Effect of Ingested Thermally Oxidized Corn Oil on Fat Composition in the Rat. (26342)

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Previous studies(1,2,3) have shown that rats fed diets which contained oxidized oils did not grow as well and had larger or heavier livers than those fed fresh oils. Although thermally oxidized corn oil can be hydrolysed by pancreatic lipase *in vitro*(4), its rate of absorption from the intestinal tract was significantly less than that of fresh oil. The present study is concerned with the effects of hydrogenation, and of dilution of oxidized with fresh corn oil on absorption, growth, liver weight, and carcass and fecal fat composition.

Methods. A basal diet(5) which contained 12% of fat was used in all experiments. The test fat provided 10% and fresh cottonseed oil 2% of the basal diet. The diets were stored under nitrogen and refrigerated to minimize autoxidation of fat. Groups of 5 male weanling rats each were kept for 59 days in single cages, weighed on alternate days, and arbitrarily restricted to the same amount of food intake as those fed a basal diet containing 12% of a light-colored, refined corn oil. Samples of the feces were collected and frozen. After the test period the animals were sacrificed, the livers removed and weighed, and the carcass frozen and stored at −20°C until required for analysis. The carcass and fecal fats were extracted as previously described(6).

Aliquots of the fat samples were converted to their corresponding methyl esters prior to gas liquid chromatographic analysis. The procedure adopted was carried out as follows: Approximately 1 g of the fat to be esterified, 25 to 30 ml of methanol (anhydrous), and 50 to 100 mg of dry sodium methoxide were added to a small flask, refluxed for 45 minutes, poured into a separatory funnel, and diluted with 100 ml of water. The methyl esters were removed by extraction with petroleum ether (Skellysolve F), washed with water, and dried over sodium sulfate. Solvent was removed under vacuum on a rotary evaporator. Duplicate samples of methyl esters were prepared from all fats and stored under nitrogen in a refrigerator until analyzed. Wijs iodine values(7) and acetyl values(8) were determined in duplicate. The samples of oxidized corn oil, hydrogenated oxidized corn oil, and fatty acids of oxidized corn oil were prepared as previously described(9). The percentage of hydroxylated material in the dietary fats varied from 0 to 36.6%. This hydroxylated material was essentially a high molecular weight, polymeric material produced during thermal oxidation of corn oil. The remaining component acids are tabulated in Table I.

Gas chromatographic analyses of the methyl esters were carried out on an instrument constructed in our laboratory, which employs a thermal conductivity detector system. A 4 foot, ¼ inch internal diameter copper column was packed with 60-80 mesh Chromasorb impregnated with 30% by weight of succinic anhydride diethylene glycol polyester. The general method for preparing this type of column has been published(10).

Analyses were usually run at 190-205°C with a helium pressure of 11 lb per square inch, which resulted in a flow rate of about 60 ml of gas per minute. For a typical analysis of fatty acid methyl esters, 1-5 μl of the ester was injected into the column with a micro-syringe. The analysis required from 30 to 60 minutes to complete, depending upon the operating column temperature selected. Identification was based on comparison of retention time of the esters with those of authentic samples. The composition of each mixture analyzed was calculated on the basis of the area under each peak, as found by the triangulation method.

Results and discussion. Rats fed 10% of the diet as hydrogenated oxidized oil exhib-

TABLE I. Comparison of Dietary Supplement Fatty Acid Composition.

Fatty acid component	Fresh corn oil	Oxidized corn oil	Hydrogenated oxidized corn oil	Fatty acids from oxidized corn oil	10% oxidized fatty acids + 90% fresh fatty acids from corn oil
Linoleic	54.4	14.6	—	14.6	50.5
Oleic	28.8	28.3	.5	28.3	28.7
Stearic	2.3	3.6	46.5	3.6	2.5
Palmitic	13.0	16.4	16.4	16.4	13.3
Myristic	.2	—	—	—	.2
Lauric	.4	—	—	—	.3
Hydroxy acids	—	36.6*	36.6	36.6	3.6
Iodine value	126.0	71.0	55.0	71.0	120.0

* Determined as percentage of hexane-insoluble fatty acids.

ited less growth depression and less liver enlargement than those fed the non-hydrogenated material (Table II). The feeding of 10% of oxidized corn oil resulted in a statistically significant increase in liver weight and a decrease in weight gain when compared to the effects of feeding fresh corn oil. No significant differences in either liver weight or weight gain were observed when the animals were fed a mixture containing 10% of the fatty acids from oxidized and 90% from fresh corn oil. The effect of addition of such a small amount of oxidized material may not have become apparent in the short experimental period.

