Original Article

Effect of dietary cholesterol, *trans* and saturated fatty acids on serum lipoproteins in non-human primates

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Nine cynomolgus monkeys were rotated randomly through four dietary treatments with each treatment lasting 6 weeks. A wash-out period of 4 weeks was maintained between each dietary rotation. The animals were fed diets containing 32% energy fat derived from palm olein (POL), lauric-myristic-rich oil blend (LM), American Heart Association (AHA) rich oil blend and hydrogenated soybean oil blend (*trans*). Diets were fed with (phase 1) or without (phase 2) the addition of dietary cholesterol (0.1%). In phase 1, when animals were fed without dietary cholesterol, plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) was significantly raised and high-density lipoprotein cholesterol (HDL-C) was significantly depressed by the *trans* diets relative to all other dietary treatments. The resulting LDL-C/HDL-C ratio was also significantly increased. The LM diet increased TC significantly relative to the AHA diet while LDL-C was significantly increased compared to both POL and AHA. Apolipoprotein (apo) B was not affected significantly by these dietary treatments. Apo A1 was significantly increased by POL relative to all other dietary treatments. The *trans* diet reduced apo A1 and the resulting apo B/A1 ratio was increased significantly by trans relative to all other dietary treatments. Addition of 0.1% dietary cholesterol to these diets almost doubled the plasma TC and LDL-C in all dietary treatments. However, HDL-C was only marginally higher with the addition of dietary cholesterol. The LM + C (cholesterol added) diet resulted in the highest TC and LDL-C that was significant compared to all other dietary treatments. *Trans* + C increased TC compared to POL + C and AHA + C diets while increases in the LDL-C did not attain significance. The addition of dietary cholesterol did not affect HDL-C between treatments whereas plasma triglycerides were significantly increased by the *trans* + C diet relative to all other treatments. Both the *trans* + C and LM + C diets increased apo B and decreased apo A1 relative to the POL + C and AHA + C diets. The resulting apo B/A1 ratio was similarly altered. These results affirm that the lauric + myristic acid combination, along with *trans* fatty acids, increased lipoprotein-associated coronary heart disease risk factors compared to either POL or AHA.

Introduction

Fats are an integral part of our diets. They provide a dense source of calories for our metabolic requirements, supply essential fatty acids and assist in the absorption of fat-soluble vitamins. The quality and quantity of fat in the diet influences serum lipid concentrations. Serum lipids play an important role in the pathogenesis of many disorders, such as atherosclerosis, cancer and diabetes. A desirable serum lipid profile is one that has low plasma total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) and a high level of high-density lipoprotein cholesterol (HDL-C).[1]

High levels of TC are a risk factor for coronary artery disease and saturated fatty acids have been positively implicated. These include lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids. Palm kernel and coconut oil contain more than 65% of their fatty acids as lauric and myristic acids, the combination of which has been reported to be atherogenic.[2,3]

In comparison, a number of studies[4–7] have reported that palmitic acid is less cholesterolaemic than a lauric + myristic acid (C12:0 + 14:0) combination in diets that are low in dietary cholesterol (<250 mg/day). To ascertain the effects of specific fatty acids, investigations using blends of edible oils in which the total saturates, monounsaturated and polyunsaturated, were held constant have been attempted. The exchange of dietary 16:0 for monounsaturated oleic acid (C18:1 n-9) has been reported by Sundram *et al.* to not result in significant differences in plasma TC responses.[8] Grundy and Vega[9] and Denke and Grundy[10] have also reported that C18:0 is basically a neutral fatty acid. Hence it appears from these studies that the cholesterolaemic effects of the saturated fatty acids may reside in the 12:0 + 14:0 combination.

Trans fatty acids may occur naturally, albeit in small concentrations, but the principal source of *trans* fatty acids in the human diet is through the consumption of hydrogenated fats. Hydrogenated vegetable oils in shortenings and margarines are important components of the diet in many industrialised societies. Hydrogenation converts liquid oils to solid fats, and this process protects fats from oxidation while adding texture by imparting plasticity.

