Replacement of dietary fat with palm oil: effect on human serum lipids, lipoproteins and apolipoproteins

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Thirty-eight male volunteers participated in a double-blind cross-over trial evaluating the effect of replacing the usual sources of saturated fat in the Dutch diet (animal fats and hydrogenated oils) by palm oil, which is virtually free of cholesterol and *trans*-fatty acids, on serum lipids, lipoproteins and apolipoproteins. Maximum (about 70%) replacement had no significant effect on serum total cholesterol or most lipoprotein fractions, but resulted in an 11% increase in serum high-density-lipoprotein $(HDL)_2$-cholesterol relative to the control ($P_2 = 0{\cdot}01$). The palm-oil diet also caused an 8% decrease in low-density-lipoprotein (LDL):$HDL_2 + HDL_3$-cholesterol ratio ($P_2 = 0{\cdot}02$) as well as a 9% decrease in triacylglycerols in the low-density-lipoprotein fractions ($P_2 = 0{\cdot}01$). Palm oil consumption resulted in a 4% increase in serum apolipoprotein AI ($P_2 = 0{\cdot}008$) and a 4% decrease in apolipoprotein B ($P_2 = 0{\cdot}01$) relative to the control diet; the B:AI apolipoprotein ratio was decreased by 8% ($P_2 < 0{\cdot}0001$). These results were not significantly affected by the different lipoprotein E phenotypes of the volunteers. Although the observed differences were relatively modest, the present study, nonetheless, indicates that dietary palm oil, when replacing a major part of the normal fat content in a Dutch diet, may slightly reduce the lipoprotein- and apolipoprotein-associated cardiovascular risk profiles.

Palm oil: Serum lipids: Lipoproteins: Apolipoproteins: Phenotypes: Dietary response

The contributory role of dietary oils and fats in the aetiology of cardiovascular diseases and their ability to influence serum cholesterol levels is well established. Polyunsaturated fatty acids (PUFA) decrease (Spritz & Mishkel, 1969; Vega *et al.* 1982), whereas saturated fatty acids (SFA) increase (Keys *et al.* 1965*a*, *b*; Pownall *et al.* 1980; AMA Council on Scientific Affairs, 1983) serum and low-density-lipoprotein (LDL)-cholesterol levels. Recent evidence (Mensink & Katan, 1989; Ginsberg *et al.* 1990) also suggests that the substitution of monounsaturated fatty acids (MUFA) for SFA has a similar cholesterol-lowering effect.

Among SFA the potential to raise serum cholesterol levels is variable. Thus, it has repeatedly been shown that stearic acid ($C_{18:0}$) does not raise serum cholesterol (Keys *et al.* 1965*a*, *b*; Bonanome & Grundy, 1988). The major SFA in the human diet, however, is palmitic acid ($C_{16:0}$) which together with lauric ($C_{12:0}$) and myristic ($C_{14:0}$) acids, is considered hypercholesterolaemic (Hashim *et al.* 1960; Grande, 1962; Hegsted *et al.* 1965). It should be realized that these types of studies did not take into account the various sources

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of these saturates which in the Western hemisphere are chiefly derived from animal fats and hydrogenated vegetable oils. Unfortunately, the consumption of animal fats is associated with increased cholesterol intake, while hydrogenated oils contain considerable amounts of *trans*-fatty acids which were recently shown to have adverse effects on the serum cholesterol profile (Mensink & Katan, 1990). Therefore, edible oils rich in saturates, but free from cholesterol and *trans*-fatty acids, may represent preferred sources of saturates when included in the human diet.

Palm oil contains approximately (g/100 g): palmitic acid 45, stearic acid 5, oleic acid 40 and linoleic acid 10, while being virtually free from cholesterol and *trans*-fatty acids. In addition, palm oil contains a high content of tocotrienols which have been reported to suppress cholesterol synthesis in an animal model (Qureshi *et al.* 1986). Thus, it is difficult to predict the likely effect of this oil on plasma lipids. In spite of its growing importance as an edible oil (Mielke, 1987), scientific evidence evaluating the role of palm oil in human nutrition is rather limited (Anon, 1987). We, therefore, felt it pertinent to investigate the effect of a diet in which the main fat components have been replaced by palm oil, on the various aspects of the cardiovascular risk profile. In the present paper we report on the effect of a diet in which the main fat components have been replaced by palm oil on serum total-(TC) and lipoprotein-cholesterol and triacylglycerols (TG), and on serum apolipoproteins. Results for other variables of interest, such as blood pressure, coagulation, fibrinolysis and platelet functions will be reported separately.

MATERIALS AND METHODS

Experimental design

The research protocol was approved by the Medical Ethical Committee of the University of Limburg, Maastricht, The Netherlands. Written informed consent was obtained from all participating volunteers.

The study was designed as a double-blind cross-over trial consisting of two periods of 6 weeks each, preceded by a run-in period of 3 weeks and interrupted by a wash-out period of 3 weeks.

To assess the effect of maximal palm oil substitution in a normal Western diet a series of food products, containing palm oil as the major fat source, and a second series of control products, containing the usual fats and oils while excluding palm oil, were prepared and distributed to the volunteers.

During the run-in period all volunteers consumed control products which were formulated to reflect the habitual fat consumption pattern in the Maastricht region of The Netherlands. At the end of this period volunteers entered into the first experimental period, wherein 50% were assigned the palm-oil products and the remaining 50% continued to consume the control products. During the wash-out period all volunteers resumed consumption of the control products, whereas on entry into the second experimental period volunteers who consumed the palm oil products in the first period now consumed the control products and vice versa. Maximum care was taken to ensure that both the volunteers and investigators remained unaware of the types of products the volunteers consumed.

