

DOES PALM MID FRACTION AFFECT ADULT SATIETY?

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ABSTRACT

Dietary fats with different melting characteristics, fatty acids chain length and positional distribution may affect postprandial gut hormones and satiety response. We investigated the effects of palm mid fraction (PMF) (POP-rich), shea stearin (SS) (SOS-rich) and high oleic sunflower oil (HOSF) (OOO-rich) with either palmitic, stearic or oleic acid predominance at the sn-1 and sn-3 positions on gut hormone concentrations and satiety. A randomised, double-blind crossover (3 × 3 arms) orthogonal Latin-square study was conducted on 36 healthy adults (18 males, 18 females; average aged 23 years). Each subject received ~50 g of test fat incorporated in a muffin in random order, two weeks apart, over a six-week period. Blood samples were collected for a 3-hr period. We found that PMF- and HOSF-rich diets with either palmitic or oleic acid at the sn-1 and sn-3 positions exerted significantly higher ($P < 0.05$) postprandial glucose dependent insulinotropic polypeptide (GIP) compared to SS-rich diet. However, plasma glucagon like-peptide 1 (GLP-1), peptide YY (PYY), ghrelin and visual analogue scale (VAS) ($P > 0.05$) were not affected. These results suggested that PMF- and HOSF-rich diets increased the secretion of GIP that may promote satiety response in human adults.

Keywords: ghrelin, glucagon like-peptide 1, glucose dependent insulinotropic polypeptide, gut hormone, palmitic acid.

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INTRODUCTION

Obesity is one of the greatest public health challenges of the 21st century. In 2016, the World Health Organization reported that 1.9 billion adults globally (age ≥ 18 years) were overweight and at

least 650 million adults were obese. In addition, the prevalence of obesity and severe obesity are forecasted to have an increment of 33% and 130%, respectively in the next two decades (Finkelstein *et al.*, 2012). One of the reasons that causes obesity to develop and perpetuate rapidly is gut hormone dysregulation (Lean and Malkova, 2016). Gut hormone is vital in regulating the haemostasis of food intake, energy and glucose.

Fat in the gastrointestinal tract reduces hunger and curbs food intake by eliciting satiety signals. These signals are evoked by entry of dietary triacylglycerol (TAG) or fatty acids into the small intestine (Maljaars *et al.*, 2009). Gut hormones are satiety signals that are released from the gastrointestinal tract which modulate the activity of appetite centres within the brain. Examples of gut hormones include glucose dependent insulinotropic polypeptide (GIP), glucagon like-peptide 1 (GLP-1), peptide YY (PYY), cholecystokinin and ghrelin.

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GIP and GLP-1 are incretin hormones secreted from the intestine, they stimulate the release of insulin from pancreatic β -cells upon ingestion of glucose (Yabe and Seino, 2011). PYY is synthesised and released in response to food intake primarily from the endocrine L-cells, especially ileum, colon and rectum (Ueno *et al.*, 2008). PYY is first isolated as a 36-amino acid peptide from porcine upper small intestine (Suzuki *et al.*, 2010). Ghrelin is an orexigenic gut hormone of 28-amino acid gastrointestinal peptide which is secreted by endocrine cells in the gastrointestinal tract, primarily the fundus of the stomach (Mittelman *et al.*, 2010). Ghrelin circulates in both acylated (active) and de-acylated forms in which the former appears to be responsible for signalling hunger (Hosoda *et al.*, 2004).

These signals play a fundamental role in regulating food intake and satiety as well as in energy balance. Gut hormones release has been shown to play a role in the prevalence of obesity through food intake reduction and appetite satisfaction (Little *et al.*, 2007). Release of gut hormones could be regulated by the consumption of dietary fats which give a greater postprandial satiety (act of fullness) which in turn can facilitate weight loss or curb weight gain. In addition, GIP and GLP-1 have been implicated in the treatment of patients with diabetes (Yabe and Seino, 2011).

Postprandial studies involving the effects of dietary fats on gut hormone have been extensively studied over the recent years. De Silva *et al.* (2011) demonstrated that a combined administration of PYY₃₋₃₆ and GLP-1₇₋₃₆ amide to fasting human subjects lead to reductions in food intake and subsequently energy intake and this was supported by a meta-analysis which was carried out by Verdich *et al.* (2001). Furthermore, a study conducted by Thomsen *et al.* (1999) had shown that postprandial GLP-1 and GIP responses were higher after a meal of monounsaturated fat (olive oil) as compared to a saturated fat (butter). The finding indicated that postprandial GIP and GLP-1 secretion were stimulated by monounsaturated fat intake. Meanwhile, Poppitt *et al.* (2006) found that high-fat meals had no significant effects on postprandial ghrelin levels in a group of healthy men.