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The effects of *trans* fatty acids on plasma lipoproteins and associated coronary heart disease (CHD) risk factors have become the subject of intense debate. Several reports demonstrate that modest intake of *trans* fatty acids can negatively affect lipoproteins by increasing LDL-C and decreasing HDL-C relative to the *cis* isomers.[11–13] *Trans* fatty acids have been compared to saturates primarily because their structure and metabolic impact on cholesterol metabolism are often thought to imitate the dietary saturated fatty acids that they were designed to replace.[8] Several studies reported that consumption of *trans* fatty acids is more detrimental than saturated fatty acids because of their negative impact on the LDL/HDL-C ratio and lipoprotein (a) concentration.[14–16] Consumption of *trans* 18:1, especially the elaidic acid isomer (t18:1 n-9) in the range of 2–7% energy (% en), has been demonstrated to substantially elevate the LDL/HDL-C ratio, primarily because it typically depresses HDL-C while raising LDL-C.[17] By contrast, saturated fatty acids that raise LDL-C also raise HDL-C.[18]

In spite of the large volume of information on the cholesterolaemic effects of individual fatty acids (including *trans*), their interactions with dietary cholesterol appears to be less clearly defined. This information is considered important to allow proper recommendations to formulate dietary guidelines for the population. This study was therefore designed in non-human primates to evaluate the cholesterolaemic response of different fatty acid combinations either in the presence or absence of dietary cholesterol.

Materials and methods

A total of nine *Cynomolgus* monkeys were used in this study, which was divided into two phases. In each phase, animals were randomly rotated through four dietary treatments, with each dietary period lasting 6 weeks. Semipurified diets (in accordance with the American Institute of Nutrition specifications) were formulated as follows (g/kg diet): casein, 160; dextrose, 190; cellulose, 80; vitamin mix, 5; salt mix, 46; choline bitartrate, 2; corn starch, 220; dietary cholesterol, 1; commercial monkey pellet, 125; ascorbic acid, 1; dietary oil, 170. These diets provided 32% energy as fat derived from four different dietary oil blends formulated for the purposes of this study. In the first phase, all diets were formulated without the addition of dietary cholesterol whereas in the second phase, 0.1% dietary cholesterol (1 g/kg diet) was added to make them atherogenic. All animals were housed individually in stainless steel cages kept in a temperature-controlled room (24°C–27°C) and fed ad libitum these formulated diets for the prescribed periods of 6 weeks each. At the end of each dietary period, the animals were placed on a wash-out period of 4 weeks during which they were fed commercial monkey pellets.

The following oil blends were used to provide the fat source during the experimental periods. Palm olein (POL) was used without blending as a source of palmitic acid. An American Heart Association (AHA)-type blend containing equal proportions of saturated, monounsaturated and polyunsaturated fatty acids was formulated using 50% soybean oil and 50% palm olein. The *trans*-rich oil was derived from partially hydrogenated soybean oil (melting point 35°C), which contained 39% *trans* fatty acids, predominantly as elaidic acid. This was remixed with native refined soybean oil so that a final *trans* fatty acid content of 29.2% was achieved in the blend (TR). The high lauric–myristic fatty acid blend (LM) was a mixture of palm kernel, coconut and corn oils in the ratio 2.0:2.5:5.5. We also ensured that the linoleic acid content of this blend matched closely to that of the *trans*-rich oil (LM = 15.1%; TR = 17.6%). All the ingredients were weighed and mixed together in a large mixing bowl before pelletising. The oil-enriched pellets were then baked, packed and ready to be fed to the monkeys. The fatty acid profile of the dietary oil blends (Table 1) and the resulting energy (%) content is given in Table 2.

Laboratory methods

At the end of the 6-week feeding period, following an overnight fast (14–16 h), the monkeys were anaesthetised with an intramuscular injection (0.2 mL/kg bodyweight) of Zoletil 50 (Virbac Laboratories, Carros, France). A 10 mL blood sample was drawn by venous puncture of the femoral vein and placed into tubes containing ethylenediaminetetraacetic acid solution kept on ice. Plasma was isolated by centrifugation at 3000 *g* for 20 min and aliquoted for the various analyses. For the isolation of lipoproteins, 2.5 mL plasma was used and very low-density lipoprotein (VLDL), LDL and HDL were prepared by gradient density ultracentrifugation, as described previously.[19] The cholesterol content in these lipoprotein fractions and in plasma was analysed enzymatically using a clinical autoanalyser (Express 550; Ciba-Corning, Oberlin, OH, USA) according to standardised protocols in our laboratories.[8] Similarly, plasma TG and HDL-C content were also analysed enzymatically on the same autoanalyser. Apolipoprotein (apo) A1 and apo B were measured using diagnostic kits from Sigma Dignostics (St Louis, MO, USA). The analytical procedures prescribed in theses kits were adhered to without any modifications.