Screening, selection and randomization of volunteers

Male volunteers (120) were recruited from the general population of Maastricht and screened for eligibility 10 weeks before starting the run-in period. Only volunteers fulfilling the following selection criteria were allowed to enter the study: (1) aged between 19 and 45 years, (2) non-obese; body mass index (BMI) $< 28\ kg/m^2$, (3) clinically healthy (no

Table 1. *Characteristics of volunteers at the start of the run-in period (week −3)*
(Mean values with their standard errors for thirty-eight subjects)

Characteristic	Mean	SEM	Range
Age (years)	35·7	0·85	19–45
BMI	23·8	0·31	20·1–26·4
Serum cholesterol (mmol/l)	5·13	0·13	3·50–6·40
HDL-cholesterol (mmol/l)*	1·08	0·04	0·70–1·80
Serum triacylglycerols (mmol/l)	0·99	0·06	0·27–2·08

BMI, body mass index (body-weight (kg)/height^2 (m)); HDL, high-density-lipoprotein.
* Measured enzymically in serum after heparin–manganese precipitation of very-low-density- and low-density-lipoproteins.

medication, absence of glucose and protein in urine), (4) normotensive (systolic blood pressure between 100 and 140 mm Hg; diastolic blood pressure between 40 and 90 mm Hg), (5) normolipidaemic (serum TC < 6·8 mmol/l; serum TG < 2·0 mmol/l), (6) non-smokers (fewer than seven cigarettes per week), (7) willingness to participate in the study.

A total of fifty-six volunteers were judged eligible and entered the study at −3 weeks (start of the run-in period). The dietary history of each volunteer was recorded by a dietitian and the polyunsaturated:saturated (P:S) fatty acid ratio of the diet was calculated using the BECEL® computerized program (BECEL®, 1988). Blood was also sampled for serum TC, high-density-lipoprotein-cholesterol (HDL-C) and serum TG on the day of entry into the run-in period.

The final volunteer population (forty persons) was selected on the basis of individual data for TC as the primary selection criterion with HDL-C and TG as the secondary criteria. Volunteers reporting a P:S ratio different from the habitual dietary pattern in The Netherlands were excluded. The selected volunteers were then stratified over two groups of twenty persons on the basis of the previously stated criteria. Of these, one person dropped out of the study because of job commitments requiring travel outside the Maastricht area, and a second volunteer was excluded after he was prescribed medication for treatment of gout by his physician. The entry characteristics of the thirty-eight remaining men who completed the study are given in Table 1.

Because of optimization in the laboratory, only five volunteers were permitted each day, from Tuesday to Friday. Mondays were excluded to provide a safeguard against unusual activities during the weekend. Within each day, volunteers were subdivided between the two groups in such a manner that equal numbers of volunteers were assigned to the two dietary groups by the end of each second day.

Composition of dietary supplements

Dietary products contributing a high fat intake in the Dutch diet were identified through a sample dietary survey. Technology permitting, a series of palm-oil-based products was formulated replacing the normal fat component in these products. A second series of the same products, reflecting normal compositions but containing little or no palm oil, was also prepared and used as the control products in this study. Broadly, the products included margarines, frying and bakery fats, snack foods (hamburgers, chicken nuggets, meat croquettes), dairy products (cheese, chocolate milk, ice cream, dairy products), bakery products, and chocolate spread. The characteristics of these products are described elsewhere (Sundram *et al.* 1990*a*). All food products were individually packed in identical packaging material and bar coded, thus offering complete blindness.

A special 'shop' with appropriate displays of the coded products was manned by dietitians. Volunteers had access to items in the 'shop' from Monday to Friday. A computer program was specially designed to keep a complete record of food items handed out to the volunteers. Since each product was bar-coded, the computer automatically decoded the information and only allowed passage of the correct dietary type according to preprogrammed stratification information. Product composition data, previously entered into the computer, enabled the dietitians to monitor continuously for deviations in the P:S ratio of the volunteers' diets. Food products from the 'shop' were made available to the whole family free of charge, in order to optimize compliance.

Compliance measurements

Since our objective was to investigate the effect of maximum palm oil consumption in a free-living population, we deliberately abstained from any interference with respect to the volunteers' food selection and preparation. However, a strict check on compliance by the volunteers was monitored as follows: all volunteers were instructed to maintain a diary to monitor any occasional deviations from their normal dietary patterns. The diaries were checked at least once weekly by the dietitian. Each week the volunteers were given a list of products that were available to them from the 'shop'. However, if they had purchased any comparable product elsewhere they were asked to indicate dates, brand name and quantity on a 'shadow list'. This enabled compliance to be calculated for each of the products provided, as well as any changes that may have occurred resulting from the use of the 'shadow products'. During the 5th week of each experimental period the volunteers were subjected to a dietary history interview by the dietitian. This permitted calculation of the average nutrient intake during each experimental period and comparison for deviations from their habitual dietary patterns.

During the 4th week of each experimental period volunteers were instructed to collect a duplicate portion of all foods and liquids consumed over a 48 h period; these were brought to the laboratory for subsequent processing. The duplicate portions were weighed and then homogenized in a food mixer. Approximately 750 g of the homogenized mixture was collected in lidded tins and frozen at $-20°$ until required for analysis.

Fatty acid composition of serum TG was analysed at the end of each experimental period and used as an additional compliance measurement.

Blood sampling procedures

Volunteers reported to the attending physician at 3-week intervals beginning at -3 weeks and ending on the 15th week. All blood samplings were performed between 08·00 and 12.00 hours under fasting conditions (no food after 22.00 hours the previous evening) and after 24 h abstinence from alcohol. Body-weights were measured with volunteers wearing minimum indoor clothing and without shoes. The volunteers then took a 10 min rest in a recumbent position. With the volunteers still in a recumbent position, a forearm vein was punctured under minimal stasis using a 19G butterfly venisystem. After four blood samples had been taken for other measurements (about 50 ml in total, results to be reported elsewhere), two 10 ml samples of blood were collected in two Monovette® tubes (051104; Sarstedt, Numbrecht, Germany). Blood in the first monovette was allowed to clot for 2 h at room temperature (about 24°) and serum prepared for the analysis of TC, HDL-C, TG and the isolation of lipoprotein classes by ultracentrifugation. The second monovette was placed in a water-bath at 37·5° and blood allowed to clot for precisely 60 min. Serum from this preparation was utilized for the analysis of thromboxane B_2 (to be reported elsewhere), serum fatty acids and apolipoproteins. Spare serum was collected whenever possible and stored at $-80°$ for later use. This bleeding procedure was carried out during 0, 6, 9 and 15

weeks only. During weeks −3, 3 and 12, only 10 ml blood was drawn from the volunteers and used for the analysis of TC, HDL-C and TG. Serum was prepared by centrifugation at 1200 *g* and 20° for 15 min.