It was clearly seen that the concentration of gut hormone released and circulated in the body were correlated with the types of dietary fat intake (Sun *et al.*, 2019). Differences in chain length, degree of saturation, emulsification as well as emulsion stability may also influence the efficiency of satiety (Bosscher and Viberg, 2009). The induction of physiological satiety signals may well depend on the composition of fatty acids in the particular fats used (Lawton *et al.*, 2000). Every type of dietary fat has its own unique TAG composition with different positional distribution of fatty acids.

The positional distribution of fatty acids in TAG marked its importance when the food industries are seeking for cocoa butter equivalent (CBE) as alternative to cocoa butter that is expensive and low in production. Palm oil, illipe and shea were listed in the European Chocolate Directive 2000/36EEC (EC, 2000) as CBE. Besides, vegetable oils such as palm mid fraction (PMF), kokum, mahua, mango fats, olive oil and teaseed oil have also been used for CBE preparation.

Cocoa butter is composed of three main TAG: 1, 3-dipalmitoyl-2-oleoylglycerol (POP); 1(3)-palmitoyl-3(1)-stearoyl-2-oleoylglycerol (POS) and 1,3 distearoyl-2-oleoylglycerol (SOS), with oleic acid in the *sn*-2 positions (Ong and Goh, 2002). The stearic to palmitic acid ratio in cocoa butter is 1.3:1.0. It is therefore imperative that a vegetable oil which has TAG with oleic acid in the 2-position, can be used for CBE preparation. The availability of palm oil and its fractions with a similar chemical composition (predominantly POP) to cocoa butter (Dian *et al.*, 2017; Edem, 2002) has made these palm-based oils suitable for CBE production (Zaliha and Norizzah, 2012).

To date, the effects of different types of fatty acids at the *sn*-1 and *sn*-3 positions of the TAG molecule, especially with the CBE type of fats on postprandial gut hormone concentrations have not been explored extensively. Therefore, this study was conducted to investigate the effects of CBE type of fats, namely PMF and shea stearin (SS) with either palmitic- or stearic acid that is predominantly present at the *sn*-1 and *sn*-3 positions of the TAG backbone, on gut hormone concentrations and satiety response compared to HOSF (oleic acid rich fat) that served as a control.

METHODS

Subjects

This study was approved by Research and Ethics Committee, International Medical University, Kuala Lumpur, Malaysia and was registered at ClinicalTrials.gov (a world database of privately and publicly funded clinical studies) as NCT01428960 (<https://clinicaltrials.gov/ct2/show/NCT01428960>). A total of 36 healthy adult males (n=18) and females (n=18), aged 25-50 years old (Table 1) were recruited to participate in this study. A health screening was conducted and the following data were collected: a) physical examination [height, weight, body mass index (BMI) and blood pressure]; b) fasting serum lipid profile; c) plasma glucose determination; d) liver function tests (serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase); and e) kidney function tests (serum creatinine). Only volunteers with BMI 18.5-25.0 kg

m⁻², normotensive (systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg, normolipidemic [total cholesterol <6.2 mM litre⁻¹ (<240 mg dL⁻¹), fasting TAG <1.70 mmol litre⁻¹ (<150 mg dL⁻¹)], Non-diabetic: fasting glucose 4.0 mmol litre⁻¹ to 7.0 mmol litre⁻¹ were recruited into the study. The exclusion criteria were subjects who smoke, taking cholesterol or blood glucose medication, consume alcohol, with a history of blood-clotting problem, non-availability during the intervention; and for women who were pregnant or lactating.

Study Design

A randomised, double-blind crossover (3 × 3 arms) orthogonal latin-square design was used. Each subject received three experimental test meals in a random order, two weeks apart, over a six-week period. Each set of test meal contains a muffin and a milkshake that provided 875.6 kcal energy, 16 g protein, 83 g carbohydrate and 53 g test fat. Milkshake was prepared to aid the test muffin intake as part of the standard test meal set. For each postprandial sampling, blood collections were conducted at zero min and at every 30 min intervals for 3 hr.

Test Fats

Test fat, high oleic sunflower oil (HOSF) was obtained from Intercontinental Specialty Fats Sdn. Bhd.; whereas PMF (iodine value=34.9) and SS (iodine value=34.1) were obtained from Wilmar PGEO Edible Oils Sdn. Bhd. The latter two test fats were blended with a small amount of sunflower oil (Mazola, Switzerland) to standardise the content of

linoleic acid across the diets to 7%. These test fats were incorporated into muffins, labelled with a code and stored frozen until being consumed within six weeks.

