Original Article

Effect of palm olein oil in a moderate-fat diet on plasma lipoprotein profile and aortic atherosclerosis in non-human primates

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Several studies have reported on the effect of palm olein oil (PO; palmitic acid content ~38%) incorporation into the diet on blood cholesterol concentration. Information on the effect of PO on atherosclerosis is, however, lacking. In vervet monkeys (*Cercopithecus aethiops*), low-density lipoprotein cholesterol (LDL-C) concentrations can be modulated by the type and amount of fat in the diet. The vervet is a proven model for both the type and composition of human atherosclerotic lesions. The aim of this study was to determine the effect of PO in a moderate-fat moderate-cholesterol diet (MFD) on plasma lipoproteins and the progression of atherosclerosis in a non-human primate model after 25.5 months of dietary exposure. Thirty adult male vervets, never exposed to a Western-type atherogenic diet, were stabilised on a MFD (28%E fat; 26 mg cholesterol/1000 kJ) with a polyunsaturated to saturated fatty acid (P/S) ratio of 0.4 for six weeks. Baseline LDL-C, high-density lipoprotein (HDL)-C and bodyweight were used to stratify the vervets into three comparable groups of 10 each. One group continued with the MFD in which 11.0%E was derived from lard (AF). In the other two groups, the AF was substituted isocalorically with either sunflower oil (SO) or PO. Plasma lipids were measured at 6-monthly intervals and atherosclerosis was assessed in the aorta and in five peripheral arteries after 25.5 months of dietary exposure. The frequency of atherosclerosis in peripheral arteries and aortas was low. PO, relative to SO and AF, significantly reduced the risk for developing early lesions in peripheral arteries ($P = 0.0277$ and $P = 0.0038$, respectively) and, relative to AF, in aortas ($P = 0.0335$). The cholesterolaemic effect of MFD-PO was not significantly different from MFD-SO and MFD-AF. However, at 24 months the plasma total cholesterol concentration with MFD-AF was significantly higher than with MFD-SO ($P = 0.0256$). It is confirmed that a MFD with PO is no different from AF or SO in its cholesterolaemic effect. The anti-atherogenic efficacy of a MFD with PO, relative to SO and AF, was demonstrated in a non-human primate model of atherogenesis.

Key words: aorta, atherosclerosis, lard, moderate-fat moderate-cholesterol diet, non-human primate, palm olein oil, peripheral artery, plasma lipid, sunflower oil.

Introduction

Dietary fat and cholesterol affect plasma low-density lipoprotein cholesterol (LDL-C) concentrations. Whereas a high LDL-C concentration is associated with an increased risk of coronary heart disease in humans, high-density lipoprotein cholesterol (HDL-C) concentrations show a negative association.[1] The cholesterol-raising property of saturated fat, which is implicated as a risk factor in hypercholesterolaemia and cardiovascular disease,[2] is generally attributed to myristic and palmitic acids. Although palmitic acid is the most abundant saturated fatty acid in the diet, its effect on plasma cholesterol concentration appears to depend on the cholesterol content of the diet.[3,4]

Oil of palm is a major source of the world's supply of oils and fats. Its palmitic acid content (~43%) constitutes the bulk of the total saturated fraction of ~49%. Several studies investigated the cholesterolaemic effect of diets containing palm oil.[5–13] Based on re-evaluation of accumulating data, it appears that in normocholesterolaemic humans when dietary cholesterol intake is ≤300 mg/day, palmitic acid minimally affects cholesterol concentrations.[3,4] Palmitic acid appears to increase plasma cholesterol concentrations only when cholesterol intake is >400 mg/day, or when hypercholesterolaemic humans are studied.[3,4]

In contrast to several reports on the cholesterolaemic effect of palm oil, information on its effect on atherosclerosis *per se* is lacking. Atherosclerosis is a multifactorial disease; its progression is slow and it develops over many decades starting silently with intra- and extracellular accumulations of lipid, derived mainly from LDL, in the intimas of arteries. Human atherosclerotic lesions are defined according to microscopic and chemical composition and are classified into eight lesion types from initial to advanced complicated lesions.[14–16] Early lesion types I to III and early advanced

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type IV (atheroma) rarely account for symptomatic obstruction of arteries and are therefore clinically silent. Further progression to the more advanced 'unstable' type VI (complicated fibroatheroma), is described as the conversion from clinically silent to overt disease. Advanced lesion types V (fibroatheroma), VII (calcified) and VIII (fibrous) may be silent or overt depending on the degree of stenosis they cause.

Atherosclerotic lesions in adult African Green or vervet monkeys not only resemble that of humans[17,18] but it was recently confirmed that this non-human primate models lesion types I–VII[19] according to human lesion-type classifications.[14–16] Their cholesterolaemic response to diet is similar to that of humans, but hypertriglyceridaemia does not develop.[17–25]

Because there is a lack of information on the effect of palm oil on atherosclerosis, the aim of the present study was to determine the effect of palm olein oil in a moderate-fat, moderate-cholesterol diet on plasma lipoproteins and the progression of atherosclerosis in this non-human primate model (vervet monkey).

