EFFECT OF *TRANS* FATTY ACIDS ON PLASMA LIPID AND LIPOPROTEIN LEVELS IN CYNOMOLGUS MONKEYS

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Nine cynomolgus monkeys (Macaca fascicularis) *were fed a purified diet containing 30 en % as coconut oil for three months and then rotated through three different cholesterol-free purified diets in which 30% energy was derived from fat. The dietary fats used were partially hydrogenated soyabean oil rich in trans-fatty acids (PHSBO), palm olein (POo) and an American Heart Association (AHA) Step 1 fat blend. Total cholesterol and LDL-C increased significantly ($P < 0.02$) when the monkeys were fed the PHSBO diet as compared with the AHA diet. No significant differences in TC or LDL-C were seen as between the POo and AHA diets.*

To evaluate the effect of limited linoleic acid in the PHSBO diet, the monkeys were again fed PHSBO, but blended with safflowerseed oil (SFO) to provide linoleic acid at 3.72 en per cent. Cholesterol levels decreased substantially by comparison with the coconut oil (CNO) and PHSBO diets, suggesting that inadequate 18:2 intake was an important factor in the hypercholesterolemia induced in these monkeys.

INTRODUCTION

Hydrogenated vegetable and marine oils, used extensively in the formulation of margarines, shortenings, frying fats and cooking oils, are a major source of dietary fat in many countries. Hydrogenation results in the reduction of the highly unsaturated fatty acids by addition of hydrogen atoms to the double bonds in the fatty acid

chains. In addition, it produces shifts in the positions of the double bonds and also isomerization of the *cis* to the *trans* forms of the fatty acids. These effects of hydrogenation produce fats with a higher melting point and convert the liquid oils into semi-solid fats which can be packaged and stored for longer periods of time. In the USA, for example, out of 5 billion kilograms of edible fats and oils, 2.7 billion are partially hydrogenated (Dutton, 1979). The estimated average intake of *trans* fatty acids is 8 to 10 g per day in the United States (Senti, 1985) and as much as 17 g per day in the Netherlands (Brussard, 1986).

Naturally-occurring *trans* fatty acids are found in all fat containing products derived from ruminants, particularly those made from dairy milk fat. Unlike monogastric animals, ruminants produce *trans* fatty acids by means of the rumen flora. Dairy milk fat products can contain 1-9% *trans* fatty acids depending on the ruminant diet (Kanhai, 1988). However, the major source of *trans* fatty acids in the human diet is generally from partially hydrogenated vegetable oils used in the manufacture of margarines and shortenings. The presence of fats containing these *trans* fatty acids is of growing concern to nutritionists since they have been implicated in raising the LDL/HDL cholesterol ratio (Mensink *et al.*, 1990; Nestel *et al.*, 1992).

Studies on the effects of *trans* fatty acids have been controversial. In a well controlled human feeding study with hydrogenated fats an increase in plasma cholesterol was noted and further investigations implicated the *trans* isomers of the fatty acids as the causative agents in elevating blood cholesterol (Horlick, 1990). However, in another human study, fats containing up to 21% *trans* fatty acids and 50% 9,12 *cis* 18:2 did not elevate serum cholesterol levels (McOsker *et al.*, 1962). Similarly, when a diet containing no *trans* fatty acids was compared with one that contained 44% *trans* fatty acids no significant differences in plasma cholesterol or triglycerides were observed (Mattson, 1975). On the other hand, Vergroesen (1972) concluded that in the presence (but not in the absence) of dietary cholesterol feeding elaidic acid resulted in higher serum cholesterol levels than were obtained with fats rich in oleic and palmitic acids. In general, it was assumed that when adequate amounts of oleic and linoleic acids are available the effect of hydrogenated fat on serum cholesterol levels is negligible. In a more recent study conducted in the Netherlands it was demonstrated that high intakes of elaidic acid resulted in an elevation of LDL cholesterol (Mensink and Katan, 1990) relative to an oleic acid diet.

Because palm oil is naturally rather saturated it can be used to produce margarines and shortenings with little or no hydrogenation being required. By utilizing palm oil in such situations, both the total *trans* and total saturates in the products can be reduced. To investigate these relationships further, we conducted a study in which a partially hydrogenated soyabean oil diet was compared with a palm olein diet, as well as with the American Heart Association (AHA) step 1 diet wherein the saturated, monounsaturated and polyunsaturated fatty acids are present in the ratio 1:1:1.

