# Impact of saturated and *trans* fatty acid enriched oil blends on atherosclerosis in rabbits fed cholesterol-free diets

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Thirty six-male New Zealand White rabbits subdivided into four dietary groups (9 animals per group) were fed high fat (36% en), cholesterol-free diets for nine months. The dietary oil blends were formulated to contain high levels of the target fatty acids namely *trans*-rich (partially hydrogenated soybean oil; TRANS), *cis* monounsaturated-rich (rapeseed, sunflower seed oil and palm olein; MONO), palmitic-rich (palm olein; POL) and lauric-myristic rich (coconut, palm kernel and corn oils; LM). *Ad libitum* feeding of the rabbits resulted in normal growth throughout the nine months and no differences in the final body weights of the animals were evident at autopsy. Plasma total cholesterol was significantly elevated only by the LM enriched diet compared with all other treatments; values were comparable between the other three treatment groups. Changes in the total cholesterol were not reflected in the VLDL and LDL lipoproteins. However, HDL-cholesterol was significantly lowered by the TRANS diet compared with all other dietary groups. HDL-cholesterol was also significantly increased by the LM diet in comparison to the POL-diet. Both adipose and liver triglyceride fatty acid compositions tended to reflect the type of fatty acids fed the animals. *Trans* fatty acids were evident only in animals fed the *trans* diet and it was apparent that the *trans* fatty acids competed with linoleic acid for incorporation into these tissues. Increased concentrations of lauric and myristic fatty acids in the LM-fed animals were also evident. In the POL and high MONO fed rabbits, palmitic and oleic fatty acids (respectively) were concentrated in the adipose and liver. The diets, however, failed to induce severe atherosclerosis in this study. This can be explained, in part, by the lack of dietary cholesterol and the use of plant (rather than animal) proteins in our dietary formulations. The effect of these important atherosclerosis modulators in association with these fatty acids requires further evaluation.

**Key words: rabbits, atherosclerosis, lipids, lipoproteins, dietary fat**

## Introduction

The role of dietary fats as a determinant of plasma lipids and lipoproteins is well documented[1-3]. Plasma lipid levels are influenced not only by the amount of fat consumed but by its nature as well. The degree of saturation and unsaturation, stereo isometric differences and fatty acid chain length can all determine the response of plasma cholesterol to dietary fats. The classical studies of Hegsted, Keys and their colleagues[4-6] have dissected the cholesterolaemic effects of dietary fats into their constituent fatty acid classes. They showed that saturates were twice as effective in raising plasma total cholesterol (TC) as the polyunsaturates. Among the saturated fatty acids, only those with chain lengths 12, 14 and 16 carbons (lauric, myristic and palmitic respectively) increased TC while the 18 carbon stearic acid and the monounsaturated oleic acid were recognised as neutral. These simplified fatty acid relationships and their effects on lipoprotein classes have also come under further scrutiny recently[7]. Newer data from animal[8,9] and human[10-12] studies now suggest that the cholesterolaemic effects of the saturates are not uniform. The most potent cholesterol raising fatty acid appears to be myristic acid (14:0) while palmitic acid may behave as a neutral fatty acid under certain conditions and especially when supported by sufficient amounts of linoleic acid[7].

The fatty acid controversy has been further fuelled by the role that *trans* fatty acids play in the modulation of blood lipids and possibly atherosclerosis. *Trans* fatty acids are geometrical isomers of the unsaturated fatty acids and are produced as a result of the hydrogenation of edible oils used in margarine and shortening manufacture. Several clinical studies have shown the adverse effects of *trans* fatty acids[13-16]; they increase TC, lipoprotein Lp(a) and reduce the protective high density lipoprotein cholesterol (HDL-C). The adverse effects of *trans* on lipoproteins shown in these clinical studies have additionally been supported by the epidemiological data of Willett and coworkers[17,18]. A recent study of Sundram *et al*[19] addressed an outstanding question of whether the *trans* fatty acids are nutritionally better or worse in these regards than the dietary saturated fatty acids they were designed to replace in solid fat products. They concluded that the negative impacts of *trans* elaidic acid on the lipoprotein profile of humans were worse than the saturates of chain length 12, 14 and 16 carbons.

