SHORT COMMUNICATION

Effects of Chemically Interesterified Palm Olein on Lipid Profiles in Hamsters

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ABSTRACT

Palm olein (POo) has been perceived as atherogenic due to its high proportion of palmitic acid (41.2%) content. It is interesting that most of the palmitic acid of POo is located at stereospecific numbering *sn*-1 and *sn*-3 positions of the triacylglycerol (TAG) backbone. The present study aims to investigate the effects of positional distribution of fatty acids on the lipid profiles of POo or chemically interesterified palm olein (CIE POo) fed hamsters in comparison to high oleic sunflower oil (HOSO) fed hamsters. Male weanling Syrian golden hamsters (n=10 for each group), were fed diets formulated with the above oils for 12 weeks. There was no significant difference between CIE POo and HOSO groups for total cholesterol (TC). CIE POo with increased amount of palmitic acid (43.2%) at *sn*-2 position did not cause significant increases in TC levels compared to the HOSO group. In addition, the POo group has significantly higher high-density lipoprotein cholesterol (HDL-C) than that of the HOSO group, P = 0.011 (< 0.05) while the HOSO group has significantly lower total cholesterol (TC) levels than that of the POo group, P = 0.012 (< 0.05).
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INTRODUCTION

Interesterification of fats is a process of altering the positional distribution of fatty acids in the triacylglyerol (TAG) backbone for specific functionality and physical properties (1). The interesterification process is an alternative to the hydrogenation process, which leads to the production of trans fatty acids. Trans fatty acids have been reported to adversely impact cardiovascular health (2). There are two types of commonly used interesterification processes, namely enzymatic or chemical interesterification. The exchange of fatty acids attached to the glycerol backbone of the fat catalysed by lipase enzyme is enzymatic interesterification, whereas that catalysed by chemicals such as metal salts is chemical interesterification (3). Interesterification of palm olein for example, increases the proportion of palmitic acid in the *sn*-2 position, mimicking the fatty acid distribution of palmitic acid in breast milk, making it a suitable component in infant formulas (4).

The natural positional distribution of palmitic acid of palm oil is at the *sn*-1 and *sn*-3 positions, with only negligible amount at the *sn*-2 position (3.3%). Randomisation of palm oil increases the amount of palmitic acid at *sn*-2 to 13.6% and significantly increases its atherogenicity. Previous studies on rabbits fed diets containing 15% fat with one-third of which is a specifically prepared fat SOS (stearic-oleic-stearic), SSO (stearic-stearic-oleic), POP (palmitic-oleic-palmitic: the natural form of palm olein) or PPO (palmitic-palmitic-oleic: the CIE palm olein) showed that PPO is the most atherogenic fat with palmitic acid predominantly at *sn*-2 (5). When the sum of palmitic acid at the *sn*-2 position was doubled, a higher platelet response to adenosine 5-diphosphate (ADP) and thrombin was observed. However, no changes in plasma TAG, TC and HDL-C levels were observed.

The atherogenicity of these various fats may be related to the extent of fat absorption. Kritchevsky and coworkers (5) reported that the palmitic acid-rich fats were absorbed to a greater extent when the palmitic acid was situated at the *sn*-2 position. At *sn*-1 and *sn*-3 positions, saturated fatty acids (SFAs) are said to have neutral effects which lower plasma TC levels. On the contrary, SFAs at *sn*-2 position would generally raise plasma TC levels (7). Thus, the present study aims to determine the effects of positional distribution of palmitic acid on the plasma lipids of hamsters.

MATERIALS AND METHODS

Source of Oils and Preparation of Chemically Interesterified Palm Olein

Palm olein (POo) (Brand: Seri Murni) was obtained from Tesco Hypermarket; the chemically interesterified palm olein (CIE POo) was sourced from Oils and Fats Technology and Energy Centre (OFTEC) Unit, Malaysian Palm Oil Board; and the high oleic sunflower oil (HOSO) (Brand: Neu Vida) was obtained from Tesco Hypermarket.