MATERIALS AND METHODS

Preparation of Partially Hydrogenated Soyabean Oil (PHSBO)

PHSBO was prepared in PORIM's pilot plant using SP7-nickel catalyst with a hydrogen pressure of 20 psi and a temperature of 200°C. The stock oil was hydrogenated over a 4-hour period to an iodine value of 70.

Animals and Diets

Nine cynomolgus monkeys (*Macaca fascicularis*) weighing 2.5 ± 0.7kg (x ± S.D.) were individually housed in stainless steel cages in a room maintained at normal ambient temperatures (27°–33°C).

In Experiment I, all the monkeys were fed a purified diet containing 30% en as coconut oil (CNO) for 3 months. This is termed the run-in period. Subsequently, the animals were divided into 3 groups and each group received either the AHA blend, PHSBO, or palm olein (POo) for a 5-week period. The AHA diet was obtained by blending palm oil and soyabean oil in the ratio 1:1. At the end of 5 weeks the animals were bled and reassigned to the next group in the following

order: AHA, PHSBO and palm olein. After completion of the 3 diets in rotation, all monkeys received CNO again for 6 months.

In phase II of the experiment, after the monkeys had been stabilized on CNO for 6 months, they were fed diets containing either CNO/SFO or PHSBO/SFO each for 5 week periods. Both diets provided 3.6 en % as 18:2 from the added SFO. The proportions of major ingredients in the diets were as follows: casein 19%, sucrose 25.5%, corn starch 25.9%, fat 14.3%, cellulose 10%, salt mix 4.8% (Hegsted IV), vitamin mix 0.5%, Vitamin D_3 0.2 ml. The diets were prepared in 3 kg lots and were stored in a cold room at 4°C until use. The monkeys were fed approximately 100 kcal/kg/day and body weights were recorded once every four weeks.

Analysis of Oils

The fatty acid composition of the dietary oils was determined by analysing the methyl esters of the acids on a 60m x 0.25mm capillary column coated with 0.2 micron SP 2340 using a Perkin Elmer gas chromatogram. Helium at a flow rate of 1 ml/min was used as the carrier gas. Fatty acid methyl esters were prepared by dissolving 0.05g of the oil sample in 0.95 ml of hexane to which was added 0.05 ml of 1M methanolic sodium methoxide. The methyl esters in hexane were washed with water and finally dried over anhydrous sodium sulphate prior to injection into the GC. The *trans* fatty acid content of the partially hydrogenated soyabean oil was determined by comparing retention times with those of known standards.

Measurement of Plasma Lipids and Lipoprotein

At the end of five weeks the monkeys were fasted overnight and 10 ml blood was collected from each of them by venous puncture of the femoral vein. Lipoproteins were isolated from freshly prepared serum by sequential ultracentrifugation at densities 1.006 (VLDL), 1.063 (LDL) and 1.216 (HDL) according to the standardized techniques of Havel *et al.* (1955). The lipoprotein fractions were subsequently recentrifuged at their corresponding densities to eliminate any traces of contamination with other lipoproteins. The cholesterol contents of the individually isolated lipoprotein fractions were determined on an autoanalyser (Cobas Bio, Hoffman La-Roche, Basel, Switzerland) using a commercial enzymatic kit (Hoffman La-Roche). As usual, LDL and HDL were expressed as LDL-C and HDL-C, i.e. as cholesterol determined on the respective fractions.

Statistical Design

The statistical analysis was performed using repeated-measures Analysis of Variance of a single factor and Fisher's protected least significant difference test between periods when a diet effect was encountered. Data were considered significant at the 5% level.

RESULTS

Table 1 gives the fatty acid compositions of the dietary fats. The PHSBO diet showed a predominance of elaidic acid whilst POo was mainly rich in palmitic and oleic acids. One objective of hydrogenation was to produce as much *trans* fatty acid as possible so that the sum of the mononusaturated fatty acids (with *trans*) + the polyunsaturated fatty acids would be equal for the POo, PHSBO diets and AHA diets.

In the first experiment, total serum cholesterol ($x \pm SD$) was significantly higher on the PHSBO diet (4.36 ± 1.76 mmol/L) than during the AHA period (2.56 ± 0.64 mmol/L) *(Table 2)*. However, there was no significant difference between the PHSBO (4.35 ± 1.76 mmol/L) and POo (3.23 ± 1.36 mmol/L) groups.

