Effect of Ingestion of Isomeric Fatty Acids on Cholesterol and Lipids of Serum and Liver[1]

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Abstract

The effect of the ingestion of large amounts of the *trans, trans* isomer of linolein upon the cholesterol and lipid levels of the blood and liver was investigated using hypercholesterolemic rats. The serum levels of esterified fatty acids and cholesterol of rats fed the diets containing 30% of *trans, trans* linoleic acid glycerides and safflower oil were 15 and 25%, respectively, lower than those fed coconut oil. However, a weight loss associated with less intake of the *trans* isomer as compared with a gain with the other two fats must be considered. The serum levels of labeled cholesterol of rats administered radioactive cholesterol along with the *trans* isomer were intermediate in maximum value as compared to the levels obtained with coconut and safflower oils. These results suggest that the *trans* isomers are not as effective as the *cis* isomers in lowering the cholesterol and lipids of the blood. The livers of the former group had a lower fat content than the latter which might be accounted for in part by the lower fat intake

Introduction

THE INGESTION OF FATS containing polyunsaturated fatty acids has been shown to affect serum cholesterol levels more or less depending upon the nature and position of the double bonds. Linolenic acid appears to lower the cholesterol level as does linoleic acid while elaeostearic acid of tung oil, which contain conjugated double bonds, markedly elevates the serum cholesterol level when ingested (1,2). Since the conversion of edible oils to more solid products by hydrogenation leads to the formation of a considerable quantity of the *trans* isomers of the fatty acids, the question has arisen as to whether these isomers are objectionable for inclusion in dietary fats (3).

The *trans* polyunsaturated fatty acids have been shown to be metabolized, to provide energy and to cause changes in storage lipids but not to serve as essential fatty acids (4). The latter finding would not be particularly objectionable since the small amounts of essential fatty acids which may be required would remain after hydrogenation. However, the ingestion of large quantities of the *trans* isomers of the polyunsaturated fatty acids might influence lipid and cholesterol levels to an extent worthy of consideration. Conflicting results have been reported when a mixture of isomers were used (5,6). This study was designed to learn more about the effect of the ingestion of large amounts of *trans, trans* linoleic acid on the cholesterol and lipids of the blood and liver.

Experimental

Twelve male albino rats, weighing about 400 g, were housed in separate cages so that individual food intakes, *ad libitum*, could be determined. The experimental diets used had the following percentage composition: ground rat pellets, 67.5; cholesterol, 2.0; sodium taurocholate, 0.5 and added fat, 30.0. The fat added was either coconut oil (CNO), *trans, trans* linolein (TLO), safflower oil (SFO) or tung oil (TuO). The fatty acids of tung oil (TuFA) were substituted for the oil during the last 5 days of the 12-day period since the rats had eaten only small amounts of the tung oil mixture and the methyl esters had been reported to be not toxic (7). On alternate days the rats were weighed and blood taken from the rat's tail for the determination of esterified fatty acid (EFA) and serum cholesterol levels by the methods of Stern and Shapiro (8) and Mamose et al. (9), respectively.

Labeled cholesterol was administered to the three rats on each of the four high fat diets 3 days before sacrificing, in order to study further the effect of these dietary fats upon the relative rates of appearance and removal of the cholesterol from the blood. Each of the rats was fed by tube 30 μc of cholesterol-4-C^{14} in 0.6 ml of their dietary fat containing 2% cholesterol. Blood samples were collected at various intervals in order to follow the levels of labeled cholesterol in the blood as affected by the different dietary fats. After decapitation, the livers were removed, blotted and weighed. Duplicate 2g samples of the weighed livers were digested in 4 ml 30% KOH solution to aid in disintegrating the tissue. The mixture was then acidified with 2 ml of H_2SO_4 (1:2) and extracted with 10 ml of a modified Doles reagent (1:1 mixture of isopropyl alcohol and hexane). Aliquots of the supernatant hexane solution were used to determine the liver lipids by evaporation and weighing. Relative radioactivities of the blood samples and total activity of the livers were calculated from data obtained by direct mounting of duplicate blood or lipid extract aliquots as described previously (10).

In a second experiment, four younger rats (about 140 g) were fed each of the first three diets employed in the above study. After 4 days on the dietary regimes, the various fats containing labeled cholesterol were again administered to each rat and the activities of blood and liver followed as described above.

