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Responses of Essential Fatty Acid-Deficient Rats to Fasting-Refeeding and Partial Hepatectomy (39835)

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The proportion of arachidonate in liver phospholipids in young rats decreases from about 30 to 10-12% after 4 weeks of feeding an essential fatty acid-deficient diet (1-3). Subsequently, arachidonate decreases only slowly (3-5), and turnover of liver phospholipids appears reduced (5). It is generally assumed that a certain level of essential fatty acids (EFA) in phospholipids is required for membrane integrity, function, and formation (6). Reduced phospholipid turnover (5) might be one consequence of a decrease in EFA in membrane phospholipids.

Information on the need for EFA-containing phospholipids in membrane structure, as well as on the ability of EFA-depleted liver to form phospholipids, could be obtained by subjecting EFA-deficient rats to stimuli which normally increase phospholipid formation. Little or no response would occur if a relatively high proportion of EFA were required. Considerable response would occur if nonessential unsaturated fatty acids could replace essential fatty acids to a large extent in these functions. Stimulation of liver phospholipid formation could also produce even greater depletion of liver EFA than is usually obtained in EFA-deficient rats.

Two conditions which stimulate liver phospholipid formation are (a) partial hepatectomy (7, 8) and (b) refeeding of a high carbohydrate diet after fasting (9-11). Partial hepatectomy normally produces liver hyperplasia and hypertrophy which require formation of new phospholipid-containing membranes (8). Refeeding after fasting increases lipoprotein secretion (9). Park *et al.* (11) reported that fasting-refeeding also decreased the concentrations of arachidonate and linoleate in microsomal phospholipids, presumably as a result of increased lipoprotein secretion.

Decreases in phospholipid EFA may underlie increased activities of liver glucose-6-phosphate dehydrogenase (G6PD) and other lipogenic enzymes in rats refed high carbohydrate diets after fasting (10, 11). The activities of liver lipogenic enzymes are already elevated in EFA-deficient rats fed *ad libitum* (1). Thus, these enzyme activities might become even higher if additional decreases in phospholipid EFA were produced by fasting-refeeding of EFA-deficient rats.

In the present report, we have subjected EFA-deficient rats to fasting-refeeding or to partial hepatectomy and then have measured liver phospholipid fatty acid composition and recovery of liver and body weights. Activities of glucose-6-phosphate dehydrogenase (G6PD) and fatty acid synthetase (FAS) were also measured in the fasted-refed deficient rats.

Materials and methods. Male weanling Long-Evans or Sprague-Dawley rats (Simonsen Farms, Gilroy, Calif.) were caged individually in galvanized screen wire-bottom cages and were fed *ad libitum* either a fat-free diet or the same diet supplemented with 5% safflower oil (1). The oil replaced an equal weight of sucrose. In the fasting-refeeding study, rats were fed the fat-free diet *ad libitum* for 7 weeks after weaning, then fasted for 48 hr, and refed the fat-free diet for 1, 2, 3, and 7 days. The rats were sacrificed by decapitation without anesthesia.

For partial hepatectomy, rats were anesthetized with ether, and the median and left lateral lobes were removed (12). These two lobes are 65-70% of liver weight in both EFA-deficient and control rats. Initial liver weights were estimated by dividing the weight of the removed lobes by 0.7. The rats were fed the same diets pre- and post-

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operatively. Four to five weeks were allowed for regain of liver weight. The rats were then sacrificed under ether anesthesia without decapitation. Livers were blotted, weighed in plastic bags, frozen in dry ice, lyophilized, and extracted in 100-ml portions of chloroform:methanol (2v:1v) containing 0.1 mg of hydroquinone (13). Liver phospholipids were separated by silicic acid column chromatography. Fatty acids were determined by gas–liquid chromatography of their methyl esters, with heptadecanoic acid as internal standard (13).

For determination of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (G6PD) and fatty acid synthetase (FAS) activities, 1.0 g of liver was homogenized in 4 vol of 0.25 *M* sucrose and was centrifuged at 100,000*g* for 1 hr at 4°. Enzyme activities were assayed with an Eppendorf photometer by following NADP reduction or NADPH oxidation by aliquots of the 100,000*g* supernatant (14).