Sample Size Calculation

The sample size was calculated (n=36, 90% power) to detect a 0.5 standard deviation (SD) unit change in the area under the curve for plasma GIP concentrations with $P<0.01$. The secondary outcomes of the study were changes in GLP-1, PYY, ghrelin and visual analogue scale (VAS).

Samples Collection

The subjects were stratified randomly to one of six treatment sequences (ABC, BCA, CAB, ACB, CBA, or BAC; where A is PMF, B is SS, and C is HOSF). All subjects were requested to avoid high fat foods and strenuous exercise 24 hr before intervention day and fast overnight starting at 2200 hr. In order to avoid an over consumption of fat and energy intake, a standardised low-fat meal (containing 500-700 kcal and, 10 g fat) were provided as the evening meal to be consumed before 2200 hr and no food or drinks were allowed thereafter, except water. The participants attended the scheduled blood sampling the next morning between 0800 and 1000 hr at the Nutrition Clinic MPOB. To facilitate blood collection, 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 collection was performed using antiseptic venepuncture technique by registered staff nurses

TABLE 1. CHARACTERISTICS OF THE PARTICIPANTS

Variables	Participants		
	Women (n=18)	Men (n=18)	Total (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 litre ⁻²)	20.3 ± 1.6	21.3 ± 2.0	20.8 ± 1.8
Systolic BP (mmHg)	111.0 ± 6.1	122.0 ± 10.0	116.0 ± 9.9
Diastolic BP (mmHg)	73.0 ± 5	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: BMI - body mass index; BP - blood pressure; HDL-c - high-density lipoprotein cholesterol; LDL-c - low-density lipoprotein cholesterol; TAG - triacylglycerol. All values are means ± standard deviation.

supervised by medical officer. A total of 15 ml of fasting blood was withdrawn into blood collection tubes.

Each subject then consumed the test meal within 10 min. Each subject was given drinking water (± 250 ml) on the study day. During the intervention day, the subjects were permitted to communicate with each other, however, the conversation involving food, appetite or related issues were restricted. VAS ratings of hunger and appetite and their ability to predict subsequent food intake and *ad-libitum* lunch postprandially has been demonstrated in a number of studies (Poppitt *et al.*, 2010; Strik *et al.*, 2010). In this study, satiety feelings (hunger) were measured using VAS (100 mm) modified from Parker *et al.* (2004) in which the scales were anchored with 'not hungry at all' at one end and 'extremely hungry' at the other end. Subjects were required to mark on the scale provided that reflected their satiety feelings at that moment. Measurements were taken prior to the test meal and every 30 min with a total of seven times, up until the end of postprandial. Blood samples were collected immediately prior to the VAS measurements.

At the end of intervention day, each subject was served an *ad-libitum* lunch and a glass of drink until comfortably satisfied. The amount of food intake was measured using a weighing scale.

Analytical Methods

Plasma GIP, GLP-1, PYY and ghrelin were analysed using a sandwich-based enzyme-linked immunosorbent assay and non-radioactive kits (Millipore, USA). For the collection of blood samples for GIP, GLP-1 and PYY, 10 μ l of dipeptidyl peptidase IV per 1 ml of blood was added into ethylenediaminetetraacetic acid (EDTA) tubes prior to the study conducted. On the days of the study, 65 μ l of aprotinin was added into 2 ml EDTA tube for the collection of blood samples for ghrelin and PYY. Finally, plasma samples for ghrelin were acidified with 0.1 N of hydrochloric acid. Plasma samples were collected using an EDTA vacutainer on ice and processed within 15 min of blood collection via centrifugation at 3500 rpm, 20 min at 4°C. The cryovials containing the samples were stored at -80°C until prior to analyses.

The fatty acid composition of test fats was measured using gas-liquid chromatography on an SP-2560 column (100 m \times 0.23 mm \times 0.2 mm; Agilent Technologies) with a flame ionisation detector on an autosystem (Perkin Elmer) (Voon *et al.*, 2011). The helium carrier gas 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 system. The method of analysis was modified from American Oil Chemists' Society (AOCS) Official Method Ce 5c-93 (AOCS Official Method, 1997). Slip-melting point was determined according to AOCS Cc3b-92 (AOCS Official Method, 2017).