Fatty acid analysis

Fatty acid composition of the dietary oils and extracted dietary fats were determined following *trans*-methylation of the samples using toluene–sulphuric acid.[8] Fatty acids were then analysed as their methyl esters on a Perkin Elmer Autosystem gas chromatogram (Perkin Elmer Corporation, Norwalk, CT, USA) fitted with a 100 meter capillary column (SP2560; Supelco, Belfonte, PA, USA) and temperature programmed from 160 to 240°C at 4°C/min, as described previously. Authentic fatty acid standards were used to identify the component fatty acids of interest.

Statistical analysis

All data were checked for their frequency distribution using the Rankitts plots. Analysis of variance and the Bonferroni inequality test were used to test the differences between dietary treatments. Two-tailed tests was performed and treatments were considered significant when $P < 0.05$.

Table 1. Fatty acid composition of oil blends incorporated into diets

Fatty acid	*Trans* diet	AHA diet	POL diet	LM diet
SFA	17.8	30.0	43.7	68.1
8:0	ND	ND	ND	ND
10:0	ND	ND	ND	4.0
12:0	ND	ND	ND	37.5
14:0	ND	0.2	0.8	13.8
16:0	11.6	25.7	38.8	7.8
18:0	5.6	4.1	4.0	5.0
20:0	0.2	ND	ND	ND
22:0	0.3	ND	ND	ND
MUFA	33.2	37.4	45.0	15.8
16:1 n-9	ND	ND	ND	ND
18:1 n-9	33.2	37.4	45.0	15.8
PUFA	19.8	32.6	11.1	15.4
18:2 n-6	17.6	29.3	10.9	15.1
18:3 n-3	2.2	3.3	0.4	0.3
Trans fatty acids	29.2	ND	ND	ND
18:1 n-9t	23.1	ND	ND	ND
18:1 n-11t	3.4	ND	ND	ND
18:1 n-13t	1.6	ND	ND	ND
Unid *cis*/*trans*	1.2	ND	ND	ND
P/S ratio	1.12	1.09	0.25	0.23

Dietary composition: *trans*, 70% hydrogenated soybean oil + 30% soybean oil; AHA, 50% soybean oil + 50% palm olein oil; POL, 100% palm olein; LM, 20% palm kernel oil + 55% coconut oil + 25% corn oil. AHA, American Heart Association; LM, lauric–myristic; MUFA, monounsaturated fatty acid; ND, not detected; POL, palm olein; P/S, polyunsaturated/saturated fatty acid ratio; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; Unid, unidentified.

Table 2. Contribution of energy from individual fatty acids in the formulated diets

Fatty acid	Energy in diet (%)			
	Trans	AHA	POL	LM
SFA	5.7	9.6	14.0	21.8
8:0	ND	ND	ND	ND
10:0	ND	ND	ND	1.3
12:0	ND	ND	ND	12.0
14:0	ND	0.1	0.3	4.4
16:0	3.7	8.2	12.4	2.5
18:0	1.8	1.3	1.3	1.6
20:0	0.1	ND	ND	ND
22:0	0.1	ND	ND	ND
MUFA	10.6	12.0	14.4	5.1
16:1 n-9	ND	ND	ND	ND
18:1 n-9	10.6	12.0	14.4	5.1
PUFA	6.3	10.4	3.6	4.9
18:2 n-6	5.6	9.4	3.5	4.8
18:3 n-3	0.7	1.1	0.1	0.1
Trans fatty acids	9.3	ND	ND	ND
18:1 n-9t	7.4	ND	ND	ND
18:1 n-11t	1.1	ND	ND	ND
18:1 n-13t	0.5	ND	ND	ND
Unid *cis*/*trans*	0.4	ND	ND	ND

AHA, American Heart Association; LM, lauric–myristic; MUFA, monounsaturated fatty acid; ND, not detected; POL, palm olein; P/S, polyunsaturated/saturated fatty acid ratio; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; Unid, unidentified.

Results

From the fatty acid analysis of the diets (Table 1) it was apparent that the POL diet had significantly higher levels of palmitic acid compared to all other dietary treatments, whereas the LM diet was significantly higher in its lauric and myristic acid content. The POL and LM diets had 14 and 21.8% en as total saturates, respectively, which were significantly different from each other (Table 2). The AHA diet had equal distributions of the saturates, monounsaturates and polyunsaturates, while the TR diet was characterised by its *trans* fatty acid content, mainly as elaidic acid (7.4% en). The AHA diet was significantly higher in its content of linoleic acid (9.4% en) than all other dietary treatments tested. The POL diet provided the lowest (3.5% en.) linoleic acid content while the TR and LM were intermediate at 5.6 and 4.8% en, respectively.