When oxidized corn oil which contained approximately 36% of polymeric hydroxylated material, the corresponding fatty acids, or the corresponding oil hydrogenated to an iodine value of 55, were fed to rats at the 10% level, a distribution of the component fatty acids essentially identical to that found in animals fed 12-hydroxy stearic acid, ricinoleic acid or triricinolein(11) was found in the carcass and fecal fat (Table III). From 4.1 to 7% of hydroxy acids seemed to be deposited in the carcass fat; again very similar levels to those observed when hydroxy acids were fed to rats(11). Ricinoleic acid fed at the 10% level resulted in increased liver weights and decreased growth, although digestibility of the ricinoleic acid was 85.8% (11) and that of ingested oxidized oils varied from 77 to 86%. Ingestion of a mixture of 90% fresh oil and 10% oxidized oil produced no significant changes in fecal or carcass fatty acid composition of the rat when compared to fresh corn oil. The observation that a significant quantity of hydroxy acids is deposited when rats are fed thermally oxidized corn oil is important since it demonstrates conclusively that hydroxylated compounds present in heated fats are absorbed when fed to animals.

Summary. Five groups of weanling rats were kept for 59 days in individual cages and fed adequate diets which contained 2% cottonseed oil and 10% of the following fats: 1) corn oil, 2) oxidized corn oil, 3) hydrogenated oxidized corn oil, 4) fatty acids from

TABLE II. Comparison of Biological Response of Rat to Ingested Fats.

Diet supplement	Changes in wt (g) in 59 days	Digestibility (%)‡	Percentage of liver/body wt
Fresh corn oil	204.2 ±20.3†	100.0	3.54 ± .41
Oxidized corn oil	157.0 ± 2.5*	77.2*	4.25 ± .20*
Hydrogenated oxidized corn oil	169.5 ± .9*	86.3*	4.12 ± .30*
Fatty acids from oxidized corn oil	162.5 ±14.1*	78.8*	4.68 ± .34*
10% oxidized corn oil + 90% fresh corn oil fatty acids	196.7 ± 7.1	96.1	3.13 ± .47

* Statistically significantly different from fresh corn oil group at 95% level.
† Stand. error of mean.
‡ (Wt gain on test fat)/(Wt gain on control fat, fresh corn oil) × 100.

TABLE III. The Influence of Dietary Fat on the Carcass and Feces Fatty Acid Composition of the Rat.

Fatty acid component	Dietary supplements									
	Fresh corn oil		Oxidized corn oil		Fatty acids from oxidized corn oil		Hydrogenated oxidized corn oil		10% oxidized corn oil fatty acids + 90% fresh corn oil fatty acids	
	Carcass	Feces	Carcass	Feces	Carcass	Feces	Carcass	Feces	Carcass	Feces
Linolenic	.26 ± .55†	—	.46 ± .55	0	0 ± 0	—	0 ± 0	0	.00 ± .0	0
Linoleic	36.10 ± 2.27	17.9	15.20 ± 2.88	29.5	23.72 ± 4.07	39.8	10.28 ± 1.41	5.4	29.78 ± 4.50	18.2
Oleic	31.96 ± 1.33	34.3	44.68 ± 2.91	23.1	44.50 ± 1.41	20.2	46.25 ± 1.59	21.9	33.78 ± 1.84	33.6
Stearic	2.89 ± .83	17.8	2.59 ± .76	29.9	3.43 ± 3.41	—	4.34 ± 1.73	33.6	2.64 ± .54	8.5
Hexadecenoic	5.07 ± .02	8.8	10.08 ± .25	—	9.23 ± 2.87	—	10.47 ± 3.03	—	5.67 ± 1.45	2.5
Palmitic	21.42 ± 2.42	25.6	23.14 ± 3.30	35.7	26.33 ± 2.84	33.1	24.48 ± 1.11	37.0	24.12 ± .79	13.8
Myristic	1.61 ± .67	10.2	2.74 ± .20	1.1	2.76 ± .56	1.9	2.48 ± .71	1.9	1.95 ± .30	.8
Tetradecenoic	.10 ± 1.04	—	.87 ± .54	—	.47 ± .00	—	.53 ± .51	—	.14 ± .52	—
Lauric	.27 ± .48	2.8	.34 ± .53	1.1	.52 ± .00	5.4	.42 ± .35	.4	.45 ± .34	.5
Hydroxy acids	1.50 ± 1.04	2.50*	6.98 ± 2.66	1.93*	4.18 ± 1.12	2.84*	4.27 ± 3.01	2.59*	1.88 ± 1.95	3.86*
Iodine values	99.68 ± 3.70	103.0	77.88 ± 2.7	53.8	79.74 ± 4.83	74.9	72.57 ± 1.52	44.2	90.02 ± 4.18	89.5

* Reported as percent hydroxyl for feces fats only.
† Stand. error of mean.

oxidized corn oil, and 5) 10% oxidized fatty acids plus 90% fresh fatty acids from corn oil. All animals were restricted to the same amount of daily food intake as those fed corn oil and samples of feces collected for lipid analysis. At the end of the test period the animals were sacrificed; the carcass lipids were extracted, converted to methyl esters and subjected to gas liquid chromatographic analysis. The results indicated that hydroxy acids, originating from oxidized fats, are deposited and influence the character of the normal mixed fatty acid composition of the carcass fat.

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