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Studies on Lipid Responses to Interesterified Soya Oil-Butterfat Mixture in Hypercholesterolemic Rats and Human Subjects

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ABSTRACT

Interesterified soya-butterfat feeding significantly decreased serum cholesterol in humans and in experimental rats. This decrease was more effective than when simple mixtures of the two fats were fed. Studies with the experimental rat indicate higher rates of side-chain degradation of cholesterol as well as 7-α-hydroxylation of cholesterol when interesterified fats replace a mixed fat regimen. The lowering of serum cholesterol parallels the decrease in concentration of fully saturated glycerides and redistribution of myristic acid from the 2-position in the glyceride to 1- and 3-positions of glycerides following interesterification.

INTRODUCTION

Soyabean oil is relatively high in polyunsaturated fatty acids (50-55%) and is likely to be a potential hypocholesterolemic agent if used as a dietary oil. In India, butterfat (ghee) is the most popular dietary fat and in spite of its proven hypercholesterolemic responses in humans and experimental animals, has occupied a unique place in the Indian diet. Substitution of butterfat in part by soyabean oil has not found much favor among the butterfat-consuming population, even though a significant lowering of serum cholesterol is attained. Also unpopular is the blending of Indian ghee with vegetable oil containing relatively high polyunsaturated fatty acids because of poor acceptability by the average Indian who prefers the hydrogenated vegetable oil.

Studies with laboratory animals have revealed cholesterol-lowering action of soyabean oil when mixed with butterfat in different proportions (1,2). Myristic acid, a predominant saturated fatty acid member of butterfat, has been singled out as a major factor contributing to increase in serum cholesterol in man (3) and this has been verified in the laboratory rat (4). In natural butterfat, myristic acid is present as trisaturated glycerides, occupying exclusively the 2-position in the glyceride moieties. Data presented elsewhere tend to show that increase in serum cholesterol is not merely dependent on the content of myristic acid in the dietary fat, but is probably related to its distribution among the various types of glycerides and the position it occupies therein (1). This study was designed to modify the glyceride structure of butterfat by interesterification using Eckey's procedure (5) and to study the changes in glyceride structure with corresponding changes in lipid responses when fed to rats and humans.

EXPERIMENTAL

Serum cholesterol and triglyceride estimations were done according to standard procedures (6,7). The separation of glycerides of interesterified fat mixture was attained on thin layer chromatographic plates coated with Silica Gel G according to the procedure of Wessels and Rajgopal (8) using a solvent system of benzene/di-isopropyl alcohol (85:15). Lipase hydrolysis was done according to the Weber procedure (9), scaled up to accomodate 50-mg samples. Products of hydrolysis were isolated by thin layer chromatography (TLC) on Silica Gel G plates developed in hexane/diethyl ether/acetic acid (65:35:1), converted to methyl esters with methanol BF_3 (10). The methyl esters were separated on a 700-12 F&M gas chromatograph equipped with dual flame ionization detectors using ethylene glycol adipate polyester, 10% of which was absorbed on 60-80 mesh Chromosorb P and packed into 4-mm bore aluminum column; they were identified according to the usual procedure. Oxidation of 26-^{14}C-cholesterol by rat liver mitochondria was performed according to Kritchevsky (11) with slight modifications. 7-α-Hydroxylation of 4-^{14}C-cholesterol was done following the procedure of Shefer et al. (12). Estimations of microsomal cytochrome P-450 was done according to Omura and Sato (13).

RESULTS AND DISCUSSION

Data in Table I show the effects on serum and liver cholesterol of feeding rats soyabean oil-butterfat mixtures as interesterified mixtures having identical amounts of various saturated and polyunsaturated acids. Replacement of 50% of saturated butterfat soya oil causes significant decreases in serum cholesterol at the end of one week, but when 1:1-interesterified fat mix is fed to the rats, greater lowering of serum level results and the fall is highly significant ($p<0.05$). A comparison of effects of mixed and interesterified fats in rats maintained on a 0.5% cholesterol diet (Table II) indicates that cholesterol lowering effect of the interesterified soya-butterfat mixtures were even more marked when cholesterol was present in the diet ($p<0.001$).