During phase 1, when these animals were fed diets without the addition of dietary cholesterol, plasma TC, LDL-C and TG was significantly increased in animals fed the TR diet compared to all other dietary treatments (Table 3). No significant difference in TC and LDL-C between the POL and AHA was apparent. The LM diet resulted in a significant elevation in TC compared to the AHA diet, while LDL-C was significantly higher in the LM diet relative to both the POL and AHA diets. HDL-C was significantly decreased by the TR diet compared to all other dietary treatments and the resulting LDL/HDL-C ratio was also significantly elevated.

As a result of the addition of 0.1% cholesterol to all these diets during phase 2, TC and LDL-C were almost doubled in all the animals (Table 4). Plasma TC and LDL-C were highest and statistically significant in the LM + C diet compared to all other dietary treatments. The TR + C diet resulted in lower TC and LDL-C values than the LM + C diet, which were, however, significantly higher than the POL + C and AHA + C dietary treatments. The TC and LDL-C values after the POL + C and AHA + C diets were not significantly different from each other. HDL-C was not significantly different between all dietary treatments. The resulting LDL/HDL-C was lowest after the POL + C diet but attained significance only in comparison to the LM + C diet.

The diets influenced the plasma fatty acid composition of these animals fed the various dietary oil blends (Table 5). When fed cholesterol-free diets, plasma saturated fatty acids were increased significantly by the POL diet relative to the *trans* and LM diets but not the AHA diet. The major saturated fatty acid component was palmitic acid. The LM diet resulted in significantly higher levels of myristic acid compared to all other dietary treatments. The POL diet was also characterised by the highest level of monounsaturated

Table 3. Effect of dietary oil blends on plasma lipids and lipoproteins in *Cynomolgus* monkeys fed free-cholesterol diets

	Trans	AHA	POL	LM
TC (mmol/L)	$4.40 \pm 1.02^{a,b,c}$	$3.06 \pm 0.63^{a,d}$	3.28 ± 0.61^{b}	$3.83 \pm 0.65^{c,d}$
TG (mmol/L)	$0.75 \pm 0.39^{a,b,c}$	0.34 ± 0.12^{a}	0.42 ± 0.12^{b}	0.45 ± 0.13^{c}
LDL-C (mmol/L)	$2.94 \pm 1.10^{a,b,c}$	$1.40 \pm 0.54^{a,d}$	$1.45 \pm 0.50^{b,e}$	$2.00 \pm 0.54^{c,d,e}$
HDL-C (mmol/L)	$1.15 \pm 0.24^{a,b,c}$	1.50 ± 0.16^{a}	1.59 ± 0.21^{b}	1.63 ± 0.30^{c}
LDL/HDL ratio	$2.70 \pm 1.15^{a,b,c}$	$0.93 \pm 0.35^{a,d}$	0.99 ± 0.31^{b}	$1.27 \pm 0.40^{c,d}$
Apolipoprotein A1 (g/L)	$121.30 \pm 23.69^{a,b,c}$	$159.30 \pm 27.85^{a,d}$	$174.30 \pm 21.76^{b,d,e}$	$149.30 \pm 33.27^{c,e}$
Apolipoprotein B (g/L)	34.55 ± 7.76	34.13 ± 9.93	37.00 ± 8.83	31.20 ± 7.54
Apolipoprotein B/A1 ratio	$0.30 \pm 0.09^{a,b,c}$	0.18 ± 0.08^{a}	0.21 ± 0.04^{b}	0.21 ± 0.05^{c}

Values are mean ± SD ($n = 9$ monkeys in each group). Means with common superscripts ($^{a–e}$) are significantly different ($P < 0.05$). AHA, American Heart Association; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LM, lauric–myristic-rich oil blend; POL, palm olein; TC, total plasma cholesterol; TG, triglyceride; *Trans*, hydrogenated soyabean oil blend.