Results

As shown in Table I, a lower dietary intake of TLO as compared with that of both CNO and SFO was associated with a 5.5% loss in weight. The weight loss which occurred when TuO was the fat of the diet was reversed by substituting TuFA for the TuO. The increased intake of TuFA promoted a gain in weight similar to that of the CNO and SFO regimes during that part of the period when the FA replaced the unhydrolyzed fat. The serum EFA levels of the rats fed TLO and SFO were similar and about 15% lower than that of the rats fed CNO under the conditions described. The serum EFA levels of the rats fed TuO were approximately the same as those of the

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TABLE I

Effect of the Intake of Various Lipids Upon the Weight and Blood EFA and Cholesterol Levels of Rats

Diet suppement		Total wt. change during period	Av. serum levels during period [b]			
Lipid [a]	Amount		EFA		Cholesterol	
	g/da	g.	mEq/l	%	mg%	%
CNO	4.64	+11	16.1 ± .3	100	148 ± 7	100
TLO	3.46	−22	13.5 ± .8	84	111 ± 11	75
SFO	4.48	+13	13.7 ± 0.7	85	110 ± 12	74
TuO	2.07	−64	17.4 ± 2.2	108	269 ± 50	182
TuO	1.35	−69	11.9 ± 1.8	74	148 ± 20	100
TuFA	3.34	+5	24.7 ± 6.5	153	562 ± 54	380

[a] Last two lines show data obtained when tung oil period is divided in two parts—one covering 7 days on tung oil and the other a 5-day period when the FA of tung oil were fed.
[b] Data given is an average with standard deviations obtained from 6 determinations on each of the rats over the 12-day period.

rats fed CNO, perhaps as a result of the very low intake of TuO, since the levels were markedly elevated by an increased intake of the hydrolyzed TuO which replaced the fat.

The serum cholesterol levels were similar and appreciably lower in the rats fed TLO and SFO than in those fed CNO or TuO and TuFA, from 25 to more than 300% lower. The cholesterol levels were markedly elevated when TuFA replaced the TuO. This finding is perhaps better shown in Table II listing the average values of serum cholesterol levels obtained every other day. The final days' values are similar for TLO and SFO 129 and 123 mg%, to be compared with 157 and 664 for the CNO and the TuO regimes, respectively. The values for the latter rose rapidly with the increased intake of TuFA.

The relative radioactivities of the blood collected at the stated intervals after giving labeled cholesterol is shown in Table III. The highest cholesterol value (270 mg%) for the animals on the TLO diet was obtained from the blood collected at the 24-hour interval which was at a later time than that of the highest values for both the CNO and SFO groups, but it was intermediate between that of the CNO (295) and of the SFO (220). However, the cholesterol levels of the TLO group declined more slowly than the other two, causing the average cholesterol level to be similar to that of the CNO group, and higher than that of the SFO-fed rats. As previously reported, the TuO, which was fed for comparative purposes, promoted very high cholesterol values (1). It reached a peak shortly after its administration at a value about four times as great (1019) as the highest one associated with any of the other diets.

The relative disposition of the administered cholesterol in the liver was determined as well as the changing levels in the blood. Total liver lipids, their specific and total activities are listed in Table IV. The livers from the rats on the SFO diet had by far the greatest percentage of fat with both a specific activity (SA) and total activity greater than that of the animals on the saturated fat (CNO) diet. The lipids from the livers of the TLO group had a much higher SA than either the CNO or SFO groups, but the lower fat content resulted in a lower total

TABLE II

Blood Cholesterol Levels as Affected by Dietary Fats.

Diet lipid	Day of period						Increase during period
	2	4	6	8 [a]	10	12	
							%
CNO	122	153	160	142	155	157	40
TLO	99	89	98	129	120	129	15
SFO	103	99	120	99	119	123	10
TuO	125	131	128	208	459	664	493

[a] The TuO diet was replaced with TuFA at this time.

TABLE III

Relative Radioactivities of Blood After Administration of 30 μc of 4-C^{14} Cholesterol

Lipid fed	Relative radioactivities of blood [a]								
	Hours after administration							Period av.	Relative
	4	8	24	28	32	48	52		
									%
CNO	190	295	217	193	159	121	109	183	100
TLO	170	192	270	183	200	139	130	185	101
SFO	160	220	167	143	113	92	78	139	76
TuO	1019	.....	721	.....	464	.....	455	665	363

[a] Count/min corrected to a 7 mg sample of blood.

activity than in the latter. As expected, the TuO group had extremely high SA and total activities. The results of the second experiment were so nearly the same as in the first that the data of the two were pooled in Tables III and IV.

Discussion

Initially the purpose of this investigation was to determine whether it was nutritionally feasible to replace hydrogenation with a partial isomerization of the polyunsaturated fatty acids in order to achieve the higher melting points desired for margarines and similar products. The literature revealed that appreciable quantities of the *trans* isomers were formed during hydrogenation and that these isomers could not serve as essential fatty acids but they were metabolished similarly to other fats (4). However, the question still remained as to what effect they might have on blood cholesterol and lipid levels when consumed in rather large amounts, since the use of only relatively small amounts of mixed isomers had been investigated previously (5,6,11). Also, would the ingestion of these isomers cause the deposition of undue amounts of cholesterol or other lipids in the liver?