The liver cholesterol changes reflect a slight, but statistically significant, increase in liver cholesterol when soya oil is present in the diet, whether as mixed or in interesterified form in comparison to the butterfat-fed animals.

The results of TLC separation of various glyceride categories in mixed and interesterified fats shown in Table III indicate that there has been a marked decrease in trisaturated glycerides following interesterification of 1:1-soya-butterfat mixtures. Furthermore, when the separated glyceride fractions were subjected to deacylation with pancreatic lipase and gas liquid chromatographic (GLC) analyses were performed for fatty acids of 2-monoglycerides, as well as for those derived from the 1- and 3-positions (Tables III and IV), it appears that a shift in the position of myristic acid, partly from its original secondary position to 1- and 3-positions of glycerides other than GS_3 types, resulted during interesterification. From these results, it is reasonable to suggest that: (a) a reduction in total content of fully saturated triglycerides originally present in butterfat during interesterification may be an

TABLE I

Effect of Interesterification on Serum and Liver Cholesterol of Rats (after 6 Wk on Diet)

Dietary fat (20%)	Serum cholesterol		Liver cholesterol	
	Total mg/100 mg	Free	Total mg/g	Free
Butterfat	93.4 ± 1.5[a]	16.1 ± 0.3	2.54 ± 0.30	1.52 ± 0.66
Butterfat-soyabean oil mixture (1:1)	74.0 ± 0.8	7.2 ± 0.5	2.96 ± 0.12	2.20 ± 0.80
Butterfat-soya interesterified fat (1:1)	59.3 ± 0.3	7.0 ± 1.0	4.05 ± 0.02	3.16 ± 0.17
Soyabean oil	48.4 ± 0.3	8.1 ± 1.2	3.61 ± 0.33	1.86 ± 0.52

[a]Mean ± SEM values (10 animals/group).

TABLE II

Effect of Mixed and Interesterified Fat Diets Containing 0.5% Cholesterol on Serum Cholesterol of Rats

Diet group	Weeks on diet	Serum cholesterol	
		Total mg/100 ml	Free
Butterfat - 0.5% Cholesterol	8	203 ± 16[a]	23.6 ± 2.5
	12	204 ± 12	44 ± 1.8
Butterfat - Soybean oil 1:1 - Mixture + 0.5% Cholesterol	8	125 ± 30	12.0 ± 2.0
	12	109 ± 10	8.1 ± 0.7
Interesterified Butterfat + Soybean oil (1:1 - Mixture + 0.5% Cholesterol)	8	84 ± 8	4.6 ± 1.7
	12	78 ± 6	5.2 ± 2.0

[a]Mean ± SEM values (8 animals/group).

TABLE III

Glyceride Composition of Mixed and Interesterified Fat

	GS_3	GS_2U (sus + ssu)	GSU_2 (usu + uus)	GU_3
Butterfat-soya (1:1 mixture)	21.5 ± 2.5[a]	27.7 ± 1.3	16.9 ± 1.2	35.6 ± 1.8
Butterfat-soya (1:1 Mix—interesterified)	13.5 ± 1.2	33.2 ± 1.2	24.2 ± 2.2	30.0 ± 0.6

[a]Mean of 3 expts. ± SEM values.

TABLE IV

Redistribution of Myristic Acid following Interesterification of Butterfat with Soybean Oil

		Position of fatty acid (mixed)				Position of fatty acid		
	%	Sn-2	Sn-1 and 3		%	Sn-2	Sn-1 and 3	
GS_3	21.5	Myr[a]	Pal	St	13.6	Myr	Pal	St
GS_2U	13.5	Ol	Myr	Pal	8.5	Ol	Myr	Pal
GSU_2	16.9	Ol	Pal	Ol	24.2	Lin	Ol	Myr

[a]Myr = myristic; Pal = palmitic; St = stearic; Ol = oleic; Lin = linoleic.

