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ORIGINAL ARTICLE

Palmitic acid in the *sn*-2 position of dietary triacylglycerols does not affect insulin secretion or glucose homeostasis in healthy men and women

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BACKGROUND/OBJECTIVES: Dietary triacylglycerols containing palmitic acid in the *sn*-2 position might impair insulin release and increase plasma glucose.

SUBJECTS/METHODS: We used a cross-over designed feeding trial in 53 healthy Asian men and women (20–50 years) to test this hypothesis by exchanging 20% energy of palm olein (PO; control) with randomly interesterified PO (IPO) or high oleic acid sunflower oil (HOS). After a 2-week run-in period on PO, participants were fed PO, IPO and HOS for 6 week consecutively in randomly allocated sequences. Fasting (midpoint and endpoint) and postprandial blood at the endpoint following a test meal (3.54 MJ, 14 g protein, 85 g carbohydrate and 50 g fat as PO) were collected for the measurement of C-peptide, insulin, glucose, plasma glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1, lipids and apolipoproteins; pre-specified primary and secondary outcomes were postprandial changes in C-peptide and plasma glucose.

RESULTS: Low density lipoprotein cholesterol was 0.3 mmol/l (95% confidence interval (95% CI)) 0.1, 0.5; $P < 0.001$) lower on HOS than on PO or IPO as predicted, indicating good compliance to the dietary intervention. There were no significant differences ($P = 0.58$) between diets among the 10 male and 31 female completers in the incremental area under the curve (0–2 h) for C-peptide in nmol.120 min/l: GM (95% CI) were PO 220 (196, 245), IPO 212 (190, 235) and HOS 224 (204, 244). Plasma glucose was 8% lower at 2 h on IPO vs PO and HOS (both $P < 0.05$).

CONCLUSION: Palmitic acid in the *sn*-2 position does not adversely impair insulin secretion and glucose homeostasis.

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INTRODUCTION

Interesterified (IE) fats are widely used by the food industry to produce low trans fats with specific functional characteristics (to provide stability of emulsions and good organoleptic characteristics) especially for margarine manufacture and bakery applications.[1] Palm oil is globally the most abundant vegetable oil and its major fatty acids are oleic acid and palmitic acid, which is located almost exclusively on the outer positions of triacylglycerols (TAG) mainly as 1-palmityl, 2,3-dioleyl glycerol and 1,3 di-palmityl, 2-oleyl-glycerol. Refined palm oil is usually fractionated into high (stearin) and low melting point (olein) fractions, the latter being used as cooking oil. Palm olein (PO) has similar proportions of saturated (SFA) and monounsaturated (MUFA) fatty acid fats (~45% and 40% respectively) as animal fats such as lard. However, PO has a much lower melting point (13–15 °C) compared with lard (33 °C), whereas palmitic acid is present almost exclusively in *sn*-2 position. The process of interesterification, which involves randomly reshuffling the fatty acids in PO, produces a harder version of PO with a melting point ~33–35 °C making it suitable for food applications that require high melting point fats.

Few studies[2–5] have evaluated the health effects of IE fats and most have compared stearic acid-rich rather than palmitic acid-rich fats. Sundram *et al.*[6] reported that an IE fat made with fully hydrogenated soybean oil (IESBO) impaired insulin release, increased postprandial glucose and lowered high-density lipoprotein cholesterol concentrations compared with PO. Berry *et al.*[2] however, found no differences between randomly interesterified and native shea butter (a naturally rich source of stearic acid) on postprandial plasma glucose and insulin responses. Hayes and Pronczuk[7] have expressed caution over the use of IE fats to replace trans fats, suggesting that increasing the proportion of SFA in the *sn*-2 position may have adverse effects on insulin secretion and glucose homeostasis and lipid metabolism. Consequently, it might be of great importance to public health as IE fats are being used to replace partially hydrogenated vegetable oils, which are no longer recognized as safe.

The present study was designed to test the hypothesis that replacing native PO with IE PO (IPO) in the diet would impair insulin release in response to a meal and have an adverse effect on glucose homeostasis in healthy men and women. We evaluated insulin release by measuring the C-peptide concentration, which is a robust indicator of insulin release.[8] To compare any specific effect of palmitic acid, we included a comparison with oleic acid provided as high oleic sunflower oil (HOS).