The data of Table I might be interpreted to imply that TLO is just as effective in lowering the lipids and cholesterol levels of the blood as is the SFO; 15 and 25%, respectively, less than in the CNO fed rats—all made hypercholesterolemic by the means utilized. However, the intake of TLO was much less than that of either the CNO or SFO and the lower intake may have been an important factor. However, a lower intake of TuO was associated with both markedly elevated serum lipid and cholesterol levels, which suggests that the nature of the fatty acids may be of relatively greater importance than the intake.

In order to aid in deciding whether the lower intake of the *trans* isomer was a major factor in causing the lower serum cholesterol levels, the radioactivities of the blood and liver were determined after administration of labeled cholesterol. The cholesterol fed with TLO appears to remain in the blood over a longer period than with CNO although never reaching quite as high a level. The over-all serum cholesterol level appeared to be appreciably lower in

TABLE IV

Liver Lipids and Radioactivities After Labeled Cholesterol Ingestion

Lipid fed	Total liver lipid		Activities of liver steroids			
			Specific activities		Total per liver	
			Count/min	Relative	Count/min × 10^6	Relative
	g	%	mg	%		%
CNO	1.10 ± 0.07	8.33	796 ± 159	100	0.88	100
TLO	0.84 ± 0.16	6.42	1370 ± 228	172	1.15	131
SFO	1.48 ± 0.14	11.35	921 ± 217	116	1.36	155
TuO	0.72 ± 0.11	6.27	2238 ± 250	281	1.61	183

the SFO fed animals but not in the CNO or TLO ones. The results after administration of similar amounts of labeled cholesterol suggest that the ingestion of the *trans* isomers is not as effective as the *cis* isomers in lowering serum cholesterol levels. However, in agreement with our feeding experiment, Weigenberg and McMillan (11) found serum cholesterol levels to be similar when rabbits were fed either linoleic acid or an elaidinized linoleic acid which contained a mixture of *cis* and *trans* isomers.

Considerable interest has been aroused as to the disposition of the cholesterol when the blood levels are lowered by the ingestion of polyunsaturated fatty acids. In this study both the liver lipid level and total radioactivity was significantly increased by the *cis* isomer over that when either the *trans* isomer or CNO was fed. The cholesterol retained in the liver after the TLO feeding was intermediate in amount (131%) between that of CNO (set at 100%) and SFO (155%).

The above results suggest that the ingestion of TLO may be intermediate between that of CNO and SFO in its effect upon the production of maximum cholesterol levels in both serum and liver. Although not as effective in lowering cholesterol levels as the *cis* isomers, the *trans* isomers are apparently not any more objectionable as dietary constituents than saturated fats and less so than are fatty acids containing conjugated double bonds.

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A Rapid Method for Concentrating Highly Unsaturated Fatty Acid Methyl Esters in Marine Lipids as an Aid in Their Identification by GLC

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Abstract

The selective solubility of unsaturated fatty acid methyl esters in nitromethane at temperatures down to −20C can be used to concentrate highly unsaturated methyl esters. With a typical sample of marine oils methyl esters having an iodine value of 110–190, a concentrate can be ready for GLC analysis in an hour or less and the nitromethane layer can be injected directly for analysis in GLC apparatus with ionization detectors. Examples of the use of the method in the identification of component fatty acids in herring oil are given.

Introduction

SEVERAL METHODS are available for the concentration or isolation of highly unsaturated fatty acid methyl esters in lipids. Most commonly used are the urea complex procedure (1,2,3,), low temperature solvent crystallization (4), silver nitrate column chromatography (5,6) and countercurrent distribution (7,8). In this laboratory, where the work is chiefly concerned with marine lipids of complex fatty acid composition, a rapid method for concentration of unsaturated fatty acid methyl esters was needed in conjunction with the log-plot-separation factor procedure (9,10,11) for the provisional identification of fatty acids.

The use of nitromethane as a solvent for pilot-plant scale countercurrent distribution of marine oils methyl esters was described in an earlier communication from this laboratory (12). In the course of investigating fractions obtained from the extraction column employed, the advantages of the present method were recognized.

Cannon et al. reported the use of a nitromethane-nitroethane, pentane-hexane system for the separation of methyl esters of fatty acids (13), and Schmid et al. have determined the critical solution temperature of several fatty acid methyl esters in nitromethane (14).

Experimental

Glass-stoppered centrifuge tubes with a capacity of 2.5 ml were used for these experiments. Nitromethane, practical grade, was freshly distilled, and the first 10% of the distillate discarded. The herring oil was a commercial oil from British Columbia and the methyl esters were prepared by methanolysis using 0.5% NaOH as catalyst. An insulated beaker containing ethanol-dry ice was used for cooling the samples in the centrifuge tubes. By adding small pieces of dry ice to the beaker, any temperature in the desired range could be reached quickly and held for several minutes.

Methyl esters (0.5 ml) were added to the centrifuge tube followed by nitromethane (1.5 ml). The