The association between diet, plasma lipid concentrations and atherosclerosis has been well documented and reviewed[20]. Atherosclerotic lesions in humans and in animal experimental models appear to be related to elevated plasma cholesterol and excess fat consumption. The rabbit is a frequently used experimental model for evaluating dietary fat effects and atherosclerosis. In the present study we postulated that the adverse effects of *trans* fatty acids compared with the saturates on lipids and lipoproteins observed in a previous human study[19] may lead to the onset of atherosclerotic lesions when fed to rabbits over an extended duration. This hypothesis was therefore tested using the same fat blends as used in the above human study. The oil blends mimicked fatty acid compositions that are routinely consumed in normal human dietary situations. We however omitted the addition of dietary cholesterol in this phase of our study since dietary cholesterol significantly enhances the onset of atherosclerosis in the rabbit model.

## Materials and methods

Thirty-six male New Zealand White rabbits aged 2.5 months were randomly assigned to four different dietary groups. To each group was assigned a total of 9 rabbits and dietary feeding was commenced 15 days later at the age of three months after the rabbits had been acclimatised to their new environment in the animal experimental unit. The rabbits were fed *ad libitum* for a total of nine months a 20% (w/w) high fat diet containing the dietary oil blends whose fatty

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acid composition is described in Table 1. The *trans*-rich oil was derived from partially hydrogenated soybean oil (35° C) which consisted of almost 39% *trans* fatty acids and predominated as elaidic acid. This was remixed with native soybean oil so that a final *trans* fatty acid content of 29.2% was achieved in the blend. The high lauric-myristic (LM) fatty acid oil was a blend of palm kernel, coconut and corn oils to reflect a high level of lauric and myristic acids matched by adequate amounts of linoleic acid from corn oil to match the *cis* 18:2 content in the *trans* blend. The monounsaturated *cis* 18:1 oils blend (MONO) was a blend of rapeseed oil (30%), sunflower seed oil (25%) and palm olein (45%) whereas the 16:0-rich fat was palm olein.

**Table 1.** Fatty acid composition of fat blends incorporated into diets (g/100 g of dietary oil)

| Fatty acid | *Trans*-rich[a] hydrogenated soyabean oil | MONO-rich[b] (18:1) | Palmitic-rich[c] (16:0) | Lauric-myristic-rich (12:0+14:0)[d] |
|---|---|---|---|---|
| **SFA** | **17.76** | **23.56** | **44.56** | **60.01** |
| 8:0 | ND | ND | ND | 0.75 |
| 10:0 | ND | ND | ND | 1.91 |
| 12:0 | ND | 0.19 | 0.53 | 27.08 |
| 14:0 | ND | 0.46 | 0.84 | 14.61 |
| 16:0 | 11.60 | 19.08 | 38.94 | 10.84 |
| 18:0 | 5.62 | 3.14 | 3.96 | 4.58 |
| 20:0 | 0.24 | 0.34 | 0.29 | 0.23 |
| 22:0 | 0.30 | 0.35 | ND | 0.10 |
| **MUFA** | **33.20** | **61.01** | **44.36** | **20.87** |
| 16:1 n-9 | ND | 0.14 | 0.11 | ND |
| 18:1 n-9 | 33.20 | 60.87 | 44.25 | 20.87 |
| **PUFA** | 19.83 | 14.9 | 10.89 | 18.83 |
| 18:2 n-6 | 17.60 | 13.63 | 10.74 | 18.60 |
| 18:3 n-3 | 2.23 | 1.27 | 0.15 | 0.23 |
| ***Trans* FA** | 29.21 | ND | ND | ND |
| 18:1 n-9t | 23.11 | ND | ND | ND |
| 18:1 n-11t | 3.40 | ND | ND | ND |
| 18:1 n-13t | 1.55 | ND | ND | ND |
| Unid *cis/trans* | 1.15 | ND | ND | ND |
| **P/S ratio** | 1.12 | 0.63 | 0.24 | 0.31 |

ND, not detected; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P/S, polyunsaturated/saturated fatty acid ratio. (a) 70% hydrogenated soyabean oil (melting point 35°C), 30% soyabean oil. (b) 30% rapeseed oil, 25% sunflower seed oil, 45% palm olein. (c) 100% palm olein. (d) 45% coconut oil, 15% palm kernel oil, 40% corn oil.