Statistical Analyses

Data were analysed using a repeated measure analysis of variance (ANOVA), followed by a Bonferroni post-hoc analysis performed with GraphPad Prism Version 5 (GraphPad Software, La Jolla, CA 9203, USA) and PASW Statistics 18 to assess the significant differences between diets. The normal distribution of data was access using Shapiro-Wilk's normality test. All data were logarithmically transformed as they were not normally distributed. All data are expressed as mean with 95% confidence interval (CI). Different superscripts attached to values in the same row demonstrate that the values show differences significantly among the corresponding column ($P < 0.05$, Bonferroni multiple comparison test).

RESULTS

The fatty acid composition of the test fats is shown in Table 2. PMF and SS contained similar proportions of saturated fatty acids (SFA) (62.7%, 62.6%) and oleic acid (33.7%, 33.0%) but PMF contained much higher palmitic acid than SS (57.1% vs. 1.8%) and less stearic acid than SS (5.0% vs. 60.8%). PMF consists of POP (67.6%) while SS consists mainly of SOS (74.2%) with oleic acid in the *sn*-2 positions. The main molecular TAG species of HOSF is triolein (OOO) (66.5%).

TABLE 2. FATTY ACID COMPOSITION OF THE TEST FATS

Fatty acids	Mol %		
	PMF	SS	HOSF
C14:0	0.7 \pm 0.1	ND	ND
C16:0	57.1 \pm 0.2	1.8 \pm 0.0	4.5 \pm 0.0
C18:0	5.0 \pm 0.0	60.8 \pm 0.6	2.8 \pm 0.5
SFA	62.7 \pm 0.1	62.6 \pm 0.6	7.2 \pm 0.3
C18:1	33.7 \pm 0.1	33.0 \pm 0.5	85.3 \pm 0.4
MUFA	33.7 \pm 0.1	33.0 \pm 0.5	85.3 \pm 0.4
C18:2	3.0 \pm 0.0	2.0 \pm 0.0	7.5 \pm 0.1
PUFA	3.0 \pm 0.0	2.0 \pm 0.0	7.5 \pm 0.1
Others	0.6 \pm 0.0	1.4 \pm 0.0	ND

Note: PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil; C18:2 - linoleic acid; C14:0 - myristic acid; C18:1 - oleic acid; C16:0 - palmitic acid; C18:0 - stearic acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; ND - not detected.

The three experimental fats contain high amounts of oleic acid at the *sn*-2 position (PMF=72%, SS=80%, HOSF=88%). PMF has a melting point at ~ 31.3°C, whereas SS shows a higher melting point at ~ 38.0°C. HOSF shows a melting point at <1°C.

Table 3 shows the changes in the incretin hormone- GIP concentrations after consumption of the three experimental fats. The GIP concentrations are consistently lower ($P<0.05$) in the SS-meal group compared with the PMF- and HOSF-meal groups.

GIP responses did not differ significantly ($P>0.05$) between HOSF- and PMF- meals during the 3 hr postprandial study. However, there were significant differences ($P<0.05$) in plasma GIP levels between the test meal of SS with PMF- and HOSF- meal respectively from 60 min time points onwards. Furthermore, postprandial changes in GIP comparing HOSF and SS showed a meal \times time

interaction with $P=0.0003$ at 30 min; the difference was 75.59 pg ml^{-1} (95% CI, 3.18, 148.0).

The differences between HOSF- and PMF-meal with the SS-meal in the change of GIP at 60 min were 185.7 pg ml^{-1} (95% CI, 101.2, 270.2) and $-155.4 \text{ pg ml}^{-1}$ (95% CI, -239.9, -70.94) respectively; at 90 min 187.5 pg ml^{-1} (95% CI, 103.3, 271.7) and $-176.0 \text{ pg ml}^{-1}$ (95% CI, -260.2, -91.85); and at 120 min 152.6 pg ml^{-1} (95% CI, 67.06, 238.2) and $-138.0 \text{ pg ml}^{-1}$ (95% CI, -223.6, -52.41). Meanwhile, at 150 min, the differences between HOSF and PMF with SS were 186.5 pg ml^{-1} (95% CI, 115.8, 257.2) and $-158.0 \text{ pg ml}^{-1}$ (95% CI, -228.7, -87.27), while at 180 min 160.7 pg ml^{-1} (95% CI, 74.43, 246.9) and $-124.6 \text{ pg ml}^{-1}$ (95% CI, -210.8, -38.33), respectively. All test meals did not show a significant meal \times time interactions in plasma GLP-1 (Table 4) ($P=0.23$), PYY (Table 5) ($P=0.47$) and ghrelin (Table 6) ($P=0.48$) levels as well as in VAS scores (Figure 1) ($P=0.11$).