Materials and methods

Non-human primates

Thirty adult male African Green Monkeys (*Cercopithecus aethiops*), also called vervets, were used in this study. Male vervets were chosen because they model atherosclerosis and hypercholestrolaemia better than female vervets.[17–20] Physiological variation is also minimised by using adult males.

The acquisition of the vervets was described in detail previously.[26] Briefly, 15 male vervets were recruited from the MRC Primate Unit's in-house breeding colony. Their average age was 6.6 ± 2.4 years (range 4–10). From weaning, they received the regular high-carbohydrate (~75% energy (E)) maintenance diet.[20] The additional 15 males were caught with permission from Nature Conservation. They were first quarantined and then conditioned for 3.5 months to their new environment and high-carbohydrate maintenance diet. The 15 wild-caught vervets were estimated to be more than 4 years of age. The average bodyweight of the 30 vervets at baseline was 5.39 ± 0.92 kg (range 3.60–7.00). The vervets were housed permanently in a room within a closed unit. Individuals were kept in stainless steel cages and had access to a large exercise cage for 24 h a week. Olfactory, auditory and visual contacts with peers were not restricted. Soft tap water was available *ad libitum*. All procedures carried out with vervets, handling and care, were always by the same qualified persons.

Experimental design

The experimental design is shown in Fig. 1. The 30 vervets were stabilised for 6 weeks on a moderate-fat (28%E) moderate-cholesterol (26 mg cholesterol/1000 kJ) diet (MFD) with a polyunsaturated to saturated fatty acid ratio (P/S) of 0.4. Fasting blood samples were collected at baseline and LDL and HDL cholesterol concentrations, bodyweight and vervet origin (colony-bred or wild-caught) were used to stratify the vervets into three comparable experimental groups of 10 each. One group continued with the MFD in which 11.0%E was derived from lard (MFD-AF). In the other two groups, the lard was substituted isocalorically with either sunflower oil (SO) (MFD-SO) or palm olein oil (PO) (MFD-PO).

The three groups were fed the respective experimental diets for 24 months and fasting blood samples were collected at 6-monthly intervals (6, 12, 18 and 24 months) for lipid analysis.

Composition of diets

The MFD consisted of skimmed milk powder, egg powder, precooked maize meal and maize kernels. The main sources of fat were beef tallow as well as the experimental fat (either lard, SO or PO). No extra synthetic cholesterol was added to the diets. The diets were supplemented daily with minerals and vitamins to optimise the micronutrient intake of the vervets. Water was added to the ingredients, mixed to a stiff porridge and formed into food patties. Vervets were fed one patty in the morning and one in the afternoon to meet the energy and nutrient prescription. In addition, a piece of raw apple (~70 g) was supplied daily to each vervet. The suppliers of the eggs, beef tallow, lard, maize and fruit were kept constant.

Refined, bleached, deodorised (RBD) palm olein oil was supplied by the Malaysian Palm Oil Board (Selangor, Malaysia). Refined and deodorised pure sunflower oil (SOMOL brand) was purchased from a local supplier (Cape Oil Products, Cape Town, South Africa). Lard consisted mainly of 23.5% palmitic, 11.9% stearic, 41.8% oleic and 16.4% linoleic acids (% by weight as determined with gas-liquid chromatography). The major fatty acids in beef tallow were 27.3% palmitic, 22.7% stearic, 43.3% oleic and 2.6% linoleic. PO contained 37.9% palmitic, 4.1% stearic, 44.7% oleic and 10.2% linoleic acids. The composition of sunflower oil was 6.0% palmitic, 3.4% stearic, 29.8% oleic and 58.9% linoleic acids.

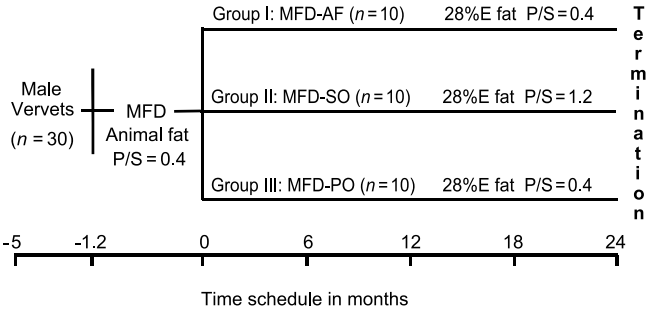

Figure 1. Experimental design to determine the effect of palm olein oil in a moderate-fat, moderate-cholesterol diet on plasma lipoproteins and the progression of atherosclerosis in vervet monkeys. AF, animal fat (lard); E, energy; MFD, moderate-fat diet; PO, palm olein oil; P/S, polyunsaturated to saturated fatty acid ratio; SO, sunflower oil.

The MRC Food Composition tables[27] were used to compile the diets and calculate the energy and nutrient content (Table 1). The total fat, cholesterol and fatty acid content of the diets were checked at regular intervals ($n = 7$) by laboratory analyses of a daily food portion of each diet, as described in detail previously.[26]

Dietary compliance was checked by weighing individual food portions before and the amount of wasted food after the vervets were fed. This was carried out on three consecutive days at regular intervals ($n = 5$).

