

Effect of Dietary Rapeseed Oil on the Fatty Acid Composition of Testes of Maturing Rats¹ (38183)

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Testicular tissue has a large concentration of polyenoic acids, but the role of these fatty acids in reproduction is not known. A possible method of study of the function of these compounds is to alter the fatty acid composition of the testis and to determine what alterations have occurred in the histological structure and physiological function of the tissue. In rat testes most polyenoic acids are of the linoleic acid (w6) family (1). Because rapeseed oil has a large concentration of fatty acids not usually encountered in diets ordinarily fed to rats, it seemed desirable to investigate the effect of this dietary fat on the composition of testicular fatty acids. Recently, Walker (2) reported that weanling rats fed rapeseed oil or corn oil containing erucic acid accumulate a small amount of 20- and 22-carbon monoenoic acids. In our studies we have fed rats rapeseed oil not only from time of weaning but also, in one group, from the time of birth by placing the mothers of the experimental rats on rapeseed oil on the day the pups were born. The fatty acid composition and the histological state of the testes of these rats and of control rats fed corn oil were compared.

Methods and Materials. Purified diets containing 51% sucrose, 24% casein, 3% minerals, 20% fat and complete vitamin mix were prepared using either corn oil or rapeseed oil.² The major fatty acid com-

position of these oils is given in Table I.

In the first experiment weanling Sprague-Dawley rats were fed the corn oil or rapeseed oil diet and maintained for 16-27 wk. One animal from each group was killed at intervals between these time periods. In the second experiment pregnant rats were maintained on laboratory chow until the day of birth of the pups. The mother rats were then fed either the corn or the rapeseed oil diet. After the pups were weaned, they were continued on the same diet given the mothers. Two rats from each group were killed at 23, 29, 35, 44, 54 and 65 days after birth. Testes of rats from each dietary group for one time period were pooled for analysis. One rat from each group was killed at 73, 77, 79 and 85 days after birth.

The testes were weighed and a testis from each sample was sectioned for histological examination. The balance of the tissue was hydrolyzed in alcoholic KOH and total fatty acids were extracted, methylated and determined by gas chromatography using methods published previously (3). Standards for identification of fatty acids by gas chromatography and for calibration of the flame ionization detector were obtained from Applied Science Laboratories, State College, Pa. Sections for histological examination were fixed in Bouin's media, stained with periodic acid Schiff stain (PAS) and counterstained with Wright's iron-hematoxylin.

Results. In neither experiment did the type of oil in the diet affect significantly the body weight or the weight of the testes. Furthermore, no alteration in the histological state of the tissue was noted in any of

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TABLE I. Major Fatty Acid Composition of Dietary Oils.

Acid	% of total fatty acids	
	Corn oil	Rapeseed oil
16:0 ^a	12.9	4.2
18:0	2.1	1.0
18:1	29.1	35.4
18:2	55.1	18.9
18:3	1.0	6.7
20:1	—	10.6
22:1	—	23.1

^a No. of carbons in chain; no. of double bonds.

the rapeseed oil-fed rats compared to the corn oil-fed rats. The fatty acid composition of the testicular lipids of rats of the first experiment is given in Table II. The rapeseed oil-fed rats had significantly greater amounts of 16:1³ and 18:1 and a significantly lower amount of 20:3 than the corn oil-fed group. There was only borderline

TABLE II. Fatty Acid Composition of Testes of Rats Fed Corn or Rapeseed Oil from Time of Weaning.

