Fatty liver produced by dietary deficiencies: its pathogenesis and potentiation by ethanol

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ABSTRACT In a study of the pathogenesis of hepatic fat accumulation under experimental conditions mimicking chronic alcoholism, rats were fed a low-fat diet, deficient in amino acids and choline, containing either ethanol or isocaloric amounts of carbohydrate. Dietary deficiencies alone produced a moderately fatty liver after 24 days. The combination of ethanol and dietary deficiencies resulted in enhanced lipid accumulation, which was apparent after only 11 days.

In an investigation of the origin of hepatic triglyceride fatty acids, the experiment was repeated after the adipose lipids had been marked by the feeding of oils containing characteristic fatty acids (linseed oil, containing linolenate, or coconut oil, containing laurate and myristate). In all animals, the fatty acid composition of the hepatic triglycerides differed markedly from that of adipose tissue; it had a larger percentage of endogenously synthesized fatty acids and a five times smaller percentage of the marker fatty acids. In addition, ethanol feeding resulted in a greater retention of the marker fatty acids in the adipose tissue.

Thus, the deposition of hepatic triglycerides produced by the feeding of deficient diets is markedly potentiated by ethanol; the triglyceride fatty acids accumulated under these conditions appear to originate, for the most part, not from mobilization of depot fat, but from endogenous synthesis.

SUPPLEMENTARY KEY WORDS adipose tissue · choline · lipotropes · methionine · laurate · myristate · linolenate

We previously reported that ethanol ingestion can produce a fatty liver in man (1, 2) and in rats (1, 3), even when the diet is fully adequate in other respects. It has also been shown, in studies utilizing diets containing 30–45% of total calories as fat, that deficiencies in dietary protein and lipotropic factors (choline and methionine) potentiate the steatogenic effect of ethanol in rats (4, 5), although others (6) have not found this effect. In humans with chronic alcoholism, liver injury is commonly produced when ethanol is ingested in conjunction with deficient, low-fat or even fat-free diets. Moreover, we have shown previously that dietary fat can be an important source for the triglyceride fatty acids that accumulate in the liver after ethanol ingestion (7, 8). We have therefore determined the extent to which deficiencies in dietary protein and lipotropic factors potentiate the effect of ethanol, even when this is given with diets almost devoid of fat, and we have also examined the origin of the fatty acids that accumulate in the liver of rats fed fat-deficient diets, with or without ethanol. A preliminary account of part of this work has been presented (9).

MATERIALS AND METHODS

Male rats of a Sprague-Dawley strain (CD) were purchased from Charles River Breeding Laboratories (North Wilmington, Mass.) in groups of weanling littermates. They were fed Purina laboratory chow and tap water ad lib. until they reached a weight of 120–150 g. They were then housed in individual wire bottom cages, and pair-fed in groups of two littermates each the low-fat liquid diet previously described (7), with the following modifications: a dextrin-maltose mixture

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(generously supplied as Dexin by the Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y.) was used instead of sucrose, and the diet was rendered deficient by reducing the amino acid, vitamin, and choline content to one-fourth that of the adequate diet, without changing the composition of the original amino acid and vitamin mixtures (7). The amino acids therefore represented only 4% of the total calories (including 0.38 mg of methionine/cal). The choline content was reduced to 0.06 mg/cal.

The only lipid in the diet was ethyl linoleate (2% of total calories), which was given to prevent essential fatty acid deficiency. The remainder of the dietary calories comprised dextrin–maltose in the control rats (94% of total), and a mixture of dextrin–maltose and ethanol (58 and 36% of total, respectively) in the alcohol-treated animals. The technique of pair-feeding was as previously described (7, 10). Unlike the adequate diet (7), the deficient regimen failed to promote growth.

Six pairs of rats were fed the diet for 5 days, eight pairs for 11 days, and nine pairs for 24 days. None of the animals died.

To determine the origin of the fatty acids accumulating in hepatic triglycerides, eight pairs of rats were given the treatment described (four pairs for 11 days and four pairs for 24 days) after a preliminary period of 21 days, during which the adipose tissue lipids had been "labeled" by the administration of nondeficient diets containing characteristic fatty acids, as described previously (7). A diet containing linseed oil (rich in linolenate, 18:3) was given to half of the rats, and one containing coconut oil (rich in laurate, 12:0, and myristate, 14:0) to the others. At the end of the experiment, the rats were killed by decapitation and the liver was quickly excised.

The total hepatic lipids were extracted, the triglyceride fraction was separated by thin-layer chromatography and its fatty acid composition was determined by gas–liquid chromatography as previously described (7). The hepatic triglycerides were quantified by determination of ester linkages (11).