Plasma lipids and lipoproteins

Isolation of plasma and plasma lipoproteins from fasting blood samples and all methods for measuring concentrations of cholesterol (C), triacylglycerol (TAG), apolipoprotein (apo) AI and LDL apo B were as described previously.[26]

Atherosclerosis

Seven weeks after the 24-month blood sampling, a standard necropsy procedure was followed as described in detail previously.[17–19] The perfusion fixation carried out under surgical anaesthesia prevented agonal intra-arterial blood clots and optimised arterial intimas for microscopy.

The aorta and five peripheral arteries (left common iliac, left subclavian, brachiocephalic, left common carotid and right common carotid) were dissected out. Aortas (with the peripheral arteries still attached) were separated from the heart and opened longitudinally on the ventral midline to the bifurcation of the common iliac arteries. This exposed the entire intima. Peripheral arteries were not opened. Fixation was completed by immersion of peripheral arteries and opened aortas in the buffered formalin solution. Peripherals were then separated from the aortas and preparation for light microscopy and staining for lipid infiltration in aortas were as described previously[17–19] and by standard histological methods. Aortas and peripheral arteries were evaluated without prior knowledge of the treatment pertaining to the specimens.

For the macroscopic evaluation of the total intimal surface, all aortas were stained simultaneously in the same solution of oil red O fat-soluble stain to demarcate lipid infiltration into fatty streaks and plaque. Surface lesions were then demarcated by direct tracing and the areas (measured in percent) free of lesions and of fatty streaks, fatty streak, fatty plaque and fibrous plaque were measured by point counting. Numerical scores were allocated to reflect severity of intimal lipid infiltration (Table 2). An overall intimal surface lesion score for each aorta was obtained by totalling the products of the lesion area times the score.

In contrast to the non-random macroscopic evaluation of the total aortic intimal surface, the evaluation by light microscopy of peripheral arteries and aortas was based on random sampling. An attempt was made to improve the result for aortic atherosclerosis by increasing the sample size from five (previous studies[17–19]) to 10 sections per aorta.

Table 1. Energy content and nutrient composition of the experimental diets per vervet per day

	Moderate-fat diet		
	AF	SO	PO
Energy (kJ)‡	2352	2348	2345
Protein (%E)	12.6	12.7	12.8
Total (g)	17.5	17.6	17.6
Animal (g)	11.3	11.3	11.3
Carbohydrate (%E)	59.2	59.2	59.3
Total (g)	73.3	73.2	73.2
Dietary fibre (g)	8.6	8.6	8.6
Fat (%E)	28.2	28.0	27.9
Total (g)†	17.9 ± 0.8	17.8 ± 1.2	17.7 ± 1.0
% Fat from oil§	39.1	39.3	39.5
Cholesterol (mg)†	60 ± 11	57 ± 8	60 ± 5
Cholesterol (mg/1000 kJ)	26	24	26
Fatty acids (g)†			
Saturated	6.48 ± 0.83	5.31 ± 0.83	6.80 ± 0.85
C14:0	0.26 ± 0.09	0.22 ± 0.09	0.25 ± 0.07
C16:0	3.52 ± 0.57	2.73 ± 0.50	4.44 ± 0.67
C18:0	2.63 ± 0.39	2.25 ± 0.41	2.01 ± 0.38
Monounsaturated	6.36 ± 1.16	6.02 ± 0.87	6.26 ± 0.92
C18:1	6.03 ± 1.09	5.87 ± 0.85	6.10 ± 0.88
Polyunsaturated	3.00 ± 0.47	6.11 ± 0.91	2.60 ± 0.61
C18:2	2.60 ± 0.33	5.95 ± 0.87	2.43 ± 0.56
Trans-fatty acids (g)	0.01	0.01	0.01
P/S ratio†	0.46 ± 0.03	1.16 ± 0.09	0.38 ± 0.06

All values as obtained from the MRC Food Composition tables,[27] except †those that were based on laboratory analyses representing the mean ± SD of seven samples. ‡Energy was calculated by multiplying the weight (g) of fat, protein, and carbohydrate + fibre by 37, 17 and 17 kJ, respectively. §Refers to all oils. AF, lard; E, energy; PO, palm olein oil; P/S, polyunsaturated to saturated fatty acid ratio; SO, sunflower oil.

Random samples for histopathology were achieved by transverse cuts extending across almost the entire aortic surface at 10 predetermined positions, regardless of the presence or absence of macroscopic lesions. The positions were spaced equidistantly from the aortic arch (proximal sites) to the iliac bifurcation (distal sites). The six proximal sites were thoracic and the four distal sites were abdominal. For histopathology of the peripheral arteries the samples were unselected as the intimas were not visible. Transverse sections for microscopy were cut at intervals of approximately 3 mm for the entire available artery length. Because the artery lengths varied slightly between cases, the total number of sections per artery differed. The frequency ratios (i.e., number of sections with atherosclerosis to total sections per artery) were compared between groups.