Fatty acid	% of total fatty acids		
	Corn oil-fed	Rapeseed oil-fed	
14:0	0.72 ± 0.005 ^a	0.63 ± 0.11 ^a	
16:0	29.6 ± 0.7	29.8 ± 1.0	
16:1	0.94 ± 0.10	1.85 ± 0.23	(<i>P</i> < 0.01)
18:0	6.0 ± 0.30	5.8 ± 0.26	
18:1	11.8 ± 0.8	14.0 ± 0.33	(<i>P</i> = 0.02)
18:2	6.4 ± 0.41	6.0 ± 1.2	
18:3	0.24 ± 0.02	0.30 ± 0.05	
20:1	0.54 ± 0.16	0.63 ± 0.12	
20:2	0.50 ± 0.09	0.78 ± 0.15	
20:3	1.70 ± 0.05	1.33 ± 0.07	(<i>P</i> < 0.01)
20:4	15.0 ± 0.53	13.3 ± 0.50	(<i>P</i> = 0.04)
20:5	0.26 ± 0.04	0.27 ± 0.06	
22:1	0.25 ± 0.15	0.33 ± 0.08	
22:4	2.38 ± 0.22	2.15 ± 0.19	
22:5	18.2 ± 0.45	17.2 ± 0.73	
22:6	1.5 ± 0.41	2.0 ± 0.17	
>22:6	4.0 ± 0.17	3.83 ± 0.2	

^a Mean ± standard error of the mean of 6 rats.

³ No. of carbon atoms in chain; no. of double bonds.

TABLE III. Fatty Acid Composition of Testes of Rats Fed Corn or Rapeseed Oil from Day of Birth.

Fatty acid	% of total fatty acids		
	Corn oil-fed	Rapeseed oil-fed	
14:0	1.1 ± 0.17 ^a	0.97 ± 0.07 ^a	
16:0	30.9 ± 0.81	30.1 ± 1.2	
16:1	1.3 ± 0.13	1.5 ± 0.11	
18:0	7.5 ± 1.1	6.8 ± 0.68	
18:1	11.2 ± 0.78	14.8 ± 1.27	(<i>P</i> = 0.03)
18:2	6.4 ± 0.67	5.2 ± 0.45	
18:3	0.24 ± 0.03	0.77 ± 0.18	(<i>P</i> = 0.01)
20:1	0.40 ± 0.11	0.78 ± 0.10	(<i>P</i> < 0.001)
20:2	0.52 ± 0.06	0.67 ± 0.19	
20:3	1.20 ± 0.12	1.16 ± 0.15	
20:4	14.2 ± 1.0	13.3 ± 1.0	
22:4	2.4 ± 0.10	2.3 ± 0.13	
22:5	See Table 4		
22:6	1.4 ± 0.15	2.5 ± 0.34	(<i>P</i> < 0.001)
>22:6	4.2 ± 0.27	4.0 ± 0.31	

^a Mean ± standard error of the mean of 10 samples.

significance (*P* = 0.04) to the lower value for 20:4 in the rapeseed oil-fed group.

A similar summary is given in Table III for the rats fed these oils from the day of birth, (i.e., pups born to mothers which were placed on these diets on the day of delivery). Significantly larger amounts of 18:1, 18:3, 20:1 and 22:6 were present in testes of rapeseed oil-fed than in corn oil-fed rats. The concentration of 22:5 increases markedly with age in testes of growing rats; therefore, data for concentrations of this polyenoic acid are given separately in Table IV. The amount of 22:5 was greater in the corn oil than in the rapeseed oil-fed animals at each time period tested. If the paired data are treated statistically, the *P* value is less than .001.

Discussion. No effect either on weight gain or on weight of testes was observed in our experiments. Beare *et al.* (4) had observed smaller weight gains in rats fed rapeseed oil compared to rats fed corn oil, but the rapeseed oil used by these investigators contained over 40% erucic acid compared to 23% in the oil used in the present studies. In general, the differences in weight gains were explained by the investigators

TABLE IV. Comparison of Amount of 22:5 in Testes of Corn Oil- and Rapeseed Oil-Fed Rats.