Analytical methods

Lipids and lipoproteins. Isolation of serum lipoprotein classes was performed using a Beckman SW41 rotor in a Beckman L5-65 ultracentrifuge according to a slightly modified method of Terpstra *et al.* (1981). The isolation procedures were always started within 5 h of sampling blood from the volunteers.

Briefly, 2·0 ml serum was prestained with 0·2 ml Sudan Black B (S-2380; Sigma, St Louis, MO, USA), in ethylene glycol (822329; Merck, Darmstadt, Germany) and the density (ρ) of this prestained serum was raised to $\rho 20$ 1·250 g/ml using solid potassium bromide (4905; Merck), sodium chloride (6404; Merck) and sucrose (7651; Merck) in a Beckman Ultraclear® (no. 344060) centrifuge tube. The prestained serum was then sequentially layered with salt solutions $\rho 20$ 1·225 g/ml (11·42 g NaCl and 315·54 g KBr/l) followed by salt solution $\rho 20$ 1·100 g/ml (11·42 g NaCl and 133·48 g KBr/l) and overlayered with distilled water. All solutions contained 0·112 g EDTA (disodium salt; 8418; Merck)/l. The layering was performed using a peristaltic pump (10200; LKB, Perpex, Sweden) with controlled displacement speeds that were adjusted so as not to disturb the salt gradients. Tubes were centrifuged for 22 h at 40000 rev./min and 20°. By this technique seven different fractions of prestained lipoproteins were harvested at the following density ranges ($\rho 20$ g/ml): very-low-density-lipoprotein (VLDL) < 1·006, intermediate-density-lipoprotein (IDL) 1·006–1·019, LDL 1·019–1·055, high-density-lipoprotein $(HDL)_1$ + atherogenic lipoprotein (Lp(a)) 1·055–1·085, HDL_2 1·085–1·120, HDL_3 1·120–1·210 and a bottom fraction (bf) > 1·210.

Customarily ρ 1·063 is taken as the demarcation between LDL and HDL. However, this results in the inclusion of appreciable amounts of Lp(a) into the HDL. By interposing an extra fraction with $\rho 20$ 1·055–1·085, Lp(a), when present, is largely separated from LDL and HDL_2. According to Schmitz & Assmann (1982), HDL_1 floats as a single peak at ρ 1·08–1·09 g/l. Therefore, our $\rho 20$ 1·055–1·085 fraction would actually consist of both Lp(a) and HDL_1. For convenience, however, this fraction will be termed HDL_1. Because HDL are heterogeneous, several subfractions can be distinguished and the most important of these are HDL_2 and HDL_3.

Before the actual study, a pilot trial was performed during which serum as well as dummy salt solutions mimicking human serum were used to establish the density ranges of each lipoprotein class using a densitometer. Based on these results, a Perspex mould was constructed which indicated the level of each salt gradient to be layered, as well as the expected distribution ranges of each lipoprotein class in the ultracentrifuge tube. These indicator levels were marked off on each ultracentrifuge tube and served as a guide for harvesting the lipoprotein class by aspiration with a pasteur pipette. Prestaining with Sudan Black B further helped in maximizing recovery at the correct density ranges.

Cholesterol and TG concentrations in each of these lipoprotein fractions and in serum were determined on an autoanalyser (Cobas Bio; Hoffman-La-Roche, Basel, Switzerland) using commercial enzymic kits (TG, 1086; Roche Diagnostica, Basel, Switzerland; TC, Boehringer Monotest® cholesterol, Boehringer Mannheim, GmbH). Recovery rates of the lipoproteins were calculated as the sum of both cholesterol and TG contents of the fractions and were expressed as a percentage of the serum total cholesterol or triacylglycerols.

The calculated recovery rates from the seven lipoprotein fractions during the entire study (n 153) were 100·12 (SE 0·36) and 95·99 (SE 0·77)% for cholesterol and TG respectively. Prestaining the serum with Sudan Black B did not interfere with TG analysis.

Apolipoproteins. Serum apolipoproteins AI, AII and E were determined according to the method of Alvers *et al.* (1976), while Apo B was evaluated by radial immunodiffusion according to Havekes *et al.* (1981). Apolipoprotein E phenotypes were measured directly in serum as described by Havekes *et al.* (1987).

Duplicate portion analysis. Frozen food duplicate portions were thawed and a sample of about 500 g was freeze-dried. A 20 g freeze-dried sample was Soxhlet-extracted with light petroleum (b.p. 40–60°) for quantification of fat content. Due to a technical error in the laboratory, determinations from six duplicate portions were omitted. A portion of the fat extracted was methylated for fatty acid analysis (Rand *et al.* 1986) of these duplicate portions. An extensive comparison of fats extracted from the duplicate portions by the Soxhlet method with fats extracted from the duplicate portions by the Bligh & Dyer (1959) method did not reveal any differences in the fatty acid composition of these duplicate portions. Cholesterol content of the duplicate portions was analysed by gas–liquid chromatography (Bovenkamp & Katan, 1981).

Fatty acid composition of serum TG. Serum lipids were extracted by the Bligh & Dyer (1959) method and TG isolated by thin-layer chromatography, scraped, methylated, and the fatty acids were analysed (Rand *et al.* 1986). For this purpose, serum collected at the end of weeks 6 and 15 were used.

Statistical methods

Distributions were visually checked for normality and detected outliers were deleted only when clear evidence existed of faulty recording or analytical errors. Results are presented as means with their standard errors (geometric means for log-transformed data). These means are calculated for week 0, and for the completion of the palm oil and control periods.

Testing the palm oil *v.* control effect was done using the two-sided paired *t* test modified for the cross-over design as described by Armitage & Berry (1987). Values for $P(P_2) < 0{\cdot}05$ were considered significant.

Preceding these analyses, tests for treatment × period interaction were performed (Armitage & Berry, 1987). If this yielded a significant result at the 5% level, the comparison of palm oil *v.* control was planned using first-period results only. However, no significant treatment × period interaction was observed in the present study.