Commercial rabbit diet (in pellet form) was used as the basal diet. The commercial pellets were obtained as a single production batch for the entire study duration to reduce variations in their nutrient content. This contained 2.5% fat, 16.0% protein, 48.8% carbohydrates, 18% fibre, 12.8% moisture and 1.9% vitamins, minerals (including calcium) and trace elements required for optimum maintenance of the rabbits. The protein source in this basal diet was mainly of vegetable (soy) origin. Incorporation of the oil blends into the basal rabbit pellets to increase the fat energy density of the diets was achieved as follows: 200g of each oil blend was first dissolved in 120ml of diethyl ether and this was mixed with 1.0 kg of the rabbit pellet. This was stirred in a large vessel and the diethyl ether was air-dried. The oil enriched pellets were further oven dried to remove any traces of diethyl ether in the feed. The resulting diet contained 36.4% en as fat, 50.6% en as carbohydrates and 13% en as proteins. Their fatty acid composition is given in Table 2. All animals were weighed at regular monthly intervals and monitored for their general well being and feed intakes.

At the end of nine months feeding, the rabbits were fasted overnight, anaesthetised with sodium pentabarbital and had their blood collected by heart puncture. The animals were then killed by an overdose of the anaesthesia (100mg/kg body weight) and autopsied to remove the various organs of interest. The entire aorta from the aortic valve to the bifur-cation was removed quickly and placed in cold physiological saline at 4°C. The heart, kidney, liver, spleen, pancreas and lungs were removed for weight determination and samples were preserved in 10% neutral buffered formalin (pH 7.2) for histopathological examination. The aorta was opened along the mid-dorsal line to expose the intimal surface. It was then divided into arch, ascending, descending and abdominal aorta. Samples from the aorta and liver were preserved on 4% ice-cold glutaraldehyde for electron microscopy (at a later date). The aorta was examined macroscopically; portions were pinned on a wax board and stained with Oil Red O for delineation of atheromatous deposits in the intima.

Lesions of the intimal surfaces were evaluated by microscopic examination from tissues and from constant sites from the arch, ascending and abdominal aorta, without regard to the presence or absence of gross pathology. Tissue was stained with both haematoxylin and eosin as well as with Oil Red O after cryostat section. The tissue sample at each site consisted of a transverse section of the aorta. Involvement of the aorta by atheroma was assessed by depth of lipid infiltration. Samples from other organs were stained with haematoxylin and eosin, and the general pathological alteration was documented. In addition, specific evidence of fatty change and fatty infiltration was studied.