TABLE 3. POSTPRANDIAL GLUCOSE DEPENDENT INSULINOTROPIC POLYPEPTIDE CONCENTRATIONS IN THE THREE EXPERIMENTAL GROUPS

Time (min)	GIP (pg ml ⁻¹)		
	PMF	SS	HOSF
Fasting	65.15 (49.62, 80.67)	59.60 (46.38, 72.83)	60.24 (51.29, 69.20)
30	339.51 (284.27, 394.75) ^a	275.33 (225.06, 325.60) ^b	350.92 (303.79, 398.05) ^a
60	498.00 (434.56, 561.45) ^a	342.58 (294.51, 390.64) ^b	528.30 (467.69, 588.90) ^a
90	561.79 (492.62, 630.96) ^a	385.74 (338.97, 432.52) ^b	573.24 (513.00, 633.48) ^a
120	569.97 (498.80, 641.14) ^a	431.98 (379.90, 484.06) ^b	584.62 (521.86, 647.39) ^a
150	585.12 (523.33, 646.91) ^a	427.15 (376.51, 477.78) ^b	613.61 (548.06, 679.15) ^a
180	593.83 (518.44, 669.22) ^a	469.25 (405.75, 532.76) ^b	629.93 (558.09, 701.77) ^a

Note: GIP - glucose dependent insulinotropic polypeptide; PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil. Different superscript letters in the same row showed significant differences ($P<0.05$, Bonferroni multiple comparison test) between corresponding columns. Values are geometric means; 95% confidence interval (CI) in parentheses. $n=36$ for all test diets. Data were log-transformed, performed by repeated-measures ANOVA and showed a diet \times time interaction ($P=0.000$).

TABLE 4. POSTPRANDIAL GLUCAGON LIKE-PEPTIDE 1 CONCENTRATIONS IN THE THREE EXPERIMENTAL GROUPS

Time (min)	GLP-1 (pM)		
	PMF	SS	HOSF
Fasting	3.69 (1.56, 5.83)	3.00 (1.87, 4.13)	2.61 (2.25, 2.97)
30	8.10 (5.19, 11.00)	8.16 (5.41, 10.91)	8.03 (6.37, 9.69)
60	6.40 (4.72, 8.08)	5.59 (4.31, 6.87)	6.40 (4.78, 8.02)
90	7.78 (5.55, 10.01)	6.27 (4.98, 7.57)	8.05 (6.68, 9.43)
120	7.86 (6.26, 9.47)	5.94 (4.74, 7.13)	7.57 (6.03, 9.11)
150	7.33 (6.09, 8.57)	6.05 (4.90, 7.20)	7.62 (6.30, 8.93)
180	7.44 (5.95, 8.94)	6.36 (5.09, 7.64)	7.77 (6.34, 9.20)

Note: GLP-1 - glucagon like-peptide 1; PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil. Values are geometric means; 95% confidence interval (CI) in parentheses. $n=36$ for all test diets. Data were log-transformed, performed by repeated-measures ANOVA and showed a diet \times time interaction ($P=0.227$).

TABLE 5. POSTPRANDIAL PEPTIDE YY CONCENTRATIONS IN THE THREE EXPERIMENTAL GROUPS

Time (min)	PYY (pg ml ⁻¹)		
	PMF	SS	HOSF
Fasting	72.39 (62.06, 82.71)	73.37 (66.50, 84.01)	76.63 (62.72, 86.75)
30	124.83 (108.78, 140.88)	120.77 (108.17, 137.94)	127.05 (103.60, 145.93)
60	134.85 (117.83, 151.88)	122.57 (109.76, 137.90)	128.58 (107.24, 147.39)
90	136.44 (120.18, 152.70)	125.99 (115.44, 140.00)	133.31(111.98, 151.18)
120	139.86 (122.53, 157.19)	130.30 (119.41, 143.84)	137.49 (116.77, 155.57)
150	140.11 (123.12, 157.12)	131.81 (118.23, 148.42)	135.67 (115.21, 153.10)
180	138.20 (121.23, 155.18)	133.96 (119.59, 149.06)	135.46 (118.87, 151.33)

Note: PYY - peptide YY; PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil. Values are geometric means; 95% confidence interval (CI) in parentheses. $n=36$ for all test diets. Data were log-transformed, performed by repeated-measures ANOVA and showed a diet \times time interaction ($P=0.472$).

