Fatty Acids in Tissue Lipids of Rats Fed *Sterculia foetida* Oil

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Increased amounts of fat were found in the liver and in the hardened heart of female rats that ingested 2% dietary *Sterculia foetida* oil for 34 weeks. Adipose contained the highest level of Halphen positive material, followed by liver and heart tissue. Components having a retention time corresponding to a C_{18}–C_{14} fatty acid were found in the tissue lipids of animals that received the 2% dietary *S. foetida* oil. Dietary *S. foetida* oil caused increased levels of saturated fatty acids and decreased levels of unsaturated fatty acids in heart, liver, and particularly in the adipose tissue. Higher levels of linoleate and lower levels of arachidonate in both heart and liver suggested that the mechanism of conversion of linoleate to arachidonate was inhibited.

It has been known for a long time that ingestion of cottonseed oil (CSO), *Sterculia foetida* oil (SFO), or materials that contain these oils causes changes, including increased saturation, in the body fat of pigs, cows, and hens (Phelps *et al.*, 1965). Reiser *et al.* (1962), Friedman and Mohr (1968), Burns *et al.* (1969), and Coleman and Friedman (1969) reported that SFO alters the tissue lipids of rats. Recently, Lee *et al.* (1968) reported that increased amounts of endogenous fat were found in the liver tissue of rainbow trout that received SFO and *Hibiscus syriacus* oil. The reported lipid changes were believed to be related to the cyclopropenoid fatty acids (CPA) contained in these oils. Raju and Reiser (1967) and Allen *et al.* (1967) have suggested that the increase in saturation of animal tissue lipids might result from the binding of the thiol groups of acyl desaturase by the cyclopropene function, thus inhibiting desaturation of saturated fatty acids to the corresponding monoenes.

Cottonseed oil and *S. foetida* oil contain 7-(2-octyl-1-cyclopropenyl) heptanoic acid and 8-(2-octyl-1-cyclopropenyl) octanoic acid, commonly referred to as malvalic and sterculic acids, respectively. The combined level of these fatty acids in CSO and SFO is approximately 0.04 to 2% and 44 to 72%, respectively (Phelps *et al.*, 1965).

The above mentioned reports on exposure of mammalian systems to CPA-containing material have been of a preliminary nature, and have produced only meager data concerning changes in fatty acid distribution due to exposure for more than 6 weeks. Therefore, the results of a study showing lipid alterations in several rat tissues due to ingestion of SFO for 6, 12, and 34 weeks are reported here.

EXPERIMENTAL

Extraction and Analysis of SFO. *S. foetida* oil was prepared by Soxhlet extraction of the ground kernels (S. B. Penick Co., 100 Church St., New York, N.Y., 10008), using petroleum ether (b.p. 30°–60° C). The solvent was removed from the oil in a rotary evaporator under reduced pressure at a temperature not exceeding 40° C. The oil was analyzed by the HBr titration method of Harris *et al.* (1964) and by the Halphen test of Bailey *et al.* (1965). The oil was found to contain from 44 to 46% CPA.

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Experimental Animals. Female weanling rats of the Holtzmann strain, weighing 45 to 55 g, were used for two chronic (6 to 34 weeks) feeding studies. In addition, male rats of an Osborne-Mendel derived strain (FDA, Division of Nutrition colony), weighing 400 to 450 g, were used for a single feeding study after being on the basal diet (containing 4% safflower oil) for 6 months. The rats were housed individually in stainless-steel cages, and allowed food and water *ad libitum*. Room temperature (23° C) and relative humidity (50%) were automatically controlled.

Diets. The rats were fed the following basal diet (in percent): vitamin-free casein, 18.0; sucrose, 76.0; salts, 4.0 (Jones-Foster Salt Mix, General Biochemicals, Chagrin Falls, Ohio); and vitamin mix, 2.0 (Vitamin Diet Fortification Mixture, Biochemicals Corp., Cleveland, Ohio). Safflower oil or safflower oil and SFO (1:1) were substituted for sucrose on an equal weight basis, such that the final oil concentration was 4.0%. The diets also contained 125 mg of the antioxidant Santoquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyl quinoline, Monsanto Co., St. Louis, Mo.) per kg of diet.