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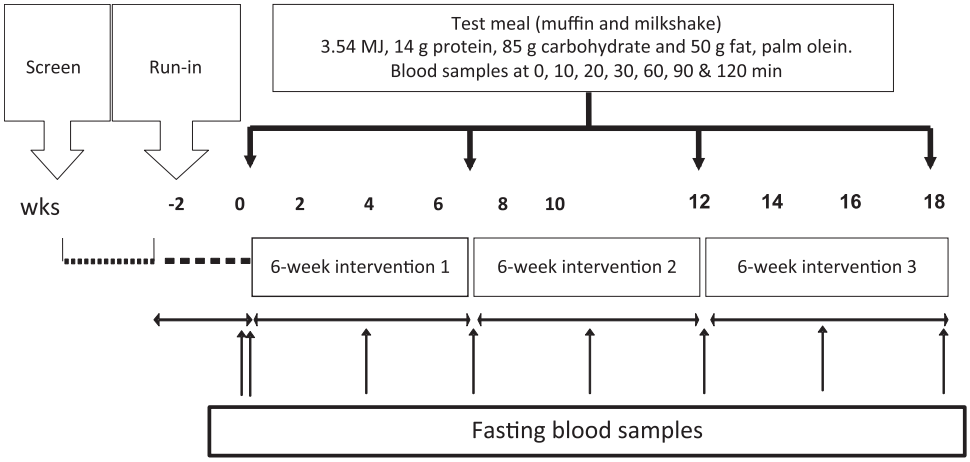

Figure 1. Outline of study design.

SUBJECTS AND METHODS

Study design and participants

A single-blind cross-over design compared three isoenergetic diets where 20% fat energy was provided by PO (iodine value 56, Intercontinental Specialty Fats, Selangor, Malaysia), IPO (iodine value 56, Wilmar PGEO Edible Oils, Johor, Malaysia) or a HOS (a 5:95 blend of high linoleic acid: high oleic acid sunflower oil, Intercontinental Specialty Fats). It was estimated that 20% of the dietary energy from the fats was the maximum amount of fat that could be exchanged in the diet as the remaining 10% of the energy was present as intrinsic fat in nuts, soy, chicken and fish. The TAG molecular species of the oils[9] and their fatty acid composition of the TAG and the proportion in the *sn*-2 position were analyzed.[10] The proportion of palmitic acid in the *sn*-2 position was 45.9% vs 9.8 mol % in IPO vs PO compared with 0.5 mol% in HOS. Measurements of the melting point by differential scanning calorimetry and solid fat content by nuclear magnetic resonance spectroscopy were conducted by Reading Scientific Services Ltd (Reading, UK). PO and HOS were fully melted and contained no solid fats above 32 °C, whereas the solid fat content for IPO was 11.8% at 32 °C, 5.9% at 37 °C and 1.3% at 42 °C, respectively.

Healthy men and women (20–50 year; BMI >18.5— $<30\ kg/m^2$) were recruited by advertisements within the Malaysian Palm Oil Board (MPOB Kajang, Malaysia), and nearby governmental and corporate institutes. Exclusion criteria were: medical history of cardiovascular disease, cancer or diabetes; metabolic syndrome as defined by the International Diabetes Federation (http://www.idf.org/metabolic-syndrome), current use of medications to lower BP or lipids, insulin/glucose modulating medication, serum total cholesterol >7.0 mmol/l, serum TAG >3 mmol/l or an alcohol intake exceeded 21 units/week for males and >14 units/week for females. Potential participants attended a clinic in the fasting state for measurement of height, weight, waist circumference, seated BP, collection of blood samples to confirm normal liver function, glucose, lipids and hematology.

Following a 2-week run-in period on the control diet (PO) participants were randomly allocated by a computer program to one of the six orthogonal treatment sequences with each treatment period lasting 6 week in consecutive order without a washout period between treatments (Figure 1). Measurements were made at the end of the run-in period and at the end of each treatment. The primary outcome was a change in the postprandial increment in C-peptide. Sample size calculations were based on 48 participants completing the study, with 80% power to detect a 30% difference in C-peptide release at $P=0.01$ between diets. This estimate was based on a mean plasma C-peptide increment of 1049 pmol/l at 2 h with a within-subject s.d. of 490 for healthy European men and women[11] measured in our laboratory. To allow a 10% drop out rate, the recruitment target was set at 54 participants. Secondary outcomes were changes in glucose and insulin. The serum lipid profile was measured to confirm compliance to dietary intake. All other outcomes were regarded as exploratory. The study received ethical approval and registered with the National Medical Research Register (NMRR-10-933-7221) on 17 February 2011 and the Current Controlled Trials Database (http://www.controlled-trials.com/ISRCTN16669399) on 19 September 2011. Recruitment was initiated in February 2011 and the dietary intervention completed in July 2011. Participants gave informed consent in writing and received a small remuneration for taking part in the study. Participants were blinded to treatment allocation and so was the laboratory (KingsPath, the King's College Hospital, London) that measured the primary and secondary outcomes.