Microscopy was carried out by a private pathologist who examined coded sections of aortas and peripheral arteries without knowledge of the experimental design. Atherosclerosis of aortas and peripheral arteries was described according to unequivocal morphological components that define the composition and classification of human types I–VIII[14–16] (Table 3) as applied by Fincham *et al.*[19]

Ethics

The study was approved by the Ethics Committee for Research on Animals of the South African Medical Research Council. The MRC's guidelines on ethics for medical research and the national code for animal use in research, education, diagnosis and testing of drugs and related substances in South Africa was followed.

Statistical analysis

Data were analysed with the SAS package for Unix (version 8; SAS Institute, Cary, NC, USA). A repeated-measures analysis of variance was used to model the plasma lipid profile on the experimental factors group, time, group–time interaction, as well as the baseline measurement as covariate (Table 4). When a significant group–time interaction for a specific variable was detected a post hoc multiple comparison, taking the slope of the baseline measurement into account, was carried out for the specific variable at each time interval (6, 12, 18 and 24 months).

The variables of macroscopic aortic atherosclerosis over the entire intimal surface (Table 5) were compared by analysis of variance when data were normally distributed (overall *F*-test). Data were tested for homogeneity of variance by using the Levene test and where there was a difference in variance the Welch test was used to indicate group differences.

The frequency ratios (positive counts) of peripheral and aortic atherosclerosis were compared by applying a binary logistic regression model using generalised estimation equations, logit link functions and an exchangeable working correlation matrix to adjust for clustering within individuals

Table 2. Macroscopic measurements of aortic intimal surface lesions†

Lesion	Characteristics	Numerical score	Stain‡
Lesion-free	Healthy intima with no blemishes	0	Unstained
Fatty streak§	Flat and pale red	0.5	ORO
Fatty plaque¶	Slightly raised; moderately red to distinctly elevated; intensely red	1	ORO
Fibrous plaque††	Non-lipid, raised, tough, nodular, corrugated areas; white and hard when mineralised; distinctly recognizable without staining	2	Unstained

Measurements of lesions are the percentage area of the intimal surface. Adapted from Fincham †*et al.*[17] ‡Conventional ORO staining method, all aortas stained simultaneously in the same solution. Includes lesion types §I, ¶II, III and IV, and ††VII and VIII (see Table 3). ORO, oil red O lipid stain.

Table 3. Classification of atherosclerotic lesion types based on microscopic and chemical composition†

Type	Lesion	Description
I	Initial	Lipoprotein accumulation in intima; lipid in macrophages; these changes discernible only microscopically or chemically; no tissue damage
II	Fatty streak	Lipoprotein accumulation in intima; lipid in macrophages and smooth muscle cells; quantities large enough to be visible to the naked eye but still no tissue damage
III	Pre-atheroma	All type II changes plus multiple deposits of pooled extracellular lipid; microscopic evidence of tissue damage and disorder
IV	Atheroma	All type II changes plus confluent mass of extracellular lipid (lipid core) with massive structural damage to intima
V	Fibroatheroma	All type IV changes plus development of marked collagen and smooth muscle cell increase above lipid core
VI	Complicated fibroatheroma	All type V changes plus a thrombotic deposit, and/or haemorrhage, and/or erosion or fissure
VII	Calcific	Any advanced lesion type composed predominantly of calcium; substantial structural deformity
VIII	Fibrotic	Any advanced lesion type composed predominantly of collagen; lipid may be absent

†Adapted from Table 1 of Stary.[14]

Table 4. Effect of a moderate-fat diet containing either of the experimental fats (lard, sunflower oil or palm olein oil) on plasma lipids, lipoproteins and apolipoproteins over 24 months

