The role of liver and adipose tissue in the pathogenesis of the ethanol-induced fatty liver

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SUMMARY In vivo incubation of paired epididymal fat pads with medium containing palmitic acid-1-C^{14} was employed to label adipose tissue triglycerides. The administration of a single dose of ethanol to normal rats so treated produced an elevation of liver triglyceride, judged by comparison with control rats given glucose isocalorically. The radioactivity of liver triglyceride was proportionately elevated, while the specific activity was unchanged. The loss of radioactive triglyceride from adipose tissue was not significantly different in the ethanol group from that in the controls.

These studies demonstrate that adipose tissue triglyceride is mobilized at a normal rate during the development of this type of hepatic steatosis. Specific activity data suggest nevertheless that adipose tissue triglyceride fatty acids constitute the principal source of the fatty acids of the accumulated hepatic triglycerides, indicating an altered hepatic metabolism of triglycerides (or fatty acids) as the cause of the accumulation.

It is generally agreed that there is a close relationship between alcoholism, fatty metamorphosis of the liver, and cirrhosis (1–3). Although ethanol is obviously an important etiological factor, the precise manner by which it produces fatty infiltration is unknown (3).

At the present time, it is widely considered that the hepatic steatosis of alcoholics, and its sequela, cirrhosis, may be related to a decrease in adequate food consumption, particularly of lipotropic agents (1, 2) and is therefore a deficiency disease. This concept was supported by studies on long-term alcohol feeding which demonstrated that ethanol increases choline requirements (4). The fatty liver in this condition is readily corrected by the administration of choline or other lipotropic agents (5). Klatskin (1) has presented evidence that alcohol may have some direct effect on the liver independent of dietary factors.

Recent studies (6–13) have amply demonstrated that a single intoxicating dose of ethanol or various commercial alcoholic beverages (14) to normal rats produces an acute, reversible, fatty liver. Considerable differences in opinion exist, however, regarding the pathogenesis of the triglyceride accumulation. Current concepts regarding its genesis include increased hepatic synthesis of lipid (15), increased mobilization of free fatty acids (FFA) from adipose depots (8–10), and decreased intrahepatic utilization of triglyceride (7, 12). Another factor in the development of the acute ethanol-induced fatty liver could be failure in the hepatic triglyceride secretory mechanism (16). The metabolic effects of ethyl alcohol have recently been reviewed (17).

The development of the technique of in vivo incubation of paired epididymal fat pads of rats for the selective labeling of adipose triglyceride has been reported (18). This technique, which offers a rapid and convenient way of labeling adipose triglyceride in an intact animal, was employed to evaluate the significance of increased mobilization of FFA in the genesis of a fatty liver in rats exposed to ethanol. Hepatic lipid metabolism was also studied to investigate the contribution of altered liver triglyceride metabolism to the development of the fatty liver.

METHODS

Male rats (Holtzman Co., Madison, Wis.) weighing about 130 g and previously maintained on a Purina Laboratory Chow diet were employed. After 8 hr of fasting, the animals were lightly anesthetized with ether and both epididymal fat pads were exteriorized and incubated at 37° in a glass well incubator for 20 min according to the procedure of Stein and Stein (18).

The incubation medium consisted of plasma obtained from normal rats in which palmitic acid-1-C^{14} (Nuclear-Chicago) had been incorporated into the albumin

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TABLE 1 EPIDIDYMAL FAT PAD WEIGHT AND TRIGLYCERIDE CONTENT OF FIVE NORMAL RATS FOLLOWING IN VIVO INCUBATION*

Fat Pad Wet Weight		% Body Weight		Triglyceride	
Right	Left	Right	Left	Right	Left
mg				*mg*	
356 ±42	345 ±40	0.27 ±0.02	0.27 ±0.02	184 ±45	183 ±45

* Values are expressed as means ± standard errors.

fraction according to the procedure of Borgström and Olivecrona (19). The incubation volume was 5 ml, containing approximately 8 μc of palmitic acid. The purity of the palmitic acid sample was established by thin-layer chromatography (20) and subsequent radioautography.

At the end of incubation, the fat pads were washed with saline to remove surface radioactive contamination. The right pad was rapidly removed and weighed, the lipids were extracted with chloroform–methanol (21). The left pad was replaced in the abdominal cavity and the incision closed.