Diets were prepared once a week and stored at 5° C prior to feeding. Rats were given fresh diet every other day and weighed once a week.

Feeding and Sacrifice. In the first chronic study involving 25 rats in the test group and 26 rats in the control group, 5, 6, and 8 rats were sacrificed at random from each group at 6, 12, and 34 weeks, respectively. In the second chronic study involving 20 rats each in the test and control groups, rats were sacrificed from each group at 34 weeks only.

In the single feeding studies, rats were orally intubated with 5 ml of safflower oil or 5 ml of SFO per kg of body weight, and two rats each were sacrificed at time intervals of 2, 3, and 4 hr.

Tissue Analysis. Rats were killed by decapitation, after which adipose tissue, heart, and liver were rapidly removed, frozen in Freon-12 (Virginia Chemicals Inc., Portsmouth, Va.), and stored at −140° C until analyzed.

Tissues from five to eight rats (first chronic study) were analyzed individually or in pools at 6, 12, and 34 weeks (Table I). In the second chronic study, lipid analyses were done on pooled tissue only.

Total lipids were determined gravimetrically on extracts obtained by the extraction procedure of Folch *et al.* (1957). Cholesterol was determined on the extracts by the procedure of Abell *et al.* (1952). Lipid phosphorus was done by the acid digestion procedure of Lowry *et al.* (1954) and with the use of the Elon-Molybdate color reagent (Standard Methods of Clinical Chemistry, 1953). Neutral lipids were determined by difference. Liver protein was determined by the procedure of Layne (1957). The Halphen test of Bailey *et al.* (1965) and

Table I. Lipid Analyses of Female Rat Tissues[a]

Time, weeks	Tissue	Mg/g of Wet Tissue[b] Total Lipids Cont.	Total Lipids Exptl.	Phospholipid Cont.	Phospholipid Exptl.	Cholesterol Cont.	Cholesterol Exptl.	Neutral Lipid[c] Cont.	Neutral Lipid[c] Exptl.
6	Liver[d]	44.4	40.4	19.4	22.3	1.6	1.4	23.4	16.6
		±3.1	±0.9	±0.5	±1.3	±0.1	±0.0	±2.6	±1.8
12	Liver[e]	41.8	40.5	25.7	32.0[f]	1.7	1.4[f]	14.4	6.8
		±1.9	±1.6	±0.3	±1.6	±0.0	±0.1	±1.8	±3.7
34	Liver[g]	69.4	77.9	32.3	34.4	2.5	2.2	34.6	41.3
		±4.7	±3.5	±1.5	±2.0	±0.2	±0.1	±6.2	±4.5
	Liver[h]	67.7	75.8						
34	Heart[i]	37.3	49.8[f]	21.4	27.1[f]	0.7	0.6	15.2	22.1[f]
		±1.1	±2.9	±0.5	±0.7	±0.0	±0.0	±0.8	±1.5
	Heart[j]	40.9	53.4						

[a] Rats fed 4% safflower oil (control) or 2% *S. foetida* oil + 2% safflower oil (experimental); data from first chronic study, unless otherwise indicated. [b] Mean ± standard error of the mean. [c] Determined by difference. [d] Five animals (two pools of two and one individual) for control and experimental. [e] Six animals (three pools of two) for control and experimental. [f] The difference between the means of the control and experimental samples is significant at $P \leq 0.05$. [g] Five animals each, individually analyzed for control and experimental. [h] Pools of four animals (control) and seven animals (experimental) from second chronic study. [i] Eight animals each, individually analyzed for control and experimental. [j] Pool of six animals each for control and experimental from second chronic study.