TABLE 6. POSTPRANDIAL GHRELIN CONCENTRATIONS IN THE THREE EXPERIMENTAL GROUPS

Time (min)	Ghrelin (ng ml ⁻¹)		
	PMF	SS	HOSF
Fasting	1185.10 (940.71, 1399.47)	1158.76 (920.98, 1396.54)	1170.09 (943.74, 1426.45)
30	1117.15 (847.88, 1297.60)	1095.31 (854.51, 1336.11)	1072.74 (887.06, 1347.24)
60	918.07 (606.02, 1049.63)	934.10 (705.37, 1162.83)	827.82 (678.18, 1157.96)
90	927.25 (640.75, 1068.48)	924.21 (688.45, 1159.97)	854.62 (696.46, 1158.03)
120	871.49 (588.39, 1038.16)	777.07 (565.22, 988.92)	813.28 (633.00, 1109.98)
150	879.15 (535.05, 975.10)	894.24 (666.23, 1122.24)	755.0 (637.62, 1120.68)
180	860.09 (577.94, 1043.13)	899.45 (664.98, 1133.92)	810.54 (605.58, 1114.59)

Note: PMF - palm mid fraction; SS - shea stearin; HOSF - high-oleic sunflower oil. Values are geometric means; 95% confidence interval (CI) in parentheses. $n=36$ for all test diets. Data were log-transformed, performed by repeated-measures ANOVA and showed a diet \times time interaction ($P=0.477$).

DISCUSSION

PMF and SS are widely used as CBE due to their excellent mouth-feel effects arising from their respective melting point of 31.3°C and 38.0°C. PMF displayed a steep melting profile, which displays a narrow melting interval around 35.0°C that make them useful to produce confectionary fats, being the source of palmitic acid-rich disaturated TAG in the formulation of CBE (Salas *et al.*, 2009).

GIP is a 42-amino-acid hormone secreted from K cells of the upper small intestine while GLP-1 is a 31-amino-acid hormone produced from L cells of the lower intestine and colon (Karhunen *et al.*, 2008). Both of these hormones are incretin hormones that stimulate insulin secretion from pancreatic β cells. GIP and GLP-1 exert their effects by binding to their specific receptors, GIP receptor and GLP-1 receptor which belong to the G-protein coupled receptor family. Through the receptor binding activation, it activates and increases the level of intracellular cyclic adenosine-3,5-monophosphate in pancreatic β cells and hence stimulates insulin secretion glucose-dependently (Yabe and Seino, 2011).

In this study, palmitic acid at *sn*-1 and *sn*-3 positions of PMF exerted similar postprandial GIP and GLP-1 profile as compared to HOSF (oleic acid at the *sn*-1 and *sn*-3 positions). Our results suggest that relative absorption of palmitic acid in PMF was similar to that of oleic acid in HOSF. In other words, different types of fatty acid that are situated at the *sn*-1 and *sn*-3 positions have similar effects in terms of metabolic absorption. Our study is in line with Filippou *et al.* (2014) that showed that TAG containing a high amount of oleic acid (palm olein and HOSF) situated at the *sn*-2 position had raised GIP secretion in a group of healthy individuals.

Entry of dietary fat into the small intestine induces the release of gut hormones and thus, evokes satiety in the gastrointestinal tract (Karhunen *et al.*, 2008; Suzuki *et al.*, 2010). The marked difference of GIP release between HOSF-(OOO) and PMF- (POP) with SS-meal (SOS) could be due to different degree of saturation of fatty acids (Diakogiannaki *et al.*, 2012; Thomsen *et al.*, 1999). The relative difference in the release of GIP between the three test meals could be due to difference in fat absorption of postprandial TAG response

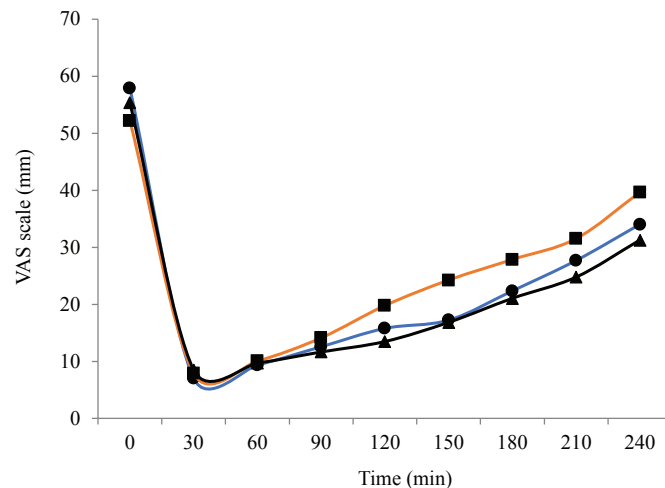


Figure 1. Postprandial concentrations of visual analogue scale (VAS) ($n=36$) following three test meals containing 53 g test fat from high-oleic sunflower oil (HOSF) (●), shea stearin (SS) (■) and palm mid fraction (PMF) (▲). Deviations from postprandial values were performed by repeated measures ANOVA; meal \times time interaction $P=0.11$, followed by a Bonferroni multiple-comparison test. VAS, visual analogue scores.