RESULTS

No major problems were encountered during the study. Acceptance of the food products by the volunteers was excellent, although some of the products were reported by the volunteers to have slightly different sensory properties than normally perceived. Nevertheless, these differences in the sensory properties (taste and texture) did not compromise the consumption of these products as reflected by our dietary records and interviews. Of the forty volunteers enrolled into the study, one person dropped out because of job commitments requiring travel outside the Maastricht area, and a second volunteer was excluded after he was prescribed medication for treatment of gout by his physician.

Dietary composition and compliance

The results of the dietary history interviews conducted by the dietitians at the start of the run-in period reflect the habitual dietary consumption patterns of the volunteers. Results are shown in Table 2. The results of dietary history interviews conducted during the 5th week of the palm oil and control periods respectively are also given in Table 2. Mean dietary energy (MJ/d) and fat energy % (en %) were comparable between volunteers

Table 2. *Nutrient intake immediately before (habitual) and during the replacement of dietary fat with palm oil as calculated from repeated dietary histories**
(Mean values with their standard errors for thirty-eight subjects)

Variable	Habitual		Palm oil (PO)		Control (CT)		(PO − CT)		Statistical significance of difference: P_2
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Energy (MJ/d)	13·3	0·43	14·6	0·44	14·4	0·48	0·1	0·16	NS
Fat energy (%)	39·2	0·81	41·0	0·78	41·1	0·69	−0·1	0·36	NS
Fat energy, PO (%)	—		28·7	0·55	NIL		—		—
PUFA (% energy)	7·0	0·28	6·8	0·20	6·9	0·21	−0·2	0·14	NS
SFA (% energy)	16·7	0·39	17·2	0·33	16·3	0·28	0·9	0·21	< 0·0001
MUFA (% energy)	15·5	0·45	17·1	0·38	17·8	0·37	−0·8	0·23	0·001
P:S ratio	0·40	0·02	0·40	0·01	0·43	0·01	−0·04	0·01	0·001

PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; P:S, polyunsaturated:saturated fatty acids; NS, not significant.
* For details of subjects and procedures, see Table 1 and pp. 678–682.

consuming the palm-oil and control diets, but were increased in comparison with the habitual diet. However, this did not result in significant changes in body-weight during the study (palm oil −0·1 (SE 0·14) kg, control −0·3 (SE 0·18) kg; within-group and between-groups differences were not significant). Differences in the energy derived from the various fatty acids were also evident from these dietary interviews. Palm-oil consumption resulted in a higher intake of energy from the saturates in comparison with both the control and habitual diets ($P_2 < 0·0001$). A significant difference in the energy derived from polyunsaturates was, however, not evident. Subsequently, the P:S ratio of the diet during the palm-oil period was significantly lower ($P_2 = 0·001$) than that of the diets during the control and run-in periods.

From duplicate-portion analysis it appeared that during the palm-oil period the diet contained 120 (SE 5·8) g fat/d, which was equivalent to 40·5 (SE 1·4)% of the daily energy intake of which 28·7 (SE 0·55)en % was derived directly from palm oil. A slightly lower fat consumption (113 (SE 6·8) g/d) was observed during the control period, accounting for 39·4 (SE 1·5)% of the daily energy intake. These differences in fat content and fat-en % were not significant between dietary treatments. Cholesterol intake (mg/d) determined from these double portions was significantly lower ($P_2 = 0·004$) during the palm-oil diet (193 (SE 15); n 35) than the control diet (259 (SE 24); n 34).

Energy (MJ) estimated from the dietary histories was higher than that determined from the duplicate portions during both dietary periods. There was a poor correlation between the two methods for the estimation of daily energy intake. However, when fat-en % was considered, the values determined by the dietary histories (X 40·23 (SE 0·52) en %) and the duplicate portions (Y 39·92 (SE 1·01) en %) were correlated significantly: $Y = 16·80 + 0·575\ X$, r 0·30, n 77, $P = 0·008$).

Fatty acid composition of the duplicate portions (Table 3) collected over a 48 h period showed significant differences between the palm-oil and control diets. Indeed all fatty acids except $C_{12:0}$, $C_{18:1(n-9)}$, $C_{20:0}$ and the minor fatty acids (less than 0·1 g/100 g total fatty acids) were significantly different between treatments. In these duplicate-portion analyses, neither the sum of the PUFA nor the MUFA were significantly different between treatments. However, the palm-oil diet resulted in a higher SFA content ($P_2 < 0·0001$) than

Table 3. *Fatty acid composition (g/100 g total fatty acids) of lipids extracted from duplicate portions collected over 48 h period for subjects given the control diet or the diet in which dietary fat had been replaced by palm oil**

(Mean values with their standard errors; no. of subjects in parentheses)

Fatty acid	Palm oil (36) Mean	Palm oil (36) SEM	Control (38) Mean	Control (38) SEM	Statistical significance of difference: P_2
10:0	0·2	0·02	0·4	0·04	< 0·0001
12:0	1·8	0·21	2·0	0·21	NS
14:0	2·4	0·11	4·7	0·25	< 0·0001
15:0	0·1	0·02	0·4	0·03	< 0·0001
16:0	28·6	0·67	20·8	0·64	< 0·0001
16:1($n-7$)	1·4	0·08	2·7	0·12	< 0·0001
17:0	0·3	0·01	0·6	0·03	< 0·0001
18:0	6·9	0·17	8·6	0·20	< 0·0001
18:1($n-9$)	38·7	0·37	37·2	0·66	NS
18:2($n-6$)	14·3	0·59	12·7	0·60	0·02
20:0	0·5	0·03	0·6	0·06	NS
18:3($n-3$)	2·0	0·09	2·6	0·14	< 0·0001
20:1($n-9$)	0·8	0·05	1·8	0·13	< 0·0001
22:0	0·2	0·03	0·4	0·03	< 0·0001
22:1($n-9$)	0·2	0·03	0·6	0·07	< 0·0001
SFA	41·1	0·61	38·6	0·88	0·002
PUFA	16·4	0·63	15·4	0·67	NS
MUFA	41·1	0·44	42·3	0·56	NS
P:S ratio	0·4	0·02	0·4	0·03	NS
($n-6$):($n-3$) ratio	7·4	0·37	5·1	0·28	< 0·0001

SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; P:S, polyunsaturated:saturated fatty acids; NS, not significant.