Experimental diets

The experimental diets were modeled on a diet supplying 15, 30 and 55% energy as protein, fat and carbohydrates, respectively. The major source of carbohydrate in the diet was rice, and the major source of animal protein was fish and chicken. The diets contained no pork (as most of the participants were Muslim who also abstained from alcohol) and intakes of dairy produce were minimal. An allowance of 50 g oil was used for daily food preparation allowing for a 10% loss in cooking this supplied 45 g/day or 20% energy. The subjects were provided with three meals (breakfast, lunch and dinner) per day (Monday–Friday) on 10-day rotating menu. Breakfast and lunch were served in the dining hall of the MPOB, and dinner was packaged into subject identity-labeled containers to be taken home. No meals were provided at weekends but participants were provided with a supply of appropriate test oil (450 ml/weekend) for home food preparation with sufficient allowance for other household members. Habitual dietary energy intakes were assessed from weighed 3-day diet diaries using NutritionistPro software (AXXYA Systems LLC., Stafford, TX, USA) prior to the start of the study. The daily meal plan was designed to supply 8.36 MJ/day (2000 kcal), and differences in energy requirements between participants were adjusted by providing muffins (0.52 MJ each) that had a similar macronutrient profile to the experimental diet but with the fat supplied exclusively by the relevant test oil for each test period. Each food serving was weighed to provide standardized portions and plate waste recorded. Participants were requested only to consume the food provided and to avoid other foods during the week days. To acclimatize the participants to feeding regime and to ensure that the dietary amounts provided were appropriate to meet their energy needs, the participants completed a 2-week run-in period on the control diet (PO).

Duplicate portions of the 10 daily menus on the control diet (PO) were collected for chemical analysis. Protein content was measured by the Kjeldahl method, net metabolizable energy was determined by bomb calorimetry after adjustment for nitrogen content on a Parr 6100 Calorimeter (Parr Instrument Company, Moline, IL, USA), fat content by the Soxhlet method, and fatty acids and cholesterol by capillary gas chromatography. Carbohydrate intake was estimated by difference using Atwater factors of 9 kcal/g for fat and 4 kcal/g for protein. For the estimation of the meals on the IPO and HOS diets it was assumed that the test fats would provide two-thirds of the fat intake. Table 1 shows the composition of the diets determined by chemical analysis compared with the targets.

Measurements and blood sampling

Participants were advised to keep their weight stable throughout the 21-week study period; for this purpose body weight was measured every

Table 1. Composition of the experimental diets determined by chemical analysis compared with the dietary targets[a]

	Palm olein (control)		*Interesterified palm olein*		*High oleic sunflower oil*	
	Target	*Analyzed*	*Target*	*Analyzed*	*Target*	*Analyzed*
Energy (MJ/day)	8.37	7.59 ± 0.15	8.37	7.66 ± 0.23	8.37	7.63 ± 0.24
Protein (% energy)	15	18.1 ± 0.1	15	18.1 ± 0.1	15	18.0 ± 0.1
Carbohydrates (% energy)	55	55.1 ± 0.1	55	55.1 ± 0.1	55	55.1 ± 0.1
Fat energy (% energy)	30	27.1 ± 0.1	30	27.1 ± 0.1	30	27.1 ± 0.1
SFA (% energy)	12	11.0 ± 0.05	12	11.2 ± 0.1	4	4.4 ± 0.03
MUFA (% energy)	12	10.5 ± 0.03	12	10.3 ± 0.1	20	17.1 ± 0.1
PUFA (% energy)	4	4.1 ± 0.01	4	3.9 ± 0.01	4	4.1 ± 0.02
Cholesterol (mg/day)	250	206 ± 55	250	206 ± 55	250	206 ± 55
SFA (% energy)	12	11.0 ± 0.05	12	11.2 ± 0.1	4	4.4 ± 0.03
12:0		0.2 ± 0.004		0.2 ± 0.006		0.2 ± 0.005
14:0		0.4 ± 0.005		0.4 ± 0.008		0.3 ± 0.01
16:0		9.0 ± 0.02		9.1 ± 0.1		2.9 ± 0.004
18:0		1.3 ± 0.01		1.4 ± 0.02		1.1 ± 0.009
MUFA (% energy)	12	10.5 ± 0.03	12	10.3 ± 0.1	20	17.1 ± 0.1
16:1n-7		0.4 ± 0.01		0.4 ± 0.01		0.4 ± 0.01
18:1n-9		9.9 ± 0.03		9.7 ± 0.1		16.4 ± 0.1
18:1n-7		0.3 ± 0.01		0.3 ± 0.01		0.3 ± 0.01
PUFA (% energy)	4	4.1 ± 0.01	4	3.9 ± 0.01	4	4.1 ± 0.02
18:2n-6		3.4 ± 0.0002		3.2 ± 0.01		3.3 ± 0.02
18:3n-3		0.2 ± 0.003		0.2 ± 0.01		0.2 ± 0.01
20:5n-3		0.1 ± 0.002		0.1 ± 0.003		0.1 ± 0.004
22:6n-3		0.2 ± 0.004		0.2 ± 0.01		0.2 ± 0.01