TABLE V

Effect of Trimyristin Addition to Soybean Oil on Serum and Liver Cholesterol of Rats (4 Wk on Diet)

Dietary regimen	Serum cholesterol		Liver cholesterol	
	Total mg/100 ml	Free	Total mg/g	Free
25% Soyabean oil	50.6 ± 8.4[a]	8.1 ± 0.7	2.61 ± 0.02[a]	1.80 ± 0.05
22.5% Soyabean oil + 2.5% Trimyristin	72.6 ± 1.5	9.6 ± 1.5	2.90 ± 0.11	2.31 ± 0.06
20% Soyabean oil + 5% Trimyristin	92.8 ± 2.1	11.5 ± 0.8	5.01 ± 0.27	4.26 ± 0.10
M. 5% Soyabean oil + 7.5% Trimyristin	118.5 ± 0.9	13.1 ± 1.4	12.10 ± 0.33	8.60 ± 1.54
17.5% Soya + 7.5% Trimyristin (interesterified)	72.5 ± 2.1	8.5 ± 1.5	2.76 ± 0.16	2.00 ± 0.06

[a]Mean ± SEM values (6 rats/group).

important factor in the observed lowering of serum cholesterol, and (b) a shift of myristic acid from its original 2-position to 1- and 3-positions also contributes to the decrease in serum cholesterol when interesterified fats were fed.

That the trisaturated glyceride content of a dietary fat can predominently influence the serum cholesterol level was clearly established from studies feeding soyabean oil mixed with 2.5, 5 and 7.5% trimyristin. The results in Table V indicate that a predominant rise in serum cholesterol may be achieved by increasing the trisaturated glyceride content of the dietary fat. Interesterification of soyabean oil-trimyristic mixture produced a fat which, when fed to rats, showed a marked decrease in serum cholesterol compared to the same mixture of trimyristin-soya oil. This experiment gives considerable support to the hypothesis that, in butterfat, the presence of fully saturated glycerides is one of the major factors in raising blood cholesterol and, second, alteration of the structure of the glycerides with respect to the position of myristic acid is associated with lowering of serum cholesterol following interesterification of butterfat with soya oil.

Effect of Feeding Interesterified Soya-Butterfat Mixture to Human Subjects

The marked serum cholesterol lowering effect achieved in rats with interesterified soya-butterfat mix prompted us to try its effect in humans with hypercholesterolemia. In hospitalized patients with or without evidence of coronary heart disease (CHD) who had high levels of serum cholesterol, caloric restriction as prescribed by the physician was followed, but the dietary fat comprised entirely 1:1-soya-butterfat interesterified mixture. The level of intake of fat varied from 15 to 25% of the total calories administered to the patients. Body weights were taken at regular intervals and subjects showed no adverse effects. The average cholesterol content of the diet given to patients varied from 60 to 82 mg/day, whereas no dietary alterations in cholesterol content were made in the diet of the subjects with no evidence of CHD, the average intake varying from 180 to 450 g/day. During the first six weeks of feeding the interesterified fat, a sharp decline in serum cholesterol was noticed (Fig. 1) and the trend continued until the end of the test-fat feeding period. From a comparison of the slope of the curves for interesterified and mixed fat diet regimens, it was observed that hypocholesterolemic responses with the interesterified regimens was significantly greater throughout the experimental period. Triglyceride level of serum decreased simultaneously during the initial phase, although during the second six-week period, soya-butterfat mixture as well as interesterified fat feeding, there appeared a tendency for a gradual rise in serum triglyceride level, but values were always below the initial level. Thus lipid responses observed in experimental rats fed an interesterified soya-butterfat mixture were well documented in hypercholesterolemic human subjects with or without evidence of CHD. It is notable that interesterified soya-butterfat mixtures always produced a greater effect than simple mixtures of the two fats in identical proportions.

Studies on mitochondrial rates of 26-^{14}C-cholesterol in rats clearly indicate higher rates of side-chain degradation of cholesterol when rats were fed the interesterified fat mixture in comparison to the mixed fat diet regimen (Fig. 2). A comparison of the activity of the microsomal enzyme 7-α-hydroxylase in liver of rats fed the two experimental diets also revealed that conversion to 7-α-hydroxycholesterol was invariably greater when the rats were maintained on the soya-butterfat interesterified diet regimen (Fig. 3). Cholesterol 7-α-hydroxylase has been considered by several investigators as the rate-limiting enzyme in the pathway of conversion of cholesterol to bile acids (14,15). In scorbutic guinea pigs, it was found that the regulation of activity of this rate-limiting enzyme is due to a decrease in the microsomal content of cytochrome P-450 (16). Several other studies have indicated that the level of cytochrome P-450 may influence and even grossly reduce