* For details of subjects and procedures, see Table 1 and pp. 678–682.

the control diet. Nevertheless, these shifts in the different fatty acid classes did not cause significant differences in the calculated P:S ratio. A higher ($n-6$):($n-3$) fatty acid ratio was evident on the palm-oil diet.

Fatty acid composition of serum TG is reported in Table 4. Diet-induced changes in these fatty acid compositions were evident. Both palmitic ($P_2 < 0·0001$) and oleic ($P_2 = 0·02$) acids in serum TG were significantly elevated due to the palm-oil diet compared with the control diet. On the other hand, the palm-oil diet caused significant reductions in the serum TG fatty acids, 16:1 ($n-7$), 17:0, 18:2 ($n-9$), 18:3 ($n-3$), 20:1 ($n-9$) and 21:0, relative to the control diet. The sum of serum TG-SFA was significantly increased ($P_2 = 0·01$) on the palm-oil diet in comparison with the control diet, whereas no significance was detected in the sum of the PUFA, although a tendency of the PUFA to be elevated after the control diet was noted.

Serum TC, HDL-C and TG

Initial values (week zero) of the volunteers enrolled in the study (n 38) were as follows (mmol/l); serum TC 5·11 (SE 0·11), HDL-C 1·13 (SE 0·03), serum TG 1·028 (SE 0·056). For these variables there was hardly any diet-induced difference between the palm-oil and control diets respectively: serum TC (4·94 (SE 0·12), 4·92 (SE 0·12); HDL-C (1·15 (SE 0·04), 1·11 (SE 0·04); serum TG (0·962 (SE 0·055), 1·007 (SE 0·056)).

Table 4. *Fatty acid composition (g/100 g total fatty acids) of serum triacylglycerols after dietary treatments for subjects given the control diet or the diet in which dietary fat had been replaced by palm oil**

(Mean values with their standard errors; no. of subjects in parentheses)

Fatty acid	Palm oil (38)		Control (38)c		Statistical significance of difference: P_2
	Mean	SEM	Mean	SEM	
14:0	1·0	0·08	1·3	0·13	0·01
16:0	23·2	0·28	20·9	0·42	< 0·0001
16:1($n-7$)	4·5	0·17	5·4	0·17	< 0·0001
17:0	0·6	0·05	0·8	0·05	0·0007
18:0	3·2	0·17	3·3	0·14	NS
18:1($n-9$)	41·4	0·38	40·1	0·46	0·02
18:2($n-9$)	0·3	0·06	0·8	0·08	< 0·0001
18:2($n-6$)	17·9	0·51	18·2	0·69	NS
18:3($n-6$)	0·4	0·04	0·5	0·05	NS
20:0	0·1	0·02	0·2	0·03	NS
18:3($n-3$)	1·7	0·06	2·1	0·09	< 0·0001
20:1($n-9$)	0·5	0·02	0·7	0·05	0·0005
21:0	0·2	0·08	0·4	0·12	0·04
20:3($n-9$)	0·2	0·05	0·3	0·06	NS
20:3($n-6$)	0·2	0·02	0·2	0·03	NS
20:4($n-6$)	1·2	0·06	1·2	0·05	NS
20:5($n-3$)	0·3	0·04	0·3	0·03	NS
24:0	0·1	0·06	0·1	0·06	NS
22:4($n-6$)	0·2	0·03	0·2	0·02	NS
22:5($n-3$)†	0·4	0·03	0·5	0·03	NS
22:6($n-3$)	0·6	0·06	0·7	0·05	NS
SFA	28·5	0·37	27·1	0·48	0·01
PUFA	23·6	0·60	25·0	0·80	NS
MUFA	46·4	0·41	46·2	0·52	NS
($n-6$):($n-3$) ratio	8·4	0·29	7·2	0·33	0·004
SCFA	1·0	0·08	1·4	0·15	0·01

SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SCFA, shorter-chain fatty acids (10:0+12:0+14:0); NS, not significant.

* For details of subjects and procedures, see Table 1 and pp. 678–682.

† May contain some 24:2($n-6$) fatty acid.

Cholesterol and TG contents of serum lipoproteins

Cholesterol values for serum and lipoprotein fractions at the completion of the palm-oil and control treatments are given in Table 5. For most of the lipoprotein fractions, cholesterol levels were essentially similar after the palm-oil and control treatments. However, palm-oil consumption resulted in a significant increase (9·7%; $P_2 = 0·01$) in the HDL_2-C compared with the control treatment.

The ratios of cholesterol in various lipoprotein classes were calculated to provide a better indicator of the dietary effect (Table 6). Compared with the control treatment, the palm-oil diet caused a significant reduction (7·2%; $P_2 = 0·02$) in the LDL-cholesterol (LDL-C):HDL_2+HDL_3 (HDL_{2+3})-C ratio. A similar statistically-significant reduction (7·6%; $P_2 = 0·02$) in the IDL-cholesterol (IDL-C)+LDL-C:HDL_{2+3}-C ratio was again evident on the palm-oil diet compared with the control diet. The TC:HDL_{2+3}-C ratio, although lower in the palm-oil diet than the control diet, did not attain significance.

Table 5. *Serum total (TC) and lipoprotein-cholesterol levels (mmol/l) before and after dietary treatments for subjects given the control diet or the diet in which dietary fat had been replaced by palm oil**

(Mean values with their standard errors; no. of subjects in parentheses)

	Initial (38)		Palm oil (PO) (37)		Control (CT) (38)		(PO−CT) (37)		Statistical significance of difference:
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P_2
Serum TC	5·11	0·11	4·94	0·11	4·92	0·12	0·02	0·10	NS
VLDL	0·42	0·03	0·36	0·03	0·38	0·03	−0·03	0·03	NS
IDL	0·29	0·03	0·23	0·02	0·25	0·02	−0·02	0·02	NS
LDL	3·04	0·10	2·84	0·10	2·91	0·11	−0·10	0·08	NS
HDL_1	0·20	0·02	0·19	0·01	0·17	0·01	0·01	0·01	NS
HDL_2	0·31	0·02	0·31	0·02	0·28	0·02	0·03	0·01	0·01
HDL_3	0·83	0·02	0·81	0·02	0·78	0·02	0·03	0·02	NS
Bf	0·19	0·01	0·17	0·01	0·16	0·01	0·01	0·01	NS
Recovery (%)	102·9	0·89	98·83	0·51	100·25	0·67	−1·37	0·87	NS

VLDL, very-low-density-lipoprotein; IDL, intermediate-density-lipoprotein; LDL, low-density-lipoprotein; HDL, high-density-lipoprotein; Bf, bottom fraction; NS, not significant.