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. [a]Mean values ± s.d. Duplicates of the meals fed on the control diet (PO) were collected for chemical analysis. For the estimation of the meals on the IPO and HOS diets it was assumed that the test fats would provide two-thirds of the fat intake.

2 week . Blood samples were collected on two occasions at baseline, and at the midpoint and at the end of each treatment period. On the day prior to each blood sample, participants were also asked to refrain from strenuous exercise, caffeine and alcohol intake. They were given a standard, light meal of low fat noodle soup (2 MJ), which they were asked to consume before 2200 and then fast overnight (but allowed to drink water *ad libitum*), until they attended the metabolic facility between 0800 and 1000 the following day. An indwelling intravenous cannula (Cat no. 381223, Beckton Dickinson, Oxford, UK) was inserted into the forearm and blood was collected by syringe and dispensed into appropriate uncapped blood collection containers; two fasting samples were collected 5 min apart. A test meal comprising a muffin and milkshake (3.54 MJ, 14 g protein, 85 g carbohydrate and 50 g fat as PO) was consumed within 10 min. The same test meal challenge was used on all treatments. Further venous blood samples were collected at 10, 20, 30, 60, 90 and 120 min following the test meal. Participants had access to water to sip as required (up to 150 ml) over the 2- h period following the meal. For the midpoint visit fasting blood samples were collected using a venous butterfly needle.

Blood samples for serum C-peptide, insulin and lipid analysis were collected into gel separator serum tubes (Beckton Dickenson). Vacutainers were allowed to stand for 30 min at room temperature before centrifugation at 1500 *g* for 15 min at 4 °C. Blood samples for plasma glucose analysis were collected into 2 -ml fluoride oxalate tubes (Beckton Dickinson) and centrifuged at 1500 *g* for 15 min at 4 °C. Samples for plasma GIP and GLP-1 analysis were collected into K2 EDTA tubes (Beckton Dickinson) and pre-prepared with DPP-IV inhibitor (Millipore Corporation, Merck, Middlesex, UK). Samples were separated within 1 h of collection and stored at − 80 °C pending shipment by air on cardice to the UK for analysis.

Analytical methods

The following analyses were conducted by KingsPath at the King's College Hospital (Clinical Pathology Accreditation No 1245): C-peptide and insulin were analyzed on a Siemens Immulite 2000 analyzer using reagents supplied by Siemens Medical Solutions Diagnostics Europe Ltd (Bayer House, Berkshire, UK); interassay precision estimates for insulin were $< 5\%$ for insulin and $< 3\%$ for C-peptide. GIP and GLP-1 were determined by ELISA kits from Linco Research (St Charles, MI, USA); total cholesterol, high-density lipoproteins cholesterol and TAG concentrations using fully enzymatic procedures were analyzed on a Siemens ADVIA 2400 automated chemistry analyzer (Siemens Medical Solutions Diagnostics Europe Ltd). The following analyses were conducted at MPOB on a Hitachi 902 autoanalyser (Roche Diagnostics Gmbh, Mannheim, Germany): plasma glucose using the Glucose GOD-PAP reagent (Roche Diagnostics Limited, West Sussex, UK) and non-esterified fatty acids using reagents supplied by Wako Pure Chemical Industries Ltd. (Osaka, Japan); and apolipoprotein B using the Tina-quant apolipoprotein B version 2 reagent (Roche Diagnostics Limited, West Sussex, UK) and Tina-quant apolipoprotein (a) reagent (Roche Diagnostics Limited). Low-density lipoprotein sub-fractions were analyzed using the Lipoprint Lipoprotein Subfractions Testing System (Quantimetrix, Los Angeles, CA, USA). Apolipoprotein A1 concentrations were determined on an ILAB-650 (ILAB, Instrumentation Laboratories, Cheshire, UK) at King's College London using reagents supplied by Randox Laboratories Limited (County Antrim, UK).

