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Effects of Ethanol Intake on Lipid Metabolism in the Lactating Rat

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TAVARES DO CARMO, M. G., C. M. OLLER DO NASCIMENTO, A. MARTÍN-HIDALGO AND E. HERRERA. *Effects of ethanol intake on lipid metabolism in the lactating rat.* ALCOHOL 13(5) 443–448, 1996.—Female rats receiving alcohol (20%) in drinking water during lactation (AL) were compared to pair-fed animals (PF) and normal controls (C) fed ad lib. All animals were killed on the 12th day of lactation. When compared to C rats, food intake decreased in both AL and PF groups, and this effect was followed by a lower body weight and mammary gland (MG), liver, and parametrial adipose tissue weights. Mammary glands triacylglyceride concentration (TG) was much lower in PF than in AL, although in the latter, values did not reach those of C, and had higher liver TG concentration than any of the other groups. Both PF and AL rats had lower plasma TG, glycerol, and free fatty acid concentrations and higher β;-hydroxybutyrate concentration than C rats. When compared to C rats, the rate of lipogenesis in MG was higher in both PF and AL rats, whereas in liver it was higher in PF and lower in AL rats, and in adipose tissue it was higher in PF and unchanged in AL rats. The appearance of ^{14}C lipids 4 h after oral [^{14}]triolein in both MG and liver was lower in AL and PF rats and only lower in adipose tissue of AL rats as compared to the C rats. Lipoprotein lipase and hormone-sensitive lipase activities were lower in MG in both PF and AL rats than in C, whereas in adipose tissue the activity of lipoprotein lipase did not differ between AL and C rats and the activity of HSL was lower in the former. These findings therefore show that in spite of reduced uptake of orally administered triglycerides due to decreased LPL activity, maternal alcohol feeding during lactation in the rat preserves the mammary gland triglyceride content thanks to enhanced lipogenetic activity. On the other hand, it causes liver triglycerides accumulation, probably as a result of the decreased rate of triglycerides released into circulation, and these changes are not caused by the reduced food intake of the animals.

Alcohol and lactation Undernutrition Mammary gland Rat Lipoprotein lipase Hormone-sensitive lipase Metabolism of oral [^{14}]triolein

DURING lactation, lipid metabolism is regulated in such a way as to direct circulating substrates (triacyglycerols and nonesterified fatty acids) to the mammary gland for milk fat synthesis (20,27). In addition to hyperphagia and enhanced absorption of dietary lipids in lactation, which results in greater availability of chylomicrons for the gland, the lipid present in milk not being synthesized within the gland comes from two other sources: 1) hepatic triacylglcerols secreted as very-low-density lipoproteins (VLDL) and 2) free fatty acids (FFA) released from adipose tissue triacylglycerols stores (15,29). The uptake of triacylglycerols (chylomicrons and VLDL) by the mammary gland is dependent on their initial hydrolysis by the enzyme lipoprotein lipase (LPL) (5), and this activity is increased in the mammary gland of lactating rats compared to nonlactating animals (10,29). In addition, during lactation in rat, maternal metabolic changes tend to favour the depletion of fat stores, as indicated by the reduction in adipose tissue LPL activity (14) and increase in adipose tissue lipolysis (3,28).

Maternal ingestion of ethanol during pregnancy and lactation results in important metabolic and physiological modifications in the lactating mother, including a decrease in milk

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production and a change in milk composition (25). We have recently reported that ethanol intake during lactation alters lipid metabolism, as indicated by an increase in the rate of lipogenesis in vivo in the mammary gland (measured with ^3H_2O) (23).

The aim of the present investigation was to examine the effects of chronic ethanol drinking on lipid metabolism during lactation in the rat and to differentiate its direct effects from those produced as a result of the reduced food intake that it causes.

METHOD

Female Wistar rats were mated with mature males of the same strain on reaching a weight of 150–180 g, and gestation was timed by the appearance of spermatozoids in vaginal smears. Pregnant rats were housed in individual cages in a light cycle- and temperature-controlled condition (0800–2000 h light/dark cycle; 24 ± 1°C), and fed Purina chow pellets and water ad lib. The day of parturition was considered day 0 of lactation and the litters were restricted to 8–10 pups per dam. On the first day postpartum lactating dams were divided into three groups: 1) alcohol-treated rats (AL), which received 20% ethanol diluted in drinking water and food ad lib until sacrifice; 2) pair-fed group (PF), as a nutritional control, which received the same amount of solid diet per day and per 100 g body weight as consumed by the alcohol-treated animals during the previous day, but divided into two equal portions and given at the onset of the dark and light period, and water ad lib; and 3) control animals (C), which received both solid diet and tap water ad lib and were handled in the same way as the ethanol-treated rats. Daily food and liquid intake and body weight were measured throughout the treatment period. All animals were studied at day 12 of lactation.