CIE method was adapted from Lida et al. (2002). Palm oil was heated and dried for 30 min at 110°C under vacuum. Sodium methoxide (0.2%) was added to catalyse the reaction. After 60 min of stirring at a constant speed of 3000 rpm, the oil was then cooled to 60°C-70°C. A citric acid solution (20%) was then added to deactivate the residual catalyst. The oil was then rinsed several times with hot water (70°C-80°C) to remove soap by-products. The interesterified (IE) oil was then dried under vacuum at 110°C for approximately 60 min. One percent bleaching earth was then added to the dried IE oil to lighten its colour. The oil and bleaching earth were left to react for 30 min at 90°C-100°C followed by cooling of the mixture to 60°C. The mixture was then filtered to separate the oil and the bleaching earth. The bleached IE oil was then refined to remove free fatty acids using short path distillation method at 240°C

Determination of Fatty Acid Compositions (FAC) of Dietary Fats with and without Transmethylation

Fatty acid composition of the dietary oils and extracted dietary fats were determined following transmethylation of the samples using toluene–sulphuric acid. Fatty acids were then analysed as their methyl esters on a Perkin Elmer Autosystem gas chromatography system (Perkin Elmer Corporation, Norwalk, CT, USA) fitted with a 100 meter capillary column (SP2560; Supelco, Belfonte, PA, USA) and temperature programmed from 160 to 240°C at 4°C min-º. Authentic fatty acid standards were used to identify the component fatty acids of interest (12).

Animals and Monitoring Parameters

Thirty 8-9 weeks old weanling male specific pathogen free (SPF) Golden Syrian hamsters were sourced from Janvier Laboratory, France, and acclimatised for 2 weeks at the animal facility. They were divided into three different dietary groups of ten animals each. Respective groups were fed formulated diets (Altromin, Germany) (Table I) for 12 weeks. These diets differ only in the types of dietary fats (all with 31% energy level), namely i) palm olein (POo) ii) chemically interesterified palm olein (CIE POo) and iii) high oleic sunflower oil (HOSO). The hamsters were housed two in a cage in an air-conditioned temperature-controlled room at 18 ± 1o C with a 12-hour light-dark cycle. Feed and water were given ad libitum. Body weight was recorded on a weekly basis. The animals' general health was monitored throughout the experimental period. Feed consumption was recorded over five consecutive days on week-7 to gauge the average feed intake of different diets. The reason why feed consumption was not recorded since the beginning of the study was that the readings tend to fluctuate a lot when new feed was introduced to the animals. Animal ethics approval (AUP: R031/2014) was obtained from the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The chow diet control group was excluded in the present study as the focus was to compare the redistribution of the palmitic content at *sn*-2 position, in comparison to the POo group (the control group). HOSO, on the other hand, served as our non-saturated fatty acid control.

Table I: Diet Formulation

Composition	g kg-º	% En*
Dietary fat*	150	31.0
Casein	240	22.1
Corn starch	340	31.3
Choline bitartrate	2	15.6
Dextrose	170	-
Cellulose	50	-
Mineral mix	35	-
Vitamin mix	10	-
DL methionine	3	-

% En*: Percentage of energy

Blood Sampling and Plasma Lipid Analysis

Animals were anaesthetised and blood was drawn with heart puncture at the end of 12 weeks. Plasma TC, TAG, low density lipoprotein cholesterol (LDL-C), and HDL-C were analysed with enzymatic assay kits (Roche Diagnostics GmbH, Mannheim, Germany), using a clinical chemistry autoanalyser (Roche/Hitachi 902, Japan).

Statistical Analysis

Statistical differences were determined using one-way analysis of variance (ANOVA), followed by Tukey HSD post-hoc test. A value of $p < 0.05$ was considered to be significant. All analyses were performed using the Statistical Package for Social Science (SPSS, Version 20.0, SPSS Inc., Chicago, USA).

RESULTS

Fatty Acid Composition (FAC) of Dietary Fats

All three dietary oils were analysed for their fatty acid composition (FAC) prior to diet formulation (Table II). Both POo and CIE POo had relatively higher palmitic acid content (16:0) compared to HOSO; while HOSO had the highest oleic acid content (18:1) among all. The linoleic acid (18:2) content was similar in all three dietary oils.