**Table 2.** Fatty acid composition (%) and energy (%) of diets.

| **Fatty acid** | **TRANS** | | **MONO** | | **POL** | | **LM** | |
|---|---|---|---|---|---|---|---|---|
| | FAC % | en % | FAC % | en % | FAC % | en % | FAC % | en % |
| SFA | 21.0 | 7.6 | 26.2 | 9.4 | 44.2 | 15.9 | 57.4 | 20.7 |
| 8:0 | 0.3 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.8 | 0.3 |
| 10:0 | 0.1 | - | 0.1 | - | 0.1 | - | 1.7 | 0.6 |
| 12:0 | 1.9 | 0.7 | 2.1 | 0.8 | 2.4 | 0.9 | 25.2 | 9.1 |
| 14:0 | 0.8 | 0.3 | 1.2 | 0.4 | 1.5 | 0.5 | 13.4 | 4.8 |
| 16:0 | 12.3 | 4.4 | 18.9 | 6.8 | 36.0 | 13.0 | 11.8 | 4.3 |
| 18:0 | 5.1 | 1.8 | 3.0 | 1.1 | 3.3 | 1.2 | 4.2 | 1.5 |
| 20:0 | 0.2 | - | 0.3 | 0.1 | 0.3 | 0.1 | 0.2 | 0.1 |
| 22:0 | 0.3 | 0.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.1 | - |
| MUFA | 31.6 | 11.4 | 56.0 | 20.2 | 41.1 | 14.8 | 20.6 | 7.4 |
| 16:1n-9 | ND | ND | 0.4 | 0.1 | 0.1 | - | 0.4 | 0.1 |
| 18:1n-9 | 31.6 | 11.4 | 55.6 | 20.0 | 41.0 | 14.8 | 20.2 | 7.3 |
| PUFA | 21.4 | 7.7 | 17.1 | 6.2 | 13.9 | 5.0 | 20.5 | 7.4 |
| 18:2n-6 | 19.5 | 7.0 | 16.0 | 5.8 | 13.7 | 4.9 | 20.3 | 7.3 |
| 18:3n-3 | 1.9 | 0.7 | 1.1 | 0.4 | 0.2 | 0.1 | 0.2 | 0.1 |
| TRANS | 25.1 | 9.1 | ND | ND | ND | ND | ND | ND |
| 18:1n-9t | 19.9 | 7.2 | ND | ND | ND | ND | ND | ND |
| 18:1n-11t | 2.9 | 1.0 | ND | ND | ND | ND | ND | ND |
| 18:1n-13t | 1.3 | 0.5 | ND | ND | ND | ND | ND | ND |
| Unid *cis/ trans* | 1.0 | 0.4 | ND | ND | ND | ND | ND | ND |

SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids

**Table 3.** Effect of dietary oil blends on rabbit lipids and lipoproteins following 9-month dietary feeding

| | TC mmol/L | TG mmol/L | VLDL-C mmol/L | LDL-C mmol/L | HDL-C mmol/L | LDL/HDL-C RATIO |
|---|---|---|---|---|---|---|
| TRANS | $2.41 \pm 0.45^{a}$ | $0.99 \pm 0.18$ | $0.44 \pm 0.09$ | $1.33 \pm 0.38$ | $0.64 \pm 0.30^{a,b,c}$ | $2.08 \pm 0.46^{a,b,c}$ |
| MONO | $2.44 \pm 0.57^{b}$ | $0.86 \pm 0.18$ | $0.39 \pm 0.09$ | $1.25 \pm 0.27$ | $0.80 \pm 0.38^{a}$ | $1.56 \pm 0.39^{b}$ |
| POL | $2.52 \pm 0.45^{c}$ | $1.01 \pm 0.20$ | $0.46 \pm 0.11$ | $1.28 \pm 0.33$ | $0.76 \pm 0.21^{b,d}$ | $1.68 \pm 0.32^{c}$ |
| LM | $2.78 \pm 0.34^{a,b,c}$ | $0.96 \pm 0.45$ | $0.48 \pm 0.20$ | $1.32 \pm 0.48$ | $0.89 \pm 0.25^{c,d}$ | $1.48 \pm 0.37^{a}$ |

Values are means ± SD. (n=9 rabbits per group); Means with common superscript are significantly different ($P < 0.05$)