In all experiments, each result was compared to the corresponding control, and the mean of the individual differences was tested for significance by Student's t test (12).

RESULTS

As indicated in Fig. 1, the feeding of a deficient diet alone resulted in a moderate accumulation of hepatic triglycerides, which became manifest only after 24 days. After 5 and 11 days, the mean hepatic triglyceride values were not significantly different from those found previously in rats fed a similar low-fat diet, but which contained adequate amounts of amino acids and vitamins (7). The combination of ethanol and dietary deficiencies, however, resulted in a much more striking increase of hepatic triglycerides. As shown in Fig. 1, this increase was significant after only 11 days. After 24 days of feeding of the deficient, ethanol-containing diet, the average hepatic triglyceride concentration was

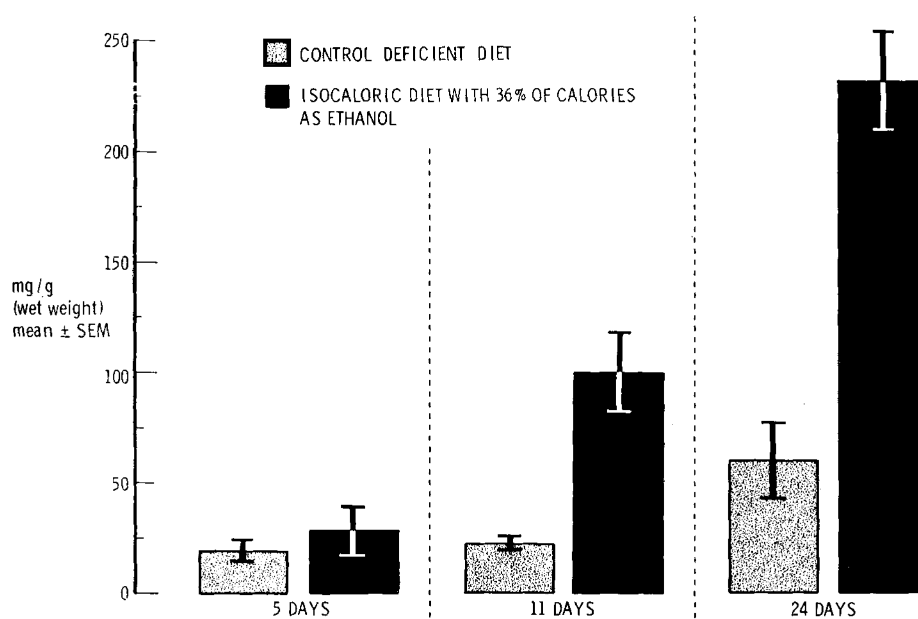

Fig. 1. Hepatic triglycerides in rats fed deficient diets (with ethanol or isocaloric carbohydrate) for various periods of time.

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TABLE 1 EFFECT OF DEFICIENT DIETS (WITH OR WITHOUT ETHANOL) IN RATS WHOSE ADIPOSE TISSUE HAD BEEN "LABELED" WITH LAURATE PLUS MYRISTATE (12:0 + 14:0)

Pair of Littermates	Duration of Treatment	Ethanol	Tissue	Fatty Acid Composition					
				12:0+14:0	16:0	16:1	18:0	18:1	18:2
				% of total fatty acids					
A	11 days	−	Liver triglycerides	4.0	44.9	9.2	1.3	39.6	1.3
		−	Adipose tissue lipids	23.1	23.8	15.8	3.3	24.3	9.1
		+	Liver triglycerides	8.2	28.2	11.8	4.1	45.0	2.7
		+	Adipose tissue lipids	26.3	21.9	9.9	2.2	32.5	6.6
B	11 days	−	Liver triglycerides	4.8	36.5	9.7	4.8	39.7	3.2
		−	Adipose tissue lipids	25.9	23.5	12.3	1.4	30.2	5.6
		+	Liver triglycerides	7.1	35.5	11.3	3.6	40.4	2.1
		+	Adipose tissue lipids	24.5	24.1	12.7	2.3	30.9	5.0
C	24 days	−	Liver triglycerides	2.8	31.4	11.7	5.2	45.0	4.0
		−	Adipose tissue lipids	15.6	27.7	18.2	1.7	32.0	4.8
		+	Liver triglycerides	4.4	31.0	14.9	2.8	43.7	3.4
		+	Adipose tissue lipids	31.2	18.9	6.2	0	39.5	4.2
D	24 days	−	Liver triglycerides	1.7	40.3	7.9	3.4	42.1	4.4
		−	Adipose tissue lipids	15.5	26.4	18.5	1.9	32.6	4.1
		+	Liver triglycerides	2.9	32.9	15.9	2.9	40.6	4.7
		+	Adipose tissue lipids	26.1	26.0	19.5	0	23.9	4.3