Variable	Group (n = 10)	Time (months)					Significance of effects (P-value)			
		BL	6	12	18†	24	BL-value	Group	Time	Gr-Time
Plasma-C (mmol/L)	MFD-AF	5.33 ± 0.92[2]	5.55 ± 0.70	6.49 ± 1.13	5.64 ± 0.82	6.16 ± 1.40‡	<0.0001	0.5486	0.0022	0.0337
	MFD-SO	5.20 ± 0.97	5.77 ± 2.04	5.87 ± 2.08	5.35 ± 1.41	5.04 ± 1.10				
	MFD-PO	5.31 ± 1.05	5.61 ± 0.88	5.76 ± 0.90	5.39 ± 0.77	5.56 ± 0.87				
VLDL + IDL-C (mmol/L)	MFD-AF	0.21 ± 0.04	0.24 ± 0.08	0.63 ± 0.49	0.51 ± 0.26	0.64 ± 0.57	0.0027	0.9843	0.0012	0.1933
	MFD-SO	0.23 ± 0.13	0.46 ± 0.52	0.58 ± 0.63	0.68 ± 0.66	0.45 ± 0.31				
	MFD-PO	0.26 ± 0.19	0.40 ± 0.22	0.56 ± 0.27	0.68 ± 0.48	0.61 ± 0.36				
LDL-C (mmol/L)	MFD-AF	2.55 ± 0.62	3.05 ± 0.65	3.62 ± 1.21	2.83 ± 0.83	3.27 ± 1.16	<0.0001	0.2140	<0.0001	0.2399
	MFD-SO	2.59 ± 0.85	3.06 ± 1.91	3.10 ± 1.96	2.41 ± 1.14	2.38 ± 1.03				
	MFD-PO	2.60 ± 0.77	2.90 ± 0.80	3.02 ± 0.79	2.46 ± 0.56	2.78 ± 0.82				
HDL-C (mmol/L)	MFD-AF	2.40 ± 0.59	2.05 ± 0.52	1.99 ± 0.59	2.06 ± 0.49	1.87 ± 0.52	<0.0001	0.4186	0.0153	0.9425
	MFD-SO	2.23 ± 0.55	2.07 ± 0.65	2.03 ± 0.78	2.02 ± 0.61	1.95 ± 0.52				
	MFD-PO	2.24 ± 0.44	2.13 ± 0.44	2.00 ± 0.48	2.00 ± 0.32	1.94 ± 0.48				
LDL-C : HDL-C	MFD-AF	1.11 ± 0.36	1.64 ± 0.78	2.24 ± 1.93	1.54 ± 0.99	2.03 ± 1.55	<0.0001	0.2390	0.0237	0.3951
	MFD-SO	1.26 ± 0.68	2.18 ± 3.23	2.48 ± 3.85	1.57 ± 1.73	1.42 ± 1.13				
	MFD-PO	1.19 ± 0.39	1.44 ± 0.61	1.64 ± 0.78	1.28 ± 0.41	1.53 ± 0.68				
Plasma-TAG (mmol/L)	MFD-AF	0.35 ± 0.07	0.40 ± 0.10	0.54 ± 0.13	0.52 ± 0.12	0.52 ± 0.21	0.0121	0.4773	0.0009	0.5218
	MFD-SO	0.44 ± 0.11	0.41 ± 0.12	0.48 ± 0.12	0.63 ± 0.13	0.57 ± 0.23				
	MFD-PO	0.45 ± 0.12	0.53 ± 0.20	0.53 ± 0.14	0.62 ± 0.24	0.67 ± 0.39				
LDL apo B (g/L)	MFD-AF	0.27 ± 0.06	0.32 ± 0.08	0.35 ± 0.10	0.31 ± 0.11	0.37 ± 0.10	<0.0001	0.1127	0.0018	0.2895
	MFD-SO	0.28 ± 0.08	0.32 ± 0.20	0.32 ± 0.17	0.26 ± 0.11	0.28 ± 0.08				
	MFD-PO	0.29 ± 0.07	0.31 ± 0.10	0.33 ± 0.07	0.26 ± 0.06	0.32 ± 0.08				
Plasma apo AI (g/L)	MFD-AF	1.29 ± 0.26	1.50 ± 0.26	1.64 ± 0.35	1.58 ± 0.28	1.55 ± 0.33	<0.0001	0.3939	0.0044	0.4974
	MFD-SO	1.22 ± 0.28	1.53 ± 0.37	1.62 ± 0.45	1.61 ± 0.43	1.62 ± 0.35				
	MFD-PO	1.30 ± 0.24	1.58 ± 0.24	1.66 ± 0.29	1.55 ± 0.17	1.56 ± 0.24				

Values are mean ± SD. †The results of one individual in the MFD-PO group were excluded from the l8-month data set, n = 9 (see text). apo, apolipoprotein; AF, lard; BL, baseline; C, total cholesterol; Gr-time, group–time interaction; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; MFD, moderate-fat diet; PO, palm olein oil; SO, sunflower oil; TAG, triacylglycerol; VLDL, very low-density lipoprotein. ‡P = 0.0256 (MFD-AF *vs* MFD-SO).

Table 5. Final bodyweights and macroscopic aortic atherosclerosis over entire intimal surface at termination

Variable	MFD-AF (n = 10)	MFD-SO (n = 10)	MFD-PO (n = 10)	P-value
Final bodyweight (kg)	5.62 ± 0.25	5.58 ± 0.68	5.57 ± 0.52	0.9548
Total aortic intimal area (cm^2)	26.6 ± 3.1	28.3 ± 7.2	28.6 ± 3.8	0.4513
Lesion-free (% area)†	51.2 ± 19.4	52.5 ± 19.4	46.8 ± 23.0	0.8129
Fatty streak (% area)†	37.7 ± 20.4	37.7 ± 13.7	45.9 ± 22.0	0.5479
Fatty plaque (% area)†	10.2 ± 5.7	9.7 ± 12.5	5.9 ± 1.9	0.4374
Fibrous plaque (% area)†	0.9 ± 1.0	0.1 ± 0.3	1.4 ± 2.4	0.0657‡
Overall score	249 ± 98	251 ± 157	281 ± 134	0.8353

Values are mean ± SD. †Refer to Table 2 for macroscopic lesion characteristics. ‡The data for this variable was skewed and therefore the non-parametric Kruskal–Wallis test for multiple comparisons was used to indicate group differences. AF, lard; MFD, moderate-fat diet; PO, palm olein oil; SO, sunflower oil.