**Table 4.** Adipose tissue fatty acid composition (%) of rabbits fed experimental oil blends.

| Fatty acid | | TRANS | MONO | POL | LM |
|---|---|---|---|---|---|
| **SFA** | 12:0 | ND | ND | ND | $4.62 \pm 0.89$ |
| | 14:0 | $0.66 \pm 0.16^{a}$ | $0.77 \pm 0.26^{b}$ | $0.94 \pm 0.14^{c}$ | $7.36 \pm 0.29^{a,b,c}$ |
| | 16:0 | $14.84 \pm 0.93^{a,b,c}$ | $17.79 \pm 1.67^{a,d,e}$ | $22.75 \pm 0.98^{b,d,f}$ | $9.88 \pm 1.76^{c,e,f}$ |
| | 18:0 | $5.05 \pm 0.66$ | $4.58 \pm 0.66$ | $5.20 \pm 0.68$ | $4.34 \pm 0.46$ |
| **MUFA** | 16:1 n-9 | $0.62 \pm 0.30^{a,b,c}$ | $1.24 \pm 0.30^{a}$ | $1.10 \pm 0.25^{b}$ | $1.31 \pm 0.46^{c}$ |
| | 18:1 n-9 | $39.23 \pm 15.68^{a,b,c}$ | $53.86 \pm 3.09^{a,d,e}$ | $49.83 \pm 0.79^{b,d,f}$ | $28.26 \pm 0.98^{c,e,f}$ |
| **PUFA** | 18:2 n-6 | $25.37 \pm 0.66^{a,b,c}$ | $19.09 \pm 0.98^{a,d}$ | $18.82 \pm 0.47^{b,e}$ | $32.09 \pm 2.03^{c,d,e}$ |
| | 18:3 n-3 | $1.62 \pm 0.61^{a,b,c}$ | $1.16 \pm 0.31^{a,d}$ | $0.84 \pm 0.11^{b,d,e}$ | $1.17 \pm 0.24^{c,e}$ |
| **TRANS** | 18:1 n-9t | $11.31 \pm 0.93$ | ND | ND | ND |
| | 18:1 n-11t | $0.29 \pm 0.04$ | ND | ND | ND |

Values are means ± S.D. (n=9 rabbits per group); Means with common supercript are significantly different ($P < 0.05$)

*Measurement of plasma lipids and lipoproteins*

Blood collected in tubes containing EDTA (1 mg/ml blood) was used to prepare plasma for the lipid and lipoprotein analyses. Four ml of plasma was pipetted into a Beckmann 50.3 Ti ultraclear centrifuge tube and over layered with 2.0 ml NaCl solution (d20 = 1.006 g/mL). The very low density lipoproteins (VLDL) were isolated by preparative ultracentrifuge in a Beckmann 50.3 Ti rotor in a Beckmann LM8-70 ultracentrifuge. Low density and high density lipoproteins were sequentially isolated at their respective densities (d20) of ($1.006<d<1.063$) and ($1.063<d<1.125$) on a Kbr density gradient. These lipoproteins were extensively dialysed to remove their background salt densities. The cholesterol and triglycerides content of the plasma and isolated lipoproteins was analysed enzymatically on an autosampler.

*Fatty acid analysis*

Adipose tissue and liver were first pulverised in liquid nitrogen to facilitate the extraction of its lipids using chloroform: methanol (2:1v/v) and partitioned with salt solution as described by Folch *et al*[21]. The lower chloroform layer was dried under nitrogen recovered for thin layer chromatography. The total lipids thus obtained from adipose tissue and liver were aliquoted and spotted on thin-layer plates coated with silica gel G and the lipid components were separated using hexane:diethyl ether:formic acid (80:20:2 v/v/v) as solvent system in a TLC tank saturated with the solvent vapours. Lipid components were identified after spraying the plates with 7,12-dichlorofluoresceine dye in ethanol and visualised under ultraviolet light. The triglycerides in adipose tissue were thus recovered for fatty acid analysis.

Fatty acid composition of the dietary oils, diets, adipose and liver triglycerides were determined following trans-methylation of the samples using toluene-sulphuric acid[19]. Fatty acids were then analysed by using a Perkin Elmer Autosystem gas chromatogram (Perkin Elmer Corporation, Norwalk, CT, USA) fitted with a 100-metre capillary column (SP2560, Supelco, Belfonte, USA) and temperature programmed from 160°C to 240°C at 4°C/min.

*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 $p<0.05$.

**Results**

Rabbits fed the experimental diets demonstrated normal growth throughout the nine months feeding duration. At autopsy, a diet-induced difference in body weight or in the weight of various organs was not generally evident. The diets fed to these animals had similar total fat energy densities but varied significant in the contribution of energy from the individual fatty acids (Table 3). A total of 9.1% energy as *trans* fatty acids was available for the TRANS diet and most of this was contributed as elaidic acid (18:1 n-9t; 7.2% en). The linoleic acid content in the LM and TRANS diets were matched so that differences in the cholesterolaemic and atherosclerotic effects of these fats, when apparent, could be ascribed to the target fatty acids (total *trans* in the TRANS group and lauric + myristic acids in the LM group) and not as a result of differences in the linoleic acid availability. Similarly POL was enriched in palmitic acid and oleic acids (13% en and 14.8% en) while MONO was oleic rich (20.2% en).