Results. Fasting–refeeding. The deficient rats withstood the 48-hr fast satisfactorily, regained their prefasting body weights (262 g) and liver weights (12 g) within 2–3 days of refeeding, and continued to gain. The average daily food intake was 23–30 g, both before fasting and in the refeeding period. In the first 3 days of refeeding, liver total fatty acids (mg/g of liver) showed a threefold increase above the nonfasting value (142 vs 43), with a decrease after 7 days to 62 mg/g.

Phospholipid arachidonate decreased slightly from 6.8% in the nonfasted group to 5.3% in the 3-day refed group and remained at this level in the 7-day refed group (Table 1). There was also little change in the amount of phospholipid arachidonate, which was approximately 1 mg/g before and after refeeding. The proportion of 5,8,11-eicosatrienoate, which replaces arachidonate in EFA deficiency, decreased from 20.0% in the nonfasted group to 13.0% in the 3-day refed group, but returned to prefasting values after 7 days refeeding. Eicosatrienoate (mg/g of liver) decreased in the first 3 days of refeeding. Over 90% of liver arachidonate and eicosatrienoate is in phospholipids (15). Phospholipid oleate and palmitoleate increased (data not shown) so that the ratio of unsaturated to saturated fatty acids did not change, despite the decrease in C_{20}-unsaturated fatty acids. Linoleate showed little change.

The activities of both G6PD and FAS (Table 1) decreased with fasting and increased rapidly with refeeding. The activities of the refed groups were not significantly higher ($P > 0.05$) than the activities in the nonfasted deficient rats.

Partial hepatectomy. We allowed at least 4 weeks for restoration of liver weight on the assumption that recovery might be slower in deficient rats. More than 2 weeks is required for restoration of liver weight in normal relatively young rats (12). Initially, rats fed the fat-free diet for 7 weeks postweaning were used for partial hepatectomies. In these animals, liver weights 4 weeks postoperatively were about 90% of the estimated preoperative weight. Percentages of arachidonate and linoleate in liver phospholipids were the same after the operation as before. Consequently, we depleted rats for longer periods before hepatectomy

TABLE 1. LIVER PHOSPHOLIPID POLYUNSATURATED FATTY ACIDS AND ACTIVITIES OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND FATTY ACID SYNTHETASE IN EFA-DEFICIENT RATS BEFORE AND AFTER FASTING–REFEEDING.[a]

			Day of refeeding			
	Nonfasted	Fasted	+1	+2	+3	+7
Arachidonate[b]	6.8 ± 0.6	8.6 ± 0.3	7.2 ± 0.3	6.3 ± 0.6	5.3 ± 0.5	5.4 ± 0.3
Eicosatrienoate[b]	20.0 ± 1.0	19.8 ± 1.6	15.8 ± 0.4	12.8 ± 0.6	13.0 ± 1.0	19.1 ± 1.8
Linoleate[b]	2.3 ± 0.2	1.8 ± 0.3	2.2 ± 0.1	2.6 ± 0.2	2.7 ± 0.2	2.1 ± 0.2
Glucose-6-phosphate dehydrogenase[c]	498 ± 67	239 ± 39	385 ± 23	496 ± 47	547 ± 85	586 ± 88
Fatty acid synthetase[c]	92 ± 8	29 ± 1	89 ± 5	115 ± 7	116 ± 4	105 ± 20

[a] All rats were fed a fat-free diet. Mean ± standard error. Three rats per group except for six in nonfasted group.

[b] Values are expressed as percentage by weight of total phospholipid fatty acids.

[c] Values are expressed as nanomoles of NADP reduced or oxidized per minute per milligram of protein.

in an attempt to reduce further the supply of extrahepatic EFA potentially available for liver regrowth (7).

In rats fed the fat-free diet for 11.5 weeks before the operation, liver weights at 4 weeks after the operation were 50–79% of the estimated preoperative weight (Table 2). Phospholipid arachidonate was about 4% in comparison with preoperative values of about 6%. Phospholipid linoleate did not change.

To compare the responses of both deficient and control rats to hepatectomy, another set of rats was fed the fat-free diet or the 5% safflower oil diet for 14 weeks before the operation. All rats fed the safflower oil diet regained their preoperative body weight and continued to gain. No deficient rats regained their preoperative weight although they were not losing weight before the operation. Food intakes of both nondeficient and deficient rats ranged from 0 to 17 g in the first postoperative day. By 2 days after the operation, average daily food intakes had increased to nearly 20 g, a level which was subsequently maintained. The immediate postoperative body weight losses in all rats, excluding weight of liver removed, were greater when the operation and duration of anesthesia were more prolonged.