Statistical methods

Statistical analysis was carried out using GraphPad Prism (version 6.0; GraphPad, La Jolla, CA, USA). The distributions of data were checked for normality using a D'Agostino and Pearson omnibus normality test, and logarithmic transformations or other transformations were attempted if appropriate. Data were analyzed by one-way repeated measures analysis of variance. Where the overall *F* test was significant, specific comparison between treatments were made adjusting for multiple comparisons using the Holm–Sidak test. The incremental area under the curve (iAUC) was calculated using the trapezoid rule. The homeostatic model assessment of insulin resistance (HOMA2-IR) was calculated using a software implementation of the HOMA2 model developed by the Diabetes Trials Unit, University of Oxford (http://www.dtu.ox.ac.uk/homacalculator/). Results are shown with mean (s.d.) or geometric mean (95% confidence interval (95% CI)) for log-transformed data or median (interquartile range) where transformation did not normalize the distribution.

RESULTS

Study participation, diet and compliance measures

A total of 53 subjects were randomized to treatment, 8 withdrew during the run-in period (7 withdrew consent because they were unwilling to comply with the protocol and 1 pregnancy). During

the first intervention period a further two participants withdrew (1 because of difficulty with cannulation and one for unrelated health problems, and in the second period a participant withdrew because of pregnancy and in third period a participant withdrew owing to work relocation. Supplementary Figure 1 shows the CONSORT flow chart of participants through the study. Data were available for the primary endpoint on 41 participants whose details are shown in Table 2. The participants were notably shorter in height than typical values for European and North American men and women of the same age and so their body weights appear low but typical of Malaysians living in urban areas.

Table 2. Baseline characteristics of participants who completed the study at randomization[a]

Characteristics	*Women*[b]	*Men*[c]
Age, year	29.3 ± 8.0	28.6 ± 6.3
Height, m	1.54 ± 0.05	1.70 ± 0.06
Weight, kg	54.0 ± 8.1	68.2 ± 7.3
BMI, kg/m^2	22.7 ± 3.0	23.8 ± 3.0
Waist, cm	75.9 ± 11.4	86.4 ± 4.9
Systolic BP, mm Hg	112.6 ± 8.3	122.3 ± 8.5
Diastolic BP, mm Hg	73.9 ± 7.6	76.9 ± 8.0
Plasma glucose, mmol/l	4.8 ± 0.3	5.1 ± 0.4
Serum total cholesterol, mmol/l	4.8 ± 0.7	5.3 ± 0.7
Serum HDL cholesterol, mmol/l	1.5 ± 0.3	1.2 ± 0.2
Serum LDL cholesterol, mmol/l	2.9 ± 0.5	3.7 ± 0.7
Serum triglycerides, mmol/l	0.8 ± 0.4	1.0 0.3
Total cholesterol:HDL cholesterol	3.2 ± 0.6	4.8 ± 1.1
Energy, MJ/day	6.9 ± 1.9	7.1 ± 2.0
Protein, % energy	17.1 ± 4.3	16.2 ± 4.0
Carbohydrate, % energy	53.1 ± 8.0	51.3 ± 6.1
Fat, % energy	30.3 ± 5.7	33.8 ± 4.7

Abbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein. [a]Values are means ± s.d. [b]$n = 31$. [c]$n = 10$.

Meal attendance was high (PO 95 ± 5.8; IPO 95 ± 5.3; HOS 95 ± 4.8%) and proportions of food served that was consumed was similar (PO 81.5 ± 9.8, 80.7 ± 9.5, 78.4 ± 12.2%) and there were no differences in the acceptability (PO 6.8 ± 1.5; IPO 6.3 ± 1.6; HOS: 6.7 ± 1.5) of the diets measured on a 10-point visual analog scale. Body weights were stable throughout the study.