Plasma lipids and lipoproteins were generally modulated by the diet type. Plasma total cholesterol (TC) was significantly elevated in the rabbits fed the LM diet compared to all other dietary treatments. TC values were however comparable between the TRANS, MONO and POL diets. The effect of these diets varying in their fatty acid composition was evaluated on plasma cholesterol distribution in the lipoproteins. VLDL-cholesterol was lowest in rabbits fed the MONO diet and highest in the POL group. These values did not attain significance. Similarly, LDL-cholesterol was not significantly modulated by these diets in spite of the pronounced differences in the dietary fatty acid composition. HDL-cholesterol was however lowest in the TRANS-fed animals and this lowering was significant in comparison to all other dietary treatments. HDL-cholesterol was also significantly increased by the LM diet in comparison to the POL diet. As a result of these shifts in the lipoprotein cholesterol values the ratio of LDL/HDL-cholesterol was significantly elevated by the TRANS diet in comparison to all other dietary treatments.

The adipose tissue fatty acid composition was evaluated as an index of the long-term effect of these fatty acids in these rabbits (Table 4). In general this tissue fatty acid composition reflected the type of fatty acid fed the animals. In the LM group, a significant increase in the 12:0 and 14:0 fatty acids was apparent whereas these fatty acids were present at less than 1.0% in the other dietary treatments. Palmitic acid was significantly higher in the POL fed animals while *cis* oleic acid was highest in the MONO treated animals. Rabbits fed the TRANS diet were characterised by the presence of *trans* fatty acids that were absent in all other treatments. In addition, *cis* 18:2 were significantly lower in the POL and MONO fed rabbits, signifying a lower availability of this essential fatty acid from the diet. However, the *cis* 18:2 content in the TRANS fed

**Table 5.** Major fatty acids (%) present in liver triglycerides of rabbits fed different diets for 9 months

| Fatty acid | | TRANS | MONO | LM | POL |
|---|---|---|---|---|---|
| **SFA** | 12:0 | ND | $0.26 \pm 0.09^{a}$ | $2.27 \pm 1.35^{a,b}$ | $0.20 \pm 0.04^{b}$ |
| | 14:0 | $0.74 \pm 0.46^{a}$ | $0.96 \pm 0.10^{b}$ | $5.28 \pm 1.80^{a,b,c}$ | $0.95 \pm 0.15^{c}$ |
| | 16:0 | $18.95 \pm 2.81^{a,b,c}$ | $29.80 \pm 2.36^{a,d}$ | $27.59 \pm 1.42^{b,e}$ | $31.76 \pm 1.97^{c,d,e}$ |
| | 18:0 | $13.99 \pm 2.81^{a,b,c}$ | $4.34 \pm 0.65^{a}$ | $4.50 \pm 1.37^{b}$ | $4.13 \pm 1.03^{c}$ |
| **MUFA** | 16:1 n-9 | $0.50 \pm 0.22^{a,b,c}$ | $1.31 \pm 0.34^{a}$ | $1.17 \pm 0.34^{b}$ | $1.15 \pm 0.26^{c}$ |
| | 18:1 n-9 | $17.69 \pm 2.92^{a,b,c}$ | $43.78 \pm 2.76^{a,d,e}$ | $25.27 \pm 2.89^{b,d,f}$ | $39.47 \pm 1.58^{c,e,f}$ |
| **PUFA** | 18:2 n-6 | $28.93 \pm 3.78^{a,b,c}$ | $18.97 \pm 4.92^{a,d}$ | $31.82 \pm 2.39^{b,d,e}$ | $20.92 \pm 1.60^{c,e}$ |
| | 18:3 n-3 | $1.45 \pm 0.88^{a,b,c}$ | $0.69 \pm 0.16^{a}$ | $0.85 \pm 0.19^{b}$ | $0.57 \pm 0.14^{c}$ |
| **Trans** | 18:1 n-9t | $4.78 \pm 1.99$ | ND | ND | ND |
| | 18:1 n-11t | $4.49 \pm 0.89$ | ND | ND | ND |
| | 18:1 n-13t | $1.54 \pm 0.14$ | ND | ND | ND |

Values are means ± S.D. (n=9 rabbits per group); Means with common superscript are significantly different ($P < 0.05$)

rabbits was obviously significantly lower than that of the LM fed rabbits although dietary availability was almost equally matched.