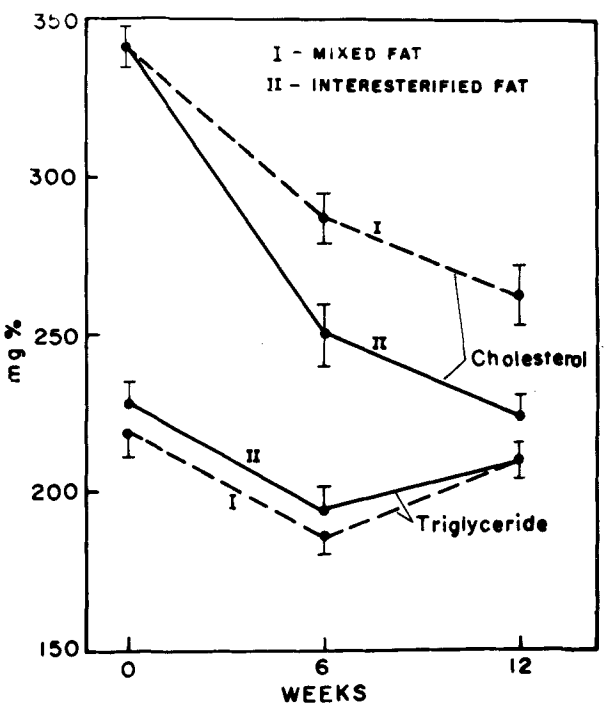

FIG. 1. Effect of feeding interesterified soyabean butterfat mixture on serum lipids of hyperlipidemic subjects.

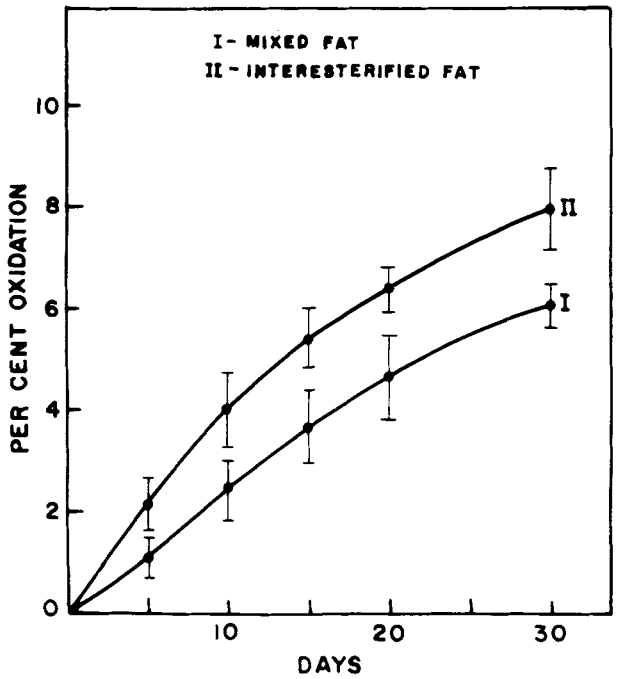

FIG. 2. Liver mitochondrial oxidation of 26-^{14}C-cholesterol in rats.

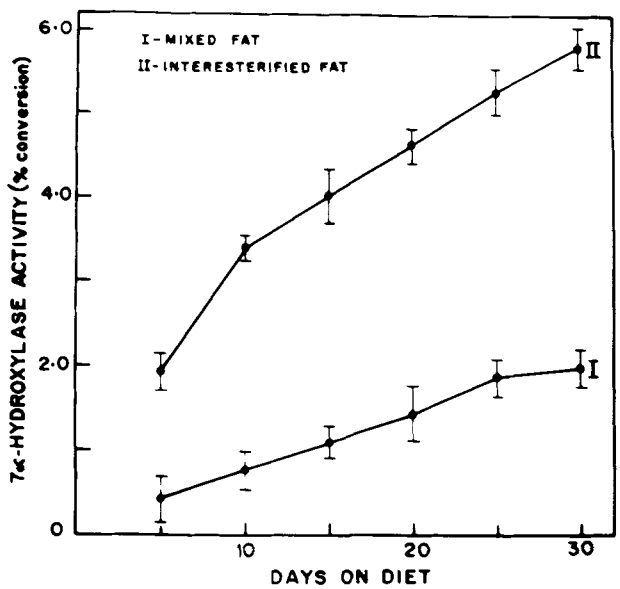

FIG. 3. Effect of feeding mixed and interesterified soyabean butterfat (1:1 mixture) on 7-α-hydroxylation of cholesterol in rat liver.