Table II. Effect of Dietary *S. foetida* Oil on Rat Weight, Heart Weight, and Liver Weight and Protein Content

Time, weeks	Dietary Oil	No. of Rats	Rat Weight, g[a]	Liver Weight, g[b]	Liver Percent of Body Weight	Liver Protein, mg/g	Heart Weight, g[b]	Heart Percent of Body Weight
6	4% Safflower	5	163	7.10	4.35	185	0.62	0.37
			±2	±0.17	±0.06	±6	±0.03	±0.01
	2% SFO[c] +	5	156	7.91	5.16[d]	191	0.61	0.40
	2% Safflower		±4	±0.28	±0.18	±2	±0.02	±0.02
12	4% Safflower	6	225	7.67	3.40	164	0.72	0.32
			±4	±0.28	±0.13	±7	±0.01	±0.00
	2% SFO +	6	218	9.57[d]	4.38[d]	171	0.78[d]	0.36[d]
	2% Safflower		±3	±0.39	±0.15	±4	±0.00	±0.01
34	4% Safflower	6	291	6.90	2.38	220	0.92	0.30
			±16	±0.36	±0.08	±5	±0.04	±0.02
	2% SFO +	8	265	12.17[d]	4.64[d]	234[d]	1.05	0.37[d]
	2% Safflower		±13	±0.48	±0.21	±3	±0.07	±0.02
34[e]	4% Safflower	8	270	6.89	2.55	172	0.90	0.33
			±6	±0.34	±0.09	±6	±0.03	±0.01
	2% SFO +	7	291	12.00[d]	4.11[d]	172	1.12[d]	0.39[d]
	2% Safflower		±7	±0.94	±0.30	±5	±0.07	±0.02

[a] Mean ± standard error of the mean. [b] Tissue wet weight. [c] *S. foetida* oil. [d] $P \leq 0.05$. [e] Data from second chronic study.

gas chromatography were used to estimate the level of cyclopropenoid material in the tissue lipids. The methyl esters were determined directly on an SE-30 column.

Gas-Liquid Chromatography. Adipose, heart, and liver tissue lipids were transesterified by the procedure of Kircher (1964), and subsequently analyzed by gas-liquid chromatography (glc).

A Varian Aerograph Model 1520 gas chromatograph equipped with flame ionization detectors was used. The columns were 9 ft × 0.25 in. o.d. stainless-steel tubing packed with 3% SE-30 on 100/120 mesh Chromosorb W (Analabs, P.O. Drawer 5397, Hamden, Conn.), and with 20% stabilized DEGS (Varian Aerograph, 2700 Mitchell Dr., Walnut Creek, Calif.) on 60/80 mesh Chromosorb W. The carrier gas was nitrogen at a flow rate of 69 ml per min. The columns were operated isothermally at 187° C for DEGS and 200° C for SE-30. The injector block and detector were at 243° and 246° C, respectively. A 1-mV recorder was used for graphic presentation. Sample components were identified by comparing their retention times to those of purified standards. Gas-chromatographic peaks were measured by peak height times retention time, according to Carrol (1961). Student's *t* test was used, when possible, to test mean differences for significance ($P < 0.05$).

RESULTS AND DISCUSSION

Total Lipids. The total lipid content of heart tissue of the 34-week animals given 2% SFO (Table I) increased 34%. Also of interest, although not significant, is the increase in lipid content of the liver tissue. The greater portion of the increase seemed to be due to increased neutral lipids. No liver lipid changes were seen at 6 weeks, whereas at 12 weeks, a significant increase in phospholipids and a small but significant decrease in cholesterol were noted in the livers of SFO-fed rats. Total heart lipids of the 6- and 12-week experimental animals are not given because the lipid content was essentially the same as that of the controls. The total heart and liver lipids at 34 weeks for both chronic studies were in good agreement. The trend toward increased liver lipids seemed compatible with the findings of Lee *et al.* (1968) that the livers of rainbow trout receiving cyclopropenoid triglycerides accumulated lipid globules.

At 34 weeks the size of the livers of the experimental animals was about double that of the controls (Table II), whereas body weights were not significantly affected. The moisture levels of the control and experimental livers were about the same (70%). Protein increased slightly.