Postoperative liver weights ranged from 83 to 100% of estimated preoperative weights for the 5% safflower oil group and from 51 to 92% for the deficient group. The same trends in liver regrowth appear if pre- and postoperative liver weights are expressed as a percentage of body weight. In nondeficient rats, the proportions of liver phospholipid arachidonate and linoleate were the same after the operation as before. In deficient rats, phospholipid arachidonate was slightly lower after the operation than before. Linoleate remained constant.

In two rats depleted of EFA for 20 weeks, the postoperative liver weights after 4 weeks were 57 and 84% of preoperative weights. There was little change in the proportions of phospholipid linoleate and arachidonate.

In all partially hepatectomized rats, the gross appearance of the liver tissue after the operation was generally similar to that of the preoperative tissue. In deficient rats, the abdominal incisions were slower to heal, with leakage of fluid and appearance of small ulcers, which eventually healed. The

TABLE 2. EFFECTS OF PARTIAL HEPATECTOMY ON BODY AND LIVER WEIGHTS AND PHOSPHOLIPID LINOLEATE AND ARACHIDONATE.[a]

	Body weight		Liver weight						Liver phospholipid fatty acids			
			Preop[b]		Postop				Preop		Postop	
Diet	Preop (g)	Postop (g)	(g calcd.)	(% of body wt)	(g calcd.)	(% of body wt)	Estimated gain in liver weight (g)	Estimated restoration of liver weight (% of initial wt)	18:2	20:4	18:2	20:4
									(percentage by wt)			
Fat-free; fed 11.5 weeks[c]	311	291	13.8	4.4	7.1	2.4	3.0	52	1.6	6.0	1.4	4.0
	316	323	12.7	4.0	10.0	3.1	6.2	79	1.5	6.1	2.0	4.2
	280	274	15.8	5.6	8.0	2.9	3.3	50	1.6	5.6	1.6	4.3
Fat-free fed 14 weeks[c]	373	360	13.4	3.6	11.2	3.1	7.2	84	1.9	6.3	2.2	4.5
	361	335	12.9	3.6	11.8	3.5	7.9	92	2.2	6.6	2.1	4.1
	341	313	11.4	3.3	9.2	2.9	5.8	81	1.5	5.4	1.5	4.3
	325	274	12.1	3.7	6.2	2.3	2.6	51	1.8	4.9	1.3	4.0
5% Safflower oil; fed 14 weeks[c]	523	546	17.2	3.3	14.3	3.0	9.1	83	10.2	27.9	10.5	30.2
	507	563	17.2	3.4	17.1	3.0	11.9	100	11.5	26.9	10.0	29.9
	435	465	13.2	3.0	12.4	2.7	8.4	94	13.2	29.4	12.5	30.0
	500	531	14.9	3.0	14.2	2.7	9.7	95	9.9	32.2	10.2	32.0
	427	442	13.5	3.2	11.8	2.7	7.8	87	12.5	31.6	12.7	28.7
	360	397	9.9	2.8	9.8	2.5	6.8	100	13.4	31.2	15.4	32.1
Fat-free; fed 20.5 weeks[c]	314	262[d]	11.7	3.7	9.8	3.7	6.3	84	1.6	4.1	1.7	2.5
	355	310	14.6	4.1	8.4	2.7	4.1	57	1.9	2.3	1.7	2.7

[a] Rats were sacrificed 30 days after hepatectomy, except as indicated.

[b] Preoperative weight was estimated on the assumption that lobes removed by hepatectomy were 70% of the initial liver weight.

[c] Length of time rats were fed the experimental diet before hepatectomy.

[d] Sacrificed at 5.5 weeks postoperation.

fur in the shaved area around the incision grew very slowly. When the incisions were made, the deficient rats bled more than the controls. The greater bleeding during the operation and slow healing of the incisions are presumably consequences of reduced prostaglandin or thromboxane formation (16). Postoperation mortality was no greater in deficient rats than in controls.

Discussion. Liver arachidonate decreases in normal rats fed a fat-free or low-fat diet after fasting, presumably because of increased lipoprotein secretion (10, 11). In contrast, we found only a slight additional decrease in the already reduced level of arachidonate in EFA-deficient rats refed after fasting, whereas eicosatrienoate decreased appreciably. These results are evidence that liver arachidonate in severely deficient rats is much less available for lipoprotein secretion, and that eicosatrienoate has replaced arachidonate for this function.