When the rat recovered from the anesthesia (about 15 min after incubation), ethanol (6 g/kg body weight as a 50% solution w/v) or isocaloric glucose was given by stomach tube. The rats were maintained with water but without food for an additional 16 hr. The rats were then anesthetized with ether and a blood sample was obtained from the inferior vena cava. Liver, left epididymal fat pad, spleen, and lung were removed, washed with saline, and weighed. Lipids were extracted with chloroform–methanol and purified (21). The triglyceride concentration of plasma, liver, right and left epididymal fat pad was determined (22). Total radioactivity of various organs and plasma and triglyceride activity of liver and fat pads were determined with a liquid scintillation counter employing a solution of 2,5-diphenyloxazole and 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene (23).

The various lipid classes were separated on silicic acid columns (24). The purity of each lipid fraction was evaluated by chromatography on silicic acid-impregnated glass paper (25) and also by thin-layer chromatography (20) and the radioactivity of each fraction was determined as previously described.

The data were analyzed statistically either by means of the "t" test, or where appropriate, such as in the analysis of all the fat pad data, by an analysis of paired observations (t test). A pooled variance was employed when the variances were homogeneous as determined by the "F" ratio. When nonhomogeneity was present, as in the case of the radioactivity of the liver triglyceride fraction, the t test was employed with the appropriate corrected degrees of freedom (26). All data were analyzed at the 95% confidence limits.

As a collateral control experiment, epididymal fat pads of rats, fasted for 8 hr, were incubated as previously described. The rats were killed at the end of the incubation. The right and left fat pads, liver, lung, and spleen were removed and weighed and lipids were extracted (21). Triglyceride concentration of right and left fat pads, total lipid radioactivity of all the organs and plasma, and triglyceride activity of fat pads were determined. Chemical and radioactive determinations were made in duplicate.

RESULTS

In agreement with previous observations (12, 27), the right and left epididymal fat pads of 5 normal rats, weighing from 110–154 g with a mean body weight of 128 g, showed a similar wet weight and triglyceride content (Table 1). The triglyceride content of the right and left fat pads comprised 49.6 and 50.8% respectively of the wet weight. The difference in triglyceride content of the left fat pad when compared to the right pad triglyceride content in the same rat ranged from −20% to +10% with a mean difference of +1.2%.

The incorporation of albumin-bound FFA into the total lipids of the epididymal pads of normal rats after a 20 min incubation averaged 2.5% (Table 2). No significant differences were observed between the right and left pad with respect to total lipid or triglyceride activity. The mean triglyceride radioactivity (cpm × 10^3) and standard error of the right fat pad were 111 ±

TABLE 2 INCORPORATION OF PALMITIC ACID-1-C^{14} INTO EPIDIDYMAL FAT PADS, LIVER, SPLEEN, LUNG, AND PLASMA DURING 20 MINUTES OF IN VIVO INCUBATION OF THE FAT PADS*

Epididymal Fat Pads				Liver	Plasma	Spleen	Lung
Right		Left					
Total Lipid	Triglyceride	Total Lipid	Triglyceride	Total Lipid			
2.20 ±0.37	2.04 ±0.30	2.81 ±0.68	2.48 ±0.64	0.0004 ±0.00008	0.0002 ±0.00005	0.0004 ±0.0001	0.0003 ±0.0001

* Values (mean ± standard error) derived from five normal rats, are expressed as per cent of palmitate in incubation medium incorporated per total fat pad; per gram of tissue (liver, spleen, lung); or per milliliter of plasma.

TABLE 3 ADIPOSE TISSUE TRIGLYCERIDE METABOLISM IN GLUCOSE- AND ETHANOL-TREATED RATS

Group & No.	Body Weight		Fat Pad Weight		Total Triglyceride		Total Radioactivity in Triglyceride*		Specific Activity†	
	Initial	Final	Right	Left	Right	Left	Right Pad	Left Pad	Right Pad	Left Pad
	g		*mg*		*mg*		*cpm* × 10^{-3}			
Glucose (8)	134 ±4	115 ±3	326 ±27	309 ±29	140 ±13	134 ±8	126 ±2.2	95 ±1.8	763 ±129	618 ±104
Ethanol (8)	130 ±2	113 ±3	317 ±15	292 ±16	145 ±15	136 ±14	109 ±1.3	82 ±1.3	821 ±109	610 ±69

Values are expressed as means ± standard errors.
* Right fat pad was removed after incubation and served as control; left fat pad was removed 16 hr later after ethanol or glucose administration.
† Cpm/mg triglyceride.