The relative size of the hearts of the 34-week animals also increased. The hearts were very firm and hard to the touch

Table III. Cyclopropenoid Fatty Acids in Rat Tissue after CPA Ingestion

Tissue[a]	Time, weeks	% CPA,[b] Halphen	% CPA, glc[c]
Adipose	34	5.1	9.9
	12	9.4	7.0
	6	3.4	4.2
Heart	34	1.5	0.9
	12	...	0.9
	6	...	0.5
Liver	34	3.6	2.5
	12	0.5	1.6
	6	...	0.4
Single Oral Dose—2 hr		0.4 (0.3)[d]	
3 hr		0.4 (0.3)	
4 hr		1.4	

[a] Data represent pooled tissue of two to eight rats per pool. (Control tissues contained negligible amounts of CPA.) [b] Calculated on the basis that SFO contains 45% CPA (HBr titration). [c] Determined directly on 3% SE-30 column. [d] Parentheses contain values of a duplicate experiment.

when compared with the controls. The increase in lipid content of this tissue was significant ($P = 0.05$). The results of Goodnight and Kemmerer (1966) might be related to this finding. They found that White Leghorn cockerels whose diet was supplemented daily with 200 mg of SFO had a significantly greater severity of aortic atherosclerosis than did the controls.

CPA in Tissues. Table III contains the results of Halphen analyses that were performed on tissue extracts from both chronic and single feeding experiments. There was a gradual buildup, with time, of cyclopropenoid fatty acids in the livers and hearts, as determined by the Halphen test and glc. (Results of both analyses are included because neither method is standard for tissue analysis.) The greatest concentration was in adipose tissue, although there was some disparity between the levels of CPA as measured by both methods at the different time intervals. CPA appeared in the liver lipids 2 hr after ingestion of a single oral dose of SFO.

Fatty Acids. Fatty acid analyses of tissue lipids from the 12- and 34-week animals revealed two unknown peaks. The equivalent chain lengths of those peaks on a 20% DEGS column was estimated to be 13.0 and 13.2. Wood and Reiser (1965) and later Chung (1966) showed that in cyclopropenoid fatty acid metabolism the C_{19} fatty acid was converted by the β-oxidation system to a C_{13} cyclopropenoid fatty acid. It was thought that a comparable degradation product could result from cyclopropene fatty acid metabolism. To characterize the material represented by the unknown peaks, efforts were made to isolate the material by preparative glc. At this time, samples of adequate purity have not been obtained, due primarily to the similarity of their vapor pressures.

As mentioned above, other investigators have reported that changes occur in the fatty acid distribution of tissue lipids of animals whose diet has been supplemented with cyclopro-

Table IV. Percent Fatty Acids[a] of Female Rats

Equivalent Chain Length	Fatty Acid	Time: 6 weeks[b] Cont.	6 weeks[b] Exptl.	12 weeks[c] Cont.	12 weeks[c] Exptl.	34 weeks[d] Cont.	34 weeks[d] Exptl.
			Adipose				
12.0	12:0	0.3	0.3	0.2	0.2	0.1	0.1
13.0	...	...	0.1	...	0.7	...	0.5
13.2	...	...	...	...	...	0.2	0.3
14.0	14:0	2.1	2.8	1.9	1.8	1.4	1.8
14.9	14:1	0.2	0.2	0.3	0.1	0.1	0.1
15.5	15:0	...	...	...	...	0.2	0.1
16.0	16:0	29.0	42.5[e]	23.9	31.9[e]	21.2	29.8[e]
16.5	16:1	5.3	0.9[e]	6.8	0.7[e]	6.0	0.6[e]
17.1	17:0	0.3	0.3	...	0.3	0.2	0.3
17.5	...	0.2	...	0.2	0.2	0.1	0.3
18.0	18:0	8.6	20.6[e]	3.9	21.1[e]	3.6	20.8[e]
18.6	18:1	27.2	12.1[e]	26.6	14.5[e]	35.0	16.2[e]
19.2	18:2	25.4	14.7[e]	34.2	20.1[e]	30.5	16.4[e]
19.9	20:0	0.3	...	0.4	0.4	...	0.4
20.5	20:1	0.6	1.6	0.5	1.1	0.3	1.3
21.9	22:0	...	...	...	...	...	...
22.3	20:4	0.4	...	0.7	...	0.3	...
	Fatty Acid Ratios						
	14:0/14:1	10.5	14.0	6.3	18.0	14.0	18.0
	16:0/16:1	5.5	47.2	3.5	45.6	3.5	49.7
	18:0/18:1	0.3	1.7	0.1	1.5	0.1	1.3
			Heart				
12.0	12:0	0.6	0.5	0.3	0.4	0.1	0.3
13.0	...	0.2	...	0.2	0.4	...	0.2
13.2	...	...	0.9	0.2	0.4	0.1	0.7
14.0	14:0	2.3	1.5	1.8	1.6	0.6	1.2
14.9	14:1	...	0.1	0.3	0.1	0.4	0.2
15.5	15:0	...	...	...	...	...	...
16.0	16:0	14.6	10.1[e]	13.4	11.4	13.8	12.4
16.5	16:1	3.5	1.0[e]	2.5	1.3[e]	1.3	0.2[e]
17.1	17:0	...	...	0.4	0.2	0.1	0.1
17.5	...	0.2	0.2	0.5	0.3	0.1	0.1
18.0	18:0	6.0	6.6	9.5	11.9[e]	22.8	26.8[e]