Fatty acid composition of liver triglycerides (Table 5) generally followed the trend seen in the adipose tissue and reflected the dietary availability of these fatty acids. Lauric and myristic acids were highest in the LM group, palmitic in the POL fed animals, *cis* oleic in the MONO fed rabbits and *trans* fatty acids were evident only in the TRANS group.

Gross pathological examination of the aorta generally showed a smooth intima with no macroscopic evidence of atheromatous lesions except in animals fed *trans*-rich (2/9) and POL-rich (1/9) diets. In these animals, fatty streaks and fibrous plaques were present in the arch and the ascending aorta. Histological examination of these grossly abnormal lesions showed moderate lipid infiltration with distinct elevation of the lesions, capped by a white fibrotic cap. In two other animals, (one each from *trans* and POL groups), there was histological evidence of mild lipid infiltration into the intima even though there was no obvious abnormality on inspection.

Most animals showed some degree of fatty change in the liver, which ranged from minimal to severe fatty change. There was no statistically significant difference between the extent of fatty change and the diet in these experimental animals. Histopathological examination of the other organs was generally unremarkable.

## Discussion

In this study rabbits were fed high-fat diets continuously for the nine-month duration. Changes in blood lipids and lipoproteins resulting from these fatty acid manipulations were evident. Although the oil blends used had very different fatty acid compositions in terms of their polyunsaturated: mono-unsaturated: saturated fatty acid ratios, they nevertheless failed to elicit the expected significant differences in plasma TC and LDL-C. However HDL-C was depressed by the *trans*-enriched diet and the resulting LDL/HDL-cholesterol ratio was significantly lower in the *trans* fed animals. The same oil blends were fed to normocholesterolaemic humans in an earlier study[19]. In these subjects *trans*-enrichment in the diet resulted in adverse lipoprotein profiles (increased TC, LDL-C, Lp(a) and decreased HDL-C) which were worse than the saturates.

In an effort to delineate the effects of the different saturated fatty acids on cholesterolaemia, Hayes and Khosla[7] have proposed that myristic acid is the most cholesterolaemic fatty acid. Its potency has been estimated to be four times that of palmitic acid that is the most abundant saturated fatty acid in the human diet. In nature, myristic acid normally coexists with lauric acid (eg. in butterfat, coconut oil and palm kernel oil) and this makes it difficult to separate the effects of lauric acid from myristic acid. In the present study the combination of lauric + myristic fatty acids did not increase TC and LDL-C compared to the other fat blends. We had increased the linoleic acid content in the LM blend and it is possible that this may have helped overcome the cholesterolaemic response of the myristic acid therein. The response between the POL (palmitic-rich) and MONO (*cis* 18:1-rich) was similar, a response that was also evident in the human volunteers[19].

The lack of cholesterolaemic response in the present study may be partially explained on the following basis. Rabbits on low-fat, cholesterol-free semi-purified diets containing casein become hypercholesterolaemic whereas normal levels of cholesterol are maintained if the casein is replaced by isolated soy protein[22]. This suggests that the mechanism of hypercholesterolaemia in rabbits may be protein dependent more than fat dependent. Apart from this fact, it is well-documented[23] that rabbits are sensitive to the addition of cholesterol in their diets and will develop cholesterolaemia and atherosclerotic lesions when exposed to dietary cholesterol for even short durations. In the present study the protein source in the basal rabbit pellet used was soy based, and no dietary cholesterol was added to the diets. This may explain the low levels of plasma lipids and the lack of response on the lipoprotein profiles even though the fatty acid compositions were significantly different.