A significant increase in LDL-C was also observed for the PHSBO (2.63 ± 1.25 mmol/L) group compared with either the AHA (1.39 ± 0.45 mmol/L) or the POo (1.58 ± 0.96 mmol/L) groups. No significant difference in LDL-C was evident between the AHA and POo fed animals. HDL-C was not significantly altered by any of these diets. The LDL/HDL ratio was significantly elevated in the PHSBO fed animals compared with those on both the AHA and POo diets. There was also a significant lowering of the LDL/HDL ratio in the POo group compared to the PHSBO period.

TABLE 1. FATTY ACID COMPOSITION (%) OF EXPERIMENTAL FAT BLENDS

Fatty Acid	AHA*	PHSBO	POo	CNO	CNO/ SFO	PHSBO/ SFO
Lauric 12:0	0.6	0.8	0.5	58.3	52.2	1.1
Myristic 14:0	0.5	0.1	0.9	19.3	16.0	0.1
Palmitic 16:0	24.7	8.8	30.0	8.9	9.6	9.4
Stearic 18:0	3.8	5.5	3.2	2.5	2.0	4.9
Arachidic 20:0	-	0.3	-	0.3	-	0.3
Palmitoleic 16:1	0.1	0.1	0.2	-	-	0.1
Elaidic 18:1 (*trans*)	-	47.6	-	-	-	45.0
Oleic 18:1 (n-9)	31.5	15.4	46.3	6.3	8.2	16.1
Linoleic 18:2 (n-6)	34.0	4.4	15.2	1.2	12.0	12.4
18:2 *trans, trans*	-	2.9	-	-	-	2.0
18:2 *cis, trans/ trans, cis*	-	1.9	-	-	-	0.8
Linolenic 18:3 (n-3)	4.6	0.3	0.3	0.2	-	0.3
Others	-	11.9	-	-	-	9.4

* abbreviations: AHA, American Heart Association fat blend; PHSBO, partially hydrogenated soyabean oil; POo, palm olein; CNO, coconut oil; SFO, safflower oil.

The fatty acid composition of the 3 dietary oils used in the second experiment *(Table 1)* was aimed at determining the effect of added 18:2 in the CNO or PHSBO diets at a level of 3.6 en per cent. The 18:2 level in both blends was kept constant. The CNO/SFO blend showed a predominance of lauric and myristic acids whilst the PHSBO/SFO blend showed a predominance of elaidic and oleic acids. CNO, as can be seen from *Table 1*, had a very low 18:2 content.

The addition of 18:2 to PHSBO resulted in a significant reduction in plasma cholesterol by comparison with the CNO period *(Table 2)*. Addition of 18:2 to CNO, however, produced no change in the total cholesterol level.

A significant difference in the total cholesterol level was noted between the CNO/SFO and PHSBO/SFO periods. The HDL-C level was lowered significantly in the PHSBO/SFO group as compared with the CNO period. The LDL-C level was significantly higher in the CNO/SFO group compared to the PHSBO/SFO group whilst there was a significant reduction in the LDL-C in the PHSBO/SFO group, compared with the periods on CNO and CNO/SFO. The ratio of LDL/HDL was also significantly lower in the PHSBO/SFO group, compared with the CNO/SFO period.

Finally there was a significant decrease in triglyceride (TG) levels in both the CNO/SFO group and the PHSBO/SFO group, as compared with the CNO group.