(continued)

Table IV (Continued)

Equivalent Chain Length	Fatty Acid	Time: 6 weeks[b] Cont.	6 weeks[b] Exptl.	12 weeks[c] Cont.	12 weeks[c] Exptl.	34 weeks[d] Cont.	34 weeks[d] Exptl.
			Heart				
18.6	18:1	6.7	3.4[e]	8.7	5.9[e]	11.8	6.0[e]
19.2	18:2	34.8	42.6	29.4	32.9[e]	23.5	25.9[e]
19.9	20:0	1.2	0.6	1.0	0.9	...	...
20.5	20:1	0.2	0.4	0.3	1.0	...	0.5
21.9	22:0	0.5	1.8	1.4	1.0	...	0.6
22.3	20:4	27.8	29.0[e]	28.1	26.7	24.8	22.8
	Fatty Acid Ratios						
	14:0/14:1	...	15.0	6.0	16.0	1.5	7.0
	16:0/16:1	4.2	10.1	5.4	8.8	10.6	62.0
	18:0/18:1	0.9	1.9	1.1	2.0	1.9	4.5
	18:2/20:4	1.3	1.5	1.0	1.2	0.9	1.1
			Liver				
12.0	12:0	0.1	0.2	...	0.2	...	0.1
13.0	...	0.1	2.5	...	0.7	...	4.8
13.2	...	...	...	...	0.4	...	0.6
14.0	14:0	0.9	2.0	0.5	1.2	0.3	0.5
14.9	14:1	...	...	0.1	0.1	0.1	0.1
15.5	15:0	...	...	...	...	0.1	0.4
16.0	16:0	17.1	18.9	19.9	20.0	18.4	19.1
16.5	16:1	2.7	0.8	2.6	0.4[e]	1.3	0.3[e]
17.1	17:0	0.2	0.2	0.2	0.3	0.4	0.2
17.5	...	0.1	...	0.2	...	0.2	0.1
18.0	18:0	16.1	10.3	20.4	28.4[e]	23.9	24.6
18.6	18:1	8.0	3.6[e]	15.7	6.7[e]	11.2	8.6[e]
19.2	18:2	18.2	25.7[e]	15.4	19.5[e]	16.0	21.8
19.9	20:0	0.9	1.6	0.6	0.5	0.7	0.4
20.5	20:1	...	...	0.5	0.5	0.2	1.0
21.9	22:0	0.8	2.0	0.6	2.5	0.3	1.1
22.3	20:4	34.0	29.0	21.3	16.6[e]	26.9	14.4[e]
	Fatty Acid Ratios						
	14:0/14:1	...	...	5.0	12.0	3.0	5.0
	16:0/16:1	6.3	23.6	7.7	50.0	14.2	63.7
	18:0/18:1	2.0	2.9	1.3	4.2	2.1	2.9
	18:2/20:4	0.5	0.9	0.7	1.2	0.6	1.5