Table 4. Effect of dietary oil blends on plasma lipids and lipoproteins (mmol/L) in *Cynomolgus* monkeys fed diets containing 0.1% cholesterol

	Trans + C	AHA + C	POL + C	LM + C
TC (mmol/L)	$7.14 \pm 2.65^{a,b,c}$	$6.28 \pm 1.82^{a,d}$	$6.47 \pm 2.14^{b,e}$	$8.11 \pm 1.98^{c,d,e}$
TG (mmol/L)	$1.29 \pm 0.85^{a,b,c}$	$0.51 \pm 0.42^{a,d}$	$0.61 \pm 0.31^{b,e}$	$0.84 \pm 0.30^{c,d,e}$
LDL-C (mmol/L)	5.16 ± 2.71^{a}	5.05 ± 1.98^{b}	4.82 ± 2.01^{c}	$6.29 \pm 1.81^{a,b,c}$
HDL-C (mmol/L)	1.43 ± 0.34	1.25 ± 0.35	1.37 ± 0.37	1.39 ± 0.50
LDL/HDL ratio	4.10 ± 3.20^{a}	4.25 ± 3.89^{b}	3.80 ± 1.70^{c}	$5.10 \pm 2.50^{a,b,c}$
Apolipoprotein A1 (g/L)	88.55 ± 18.94^{a}	$93.00 \pm 34.49^{b,d}$	$118.75 \pm 33.88^{a,b,c}$	$84.44 \pm 28.62^{c,d}$
Apolipoprotein B (g/L)	$80.00 \pm 34.42^{a,b}$	$66.00 \pm 19.53^{a,c}$	$66.44 \pm 26.20^{b,d}$	$82.44 \pm 18.09^{c,d}$
Apolipoprotein B/A1 ratio	$0.95 \pm 0.47^{a,b}$	$0.79 \pm 0.36^{a,c,d}$	$0.55 \pm 0.20^{b,c,e}$	$1.06 \pm 0.37^{d,e}$

Values are mean ± SD ($n = 9$ monkeys in each group). Means with common superscripts ($^{a–e}$) are significantly different ($P < 0.05$). AHA, American Heart Association; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LM, lauric–myristic-rich oil blend; POL, palm olein; TC, total plasma cholesterol; TG, triglyceride; *Trans*, hydrogenated soyabean oil blend.

Table 5. Plasma fatty acid profile (%) of Cynomolgus monkeys fed free-cholesterol diets

Fatty acid	*Trans*	AHA	POL	LM
SFA	29.66 ± 1.89[a]	32.65 ± 2.72[b]	35.48 ± 3.38[a,c]	27.76 ± 1.87[b,c]
12:0	0.57 ± 0.54	ND	0.2 ± 0.26	0.57 ± 0.41
14:0	0.64 ± 0.12[a]	0.37 ± 0.19[b]	0.63 ± 0.38[c]	1.26 ± 0.46[a,b,c]
16:0	16.18 ± 1.33[a]	20.90 ± 0.75	22.68 ± 0.79[a,b]	17.96 ± 1.02[b]
18:0	11.69 ± 0.84[a]	11.14 ± 2.62[b]	11.97 ± 2.21[c]	7.98 ± 1.42[a,b,c]
20:0	0.58 ± 0.14	0.10 ± 0.12	ND	Tr
24:0	ND	0.14 ± 0.10	ND	ND
PUFA	34.08 ± 1.47[a,b]	43.53 ± 2.72[a,c]	32.44 ± 3.30[c,d]	48.71 ± 1.9[b,d]
18:2 n-6	28.92 ± 1.22[a]	33.99 ± 2.18[b,c]	25.12 ± 3.75[b,d]	41.64 ± 2.39[a,c,d]
18:3 n-3	0.80 ± 0.03[a,b,c]	1.13 ± 0.14[a,d,e]	0.27 ± 0.27[b,d]	0.35 ± 0.14[c,e]
20:2 n-6	0.51 ± 0.31	0.27 ± 0.09	0.13 ± 0.17	0.44 ± 0.11
20:3 n-6	ND	1.30 ± 0.50	1.98 ± 0.72	1.47 ± 0.28
20:4 n-6	3.85 ± 0.83[a]	5.39 ± 0.58[a,b]	4.39 ± 0.62	3.98 ± 1.06[b]
22:4 n-6	ND	0.30 ± 0.06	0.14 ± 0.19	0.28 ± 0.14
22:6 n-6	ND	1.15 ± 0.09	0.40 ± 0.39	0.54 ± 0.29
MUFA	15.12 ± 1.93[a,b]	23.43 ± 2.42[a,c,d]	28.82 ± 2.84[b,c,e]	18.99 ± 1.55[d,e]
14:1 n-9	0.39 ± 0.37	ND	ND	ND
16:1 n-9	0.95 ± 0.18	0.98 ± 0.31	0.76 ± 0.39	0.53 ± 0.29
18:1 n-9	13.46 ± 2.06[a,b]	22.24 ± 2.19[a,c,d]	27.90 ± 2.45[b,c,e]	18.24 ± 1.39[d,e]
20:1 n-9	0.79 ± 0.51	ND	ND	ND
24:1 n-9	ND	0.20 ± 0.14	0.17 ± 0.22	0.21 ± 0.10
Trans	15.60 ± 2.15	–	–	–
t14:1 n-9	0.13 ± 0.30	–	–	–
t16:1 n-9	0.54 ± 0.32	–	–	–
t18:1 n-9	5.55 ± 2.42	–	–	–
t18:1 n-11	3.57 ± 1.09	–	–	–
t18:2 n-13	0.18 ± 0.24	–	–	–
t18:1 n-12	1.83 ± 1.22	–	–	–
t18:1 n-13	1.19 ± 1.09	–	–	–
18:1 11c, 14t	1.69 ± 0.17	–	–	–
18:1 12c, 15t	1.27 ± 0.13	–	–	–