TABLE 2 EFFECT OF DEFICIENT DIET (WITH OR WITHOUT ETHANOL) IN RATS WHOSE ADIPOSE TISSUE HAD BEEN "LABELED" WITH LINOLENATE (18:3)

Pair of Littermates	Duration of Treatment	Ethanol	Tissue	Fatty Acid Composition						
				12:0+14:0	16:0	16:1	18:0	18:1	18:2	18:3
				% of total fatty acids						
E	11 days	−	Liver triglycerides	0	35.5	6.6	3.3	38.8	8.6	7.2
		−	Adipose tissue lipids	1.6	23.0	12.0	2.8	30.4	12.0	18.1
		+	Liver triglycerides	0.9	31.9	4.4	4.4	33.9	11.9	12.8
		+	Adipose tissue lipids	1.4	23.0	9.3	3.1	31.6	11.7	19.6
F	11 days	−	Liver triglycerides	1.2	33.4	5.8	4.2	44.9	9.2	1.2
		−	Adipose tissue lipids	1.2	22.8	8.7	3.9	31.5	13.9	18.9
		+	Liver triglycerides	1.4	32.2	4.0	8.7	39.0	11.1	4.0
		+	Adipose tissue lipids	1.2	20.3	8.0	3.6	30.0	13.5	23.9
G	24 days	−	Liver triglycerides	3.0	27.7	14.3	6.1	41.8	6.8	0
		−	Adipose tissue lipids	0	42.3	18.5	2.8	26.1	6.2	3.9
		+	Liver triglycerides	3.2	25.4	13.9	7.2	39.7	9.1	1.3
		+	Adipose tissue lipids	1.9	23.5	12.0	1.3	31.8	13.4	16.0
H	24 days	−	Liver triglycerides	1.6	30.8	11.1	1.7	50.6	4.2	0
		−	Adipose tissue lipids	1.1	29.0	15.6	2.1	41.2	7.9	3.2
		+	Liver triglycerides	2.1	36.9	0	6.2	47.8	4.7	0
		+	Adipose tissue lipids	2.1	24.5	12.9	2.2	32.3	12.6	13.2

232.7 ± 23.1 mg/g wet weight, which is considerably more than the value of 52.1 ± 10.2 mg/g that we observed previously in comparable experiments in which rats were given the same amount of ethanol with a low-fat, nondeficient diet (7).

The type of fatty acids accumulated in hepatic triglycerides is indicated in Tables 1 and 2. The fatty acid composition of hepatic triglycerides differed strikingly from that of adipose tissue, whether the fatty liver was produced by the deficient diet alone or by the combination of deficiency and ethanol. In the rats whose adipose tissue had been "labeled" by laurate and myristate (Table 1), the liver triglyceride fatty acids had a much lower percentage of the adipose tissue marker than the depot lipids ($P < 0.001$). By contrast, liver triglyceride fatty acids had a higher proportion of endogenously synthesized fatty acids, such as palmitate (16:0) or oleate (18:1) ($P < 0.001$). Similar results were obtained in the rats whose adipose tissue had been labeled with linolenate (Table 2). The proportion of linolenate in fatty acids of liver triglycerides was, on the average, only one-fifth that found in the corresponding adipose tissue ($P < 0.01$) (in some animals, linolenate was completely absent), and endogenously synthesized fatty acids, again, usually predominated.

This difference in fatty acid composition (between

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liver triglycerides and adipose tissue lipid), although present in all animals, was usually more pronounced after 24 than after 11 days, especially in the ethanol-treated rats. In general, the ethanol-fed rats had retained a higher proportion of the marker in the adipose tissue than did the pair-fed controls (Tables 1 and 2).

DISCUSSION

The results show that even in the virtual absence of dietary fat, hepatic steatosis produced by deficiencies in dietary protein and lipotropic factors is markedly potentiated by isocaloric substitution of carbohydrate by ethanol (Fig. 1). These results contrast with those of a previous study by Best, Hartroft, Lucas, and Ridout (6) in which isocaloric replacement of dietary carbohydrate by ethanol did not aggravate the hepatic steatosis. The reason for this discrepancy is not apparent, but it could result from a difference in the mode of administration of the ethanol and the dosage. Best et al. (6) gave ethanol as part of the drinking water, a technique that resulted in low ethanol consumption (18% of total calories). By contrast, we incorporated the ethanol in a totally liquid diet, thereby overcoming the aversion of the rat for ethanol and increasing the ethanol consumption to a level of 36% of the total calories, an amount of alcohol comparable to that commonly consumed by man. The diet used by Best et al. (6) also had a higher fat content than the one given in the present study, but this probably does not explain the difference in results, since even when we used a deficient, fat-containing diet, isocaloric substitution of ethanol for carbohydrate aggravated the hepatic steatosis (5).