Both adipose tissue and liver triglycerides fatty acid compositions provided important insights into the utilisation of the dietary fatty acids pools in this animal atherosclerotic model. In these tissue samples *trans* fatty acids were evident only when animals were fed the hydrogenated oil blend. Of specific interest was the observation that the tissue linoleic and linolenic acids content were significantly lower in the TRANS than in the LM dietary group. This was in spite of the almost equal availability of these polyunsaturates in the diet from these two oil blends (TRANS, 7.7% en versus LM 7.4% en). It is now well documented that *trans* fatty acids increase essential fatty acid requirements[24,25] and therefore adequate levels of essential fatty acids must be present in the diet to overcome any adverse effects especially on the physiologically important biochemical pathways. In this model the lower tissue incorporation of linoleic and linolenic acids could signify that the *trans* had competed with these acids for some common reaction sites. The total *trans* energy available was high and in a situation where inadequate essential polyunsaturates are available adverse effects on some metabolic processes including lipoprotein cholesterol modulation and atherosclerosis may be expected.

It has been reported[26] in hamsters that dramatic hepatic changes in LDL-cholesterol metabolism occur when the diet is enriched with myristic acid. Enrichment with 18:1 n-9t (elaidic acid) however was reported to lack biochemical and physiological effects on LDL-cholesterol since in vitro ACAT cannot effectively use 18:1 n-9t for cholesterol esterification[27]. That hypothesis concerning 18:1 n-9t fatty acid contradicts recent observations in humans[13-16] wherein LDL-cholesterol was increased and HDL-cholesterol decreased. Furthermore, CETP activity increases (thereby depressing HDL-cholesterol) when *trans* are incorporated in the diet[28]. In this study LDL-cholesterol was not significantly increased by either myristic (in LM) or *trans* (in TRANS) fatty acids but HDL was depressed by the TRANS diet. We believe that this lack of effect on LDL-cholesterol and overall atherosclerotic index was due to the absence of dietary cholesterol and dietary casein (animal protein) in our diet formulation. The *trans* effect may not simply be a case of lack of activation of ACAT activity as suggested previously. The interaction of dietary cholesterol and casein along with the fatty acids of interest need to be further evaluated in a similar animal model.

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**Impact of saturated and *trans* fatty acid enriched oil blends on atherosclerosis in rabbits fed cholesterol-free diets**

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# 膳食中富含飽和脂酸與反式脂酸的油類對白兔動脈粥樣硬化的影響

## 摘要

作者把36只新西蘭白兔分成四組（9只一組），給予四種高脂肪（占總能量36%），缺膽固醇膳食9個月．膳食中的油類含大量的反式脂酸（部分氫化豆油，TRANS），順式單不飽和脂酸（菜油，向日葵子油和棕櫚油酸甘油脂，MONO），棕櫚油酸（棕櫚油酸甘油脂，POL）和月桂－豆蔻酸（椰子油；棕櫚仁油和玉米油，LM）．四組白兔進食9個月，生長正常，最后體重没有差异．結果發現只有LM組血漿總膽固醇較其他三組明顯升高；但極低密度脂蛋白膽固醇（VLDL－c）和低密度脂蛋白膽固醇（LDL－c）没有區别．TRANS組的高密度脂蛋白膽固醇（HDL－c）明顯較其他組爲低，LM組的LDL－c亦明顯高于POL組．脂肪組織和肝臟甘油三酯脂肪酸的組成隨喂養脂肪酸種類不同而异，喂養反式脂酸膳食的白兔，亞油酸競爭性地摻入這些組織中．喂養LM膳食的白兔亦摻入月桂酸和豆蔻酸在這些組織中．喂養POL和MONO膳食的白兔，棕櫚酸和油酸分别摻入這些組織中．該研究發現這些膳食不會引起嚴重的動脈粥樣硬化，可部分解釋爲我們配制的膳食缺乏膽固醇，同時應用植物（不是動物）蛋白，這個與脂肪酸有關的重要動脈粥樣硬化調節者的作用尚需進一步評估．

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