Values are mean ± SD (n = 9 monkeys in each group). Means with common superscripts ([a–e]) are significantly different ($P < 0.05$). AHA, American Heart Association; LM, lauric–myristic; MUFA, monounsaturated fatty acid; ND, not detected; POL, palm olein; P/S, polyunsaturated/saturated fatty acid ratio; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; Tr, trace amount < 0.02%.

oleic acid and this was significant compared to all other treatments. The plasma content of oleic acid was significantly higher in the AHA diet compared to the *trans* and LM diets. Plasma total polyunsaturated fatty acid, represented largely as linoleic acid, was significantly increased by the AHA and LM diets compared to the *trans* and POL diets.

With the addition of dietary cholesterol, plasma total saturated fatty acids were not significantly different between diets. However, plasma stearic acid was significantly increased by the *trans* + C diet only. Plasma monounsaturated oleic acid was highest after the POL + C diet and this was significant compared to all other dietary treatments. The AHA + C and LM + C diets relative to the *trans* + C and POL + C diets significantly increased plasma polyunsaturated fatty acids. The *trans* + C compared to the POL + C diet also significantly lowered plasma linoleic acid. *Trans* fatty acids were incorporated into the plasma of these animals during dietary rotations containing the *trans* oil blends. These fatty acids were consequently absent from the plasma when the animals were switched to other dietary treatments interjected by a 4-week wash-out period. It was therefore apparent that plasma fatty acid profiles were modulated by the different dietary oil blends, both in the absence and presence of dietary cholesterol (Table 6).

Discussion

Saturated fatty acids enhance CHD risk by increasing TC and LDL-C. Recent animal[5] and human studies[1–4,9] suggest that saturated fatty acids themselves differ in their cholesterol raising effects, the highest potency being attributed to a combination of lauric + myristic fatty acids. Apart from saturates, *trans* fatty acids are also implicated for their enhancing effects on CHD risk factors. There is increasing concern that the effects of *trans* are often equal to,[20,21] or even worse than, the saturates[19] that they were designed to replace in the first place.

The present study in *Cynomologus* monkeys compared the effects of four dietary oil blends that differed greatly in their fatty acid composition. These differences in fatty acid composition were hypothesised to result in different levels of

Table 6. Plasma fatty acid profiles (%) of *Cynomolgus* monkeys fed diets containing 0.1% cholesterol