Following chemical interesterification, the fatty acid compositions at *sn*-2 position for the respective oils were redistributed (Table III). CIE POo had 43.2%

Table II: FATTY ACID COMPOSITIONS (%) OF DIETARY OILS

FAC	POo	CIE POo	HOSO
12:0	0.20	n.d	0.25
14:0	0.90	1.00	0.05
16:0	39.90	38.80	3.85
16:1	n.d	n.d	n.d
18:0	4.00	4.40	2.10
18:1	43.60	42.20	81.75
18:2	10.80	10.10	10.60
18:3	0.20	0.20	< 0.05
20:0	0.30	0.40	0.20
20:1	0.10	n.d	0.30
22:0	n.d	n.d	0.70
24:0	n.d	n.d	0.20
Others	0	3.10	0
MUFA	43.70	42.20	82.05
PUFA	11.00	10.30	10.65
SFA	45.30	44.60	7.35

n.d – not detected; MUFA-Monounsaturated fatty acids;
PUFA-Polyunsaturated fatty acids; SFA-Saturated fatty acids

Table III. FATTY ACID COMPOSITION (%) OF CIE POo AT *SN*-2 POSITION

FAC	POo	CIE POo	HOSO
12:0	1.4	trace	0.8
14:0	1.0	1.1	1.3
16:0	12.2	43.2	7.5
16:1	n.d	trace	trace
18:0	1.2	4.0	1.3
18:1	61.6	37.6	79.35
18:2	23.0	7.5	10.45

n.d – not detected

palmitic acid content at the *sn*-2 position as compared to just 12.2% in POo and 7.5% in HOSO. Linoleic acid content was the highest in POo (23.0%) at the *sn*-2 position, followed by HOSO (10.45%) and CIE POo (7.5%).

Feed Intake and Body Weight

All hamsters remained healthy throughout the 12 weeks of feeding trial. Mean feed intake, initial and final body weights were not significantly different between the groups (Table IV).

Plasma Lipid Analysis

The POo group had significantly higher HDL-C than the HOSO group, P = 0.011 (< 0.05), while the HOSO group had significantly lower TC than the POo group, P = 0.012 (< 0.05) (Table V). However, there was no significant difference between CIE POo and HOSO groups for TC levels.

DISCUSSION

The lipid profiles and susceptibility to dietary cholesterol of the golden Syrian hamster

Table IV: FEED INTAKE AND BODY WEIGHTS

	POo	CIE POo	HOSO
Feed intake (g)	6.52 ± 0.38	6.59 ± 0.51	6.23 ± 0.94
Initial body weight (g)	123.78 ± 4.69	125.15 ± 4.55	125.81 ± 4.33
Final body weight (g)	138.84 ± 6.81	136.85 ± 6.05	139.01 ± 6.48

Table V: PLASMA LIPID PROFILES

	POo	CIE POo	HOSO
TC ($mmolL^{-1}$)	3.61 ± 0.45*	3.37 ± 0.69	2.84 ± 0.46
TAG ($mmolL^{-1}$)	1.10 ± 0.44	1.17 ± 0.43	0.91 ± 0.34
LDL-C ($mmolL^{-1}$)	0.59 ± 0.18	0.50 ± 0.16	0.48 ± 0.11
HDL-C ($mmolL^{-1}$)	2.46 ± 0.44*	2.40 ± 0.59	1.92 ± 0.42
TC/HDL-C	1.48 ± 0.13	1.42 ± 0.13	1.51 ± 0.22
LDL-C/HDL-C	0.25 ± 0.10	0.21 ± 0.05	0.25 ± 0.05

* $P < 0.05$ compared to HOSO group

(Mesocricetus auratus) are similar to those of human (8). However, much of the previous work on the effects of the dietary fat TAG structure on plasma lipoprotein composition has been done on piglets (9-10). Piglets were fed either sow's milk (55% C16:0 at *sn*-2 position), a formula containing interesterified fats (70% C16:0 at *sn*-2 position) or a palm oil (4% C16:0 at *sn*-2 position)-based formula. Animals fed both sow's milk and formula containing interesterified fats had higher levels of C16:0 incorporated into plasma TAGs and cholesterol esters four hours after feeding (11). In another study, Innis and Dyer (10) evaluated the effects of synthesised fats and the distribution of plasma lipoprotein fatty acids. New-born piglets were fed with sow's milk (55% C16:0 *sn*-2), formulas containing either synthesised fats (32% C16:0 at *sn*-2) or palm olein (4.2% C16:0 *sn*-2), for 18 days from birth. The chylomicron TAG *sn*-2 C16:0 levels corresponded to the dietary *sn*-2 C16:0 levels, and a dose-response effect was noted (sow's milk > synthesised fat-formula > palm olein-formula). The authors concluded that the *sn*-2 positions in dietary TAGs were retained during digestion and absorption, and being reassembled into chylomicron-TAG. Innis and Dyer (10) also noted that piglets fed the synthesised fat-based formula had lower chylomicron-TAG C20:4n-6 and C22:6n-3 concentrations than piglets fed palm olein based-formulas. The authors suggested that the positional distribution of dietary saturated fatty acids (SFAs) may have affected the n-3 and n-6 polyunsaturated fatty acids (PUFAs). Similar inferences were made by Renaud et al., (10) who reported that dietary fatty acids at the *sn*-2 position had an effect on lipemia, platelet aggregation, desaturation and elongation of PUFAs in rats.