[a] Determined by internal normalization of glc peaks—uncorrected; % CPA not included (see Table III). [b] Adipose: Five animals each for control and experimental (two pools of two and one individual). Heart: Four animals for control (two pools of two) and five for experimental (pools of two and three). Liver: See footnote *d*, Table I. [c] Adipose: Five animals for control (three individuals and one pool of two) and six for experimental (four individuals and one pool of two). Heart: Four animals for control and five for experimental, individually analyzed. Liver: See footnote *e*, Table I. [d] Adipose: Data represent average values obtained from a six-animal pool for control and an eight-animal pool for experimental (first chronic study), and a three-animal pool for each control and experimental (second chronic study). Heart: Eight animals each for control and experimental, individually analyzed. Liver: Data represent average values obtained from two animals (pool) for the control and six (two pools of three) experimental from first chronic study, and four animals (pool) for control and seven (pool) for experimental from second chronic study. [e] The difference between the means of the control and experimental samples is significant at $P \leq 0.05$.

penoid fatty acids. The most comprehensive account of those changes in mammals is found in the work of Reiser *et al.* (1962). They analyzed the epididymal fat pads of male weanling rats that were given a diet containing CPA for only 6 weeks, and found increased levels of saturated and decreased levels of unsaturated fatty acids. In the present study, increased levels of saturated and decreased levels of unsaturated fatty acids were found in heart and liver, as well as in adipose tissue (see Table IV). Glc analyses of adipose and liver tissue lipids of animals given a single oral dose of SFO did not show significant alterations in their fatty acid distribution and are not included.

Differences in fatty acid distribution in adipose tissue of control and experimental animals (Table IV) resulting from dietary SFO were observed at 6, 12, and 34 weeks. Significant differences were observed in the level of palmitate (33 to 47% over control), palmitoleate (83 to 90% below control), stearate (140 to 477% over control), oleate (46 to 56% below control), and linoleate (41 to 46% below control) at the time periods observed. The level of eicosenoate (20:1) in the experimental animals was about double that in the control animal tissue, and remained so for 6, 12, and 34 weeks. Arachidonate (20:4) was present in all control adipose tissue, but was not detected in experimental adipose tissue. Of the tissues examined, adipose was the only one in which the observed level of linoleate was lower in the experimental animals than in the controls. Reiser *et al.* (1962) observed these differences in adipose tissue of male rats that received 0.2% dietary SFO. The net effects of dietary SFO on this tissue at 34 weeks were higher levels of palmitate and stearate, and lower levels of palmitoleate, oleate, and linoleate, and the magnitude of the differences was also indicated by altered fatty acid ratios. The ratios of palmitate-palmitoleate and stearate-oleate were increased more than tenfold.

The effect of SFO on the fatty acid distribution in the heart lipids (Table IV) was similar to that in adipose lipids, except that the level of linoleate in experimental heart lipids was higher than that in the control at each time period. Consis-

tently significant changes were observed in the level of palmitoleate (48 to 85% below control), stearate (18 to 25% over control, except at 6 weeks), oleate (32 to 49% below control), and linoleate (10 to 12% over control, except at 6 weeks). Statistically insignificant decreases were observed in the levels of palmitate and arachidonate at 12 and 34 weeks. Unlike that in adipose tissue, the increases in saturated-unsaturated fatty acid ratios in the heart were due primarily to relatively lower amounts of unsaturated fatty acids.

The liver fatty acid distribution is presented in Table IV. The significant changes noted in this tissue were in the levels of palmitoleate (77 to 85% below control), oleate (22 to 46% below control), and arachidonate (22 to 46% below control) at 12 and 34 weeks. As in the heart, the level of saturated fatty acids remained relatively unchanged, except for the level of stearate at 12 weeks. The level of linoleate was consistently high, whereas the level of arachidonate was low. The data suggested that the mechanism leading from linoleate to arachidonate was inhibited in the heart and liver.

Reiser and Raju (1964) suggested that there might be a metabolic route (saturate-independent) for the synthesis of long chain unsaturated fatty acids, other than the simple desaturation of saturated precursors. Donaldson (1967) presented evidence that supports this hypothesis for chicks, and Raju and Reiser (1969) presented evidence for the male rat. The cumulative data of our study indicate a continued increase in the ratios of palmitate-palmitoleate and stearate-oleate. This suggests that if the saturate-independent system is present and operating, 34 weeks is not an adequate period for its induction or, more probably, its synthetic capability is considered less than the inhibitory capability of CPA.

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