Table 6. Peripheral and aortic atherosclerosis frequency ratios for the moderate-fat diet groups consuming either of the experimental fats (lard, sunflower oil or palm olein oil)

Variable	Lesion type	Frequency ratio†				
		MFD-AF (n = 10)	MFD-SO (n = 10)	MFD-PO (n = 10)	MFD (AF + SO + PO) (n = 30)	Overall group effect (P-value)
Peripheral arteries						
All peripherals (pooled sections)	Early	92:452	122:453	58:507	272:1412	0.0362
	Advanced	0:452	12:453	0:507	12:1412	ND
	All lesions	92:452	134:453	58:507	284:1412	0.0416
Left subclavian	Early	11:77	20:78	4:91	35:246	0.0860
	Advanced	0:77	2:78	0:91	2:246	ND
	All lesions	11:77	22:78	4:91	37:246	0.0926
Brachiocephalic	Early	25:98	30:102	11:109	66:309	0.0834
	Advanced	0:98	2:102	0:109	2:309	ND
	All lesions	25:98	32:102	11:109	68:309	0.0838
Left common carotid	Early	2:94	13:94	0:106	15:294	ND
	Advanced	0:94	2:94	0:106	2:294	ND
	All lesions	2:94	15:94	0:106	17:294	ND
Right common carotid	Early	0:86	8:92	1:98	9:276	ND
	Advanced	0:86	1:92	0:98	1:267	ND
	All lesions	0:86	9:92	1:98	10:276	ND
Left common iliac	Early	54:97	51:87	42:103	147:287	0.8508
	Advanced	0:97	5:87	0:103	5:287	ND
	All lesions	54:97	56:87	42:103	152:287	0.7487
Aorta						
Thoracic	Early	9:60	5:60	3:60	17:180	0.4278
Abdominal	Early	18:40	7:40	9:40	34:120	0.2120
Whole	Early	27:100	12:100	12:100	51:300	0.2030

†Number of sections with atherosclerosis *vs* total sections per artery. Lesion types: early, types I–III; advanced, types IV–VIII; all lesions, types I–VIII (see Table 3). AF, lard; MFD, moderate-fat diet; ND, not done due to extreme clustering, i.e., no response in most of the individuals and therefore no modelling was attempted (see statistical analysis); PO, palm olein oil; SO, sunflower oil.

(Tables 6,7). This analysis provides P-values (overall group effect, Table 6) and risk estimates (odds ratios) (Table 7). For certain peripheral arteries extreme clustering occurred, in that all lesion types recorded were specific to a few individuals. In these situations the estimation equation modelling was not done due to non-convergence in the modelling process and inconsistencies in the parameter estimation.

Statistical significance was defined at $P < 0.05$ and results are presented as mean ± SD.

Results

Matching

The three diets (MFD-AF, MFD-SO and MFD-PO) were applied to well-balanced adult male vervets in terms of the concentrations of total cholesterol in plasma, very low-density lipoprotein (VLDL) + intermediate-density lipoprotein cholesterol (IDL), LDL-C, HDL-C, plasma TAG, apolipoproteins AI and B, and the LDL-C : HDL-C ratio (Table 4; baseline values). The mean (±SD) bodyweights (kg) for the three

Table 7. Peripheral and aortic atherosclerosis odds ratios for the moderate-fat diet groups consuming either of the experimental fats (lard, sunflower oil or palm olein oil)

Variable	Lesion type	MFD-AF relative to MFD-SO (n = 10)			MFD-PO relative to MFD-SO (n = 10)			MFD-PO relative to MFD-AF (n = 10)		
		Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value
Peripheral arteries										
All peripherals (pooled sections)	Early	0.77	0.34, 1.72	0.5215	0.38	0.16, 0.90	0.0277	0.50	0.31, 0.80	0.0038
	All lesions	0.69	0.28, 1.72	0.4261	0.34	0.13, 0.90	0.0289	0.50	0.31, 0.80	0.0037
Left subclavian	Early	0.50	0.14, 1.80	0.2868	0.15	0.03, 0.75	0.0214	0.30	0.09, 0.98	0.0463
	All lesions	0.44	0.11, 1.67	0.2257	0.13	0.02, 0.69	0.0169	0.30	0.09, 0.99	0.0476
Brachiocephalic	Early	0.87	0.28, 2.71	0.8070	0.30	0.09, 1.04	0.0573	0.34	0.14, 0.87	0.0239
	All lesions	0.81	0.25, 2.64	0.7228	0.28	0.08, 1.01	0.0517	0.35	0.14, 0.87	0.0246
Left common carotid		ND	ND	ND	ND	ND	ND	ND	ND	ND
Right common carotid		ND	ND	ND	ND	ND	ND	ND	ND	ND
Left common iliac	Early	0.93	0.32, 2.68	0.8977	0.68	0.18, 2.58	0.5718	0.73	0.21, 2.53	0.6197
	All lesions	0.78	0.25, 2.43	0.6634	0.57	0.14, 2.32	0.4339	0.74	0.21, 2.57	0.6313
Aorta										
Thoracic	Early	1.94	0.44, 8.54	0.3802	0.58	0.15, 2.23	0.4267	0.30	0.06, 1.39	0.1231
Abdominal	Early	3.86	0.80, 18.57	0.0923	1.37	0.28, 6.62	0.6964	0.35	0.10, 1.31	0.1201
Whole	Early	2.71	0.83, 8.84	0.0980	1.00	0.33, 3.01	1.0000	0.37	0.15, 0.92	0.0335