In more recent studies by the Toronto group (13–15), rats fed deficient, low-fat diets (with or without ethanol) failed to develop marked hepatic steatosis; the hepatic triglyceride concentrations were generally 5–10 times lower than the ones observed in the present study (Fig. 1). Under these conditions, substitution of ethanol for carbohydrate did not aggravate the accumulation of hepatic triglycerides. Interpretation of these results is complicated by the high mortality (23–60%) observed in the various groups of alcohol-treated rats, which casts some doubt on the representative nature of the surviving animals and the significance of the lack of hepatic triglyceride accumulation under these conditions.

In addition to showing the potentiation by ethanol of the effects of dietary deficiencies, our results also indicated the origin of the triglyceride fatty acids that accumulate in the liver under these experimental conditions. Theoretically, these fatty acids could originate from three main sources; dietary lipids, adipose tissue fatty acids, or lipid synthesized in the liver itself (16). It has been reported that rats given a single large dose of ethanol deposit in their livers fatty acids that are derived, to a large extent, from adipose tissue (17). We confirmed these results in a previous study and also showed that in contrast to the effects of a single large dose of ethanol, prolonged ingestion of ethanol and adequate diets results in hepatic deposition of triglycerides containing fatty acids derived primarily from endogenous synthesis in rats fed low-fat diets, and from dietary fat in rats given fat-containing diets (7). Similar results were observed in men given ethanol and adequate diets (8) and in rats fed choline-deficient diets without ethanol (18).

The present investigation extends these observations to rats fed a combination of deficient diet and ethanol. Since the diet used in the present study was devoid of fat (except for the linoleate that was administered to avoid essential fatty acid deficiency), dietary lipids can be eliminated as a possible source of hepatic lipids. The fatty acids of hepatic triglycerides were also strikingly different from adipose tissue fatty acids in composition, with a much lower content of the adipose tissue marker (Tables 1 and 2); they contained instead a higher proportion of endogenously synthesized fatty acids. This observation suggests that endogenously synthesized fatty acids, rather than adipose tissue lipids, represent the main source for the fatty acids that accumulate in hepatic triglycerides of rats given deficient diets (with or without ethanol).

Despite the difference in composition between liver and adipose tissue fatty acids, liver fatty acids could have derived from adipose tissue through a process of selective free fatty acid mobilization. But since two sets of markers (linolenate and laurate plus myristate) were used, selectivity would have had to operate against fatty acids of very dissimilar structure in the two types of experiments. Furthermore, when fatty liver is produced through enhanced mobilization of peripheral fat, after epinephrine for instance, the fatty acids that accumulate in the liver do indeed resemble adipose tissue fatty acids (19). This indicates a basic difference between the fatty liver produced by enhanced mobilization of peripheral fat and that obtained under our experimental conditions. Moreover, in our previous studies on the effects of administration of ethanol with low-fat but adequate diets, we found the hepatic triglyceride fatty acids to differ in composition, not only from those of adipose tissue, but also from the circulating free fatty acids (7).

Theoretically, the difference in composition between liver and adipose tissue could be due also to a difference in the rate of hepatic metabolism of the various fatty acids. This possibility, however, is very unlikely, again in view of the fact that the adipose tissue markers used varied widely from a long-chain unsaturated fatty acid (linolenate) to shorter-chain saturated fatty acids (laurate and myristate). The most likely source, there-

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fore, for the triglyceride fatty acids of the fatty liver produced by prolonged intake of a deficient, low-fat diet (with or without ethanol) is lipogenesis in the liver itself. The mechanisms responsible for the accumulation of the fatty acids in the liver have not been investigated in the present study, but are reviewed in detail elsewhere (20).

The rats given ethanol (in conjunction with a deficient diet) differed from the controls (fed the deficient diet alone), not only in the magnitude of hepatic triglyceride accumulation (Fig. 1), but also in the composition of their adipose tissue: in the animals "labeled" both with laurate and myristate (Table 1) and with linolenate (Table 2), more of the marker was retained in the rats fed ethanol for 24 days (but not for 11 days). This difference in fatty acid composition suggests that ethanol may have decreased the turnover of adipose tissue lipids. The mechanism of this effect remains unexplained, although it may be related to the reduction in turnover of plasma free fatty acids observed in volunteers given ethanol over short periods of time (21).

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