17.9 and 122 ± 21.5 for the left fat pad. These values are not significantly different. The mean and standard error of the paired differences (× 10^3) of the fat pad activity were + 11 ± 11.2. The data are in agreement with the observation of Stein and Stein (18) that approximately 95% of the labeling of the fat pad was in the triglyceride moiety.

The exclusive labeling of epididymal lipid during in vivo incubation previously observed (18) is confirmed by the extremely low activity found in the total lipid extracts of liver, plasma, spleen, and lung.

The ethanol- and glucose-treated groups manifested a mean 14% decrease in body weight (Table 3). The weights, triglyceride concentrations, and specific activities of the epididymal fat pads in the ethanol group did not differ from those of the control rats given glucose. The mean triglyceride concentration of the left epididymal fat pad was approximately 5% less than that in the right fat pad in both groups, but this difference was not significant either within or between each group.

Every left fat pad in both the ethanol-treated and glucose groups showed a lower total triglyceride activity than did the respective right fat pad. This decline in radioactivity after glucose or ethanol administration averaged 24.1 ± 3.8% and 24.6 ± 6.2% respectively. The mean and standard error of the paired differences of the fat pad specific activity in the glucose group were −145 ± 99 and −211 ± 81 in the ethanol group. There were no significant differences between the specific activities of the right and left pads within or between the groups.

The plasma triglyceride concentration and total lipid radioactivity were not altered significantly from control values in the ethanol-treated group (Table 4). Similarly, the total lipid radioactivity of lung and spleen showed no deviation in the rats exposed to ethanol.

The administration of ethanol resulted in a 7-fold elevation in liver triglyceride content compared with the controls (Table 5). The incorporation of palmitic acid-1-C^{14} into liver triglyceride was increased 5-fold in the ethanol-treated group; there was a wide range of values in the latter group. Liver triglyceride specific activity was not significantly altered from control values in the ethanol-treated group.

DISCUSSION

The in vivo incubation of epididymal fat pads as developed by Stein and Stein (18) permitted labeling of triglyceride exclusively in a specific adipose site. Our results are in complete agreement with their observations since only a minute fraction of the radioactivity was found in liver, lung, spleen, or plasma following incubation. No significant difference was observed in the incorporation of palmitate into triglyceride of the right and left pad during the incubation in vivo. The high degree of incorporation of albumin-bound palmitic acid into triglyceride of epididymal fat pads incubated in plasma is in excellent agreement with the results employing synthetic media (18). The unaltered specific activity of the fat pads during depletion of triglyceride in both groups confirms the prior observation of the equivalent mobilization of labeled and unlabeled triglyceride (18).

Horning et al. (28) observed that the proportions of fatty acids in accumulated liver triglyceride of ethanol-treated rats were similar to those of adiopose triglyceride. Since in that study the increase in palmitic acid was similar to that in oleic or linoleic acid, the validity of utilizing palmitic acid as a tracer in these studies is

TABLE 4 PLASMA TRIGLYCERIDE CONCENTRATION AND TOTAL LIPID RADIOACTIVITY OF PLASMA, LUNG, AND SPLEEN IN ETHANOL-TREATED RATS

Group & No.	Plasma Triglyceride	Total Lipid Radioactivity		
		Plasma	Lung	Spleen
	mg/100 *ml*	*cpm/ml*	*cpm per total organ*	
Glucose (8)	48 ± 3	27 ± 8	64 ± 9	59 ± 15
Ethanol (8)	52 ± 3	33 ± 16	69 ± 14	43 ± 14

Values are expressed as means ± standard errors.

TABLE 5 INFLUENCE OF ETHANOL ADMINISTRATION ON HEPATIC TRIGLYCERIDE CONTENT AND RADIOACTIVITY

Group	Triglyceride Content	Triglyceride Radioactivity	Triglyceride Specific Activity
	mg/liver	*cpm/liver*	*cpm/mg*
Glucose	14.0 ± 1.7	138 ± 23 (71–231)*	11.0 ± 2.0
Ethanol	106.9 ± 6.3	712 ± 222 (260–2037)	7.2 ± 2.3

Values, expressed as mean ± standard error, are derived from eight animals per group.