* For details of subjects and procedures, see Table 1 and pp. 678–682.

Table 6. *Effect of replacement of dietary fat with palm oil on the cholesterol ratios of serum (TC) and lipoprotein fractions of volunteers**

(Mean values with their standard errors; no. of subjects in parentheses)

	Palm oil (37)		Control (38)		Statistical significance of difference:
	Mean	SEM	Mean	SEM	P_2
$LDL:(HDL_2+HDL_3)$	2·69	0·16	2·90	0·17	0·02
$(IDL+LDL):(HDL_2+HDL_3)$	2·91	0·17	3·15	0·18	0·02
$TC:(HDL_2+HDL_3)$	4·67	0·22	4·87	0·21	NS
$HDL_2:HDL_3$	0·38	0·03	0·36	0·02	NS
$HDL_2:LDL$	0·120	0·012	0·105	0·010	0·01

LDL, low-density-lipoprotein; HDL, high-density-lipoprotein; IDL, intermediate-density-lipoprotein; NS, not significant.

* For details of subjects and treatments, see Table 1 and pp. 678–682.

TG values for serum and lipoprotein fractions at the end of the palm-oil and control treatments are represented in Table 7. Compared with the control treatment, the palm-oil diet showed a tendency to lower TG in all lipoprotein fractions except the bf. In comparison with the observed values at the completion of the control treatment, the reduction of TG after the palm-oil treatment was significant (9·3%; $P_2 = 0·01$) in the LDL fraction.

Serum apolipoproteins

Serum apolipoproteins at week zero (initial values) and after the dietary periods are given in Table 8. After volunteers consumed the palm-oil diet, apolipoprotein AI was significantly higher ($P_2 = 0·008$) whereas the apolipoprotein B was significantly lower ($P_2 = 0·01$) relative to the values after the control diet. A diet-induced effect was not evident on

Table 7. *Effect of replacement of dietary fat with palm oil on serum and lipoprotein triacylglycerols (mmol/l) of volunteers**
(Mean values with their standard errors; no. of subjects in parentheses)

	Initial (38)		Palm oil (PO) (37)		Control (CT) (38)		(PO − CT)		Statistical significance of difference:
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P_2
Serum	1·028	0·056	0·962	0·055	1·007	0·056	−0·046	0·047	NS
VLDL	0·647	0·047	0·585	0·049	0·606	0·046	−0·031	0·042	NS
IDL	0·060	0·004	0·051	0·003	0·065	0·009	−0·015	0·008	NS
LDL	0·159	0·008	0·137	0·005	0·151	0·007	−0·012	0·005	0·01
HDL_1	0·015	0·002	0·013	0·001	0·014	0·001	−0·001	0·001	NS
HDL_2	0·025	0·002	0·023	0·002	0·024	0·002	−0·001	0·002	NS
HDL_3	0·070	0·004	0·068	0·003	0·070	0·004	−0·002	0·004	NS
Bf	0·037	0·003	0·046	0·003	0·040	0·002	0·005	0·003	NS
Recovery (%)	98·49	1·06	94·85	0·61	95·71	0·76	−0·70	0·83	NS

VLDL, very-low-density-lipoprotein; IDL, intermediate-density-lipoprotein; LDL, low-density-lipoprotein; HDL, high-density-lipoprotein; Bf, bottom fraction; NS, not significant.
* For details of subjects and procedures, see Table 1 and pp. 678–682.

Table 8. *Effect of replacement of dietary fat with palm oil on serum apolipoproteins (g/l) and their calculated ratios for volunteers**
(Mean values with their standard errors; no. of subjects in parentheses)

	Initial (38)		Palm oil (PO) (37)		Control (CT) (38)		(PO − CT) (37)		Statistical significance of difference:
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P_2
Apo AI	1·40	0·22	1·45	0·26	1·40	0·26	0·05	0·18	0·008
Apo AII	0·63	0·14	0·64	0·12	0·64	0·013	0	0·7	NS
Apo B	0·95	0·27	0·94	0·30	0·99	0·29	−0·04	0·20	0·01
Apo E (%)	116·0	2·8	112·0	2·6	116·0	3·2	−4·0	2·4	NS
Apo B:Apo AI	0·69	0·024	0·66	0·027	0·72	0·028	−0·06	0·014	< 0·0001
Apo B:Apo E	0·83	0·024	0·85	0·026	0·86	0·026	−0·01	0·026	NS
LDL-C:Apo B	1·25	0·027	1·16	0·026	1·14	0·023	0·01	0·027	NS
HDL-C:Apo AI	0·31	0·007	0·30	0·008	0·29	0·006	0·01	0·008	NS

LDL-C, low-density-lipoprotein-cholesterol; HDL-C, high-density-lipoprotein-cholesterol: NS, not significant.
* For details of subjects and procedures, see Table 1 and pp. 678–682.

apolipoproteins AII and E levels. As a consequence of these significant changes in the apolipoproteins, the apolipoprotein B:AI ratio was significantly decreased ($P_2 < 0·0001$) when compared with the control diet. The apolipoprotein B:E ratio was, however, not significantly affected by the dietary treatments.