Lesion types: early, types I–III; all lesions, types I–VIII (see Table 3). AF, lard; MFD, moderate-fat diet; ND, not done due to extreme clustering, i.e., no response in most of the individuals and therefore no modelling was attempted (see statistical analysis); PO, palm olein oil; SO, sunflower oil.

matched groups at baseline were MFD-AF, 5.22 ± 0.80; MFD-SO, 5.46 ± 1.08; and MFD-PO, 5.48 ± 0.94.

Diets and compliance

Regular laboratory analyses of daily food portions showed that a constant composition in terms of total fat and cholesterol was maintained over the 24-month experimental period. The only marked difference between the experimental diets was the higher P/S ratio due to the sunflower oil (linoleic acid, C18:2) of the MFD-SO group (Table 1). Bodyweights over the 24 months (results not shown), as well as the final bodyweights (Table 5), were not significantly different between the groups, indicating adequate energy intake. Dietary compliance during the 24 months was good and minimal food was wasted as indicated by the percentages of food wasted (mean ± SD): MFD-AF, (1.6 ± 2.0; MFD-SO, 4.5 ± 6.0; and MFD-PO, 2.7 ± 2.5.

Plasma lipids, lipoproteins and apolipoproteins

Plasma lipid and lipoprotein results for one individual in the MFD-PO group were excluded from the 18-month data set because of errors relating to analytical problems. For all the other time points the data set is complete; n = 10 per group. The effect of a moderate-fat diet containing either of the experimental fats (lard, sunflower oil or palm olein oil) on cholesterol and apolipoprotein concentrations over 24 months is given in Table 4.

The most important result was the significant group–time interaction for the plasma total cholesterol concentration, which means that there was a difference in the pattern of change over time between the three groups. Analysis for time-specific group effects showed that the mean plasma total cholesterol concentration of the MFD-AF group was significantly higher than that of the MFD-SO group at 24 months. There were no significant differences between groups at 6, 12, 18, or 24 months for the other plasma variables measured.

The baseline measurement for all the plasma variables had a significant effect on the values of the measurements at 6, 12, 18 and 24 months. The VLDL + IDL-C, LDL-C, HDL-C, plasma TAG, LDL apo B and plasma apo AI concentrations, and the LDL-C : HDL-C ratio showed a significant time effect (i.e., the overall mean for the three groups (n = 30) changed over time).

Macroscopic aortic atherosclerosis

Results of the macroscopic evaluation of aortic atherosclerosis over the entire intimal surface and physical measurements are summarised in Table 5. There was not a significant group difference in the aortic intimal areas, thus confirming close physical matching of the vervets. Mean percentages of aortic intimal areas with no lesions (47–53%), fatty streak (38–46%), fatty plaque (6–10%) and fibrous plaque (0.1–1.4%), as well as the overall score, were not significantly different between groups. Not all aortas had fibrous plaque, which was predominantly present in small localised areas in the abdominal aorta near the iliac bifurcation.

Fibrous plaque was present in 6/10 (% area for MFD-AF, 0.9–3.2), 1/10 (% area for MFD-SO, 0.8) and 4/10 (% area for MFD-PO, 1.1–7.2) cases.

Microscopic evaluation of peripheral and aortic atherosclerosis

Frequencies and odds ratios of peripheral and aortic atherosclerosis are given in Tables 6 and 7, respectively, and are grouped as early lesions (types I–III), advanced lesions (types IV–VIII) and all lesions (types I–VIII). For certain peripheral arteries extreme clustering occurred, in that lesion types recorded were specific to a few individuals and therefore statistical analysis was not attempted (see statistical analysis).

The frequency of peripheral atherosclerosis in more than 450 random sections across all five peripheral arteries (pooled sections) in each group was predominated by the frequency of early lesions; significant group effect for both early and all lesion types combined. A small number of advanced lesions (only of type IV) was present and confined to one individual in the MFD-SO group (Table 6). There was a significant reduction in the risk for developing early lesions in the pooled peripheral sections in the MFD-PO group compared to both the MFD-SO and MFD-AF groups (Table 7). The risk (odds) of early lesions in the MFD-PO group was only 0.38 and 0.50 that of the risk in the MFD-SO and MFD-AF groups, respectively. The same significant result was achieved when the combination of all lesion types in the pooled peripheral sections was considered. The risk for early lesions and the combination of all lesion types in the pooled peripheral sections in the MFD-AF and MFD-SO groups was not significantly different (Table 7).

The result of the combined peripheral atherosclerosis analysis across all five peripheral arteries does not hold for the marginal analysis of single arteries (Table 6) due to the smaller number of sections per artery (sample size). In individual arteries the frequency of peripheral atherosclerosis predominated in the left common iliac followed by brachiocephalic, left subclavian and common carotid arteries. The latter is obvious for the overall (n = 30) frequency ratios for single arteries. All aortas had early lesions, which were predominantly present in the abdominal aorta, but no advanced lesions. There was no group effect for the frequency of atherosclerosis in either of the thoracic or abdominal aorta, or in the aorta as a whole.