Table 3 shows that plasma lipids and lipoproteins did not differ between PO and IPO, but total cholesterol, low-density lipoprotein cholesterol, apolipoprotein B, total cholesterol:high-density lipoproteins cholesterol were 7, 10 and 7% and 0.17 lower, respectively on HOS than either PO or IPO (all $P < 0.01$). These lipid changes, which are in line with the predicted effects resulting from a 6% energy exchange of palmitic for oleic acid,[12] indicate good compliance to the dietary intervention. The primary outcome, postprandial changes in C-peptide, did not differ significantly ($P = 0.58$): GM (95% CI) $iAUC_{0-120\ min}$ for C-peptide in nmol.120 min/l were PO 220 (196, 245), IPO 212 (190, 235) and HOS 224 (204, 244). The maximal increments from fasting in C-peptide expressed as GM (95% CI) in pmol/l were PO 2682 (2388, 3012), IPO 2515 (2255, 2805) and HOS 2751 (2461, 3076) and did not differ ($P = 0.29$). Figure 2 shows the postprandial changes in plasma glucose, non-esterified fatty acids, GIP and GLP-1. There was a significant diet × time interaction for the postprandial increment in plasma glucose ($P = 0.007$) with the value at 2 h being 8% (95% CI: 2, 13 $P < 0.05$) lower on IPO compared with PO and HOS. Plasma non-esterified fatty acids were suppressed by the test meal to the same extent following all three diets, indicating a similar insulin-mediated action on inhibiting adipose tissue lipolysis and postprandial increases in GIP and GLP-1 did not differ. No other statistically significant differences were noted between treatments.

DISCUSSION

Replacement of PO with IPO did not adversely affect insulin release or glucose tolerance nor did it lower high-density lipoprotein cholesterol. These findings contrast to the report by Sundram *et al.*[6] that reported an impairment of fasting glucose,

Table 3. Fasting and postprandial C-peptide, insulin, glucose, lipid and lipoprotein concentrations and indices of glucose and insulin homeostasis in male and female participants on PO, IPO and HOS diets

	Run in[a]	*PO*	*IPO*	*HOS*
Fasting insulin, mU/l	7.7 (6.7, 7.7)	8.5 (7.2, 10.0)	8.5 (7.3, 9.7)	8.7 (7.3, 10.3)
Fasting C-peptide, nmol/l	0.42 (0.38, 0.47)	0.48 (0.42, 0.55)	0.47 (0.43, 0.53)	0.47 (0.41, 0.54)
$iAUC_{0-120}$ C-peptide, nmol.120 min/l	177 (161, 195)	207 (185, 232)	200 (180, 223)	215 (196,236)
Fasting glucose, mmol/l	5.3 (5.3, 5.4)	5.2 (5.1, 5.3)	5.2 (5.1, 5.3)	5.2 (5.1, 5.3)
Peak glucose, mmol/l	7.1 (6.8, 7.5)	7.2 (6.9, 7.5)	7.0 (6.7, 7.3)	7.2 (6.9, 7.5)
2 h glucose, mmol/l	6.0 (5.7, 6.3)	6.1 (5.8, 6.4)	5.7 (5.4, 6.0)[b]	6.1 (5.8, 6.4)
Glucose $iAUC_{0-120,}$ mmol.120 min/l	92 (76, 113)	88 (62, 125)	88 (70, 115)	94 (71, 113)
HOMA2-IR	0.95 ± 0.38	1.03 ± 0.53	1.06 ± 0.40	1.04 ± 0.53
Total cholesterol, mmol/l	4.96 ± 0.69	4.99 ± 0.70	4.95 ± 0.65	4.62 ± 0.64[c]
LDL cholesterol, mmol/l	3.02 ± 0.63	3.02 ± 0.65	3.02 ± 0.60	2.72 ± 0.61[c]
HDL cholesterol, mmol/l	1.43 ± 0.26	1.44 ± 0.30	1.42 ± 0.27	1.39 ± 0.26
TC:HDL-C	3.50 ± 0.77	3.52 ± 0.82	3.52 ± 0.78	3.35 ± 0.80[c]
sdLDL, %	25 ± 10	24 ± 11	23 ± 11	25 ± 12
Apolipoprotein B, g/l	0.84 ± 0.23	0.85 ± 0.26	0.83 ± 0.25	0.77 ± 0.25[c]
Apolipoprotein A1, g/l	1.20 ± 0.17	1.21 ± 0.20	1.19 ± 0.17	1.19 ± 0.16
Lipoprotein Lp(a), mg/l[d]	13 (8, 23)	11 (8, 28)	10 (7, 22)	11 (7, 18)
TAG, mmol/l	0.92 ± 0.43	0.96 ± 0.36	0.95 ± 0.39	0.91 ± 0.36
Weight, kg	57.4 ± 9.9	57.7 ± 9.9	57.6 ± 10.1	57.6 ± 10.1

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; HOMA2-IR, homeostatic model assessment of insulin resistance; HOS, high oleic sunflower; $iAUC_{0-120}$, increment al area under the curve between 0 and 120 min; IPO, interesterified palm oil; LDL, low-density lipoprotein; IQR, interquartile range; PO, palm olein; sdLDL, small dense LDL; TAG, triacylglycerol; TC:HDL-C, total cholesterol:HDL cholesterol. [a]$n = 41$(31 women, 10 men) values are mean ± s.d. or geometric mean (95% CI). [b]$P < 0.05$, compared with values for PO and HOS. [c]$P < 0.01$ compared with PO and IPO; Holm-Sidak test. [d]median (IQR).