The apo E phenotypes of our subjects were also characterized in an attempt to examine the effect of phenotype on changes induced by our test and control diets. Of the thirty-eight volunteers in the present study, eleven had the apo E 3/2 phenotype, twenty-two the apo E 3/3 phenotype and the remaining five had the apo E 4/3 phenotype. Serum and lipoprotein cholesterol at the end of week zero (initial values) were re-assigned according to the three phenotypes (Table 9). Differences between phenotypes were tested using the

Table 9. *Initial levels of serum (TC) and lipoprotein-cholesterol (mmol/l) of the volunteers* according to apo E phenotypes (phE)*
(Mean values with their standard errors; no. of subjects in parentheses)

	phE 3/3 (22)		phE 3/2 (11)		phE 4/3 (5)	
	Mean	SEM	Mean	SEM	Mean	SEM
TC	5·19	0·14	4·70[a]	0·20	5·64[a]	0·15
VLDL	0·45	0·04	0·41	0·04	0·36	0·09
IDL	0·29	0·04	0·31	0·04	0·26	0·03
LDL	3·08[b]	0·13	2·65[c]	0·12	3·71[b, c]	0·08
HDL_1	0·22	0·02	0·18	0·02	0·14	0·02
HDL_2	0·34	0·03	0·30	0·03	0·24	0·03
HDL_3	0·86	0·02	0·75	0·06	0·84	0·08
Bf	0·19	0·01	0·20	0·02	0·23	0·04

[a, b, c] Mean values with the same superscript letter were significantly different (Bonferroni adjusted *t* test): [a, b] $P_2 = 0·03$; [c] $P_2 = 0·0009$.
VLDL, very-low-density-lipoprotein; IDL, intermediate-density-lipoprotein; LDL, low-density-lipoprotein; HDL, high-density-lipoprotein; Bf, bottom fraction.
* For details, see Table 1 and pp. 678–682.

Bonferroni-adjusted *t* test. TC was lowest in phE 3/2 and highest in phE 4/3 ($P_2 = 0·03$). However, serum TC did not differ significantly between phenotypes 3/3 and 4/3 nor between 3/3 and 3/2. Similarly, LDL-C was lowest in phE 3/2, intermediate in phE 3/3 and highest in phE 4/3. These differences in LDL-C were significant for 3/2 *v.* 4/3 ($P_2 = 0·0009$) and 3/3 *v.* 4/3 ($P_2 = 0·03$). An extensive analysis of all data failed to establish any relationship between these apo E phenotypes and the effect of dietary treatments.

DISCUSSION

Through our product development techniques we were able to maximize the use of palm oil in a European type of diet. This maximization was achieved without major changes in the normal dietary consumption patterns of the volunteers with an adequate acceptance of the products.

Palm oil has a P:S ratio of 0·2 with an almost equal distribution of SFA and unsaturated fatty acids (Sundram *et al.* 1990*b*). In the present study, replacement with palm oil, calculated on the basis of several compliance indicators, was almost 70% of the daily fat intake during the experimental period. In spite of this unusually high replacement with palm oil, the overall change in dietary P:S ratio was very nominal, with the P:S ratio varying between 0·43 during the habitual diet and 0·40 during the palm oil period. This P:S ratio is well within the range normally reported for The Netherlands (Ministry of Welfare, Health and Cultural Affairs and Ministry of Agriculture and Fisheries, The Netherlands, 1988). However, the percentage energy from $C_{14:0}$ was halved (1·92 *v.* 0·98) even as the percentage energy from $C_{16:0}$ was increased from 8·5 to 11·7%.

The fatty acid composition of the duplicate portions was intended to reflect the distribution of individual fatty acids in the volunteers' diets and to act as a measure of compliance. This was achieved, as seen from the fatty acid compositional analysis. Differences in the major fatty acids of the palm-oil and control diets were obviously caused by the various products obtained for consumption from the 'shop' by the volunteers. For example, we have previously reported a higher content of shorter-chain fatty acids in the

control dairy products (Sundram *et al.* 1990*a*). This is again reflected in the duplicate-portion analysis wherein the sum of $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$ fatty acids is almost 38% higher in the control duplicate portions than that of the palm-oil duplicate portions. Similarly, palmitic acid, a major component fatty acid of the palm-oil-based products, was significantly higher in duplicate portions of the palm-oil diet than that of the control diet. The content of stearic and oleic acids were almost reflections of the amounts of these fatty acids normally expected in palm oil (Sundram *et al.* 1990*b*). These fatty acid analyses, therefore, proved to be a useful compliance check on the fat consumption patterns of our volunteers, who consumed the specially-produced food products in a free-living environment.

The fatty acid composition of serum TG proved to be a reasonable compliance check on the diets consumed by our volunteers, when comparisons were made to the fatty acid composition of the duplicate portions. This observation is further reinforced since both serum and duplicate portions were sampled during each dietary period. A significant elevation in palmitic acid content in both serum TG and duplicate-portion fatty acids, as a result of the palm-oil diet, reflects adherence to this diet by our volunteers. This observation is further enhanced by our reported fatty acid composition of the palm-oil food products themselves (Sundram *et al.* 1990*a*), wherein a consistently higher palmitic acid content was evident. In the control duplicate portions a significantly higher level of 14:0, 16:1($n-7$), 17:0 and 20:1($n-9$) fatty acids was observed. These fatty acids are also significantly elevated in the serum TG of the volunteers during the control diet relative to the palm-oil diet. As further evidence of compliance, the SFA and ($n-6$):($n-3$) fatty acid ratio showed a similar tendency to be significantly elevated in both the serum TG and duplicate-portion fatty acids during the palm-oil diet.

Serum TC was essentially similar at the completion of both the palm-oil and control treatments. Nevertheless both IDL-C and LDL-C tended to be lower after the palm-oil treatment relative to the controls. Traditionally LDL is represented by the $\rho 20$ 1·006–1·063 fraction. However, even after taking this into consideration, the sum of the IDL-C and LDL-C after the palm-oil treatment was still marginally lower than that of the control diet.

These observations seem contradictory to the findings of several workers (Laine *et al.* 1982; Baudet *et al.* 1984; Mattson & Grundy, 1985; Grundy & Vega, 1988) who reported significantly higher levels of both serum and LDL-C resulting from a palm-oil diet, high in palmitic acid. However, these comparisons were performed against dietary compositions that contained high amounts of either PUFA or MUFA. In one study (Bonanome & Grundy, 1988) the serum cholesterol content was reported to be lower on a high-stearic acid diet compared with a palm-oil diet. Our study was performed in a population whose habitual P:S ratio was 0·43. Maximal substitution with palm oil changed the P:S ratio to 0·40, while that of the control was 0·43. In themselves these changes were minimal, but we observed a shift in the type of SFA contributed by these diets: the palm-oil diet had a significantly higher content of palmitic acid (11·7 *v.* 8·5 en %), whereas the control diet had higher levels of $C_{12:0}+C_{14:0}$ and $C_{18:0}$ fatty acids. Since stearic acid is considered neutral, the real comparison lies between $C_{12:0}+C_{14:0}$ and $C_{16:0}$.