(Filippou *et al.*, 2014) as SS-meal showed a lower postprandial level of lipaemia (Sanders and Berry, 2005).

It is probable that differences in the physical characteristics of fats and changes in TAG structure may influence its metabolism (Berry, 2009) by pancreatic lipase into free fatty acids and 2-monoglyceride (Maljaars *et al.*, 2009). The liberation of free fatty acids and 2-monoglycerides are a key event preceding secretion of GIP as they act as ligands for G-protein coupled receptors and these receptors might be modulators of incretin hormone release (Diakogiannaki *et al.*, 2012). Hydrolysis is necessary to induce the effects of fat on gastrointestinal function, hormone release and satiety (Feinle-Bisset *et al.*, 2005) and these could explain the difference in GIP release between PMF- SS- and HOSF-rich test diets in this current study.

A study by Thomsen *et al.* (1999) found that GIP and GLP-1 responses were higher after an olive oil meal than after a butter meal in healthy subjects. Their findings suggested that monounsaturated-rich fat stimulated the postprandial GIP and this was also observed in the current study where the HOSF- and PMF-rich test fats exerted comparable postprandial GIP levels which were significantly higher ($P<0.05$) than corresponding levels obtained in the SS-rich test fat. This marked difference in GIP levels could be due to the relation between fatty acid composition and TAG metabolism in the postprandial state (Thomsen *et al.*, 1999). As in the current study, there was no significant difference ($P>0.05$) of postprandial GLP-1 responses between three test meals. Furthermore, we also found out that plasma level of GIP secretion by fat was still elevated after 3 hr postprandial challenge in the current study. The concentration of GIP peaks at 30-

60 min postprandially (Vilsboll *et al.*, 2003; Vollmer *et al.*, 2008) and it could stay elevated until the fifth hour (Carr *et al.*, 2008).

Blood PYY concentrations were found to rise after approximately 15 min, peaked at 1-2 hr and remained elevated for few hours thereafter (Moran and Dailey, 2009). Meanwhile, circulating ghrelin levels typically rise just before and decrease shortly and rapidly after food intake (Karhunen *et al.*, 2008; Marzullo *et al.*, 2006). Both observations of PYY and ghrelin levels were illustrated in current study (Tables 5 and 6). The present study shows no significant differences ($P>0.05$) in postprandial plasma ghrelin and PYY levels after ingestion of the three high-fat meals.

Effects of dietary fats on ghrelin release were inconsistent and contradictory as ghrelin concentrations have been shown to increase (Otto *et al.*, 2006) or decrease (Monteleone *et al.*, 2003) after an ingestion of high-fat meal. Meanwhile, a study conducted by Erdmann *et al.* (2003) revealed that a significant reduction in plasma ghrelin levels initially at 30 min postprandially in 10 healthy subjects, reaching its lowest at 180 min after the subjects were given a fatty meal (85% SFA, 6% carbohydrate, 9% protein).

Other than GIP levels, the current study found no significant differences ($P>0.05$) in the plasma levels of the other gut hormones measured. These findings agree with that of Poppitt *et al.* (2006) who reported that high (70:30) or low (55:45) SFA: unsaturated fatty acid ratios did not affect plasma ghrelin levels in healthy subjects. However, the latter study was not designed to investigate the difference between PMF-, SS- and HOSF-rich type of fats. The lack of significant difference between the three test meals in the current study may due to an increase in the suppression of ghrelin as a result of an increase

in caloric intake (~883 kcal) of a postprandial meal (Little *et al.*, 2007).

Moreover, ghrelin concentrations decrease after the consumption of PMF-, SS- and HOSF-rich test diets and the reduced ghrelin concentrations may be due to an increased fat-induced inhibition of ghrelin (Little *et al.*, 2007). The reduction in plasma ghrelin may indicate a response to the higher calorie intake that associated with high-fat diet consumption and thus, reducing the signal for additional intake of food (Hameed *et al.*, 2009; Monteleone *et al.*, 2003). It was further hypothesised that the effect of fat on ghrelin reduction was controlled by factors such as postprandial TAG levels, fat absorption and gastric emptying (Helou *et al.*, 2008).