TABLE 2. EFFECT OF DIETARY FAT ON PLASMA TC, TG, LDL AND HDL CONCENTRATIONS (mmol/L) AND LDL/HDL RATIO

Experiment and diets	Plasma Lipids				
	TC	TG	LDL-C	HDL-C	LDL-C/HDL-C
			mmol/L		
Experiment 1					
CNO (entry)	2.82 ± 1.04	0.75 ± 0.31	1.52 ± 0.84	0.98 ± 0.11	1.59 ± 0.64
1. AHA	2.56 ± 0.64[a]	0.55 ± 0.18[a]	1.39 ± 0.45[a]	0.93 ± 0.21	1.46 ± 0.33[a]
2. PHSBO	4.35 ± 1.76	1.24 ± 0.58[a]	2.6 ± 1.25[ab]	1.17 ± 0.27	2.25 ± 0.74[ab]
3. POo	3.23 ± 1.36	0.91 ± 0.51[a]	1.58 ± 0.96[a]	1.11 ± 0.15	1.39 ± 0.66[b]
Experiment 2					
4. CNO	3.29 ± 0.44[a]	1.4 ± 0.23[a]	1.8 ± 0.52[a]	1.3 ± 0.29[a]	1.54 ± 0.6
5. CNO + 18:2	3.25 ± 0.49[b]	0.81 ± 0.36[a]	1.9 ± 0.36[b]	1.2 ± 0.31	1.74 ± 0.53
6. PHSBO + 18:2	2.15 ± 0.36	0.54 ± 0.27[a]	1.0 ± 0.24[ab]	1.0 ± 0.17[a]	1.0 ± 0.2

Numbers with similar superscripts differ significantly at $P < 0.05$.

DISCUSSION

Our results demonstrate that elaidic acid fed to monkeys at 14 en % in the relative absence of 18 : 2 (1.32% en) caused an elevation in total plasma and LDL cholesterol levels in comparison with the control AHA diet. Total plasma cholesterol was comparable to that with the POo diet. Plasma triglyceride concentrations were elevated by both the PHSBO and the POo diets, compared with the AHA diet.

When SFO was added to PHSBO to increase the 18:2 level, a marked decrease occurred in the total cholesterol as well as the LDL-cholesterol levels. However, the addition of 18:2 fatty acid also brought about a decrease in the HDL-levels.

Surprisingly, addition of 18:2 at 3.6 en % to CNO did not result in lowering of total or LDL-cholesterol levels, a finding that is unexpected since the 18:2/14:0 ratio is thought to dictate the plasma cholesterol response (Hayes *et al.*, 1992). Plasma triglyceride levels were lowered significantly in both the CNO/SFO and the PHSBO/SFO groups.

Our results confirm those of Kritchevsky *et al.* (1977), who found that, when fed to vervet monkeys, semipurified diets containing 6% en as *trans* fatty acid caused an elevation in total plasma cholesterol levels. Anderson *et al.* (1961) in their human study also showed an elevation in plasma total cholesterol and triglyceride levels in volunteers fed a *trans* fatty acid-rich diet.

Mensink and Katan (1990), besides observing an increase in plasma total cholesterol, also found an elevation in the LDL cholesterol of their human volunteers fed a diet with 11% energy supplied by *trans* fatty acids, compared with an oleic acid diet. Nestel *et al.* (1992) in their study did not see any changes in total cholesterol levels in their volunteers fed an elaidic acid rich diet, but observed a significant increase in LDL-cholesterol by comparison with an oleic acid rich diet.

The study conducted by Anderson *et al.* (1961) was criticized by Mattson (1975) on the ground that the hypercholesterolemic effect of *trans* fatty acids was due to a reduction in the 18:2 content. The level of 18:2 in their test diet dropped to 12.8% from the original 22.9% (control) after hydrogenation.

The Mattson's criticism may have been warranted, because in the present study we found that increasing the 18:2 content to 3.6 en % in PHSBO by blending with SFO brought about a reduction in total cholesterol as well as LDL cholesterol levels. The argument that 18:2 is reduced upon hydrogenation and that this lack of 18:2 is causing an elevation of plasma cholesterol may be feasible (Hayes *et al.*, 1992), but both the lack of 18:2 and the presence of *trans*-18:1 may be important, because the simple lack of 18:2 in CNO did not produce the detrimental impact of PHSBO. It must be recognized that hydrogenation of unsaturated fatty acids, including 18:1, 18:2 and 18:3, will naturally be accompanied by loss of according to the degree of hydrogenation, with an accompanying rise in *trans* isomers and saturation. The important consideration here is that hydrogenated fats can raise plasma cholesterol levels (Mensink *et al.*, 1990; Nestel *et al.*, 1992; Kritchevsky *et al.*, 1977; Anderson *et al.*, 1961). In actual food industry practice, hydrogenation is a common process, but it is not a practice to increase the 18:2 concentration after hydrogenation.

Palm oil in our experiment produced plasma cholesterol and LDL-cholesterol levels comparable to those with the AHA diet, so the degree of natural saturation of palm oil would seem to give it an advantage in formulations of margarines and shortening where little or no hydrogenation would be necessary.

Further work is needed to extend these observations and their biological implications.

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