Fatty acid	*Trans* + C	AHA + C	POL + C	LM + C
SFA	32.01 ± 3.04	28.42 ± 3.60	30.20 ± 1.35	29.14 ± 1.94
12:0	0.13 ± 0.21	ND	ND	0.48 ± 0.19
14:0	0.85 ± 0.16[a,b]	0.35 ± 0.07[a,c]	0.45 ± 0.27[d]	1.54 ± 0.29[b,c,d]
16:0	19.42 ± 1.96	19.17 ± 3.08	21.76 ± 1.24	18.05 ± 0.63
18:0	11.39 ± 1.51[a,b,c]	7.67 ± 1.20[a]	7.92 ± 0.69[b]	9.07 ± 1.46[c]
20:0	0.22 ± 0.25	0.97 ± 1.71	ND	ND
24:0	ND	0.27 ± 0.12	0.07 ± 0.11	ND
PUFA	24.65 ± 3.54[a,b,c]	42.64 ± 4.91[a,d]	33.84 ± 1.92[b,d,e]	42.62 ± 2.14[c,e]
18:2 n-6	21.27 ± 3.08[a,b,c]	35.05 ± 5.71[a,d]	27.44 ± 2.02[b,d,e]	36.97 ± 1.75[c,e]
18:3 n-3	0.06 ± 0.14	0.86 ± 0.24	0.44 ± 0.25	0.56 ± 0.05
20:2 n-6	0.05 ± 0.13	0.42 ± 0.36	0.04 ± 0.11	0.44 ± 0.10
20:3 n-6	0.58 ± 0.29	0.85 ± 0.47	1.65 ± 0.31	1.38 ± 0.55
20:4 n-6	2.69 ± 0.60	3.39 ± 0.70	3.00 ± 0.34	2.65 ± 0.56
22:4 n-6	ND	0.86 ± 1.05	0.17 ± 0.33	ND
22:6 n-6	ND	1.23 ± 0.89	1.10 ± 0.93	0.61 ± 0.18
MUFA	25.99 ± 2.52[a]	28.95 ± 3.25[b]	35.82 ± 2.03[a,b,c]	25.27 ± 2.67[c]
14:1 n-9	0.18 ± 0.28	ND	ND	0.31 ± 0.32
16:1 n-9	0.88 ± 0.70	1.17 ± 0.40	1.28 ± 0.37	1.40 ± 0.35
18:1 n-9	24.80 ± 1.84[a]	27.46 ± 3.19[b]	34.40 ± 1.82[a,b,c]	23.34 ± 2.63[c]
20:1 n-9	0.13 ± 0.20	ND	0.15 ± 0.23	0.22 ± 0.18
24:1 n-9	ND	0.31 ± 0.02	0.15 ± 0.23	ND
Trans	15.88 ± 3.11	–	–	–
16:1 n-9	0.60 ± 0.41	–	–	–
t18:1 n-9	6.77 ± 1.54	–	–	–
t18:1 n-11	2.23 ± 0.26	–	–	–
t18:1 n-12	2.03 ± 0.11	–	–	–
t18:1 n-13	3.00 ± 0.86	–	–	–
18:1 12c, 15t	0.66 ± 0.14	–	–	–
Unid	0.55 ± 0.66	–	–	–

Values are mean ± SD (n = 9 monkeys in each group). Means with common superscripts ([a–e]) are significantly different ($P < 0.05$). AHA, American Heart Association; LM, lauric–myristic; MUFA, monounsaturated fatty acid; ND, not detected; POL, palm olein; P/S, polyunsaturated/saturated fatty acid ratio; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

plasma lipids and lipoproteins in these monkeys rotated sequentially through all four diets. The AHA diet contained equal proportions of saturated, monounsaturated and polyunsaturated fatty acids while the POL diet was rich in its content of palmitic and oleic acids. These fatty acid differences, however, did not elicit TC and LDL-C values that were significantly different between the POL and AHA diets. The LM diet rich in lauric and myristic acids elicited a significant increase in TC relative to the AHA diet but not the POL diet. LDL-C after the LM diet was, however, significantly increased compared to both the POL and AHA diets.

Khosla *et al.* fed monkeys AHA step-1 diets whose saturated component was formulated with either palmitic (16:0) or lauric + myristic (12:0 + 14:0) fatty acids.[22] Animals fed the 12:0 + 14:0-rich AHA diet had significantly increased TC and LDL-C levels compared to those fed the 16:0-rich AHA diet. The 16:0 diet resulted in a smaller LDL pool size, which was attributed to a significantly higher catabolic rate of LDL. They concluded that the removal of 12:0 + 14:0 from their AHA formulation improved plasma lipid profiles more favourably than the removal of 16:0. In the present study, the AHA blend made by blending palm olein resulted in a saturation content that was mostly palmitic and was almost devoid of the 12:0 + 14:0 content. This would therefore explain the similarity in our observations to the data of Khosla *et al.*[22] The hypercholesterolaemic effects of 12:0 + 14:0 relative to a 16:0-rich diet has also been reported in humans fed diets low in cholesterol content.[23–25] Moreover, in a human feeding trial,[26] an AHA diet that was palm olein based resulted in TC and LDL-C that was not significantly different compared to a POL or *cis* 18:1 diet. HDL-C was, however, significantly elevated and the LDL/HDL-C ratio significantly lowered by the AHA diet that was palm olein based.