There were no significant differences ($P>0.05$) detected in plasma PYY levels in the current study which is in agreement with the findings of Maljaars *et al.* (2009) as PYY levels were not affected or modified by the consumed test meals of different degree of fat saturation. However, specific types of fat have been hypothesised to exert different effect on plasma PYY levels (Serrano *et al.*, 1997). In another study, fasting PYY was significantly more elevated in olive oil group relative to sunflower oil group and remained so at all time points of blood sampling (30, 60, 120, 180 min) (Serrano *et al.*, 1997).

A trend of higher PYY levels was observed in the PMF-rich meal study arm as compared to SS- and HOSF-rich meal at all times where it peaked at 30 min time point followed by a plateau phase for several hours (Adrian *et al.*, 1985) as observed in the current study. The peak level of plasma PYY is released postprandially and influenced by the number of calories consumed and the composition of the food (Adrian *et al.*, 1985) with fat being the most potent macronutrient followed by carbohydrates and proteins (Onaga *et al.*, 2002). Human studies conducted by Batterham *et al.* (2003) and Batterham and Bloom (2003) had shown that higher PYY levels increase satiety and decrease food intake and these findings underscore the suggestion that dietary fat is the most effective stimulator of PYY release.

This delayed response to SOS- and OOO- as compared to POP-rich test meal may reflect the release of PYY from the L cells in the ileal and colonic mucosa after a direct stimulation of fat (Adrian *et al.*, 1985). Moreover, the magnitude of the PYY response after the ingestion of different standardised meals depends on their size (Adrian *et al.*, 1985). This could explain the non-significant differences in plasma PYY levels after subjects were given three different types of postprandial test meals in the current study. The size, the physical properties, the administration rate of the meal, and the digestive and absorptive processes along the tube may have resulted in a delayed entry of nutrients to the distal segments of intestine and to a

small amount of stimuli in contact with the mucosa at these points (Adrian *et al.*, 1985).

The lack of a significant difference in PYY or ghrelin postprandial levels after the consumption of the three test meals help to explain the similar satiety levels of VAS ratings that were found in the present study. PYY that was released postprandially will reduce appetite and inhibit food intake when administered to humans (De Silva *et al.*, 2011). Meanwhile, ghrelin secretion was shown to enhance appetite and food intake (Wren *et al.*, 2001). The effects of dietary fats on satiety and food intake were reported to rely on whether the dietary fatty acids are oxidised or stored (Flint *et al.*, 2003; Stubbs *et al.*, 1995). Friedman (1997) reported that the higher rate of oxidation of a fatty acid resulted in a greater suppression of hunger, and hence, a greater storage of fatty acids. On top of that, oleic acid was shown to be more rapidly absorbed and oxidised compared to the long-chain SFA (DeLany *et al.*, 2000). However, no significant differences ($P>0.05$) were detected in the VAS scores across the three test meals in the present study. The mean satiety VAS scores were found increased over time. SS showed a higher mean value of sensations of hunger over a period of 4 hr challenge as compared to PMF and HOSF.

The current findings are in agreement with the studies of Strik *et al.* (2010) and Casas-Agustench *et al.* (2009) that found no significant differences in satiety measures using VAS between polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and SFA rich test meals in healthy subjects. However, a study carried out by Lawton *et al.* (2000) reported that 20 healthy men and women fed with 80 g of PUFA type of fat was found more effective in decreasing appetite and increasing satiety when compared to a SFA-rich meal. In contrast to our findings, Maljaars *et al.* (2009) found that both oleic acid rich- canola and safflower oil reduced hunger and increased fullness as compared to shea oil, however, the subjects received the experimental fat through infusion in the ileum, and not through a postprandial test meal. It is important to emphasise that these earlier studies mentioned (Casas-Agustench *et al.*, 2009; Lawton *et al.*, 2000; Maljaars *et al.*, 2009; Strik *et al.*, 2010) were not designed to study the effects of PMF-, SS- and HOSF-rich type of fats with regards to the predominance of either palmitic acid or stearic acid at the *sn*-1 and *sn*-3 positions of the TAG backbone on satiety that measured by VAS scores.

CONCLUSION

In conclusion, both CBE fats (PMF and SS) performed differently in terms of satiety hormone secretion, hunger curbing and their potential in

weight management. PMF (POP-) and HOSF (OOO-rich) with palmitic- or oleic acid predominantly situated at the *sn*-1 and *sn*-3 positions raise postprandial GIP concentrations that indicating a tendency to stimulate a greater postprandial satiety compared to SS (SOS-type of fat).

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