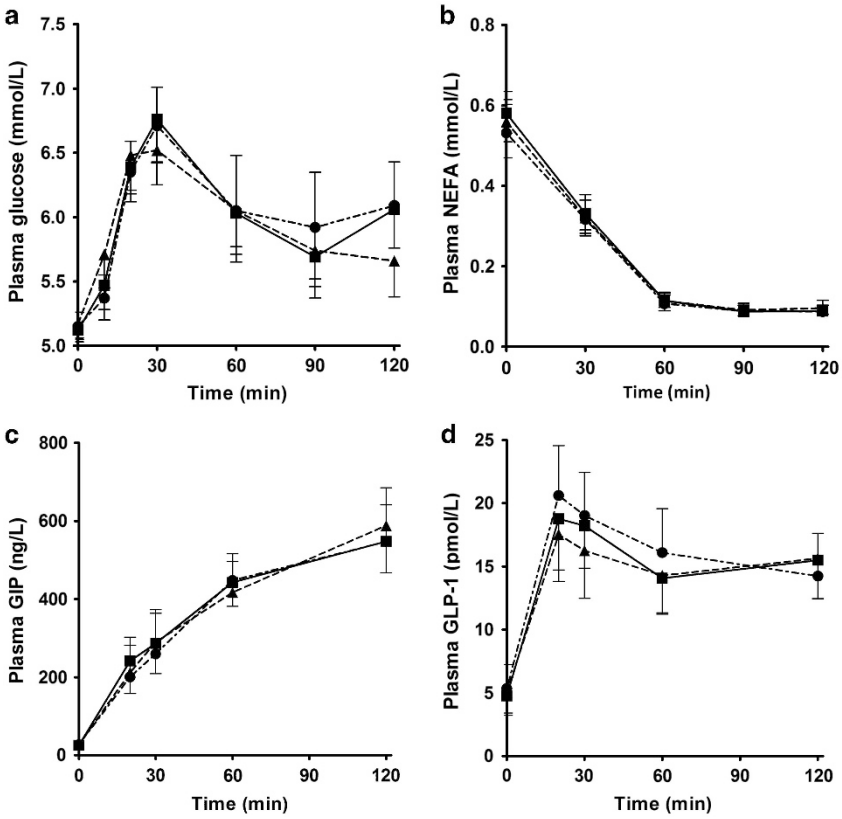

Figure 2. Changes in plasma glucose (**a**), non-esterified fatty acids (NEFA) (**b**), glucose-dependent insulinotropic polypeptide (GIP) (**c**) and glucagon-like peptide-1 (GLP-1) (**d**) in response to a test meal after 6 weeks on diet providing 20% energy palm olein (PO, ●), high oleic sunflower oil (HOS, ■) and interesterified palm olein (IPO, ▲). Values are geometric means with 95% CI; $n = 41$. Data were log transformed and analyzed by repeated measures analysis of variance. For glucose there was a significant ($P = 0.007$) meal × time interaction (3 meals, six time points) for the changes from fasting; the incremental value at 2 h is 8% lower for IPO vs PO or HOS (both $P < 0.05$). There were no other significant differences between treatments.

rising from 5.6–6.7 mmol/l, in 21 female and 11 male Malaysian participants after 4 week on a diet providing IESBO compared with PO: the proportions of energy provided as palmitic, stearic, oleic and polyunsaturated fatty acids were 5.5,12.5, 5.9 and 7.0%, respectively, on IESBO vs 12.0, 1.3, 13.6 and 3.6% on PO. They also reported that the IE fat reduced fasting insulin from 10.1–7.9 mU/l and C-peptide concentrations (13.2 pmol/l vs 16.0 pmol/l) 2 h following a meal with the same fatty acid composition as the experimental diet. We could find no evidence that IPO impaired insulin secretion. Our study differed in that it employed the same test meal challenges (PO) after each treatment period to ascertain whether there were chronic differences in response induced by the dietary change. We had previously demonstrated no difference in the postprandial increases of glucose, C-peptide and insulin between meals formulated using either 50 g PO or 50 g IPO[11] in European subjects. Consequently, the differences between our findings and those of Sundram *et al.*[6] are highly unlikely to be the result of using PO in the test meal rather than the oil from the experimental treatment.