Age of rats (days)	22:5 (% of total fatty acids)	
	Corn oil-fed	Rapeseed oil-fed
23	4.3	3.6
29	9.3	5.6
35	14.0	13.5
44	14.3	11.5
54	17.8	14.8
65	17.0	14.8
77	19.2	17.0
79	20.4	19.7
85	21.2	19.5

by the effect of rapeseed oil on food consumption.

Our studies show that starting the mother rat on rapeseed oil on the day of delivery and continuing the pups on the same diet effect on hepatic Q_{o_2} , Q_{o_2}' or $Q_{o_2}(T)$. If on the fatty acid composition of the testes not seen in the rats maintained on the rapeseed oil diet from the time of weaning. These changes were increased deposition of 18:3, 20:1 and 22:6 and decreased concentrations of 22:5. The increased 22:6 was probably due to metabolic conversion of 18:3 to 22:6; the fatty acids 20:1 and 18:3 were probably derived from the diet. Since rapeseed oil has a much higher 18:3 content than corn oil, a greater concentration of 18:3 would have been expected in the testes of rapeseed oil-fed rats in both experiments. Rats fed the rapeseed oil diet from weaning had concentrations of this testicular fatty acid similar to those of rats fed corn oil. The reason for this is not known but it is possible that much of the accumulation of this fatty acid in testicular tissue occurred in the pre-weaning period.

The decrease in concentration of 22:5 was due partly to the smaller amount of 18:2 in rapeseed oil versus corn oil (19 versus 55) and partly perhaps to the inhibition by 18:3, 20:1 and 22:1 in the rapeseed oil of the conversion of 18:2 to 22:5. It is known that linolenate and its metabolic derivatives can inhibit competitively the conversion of linoleate to longer chain, more

highly unsaturated derivatives (5). Alteration of the amount of 22:5 in the testes caused by feeding rapeseed oil is important since this is the fatty acid which accumulates in rat testes with sexual maturation of the rat (1) and which is thought to be related to the appearance of spermatids (6). It may be possible to decrease further the amount of this fatty acid in testes of rats by increasing the amount of erucic acid in the rapeseed oil and/or by starting the mother rat on the diet earlier in the pregnancy. Comparison of our results with those obtained by Walker (2) is not completely satisfactory because of the difference in oils fed the rats (that is, he did not feed corn oil alone). In his studies Walker obtained lower (but variable) concentrations of 22:5 in all groups used compared to values usually reported for rats fed corn oil. Alteration of the fatty acid composition of the testes of the magnitude obtained in our experiments did not have any effect on the histological state of the testes of rapeseed oil-fed rats, but no mating experiments were done to check fertility. No histological studies were reported by Walker (2). Previously, Carroll and Noble (7) had reported that rats maintained on diets containing 10% or more erucic acid were sterile; however, Beare *et al.* (4) reported that male rats fed a diet containing 8.5% erucic acid successfully sired three litters.

In both of our experiments there was an increase in 18:1 in testes of rapeseed oil-fed rats, due probably to the higher concentration of 18:1 in rapeseed oil compared to corn oil. Since this difference in 18:1 content in these two oils is not very large (35 versus 29) and since 18:1 (oleic acid) can be synthesized by the testis, it is not obvious why increased deposition of this fatty acid occurred.

The increase in 16:1 and decrease in 20:3 which occurred in testes of the rapeseed oil-fed rats in the first experiment were not observed in the second experiment. The reason for this discrepancy is not known.

Summary. Testes of rats fed a purified diet containing rapeseed oil from the time of weaning had more 16:1 and 18:1 and a smaller amount of 20:3 than testes fed

a similar diet containing corn oil instead of rapeseed oil. Pups nursed by mothers maintained on the rapeseed oil diet from the day of delivery and continued on the diet after weaning had larger amounts of 18:1, 18:3, 20:1 and 22:6 and lower amounts of 22:5 than pups treated similarly but with corn oil as the dietary fat. No effect was noted in either experiment on the body weight, testicular weight, or the histological state of the testes by the rapeseed oil fed at 20% of the diet.

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