Gouk et al. (11) fed C57BL/6 mouse for 15 weeks with diets fortified with POo, CIE POo and soyabean oil (SOY). The study reported that mice fed the SOY-enriched diet gained significantly higher amounts of subcutaneous fat and total fat compared with the POo group, despite similar body mass gain. Mice fed the CIE POo-enriched diet gained 14.3% more fat per food consumed when compared to the POo group, despite their identical

total fatty acid compositions. Subsequently, the author ascribed this observation to the higher content of long chain SFA at the *sn*-1, 3 positions of TAG in POo and reported that SFA of different chain lengths at *sn*-1 and *sn*-3 positions exert profound effects on fat accretion (12).

An experiment in monkeys with five-fold palmitic acid in diet did not increase blood cholesterol levels (13). The TC level was reported to decline by 22 $mgdl^{-1}$ to 183 ± 9 $mgdl^{-1}$ compared to the baseline values of 205 ± 11 $mgdl^{-1}$. Another study by Go et al., (14) reported no significant difference in serum lipids between rats fed palm oil and sunflower oil, suggesting that palm oil may not cause significant hyperlipidaemia and elevated risk of cardiovascular diseases. In contrary, studies on the effects of TAG structure on plasma lipids and atherogenic potential after long-term feeding in animal models by Kritchevsky (5) suggested that palmitic acid at the *sn*-2 position is atherogenic in rabbits.

Long-chain SFAs such as palmitic acid are not well absorbed in the lumen due to its melting point being substantially above body temperature and a strong tendency to form insoluble calcium soap with divalent cations at the alkaline pH of the small intestines (15). Therefore, it was preferential to hydrolyse unsaturated fatty acids compared to the saturated ones for absorption into the circulation portal. Considering the results from Mattson et al. (16), the absorption of SFA at the *sn*-2 position of TAG appeared to be better than those at the *sn*-1 and *sn*-3 positions. Also, the metabolism of SFA at the *sn*-2 position in blood is slower than those at *sn*-1 and *sn*-3 positions. This explains the observation where the HOSO group has lower TC levels than that of POo and CIE POo groups in the present study. Consumption of fats containing high levels of oleic acid at the *sn*-2 position of TAG had been proven to reduce LDL-C in human trials (17). However, the HOSO group (79.35% oleic acid) in the present study did not show a significant reduction in LDL-C. The absorption efficiency of palmitic acid appeared to affect the absorption efficiency of TC and thus influenced the concentration of plasma TC in the hamsters. The absorption efficiency of palmitic acid and cholesterol was reported to be significantly lower in the positional distribution of disaturated triglyceride POP (native palm oil) groups than those of disaturated triglyceride PPO (CIE POo) groups (18). This was probably due to the lower absorption efficiency of palmitic acid in POP and its free palmitic acids may react with dietary calcium and magnesium to form insoluble soaps. Karupaiah and Sundram (19) stated that the palmitic acid at *sn*-2 position resulted in higher TC and HDL-C concentrations than that of oleic acid in the same position. However, there was no significant difference in the levels of TC and HDL-C for CIE POo and POo groups in the present study. The levels of TC and HDL-C were not corresponding to palmitic acid reshuffling between CIE POo and POo.

The feed intake was greatly reduced to approximately 6.0 g daily in comparison to 10.0-12.0 g daily as in normal chow diet fed animals (not shown in this study). These formulated high fat diets (31.0% energy) probably affected the satiety centre and appetite (20).

CONCLUSION

Our study showed that the fatty acid composition but not positional distribution of fatty acids alters plasma lipid profiles, in particular both TC and HDL-C levels were increased in the POo group compared to the HOSO group. This study suggests that positional distribution may not affect plasma lipid profiles in hamsters.

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