* Figures in parentheses represent sample range. The glucose- and ethanol-treated groups had mean liver weights of 4.42 and 4.66 g, respectively.

indicated. The corresponding increase in chemical and radioactive hepatic triglyceride in the ethanol group in the present study indicates that the fat accumulated in the liver following ethanol exposure is derived to a great extent from FFA originating in adipose tissue. This finding confirms previous conclusions of Horning et al. (28).

Brodie et al. (8–10, 28) have advanced the hypothesis of Mallov and Bloch (6) that the accumulation of liver triglyceride is the result of increased mobilization of lipid in the form of FFA from peripheral adipose depots under the influence of pituitary and adrenal hormones. The observation that the fatty liver condition is prevented by adrenergic blocking agents (9), adrenalectomy (8, 9, 29), or cordotomy (8), appeared to support this concept. However, these results could be just as well explained by a decreased ability of the liver to handle a normal flow of FFA.

Brodie et al. observed a rise in plasma corticosterone, and free fatty acids, as well as a fall in adrenal ascorbic acid in ethanol-treated rats, when compared to normal values (9). However, Elko et al. found (12) that with the exception of an early intreval, *i.e.* 2 hr after glucose, the plasma FFA response in ethanol-treated rats 4, 8, and 16 hr after ethanol administration was similar to that of glucose-treated rats (12). A similar finding was reported by Nikkila and Ojala (30) of essentially identical 4-hr FFA concentrations in ethanol- and glucose-treated rats, although Mallov, in well controlled studies, has observed an increase in FFA levels in acute ethanol-treated Wistar rats (31), indicating a possible species difference.

The present findings of similar changes in body weight, fat pad weight, and epididymal triglyceride content of the ethanol group compared with controls are in agreement with previous observations (12). The similar decreases in epididymal triglyceride radioactivity in the control and in the ethanol group during the development of the fatty liver, taken in conjunction with the known normal intravascular removal rate of albumin-bound palmitate-C^{14} (32), indicate that increased mobilization of FFA is not a principal factor in the pathogenesis of the acute fatty liver following ethanol ingestion (12, 30). Since sustained elevation of plasma FFA is not observed during prolonged ingestion of ethanol, Klatskin has also concluded that it is unlikely that mobilization of fatty acids from adipose tissue is the mechanism responsible for the increase in hepatic lipids seen after prolonged alcohol administration (1).

In agreement with the concept that fatty liver is not due to increased FFA mobilization, but rather to a direct effect of ethanol on the liver, the total fatty acid content of liver slices has been observed to increase during incubation with ethanol in vitro (15). This direct hepatic effect has been credited by Lieber and Schmid to enhanced synthesis (15). Reboucas and Isselbacher (11) have presented evidence that increased lipogenesis is not a factor. We agree, for if increased triglyceride synthesis from acetyl CoA occurred, the hepatic triglyceride specific activity would be significantly lower in the ethanol group because of dilution by unlabeled newly synthesized fatty acids.

Plasma triglyceride levels do not decrease in ethanol-treated rats during the development of the fatty liver as observed in this and other studies (7, 12, 14). This finding appears to exclude failure in the hepatic triglyceride secretory mechanism (16) as a cause of the liver triglyceride accumulation.

Lipid tolerance studies demonstrated pronounced hypertriglyceridemia and excessive triglyceride accumulation in the liver in ethanol-treated rats, signifying limited ability of the liver cell exposed to ethanol to metabolize triglycerides (33). Lieber and Schmid (15) and Reboucas and Isselbacher (11) observed a decreased rate of oxidation of C^{14}-labeled palmitate.

The present data demonstrate a significant accumulation of labeled palmitate, derived from a labeled adipose depot, in the hepatic triglyceride moiety of ethanol-treated rats. The increased accumulation of triglyceride in liver can be the result of either an enhanced rate of triglyceride formation from FFA (30), or a decreased hepatic triglyceride or fatty acid oxidation (7, 12, 32), or a combination of both factors. The relative importance of these mechanisms to the pathogenesis of the acute ethanol-induced fatty liver is yet to be ascertained.

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