The *trans* diet significantly increased TC and LDL-C while depressing HDL-C relative to all other dietary treatments. In view of the present controversy surrounding *trans* fatty acids,[11–15] this finding was not surprising. Of greater concern is the observation that the *trans* diet was significantly inferior compared to the saturated LM diet in protecting against CHD risk. Sundram *et al.*[8] used identical oil blends in their human study and reported essentially similar cholesterol-raising effects for their *trans* fatty acid-rich diet compared to the LM-rich diet. It has been suggested that the

adverse behaviour of *trans*-rich fats may be overcome by ensuring that sufficient polyunsaturated linoleic acid is available. Despite the higher availability of linoleic acid in the *trans* diet compared to the POL and LM diets, plasma lipoproteins were adversely modulated. In addition, changes in the plasma fatty acid composition, accompanied by the incorporation of significant amounts of different *trans* fatty acid isomers, were obvious. In their epidemiological studies, Hu *et al.* reported that substitution of monounsaturated fatty acids for *trans* fatty acids would decrease CHD risk substantially.[27] This analysis also appears to be true in the present primate study using the AHA or POL diets as substantial sources of monounsaturated oleic acid.

Rather dramatic effects on the plasma lipid profiles were apparent in these animals when 0.1% dietary cholesterol was added to their diets. TC and LDL-C almost doubled whereas HDL-C was less-dramatically impacted upon. The AHA and POL diets did not result in TC, LDL-C and HDL-C levels that were significantly different from each other. This is contrary to present postulations about the effects of palm olein. Although it has been proposed that palm olein behaves as a neutral fat in its cholesterolaemic response, this neutrality was suggested to be conditional.[16] It was hypothesised that in hypercholesterolaemic subjects or in populations consuming diets that are rich in dietary cholesterol, palm olein (16:0-rich) would result in unfavourable plasma lipid profiles. However, when tested in the present animal study, the effects of palm olein containing a high level of dietary cholesterol (0.1%) were comparable to the AHA diet plus cholesterol diet containing a balanced fatty acid profile.

As expected the LM + C diet resulted in the highest TC and LDL-C levels. These were significantly different from all other treatments. In comparison, plasma TC in the *trans* + C diet was significantly higher than in the AHA + C and POL + C diets. These data again underscored previous observations that the lipoprotein profiles were unfavourably altered due to the consumption of *trans* or saturated fats containing 12:0 + 14:0 fatty acids but not by 16:0-rich fats.[8] *Trans* fatty acids primarily enhance CHD risk by decreasing the beneficial HDL-C. This was also apparent in this study when *trans* was administered as a cholesterol-free diet. Addition of dietary cholesterol to the *trans* diet resulted in increased levels of TC and LDL-C (also apparent in all other dietary treatments) but, surprisingly, no accompanying significant decrease in the level of HDL-C relative to other dietary treatments. The effects of *trans* fatty acids are viewed with much greater concerns than the effects of saturates as *trans* reduces HDL-C and increases TC and LDL-C, whereas in the saturates, TC and LDL-C increases are accompanied by an increase in HDL-C. In the present study, the addition of dietary cholesterol appears to have reversed the HDL-C-reducing effects of the *trans* diet. Although this observation is intriguing, we are unable to offer a credible explanation.

The above study underscores the fact that a combination of 12:0 + 14:0, as present in some naturally occurring fats such as coconut oil, palm kernel oil and butterfat, results in altered lipid and lipoprotein profiles that enhance CHD risk. In this context, the effects of *trans* fatty acids appear comparable to 12:0 + 14:0 even when the diet contains sufficient levels of linoleic acid. Addition of dietary cholesterol to the diets further increases these risk factors. Despite the large differences that are obvious in the dietary fatty acid composition of AHA and POL, they elicited comparable effects on TC, LDL-C and HDL-C both in the presence or absence of dietary cholesterol. It therefore appears that any dietary recommendations aimed at improving overall lipoprotein profiles should specifically target to reduce the consumption of *trans* fatty acids, the combination of lauric and myristic acids and cholesterol-rich foods. This has been highlighted in the recent reports of the Institute of Medicine, USA[28] and the WHO/FAO Expert Consultation.[29]

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