The findings of Keys *et al.* (1965*a*, *b*) and Hegsted *et al.* (1965) indicate that not all SFA raise cholesterol to the same extent. Bonanome & Grundy (1988) confirmed earlier findings (Keys *et al.* 1965*a*, *b*) that stearic acid is neutral in its cholesterol-raising potential compared with palmitic acid. Therefore, the true cholesterol-raising effect of the SFA is constrained in fatty acids $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$. In the present study the palm-oil diet resulted in an increase of almost 3·2 en % in $C_{16:0}$, 1·0 en % reduction in $C_{12:0}+C_{14:0}$ and 0·7 en % reduction in $C_{18:0}$ in comparison with the control diet (see Tables 2 and 3). Palm-oil consumption did not significantly affect serum TC. If stearic acid is considered neutral,

the cholesterol-modifying effect of the 1 en % decrease in $C_{12:0}+C_{14:0}$ was compensated by that of the 3·2 en % increase in $C_{16:0}$. On this assumption, the cholesterol-increasing effect of $C_{16:0}$ was about one-third that of $C_{12:0}+C_{14:0}$. This observation is in the same direction as the recent findings of Hayes *et al.* (1991) in non-human primates, wherein the hypercholesterolaemic effect of SFA was primarily due to $C_{12:0}+C_{14:0}$ and not to $C_{16:0}$.

The diets in the present study revealed a modest difference (66 mg/d) in their cholesterol content. However, Hegsted *et al.* (1965) and Anderson *et al.* (1976) demonstrated that the effect of dietary $C_{14:0}$ and $C_{16:0}$ on serum cholesterol was independent of the cholesterol content in the diet. In addition, McNamara (1990) pointed out that the serum cholesterol response to changes in dietary cholesterol intake is negligible, and averages 0·05 mmol/l for each 100 mg/d change in dietary cholesterol intake. On this basis, the difference in dietary cholesterol intake between the palm-oil and control diets (66 mg/d) could be expected to account for a difference in serum cholesterol response equivalent to 0·03 mmol/l. Therefore, the difference in dietary cholesterol in our diets would appear to be negligible.

HDL-C tended to be raised after the palm-oil diet in comparison with the control diet. This tendency occurred without a concurrent significant elevation in either serum TC or LDL-C. HDL_3-C representing approximately 70% of the HDL-C in our subjects was not altered significantly by the diets. Epidemiological studies (Rhoads *et al.* 1976; Gordon *et al.* 1977) have established an inverse correlation between coronary heart disease and the concentration of HDL-C. Results by Miller *et al.* (1981) suggest that HDL_2-C concentration may be a better reflection of this protective effect than those of HDL-C or HDL_3-C. The significant increase in HDL_2-C without compensatory increases in both serum TC and LDL-C, as observed during the palm-oil diet, is provocative especially since palm oil contains almost 50% of its fatty acid composition as saturates. The mechanism of this effect on the HDL_2 subfraction needs to be investigated.

Miller *et al.* (1981) also reported that the best discriminator between subjects with severe coronary disease and those with minimal coronary disease was the HDL_2-C:cholesterol content in the ρ 1·019–1·063 fraction, which also corresponds to our reported LDL-C measurements. Notably, the calculated HDL_2-C:LDL-C ratio in the present study is higher on the palm-oil diet (0·120 (SE 0·012)) than on the control diet (0·105 (SE 0·010)), which was significant ($P_2 = 0·01$). The calculated cholesterol ratios for LDL:HDL_{2+3} and (IDL+LDL):HDL_{2+3} were significantly lowered on the palm-oil diet. These lipoprotein ratios are good indicators of the atherogenic potential of the diet (Naito, 1985).

In the present study, serum TC following the palm-oil diet was comparable with that after the control diet, whereas a marginal decline in serum TG was observed. Furthermore, palm-oil feeding resulted in a tendency to lower cholesterol values in the IDL and LDL fractions. Similarly, TG in the IDL fraction tended to be lowered ($P_2 = 0·08$), whereas the LDL-TG concentration was significantly lower after the palm-oil diet relative to the control diet. This overall trend, especially in relation to the IDL and LDL fractions which are considered atherogenic mediators (Tatami *et al.* 1981), indicates that the consumption of palm oil does not encourage a disposition to increasing the risk of atherosclerosis in normal healthy subjects.

The response of apolipoproteins to manipulations in the diet has been previously examined (Fisher *et al.* 1983; Zanni *et al.* 1987; Denke & Breslow, 1988). There are, however, no reported values of apolipoproteins after a palm-oil diet. Our results demonstrate that palm-oil maximization in a Dutch-type diet causes a significant increase in apolipoprotein AI while reducing apolipoprotein B. This is in spite of the little change in the overall P:S fatty acid ratio between the palm-oil and control diets. These observations after the palm-oil diet lead to a significant reduction of the apolipoproteins B:AI ratio compared with the control diet. Mean apolipoprotein AI:AII ratio increased

in volunteers consuming the palm-oil diet, which suggests that HDL_2 may have increased relative to HDL_3. This is emphasized by our observation that HDL_2-C in palm-oil-consuming volunteers was significantly increased relative to the control diet.

The present study indicates that when dietary palm oil, rich in palmitic acid ($C_{16:0}$), replaced a substantial portion of the habitual fat content in a Dutch-type diet the serum TC and TG concentrations were unaffected, but the LDL:HDL_{2+3} and apolipoprotein B:AI ratios were favourably reduced. These changes may be related to the observed shift in the SFA ratio $C_{16:0}$:($C_{12:0}+C_{14:0}$) during the palm-oil diet. Nevertheless, the effect of minor components present in palm oil, although present in small quantities but having potent biological activity, cannot be ruled out. Specific studies must, therefore, be designed to evaluate these effects.

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