Differences between groups, however, were detected when the risk for developing early lesions in single arteries and in the whole aorta was considered (Table 7). There was a significant reduction in the risk for developing early lesions in the left subclavian and brachiocephalic arteries, and in the aorta (whole) in the MFD-PO group compared to the MFD-AF group (also significant for all lesion types combined). In the left subclavian artery the risk for developing early lesions was smaller in the MFD-PO compared to the MFD-SO group (also significant for all lesion types combined).

Discussion

The main objective of this study was to evaluate the effect of substituting lard (11%E; 40% of total fat) isocalorically with either SO or PO in a MFD on the progression of peripheral and aortic atherosclerosis in a non-human primate (vervet) model. After 25.5 months of exposure to the dietary regimens atherosclerosis was assessed and defined according to unequivocal morphological components, which define the composition and classification of human atherosclerotic lesion types I–VIII.[14–16]

The present study demonstrated that palm olein oil, relative to SO and AF, significantly reduced the risk for developing early lesions in peripheral arteries and, relative to AF, in aortas of vervets exposed for 25.5 months to a diet with a moderate-fat (28%E) moderate-cholesterol (28 mg/1000 kJ) content.

The frequency of peripheral and aortic atherosclerosis in relatively large random samples of microscope sections was low. Early lesion types I (initial lesion) and II (fatty streak) predominated in the peripheral arteries and the aortas as a result of the effect of a MFD, and the slow rate of natural progression of atherosclerosis.[14–16] There were no lesions beyond type II present in the aortas. However, type III (pre-atheroma) lesions and an example of an advanced lesion equivalent to type IV (atheroma) were present in peripheral arteries of the MFD-SO group; type III (pre-atheroma) lesions were present in two individuals, one of which also had type IV (atheroma) lesions.

In another study, 20 adult male vervets were fed a high-fat (34%E) high-cholesterol (98 mg cholesterol/1000 kJ) diet with a P/S ratio of 0.6 for the same period of 25.5 months. The frequency of types I–III lesions and advanced lesions of types IV, V and examples of type VIII in peripheral arteries and aortas were higher (PJ van Jaarsveld, unpubl. obs, 1999). The latter high-fat diet was similar to the atherogenic diet used previously and caused hypercholestrolaemia and accelerated atherogenesis as in other studies on vervets kept under similar experimental conditions.[17–21,23,24]

In humans, types I (initial lesion) and II (fatty streak) lesions are generally the only types that occur in infants and children, although they also occur in adults. These precursors of atherosclerosis do not obstruct or modify blood flow and are clinically silent.[14–16] Type III (preatheroma) lesions are the bridge between type II and advanced lesions and may evolve soon after puberty, and are present in young adults. Advanced lesions of type IV (atheroma) are frequent from the third decade and lesions of more advanced types (V, fibroatheroma; VI, complicated fibroatheroma; VII, calcific lesion; and VIII, fibrotic lesion) from the fourth decade of life.

Fibrous plaque (Tables 2 and 5), in small localised areas in some aortas, was identified macroscopically but not microscopically. This may be explained by the difference in the sampling method for microscopic versus macroscopic evaluation of the aorta (see Materials and methods; atherosclerosis). It further confirms the extremely low frequency of advanced lesions and the effectiveness of the MFD in slowing down progression of lesions into the more advanced stages. This

result is supported by work carried out on adult female vervets exposed to a prudent diet (29%E fat; 19 mg cholesterol/1000 kJ; P/S = 1.75) under similar experimental conditions.[17]

The results of plasma and lipoprotein total cholesterol and apolipoprotein concentrations after 6 months of dietary intervention[26] were confirmed by the results after 24 months. The effect of PO in a MFD on plasma total cholesterol, LDL-C, HDL-C, apo B and apo AI concentrations in the vervets was not significantly different from either the AF or SO moderate-fat diets during the 24 months. At 24 months it was only the mean plasma total cholesterol concentration in the group receiving AF that was significantly higher ($P = 0.0256$) than in the group receiving SO.

In conclusion, the present study demonstrated that PO lowers the risk for developing early lesions in peripheral arteries (relative to AF and SO) and aortas (relative to AF). Therefore, under the described conditions of this study in a non-human primate model of atherogenesis, the isocaloric substitution of AF with PO seems to be beneficial. Other natural components of PO, such as the tocotrienols, may have beneficial effects in slowing down progression of atherosclerosis and should be investigated.

Acknowledgements. We sincerely thank Martelle Marais, De Wet Marais, Gert Engelbrecht and Johanna van Wyk for excellent technical assistance; Charon de Villiers for management of the vervets; Amelia Damons for preparation of the food; Sonja Swanavelder and Dr Carl Lombard for statistical analysis and Dr Petro Wolmarans for help with dietary analyses. This study was supported by a research grant from the Malaysian Palm Oil Board.

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