (2011). 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, 46(4)*: 381-388.

Tholstrup, T and Samman, S (2004). Postprandial lipoprotein(a) is affected differently by specific individual dietary fatty acids in healthy young men. *J. Nutr., 134(10)*: 2550-2555.

Tholstrup, T; Sandstrom, B; Bysted, A and Holmer, G (2001). Effect of 6 dietary fatty acids on the postprandial lipid profile, plasma fatty acids, lipoprotein lipase and cholesterol ester transfer activities in healthy young men. *Am. J. Clin. Nutr., 73(2)*: 198-208.

Zampelas, A; Williams, C M; Morgan, L M; Wright, J and Quinlan, P T (1994). The effect of triacylglycerol fatty acid positional distribution on postprandial plasma metabolite and hormone responses in normal adult men. *Br. J. Nutr., 71(3)*: 401-410.