Whether lipoprotein secretion from the liver is altered by EFA deficiency is still unresolved (4, 17, 18). We did not attempt to estimate lipoprotein secretion in fasted-refed EFA-deficient rats. The changes we observed in liver total fatty acids during refeeding of EFA-deficient rats indicate that relatively normal removal of liver triglycerides was possible in deficient rats. The relative decline in total fatty acids (mg/g) from 3 to 7 days of refeeding (142 to 62 mg/g of liver) was similar to the change after 3 to 7 days of refeeding rats fed the 5% safflower oil diet before and after fasting (84 to 49 mg/g) (19). It was previously shown that phospholipid and phospholipid fatty acid concentrations were not decreased by fasting in rats adapted to a purified diet (20).

The activities of G6PD in fasted-refed EFA-deficient rats were not significantly higher than those in nonfasted EFA-deficient rats. This result is consistent with speculations of Szepesi and co-workers (21, 22) that fasting-refeeding should produce no additional increase in enzyme activity in rats fed a fat-free diet before fasting, e.g., EFA-deficient rats. Furthermore, the activities in EFA-deficient rats fed *ad libitum* are as high as those in fasted-refed rats fed an EFA-adequate diet before fasting (19).

The activity of G6PD could be controlled by dietary fat if (a) dietary fat were required for repression of genes coding for G6PD (21, 22), or (b) a less active form of G6PD were produced in animals fed diets containing fat, without appreciable change in enzyme protein level. Evidence for (b) has been obtained by Yagil *et al.* (23), Hizi and Yagil (24), and Watson *et al.* (25). Watson *et al.* also speculated that G6PD might be inactivated by increased palmitoyl-CoA derived from dietary fat, similar to the inactivation of yeast G6PD by palmitoyl-CoA (26).

The liver regrowth in EFA-deficient rats after partial hepatectomy shows that considerably new tissue can be formed even when only very low levels of arachidonate are present in phospholipids of the new tissue. This is additional evidence for the ability of unsaturated fatty acids of the oleate and palmitoleate series to substitute for EFA in functions other than formation of prostaglandins and related compounds (6, 16).

The subnormal recovery of liver weight and the maintenance of relatively constant proportions of phospholipid EFA after hepatectomy can be considered evidence for a need for EFA in cell division, either as fatty acid structures and/or as precursors of compounds such as prostaglandins and thromboxanes. More specific information on this point could be obtained by measuring cell division and associated processes such as phospholipid and DNA synthesis in the first days after hepatectomy (8). This information cannot be provided by studies with cultured cells since no cell lines specifically requiring EFA have yet been demonstrated (27–29). Thus, hepatectomy in EFA-deficient rats is a potentially important method of testing for specific functions of EFA in cell division.

Summary. Essential fatty acid-deficient rats were subjected to fasting–refeeding or partial hepatectomy to stimulate liver phospholipid and membrane formation under conditions of limited essential fatty acid (EFA) supply. Refeeding after fasting decreased the proportion of arachidonate in liver phospholipid fatty acids only slightly, i.e., from 6.8% in nonfasted rats to 5.3% after 3 or 7 days of refeeding, whereas eicosatrienoate decreased from 19.8% in

nonfasted rats to 13.0% after 3 days of refeeding, with a return to nonfasted levels after 7 days. Activities of liver glucose-6-phosphate dehydrogenase and fatty acid synthetase in fasted-refed EFA-deficient rats did not differ significantly from values in deficient rats fed *ad libitum*. Considerable increase in liver weight (ca. 3-8 g or at least 50% of the estimated preoperative weight) occurred after partial hepatectomy of EFA-deficient rats. The proportion of arachidonate in phospholipid fatty acids was slightly lower after hepatectomy than before.

These results indicate that: (a) little of the arachidonate in EFA-depleted liver is used for lipoprotein formation and transport after fasting-refeeding, whereas eicosatrienoate is available; (b) fasting-refeeding of EFA-deficient rats does not increase activities of liver glucose-6-phosphate dehydrogenase and fatty acid synthetase above the already elevated levels in EFA-deficient rats fed *ad libitum;* (c) considerable new liver tissue can be formed after partial hepatectomy even when EFA levels in phospholipids are very low.

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