TABLE VI

Hepatic Microsomal Cytochrome P-450 of Rats on Different Experimental Fat Diets

Diet	Cytochrome P-450 (n mol/mg protein)
Butterfat	1.51 ± 0.66[a]
Butterfat-soya (1:1 mix)	1.43 ± 0.72[b]
Butterfat-soya (1:1 mix—interesterified)	1.40 ± 0.68[b]
Soyabean oil	1.35 ± 0.74

[a]M ± SEM values.
[b]Not significant.

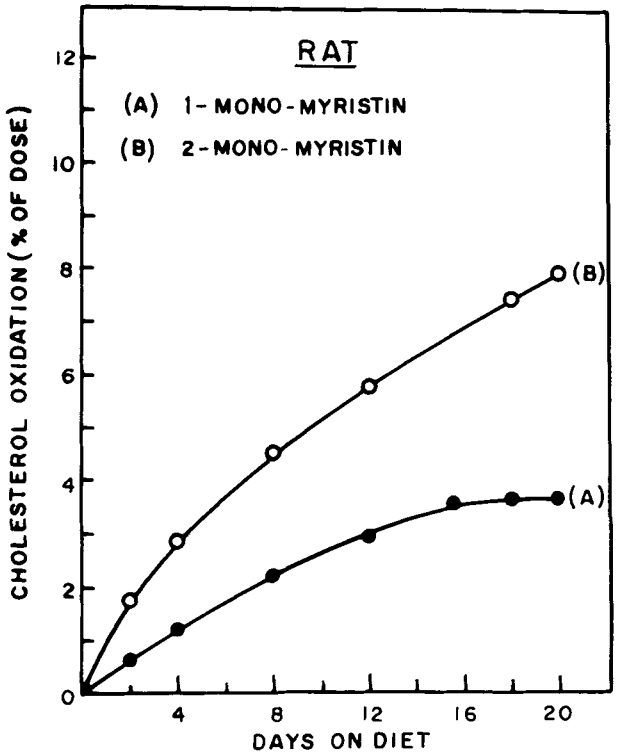

FIG. 4. Effect of 1- and 2-monomyristin on mitochondrial oxidation of 26-^{14}C-cholesterol.

the activity of the mono-oxygenases (17,18). Measurement of microsomal levels of cytochrome P-450 in rats fed mixed and interesterified fat diets, however, does not show any statistically significant differences which might explain the observed differences in cholesterol 7-α-hydroxylation on the corresponding experimental fat diets (Table VI). However, when rates of mitochondrial oxidation of cholesterol degradation were measured using radio-cholesterol, experimental rats fed 7.5% of 2-monomyristin dissolved in triolein showed greater cholesterol oxidation than rats fed the same amount of 1-monomyristin (Fig. 4). This study tends to prove that, when myristic acid is present in the 1-position in the glycerides, cholesterol breakdown is higher than when the same acid occupies the 2-position in the glyceride. The results of experiments with 7.5% level of 1- and 2-monomyristin suggest a close parallelism between serum cholesterol levels and the shift of myristic acid from its secondary position to the 1-position in interesterified fats. The lowering of serum cholesterol also resulted from the increased 7-α-hydroxylation of cholesterol in rats fed interesterified fat, irrespective of insignificant alteration in the level of microsomal cytochrome P-450. Thus, hypocholesterolemic responses to feeding interesterified soya-butterfat mixture to rats as well as humans may be attributed to a lowering of the total content of trisaturated glycerides of butterfat following the interesterification, as well as to a significant percentage of myristic acid migrating to the primary position in the glyceride, which presumably helps the breakdown of cholesterol side-chain and conversion of cholesterol to bile acids.

ACKNOWLEDGMENT

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