The scenario tested here used an intake of IPO much greater than likely to be consumed in practice. Although PO provides up to 20% of the food energy in Malaysia and Indonesia, where it is the main culinary oil, only a small fraction is used as IE fats for use in bakery products and margarine. In Europe, palm oil fractions are used in IE fats but we estimate that intakes are likely not to exceed 5% energy.

Meijer and Weststrate[13] compared IE fats with native fats at 4 and 8% energy intake, as margarines with 18 and 7% of the palmitic acid in the *sn*-2 position in 60 European subjects using a cross-over design with each diet was taken for 3 weeks but found no differences in fasting glucose and did not measure fasting insulin or postprandial changes. In the present study, there were no differences between IPO and native PO in lipid or lipoprotein concentrations. Our findings do not support the assertion[7] that palmitic acid in the *sn*-2 position has different effects on plasma lipids and lipoproteins from when it is present in the *sn*-1 or *sn*-3 position in adults and is consistent with a report by Zock *et al.*[5] The present study provided no evidence to indicate that palmitic acid in the *sn*-1 and *sn*-3 positions decrease lipids or lipoproteins because of poor absorption as it does in infants.[14] Our findings showing a small reduction in low-density lipoprotein cholesterol and apolipoprotein B, when PO was replaced by HOS, is consistent with a recent meta-analysis on the effects on palm oil on lipids.[15]

A review by Riserus *et al.*,[16] suggested that SFA adversely affect insulin release and glucose homeostasis. However, this conclusion was based on a number of small trials; the only one major study that had addressed this question was the KANWU study,[17] which reported an improvement of borderline statistical significance in insulin sensitivity when dietary SFA, mainly derived from animal fats and lard, were replaced by MUFA. More recent and larger studies[18,19] in free-living participants with features of the metabolic syndrome find no effects on insulin sensitivity when SFA were replaced with MUFA or carbohydrates. The present study assessed insulin sensitivity using HOMA2-IR, which is strongly correlated with the intravenous glucose tolerance test, as well as the response to a test meal, which also takes into account the effects of incretins such as GIP and GLP-1. Although HOMA2-IR was normal, it is interesting to note that our participants were moderately insulin resistant as indicated by the persistent elevation of plasma glucose at 2 h; this is in contrast

to the effects of an indentical test meal in healthy European subjects,[11] which caused a smaller increment in C-peptide and a return of plasma glucose concentration to baseline at 2 h. In the latter study, we reported that IPO reduced GIP release compared with PO or HOS. However, in the present study there was no evidence to suggest that IPO resulted in a chronic reduction in GIP release. However, plasma glucose at 2 h was lower following the test meal compared with PO and HOS, suggesting an improvement in glucose disposal, which might be a physiological response to chronically reduced GIP release on IPO. Despite the widespread use of palm oil in Malaysia, total fat and SFA intakes are lower (~30% and ~11% energy, respectively) than in Western countries where intakes are higher. However, the metabolic syndrome has been reported to be more prevalent among men and women of Malay ancestry.[20] The reasons for this remain uncertain but may have their origins in early life.[21] Recently, Rosquist *et al.* [22] in an overfeeding study in healthy European participants reported that replacement of SFA (palm oil) with PUFA (sunflower oil) modestly reduced body fat (−0.9%) and liver fat (−0.5%) accumulation but did not affect insulin sensitivity or fasting glucose. The design of that study is not comparable to the present study where the diets were designed to cause weight maintenance as opposed to gain. Overall, our findings are consistent with the recent studies, suggesting no specific effect of SFA on insulin sensitivity or secretion.

Strengths and limitations of the study

A strength of the present study is the relatively high intake of the test fat (20% of the dietary energy), the rigorous control of dietary intake, the relatively longer duration of the intervention period compared with other studies and a blood sampling protocol, sufficient to detect meaningful differences in insulin secretion. Our power calculations were based on 48 participants completing the study, although only 41 completed the study, which reduced the statistical power of the study from 80% power at $P = 0.01$ to $P = 0.05$ but the size effects observed were small and unlikely to change with a larger sample as planned. A limitation is that the participants were healthy and not obese so the finding may not apply to people who are obese or who have type 2 diabetes. The study was conducted in Asian subjects who traditionally consume palm oil as the major source of dietary fat, whereas in Europe it is unlikely to account for no more than 5% energy.

In conclusion, palmitic acid in the *sn*-2 position does not adversely affect insulin secretion and glucose homeostasis in healthy men and women.

CONFLICT OF INTEREST

Dr Thomas Sanders has received compensation as a member of the scientific advisory board of the Global Dairy Platform. The remaining authors declare no conflict of interest.

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