Circulating Metabolites and Tissue Lipid and Lipase Activity Determinations

Some rats from each group were killed by decapitation between 1000 and 1100 h. Trunk blood was collected in heparinized tubes for immediate plasma separation by centrifugation at 4°C, and samples of liver, parametrial adipose tissue, and mammary glands were rapidly excised and placed in liquid nitrogen and kept at −80°C until analyzed. Blood ethanol levels were determined spectrophotometrically using a commercial ethanol diagnostic Kit (Sigma, St. Louis, MO). Plasma aliquots were kept at −20°C until assayed for triglycerides (17) and free fatty acids (colorimetric Kit, Wako, NEFA C test Kit; Wako Chemicals, Germany). Other plasma aliquots were deproteinized by the Somogyi method (21) to determine glucose (6), glycerol (9), β-hydroxybutyrate (30), and acetoacetate (30) in the supernatants. Lipids were extracted and purified (8) from aliquots of mammary gland and liver for the analysis of triglycerides by enzymatic procedure after phospholipids separation (Sigma). The coefficient of variation for the above methods was always below 2%. Tissue LPL activity was measured after lipid removal with acetone/diethyl ether, as previously described (12). To measure the hormone-sensitive lipase (HSL) activity, tissue homogenates were made in 0.25 M sucrose/1 mM EDTA solution in 1 mM dithiothreitol, pH 7.4. Homogenates were centrifuged at 40,000 rpm for 45 min, at 4°C, and the clear infranatant solution was used to measure HSL as previously described (14), by using a [^{3}H]-monoacylmonoalkyl glycerol substrate (24). CV for the enzyme assays always ranged between 5% and 8%. Tissue proteins concentration was determined by the method of Lowry et al. (13).

Measurement of Lipogenesis

At 1000 h, another set of rats from each group was IP injected with 3 mCi of ^3H_2O and 1 h later the animals were killed by decapitation. Trunk blood was collected in heparinized tubes, liver and mammary glands were removed, and 1-g aliquots were saponified in 3 ml 30% KOH and lipids extracted in petroleum ether (22). The lipogenic rate was estimated as described by Robinson et al. (18).

Measurement of Tissue ^{14}C Lipid Accumulation In Vivo After Oral [^{14}C]Triolein

Another set of dams from each group was given an oral [^{14}C]triolein emulsion, prepared as described below, by means of a plastic tube connected to a syringe and without anaesthesia, as previously described (1). This treatment was carried out between 0800 and 0900 h, after which animals had access to drinking fluid but not to food. The emulsion was prepared by sonication in an Ultrasonics Ltd. sonifier (set at 15 μm, 1

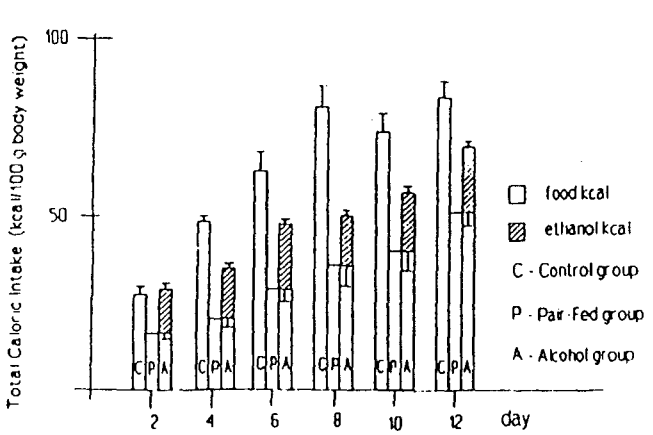

FIG. 1. Total caloric intake in the lactating rats.

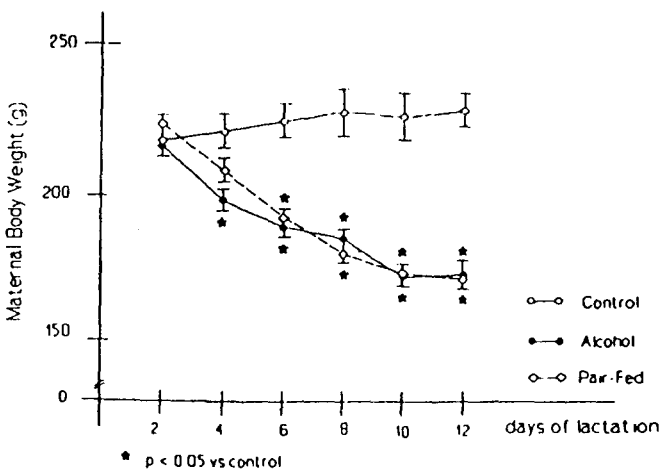

FIG. 2. Effect of maternal ethanol intake during lactation on body weight.

TABLE 1

EFFECT OF MATERNAL CHRONIC ETHANOL INTAKE ON SOME BIOCHEMICAL PARAMETERS OF THE MAMMARY GLAND, LIVER, AND PLASMA ON THE 12TH DAY OF LACTATION

	Control (C)	Pair-Fed (PF)	Ethanol (AL)
Mammary gland			
Weight (g)	10.4 ± 0.3	4.7 ± 0.2*	6.8 ± 0.7*†
Triacylglycerol (mg/g)	146 ± 5	30 ± 5*	123 ± 11*†
Liver			
Weight (g)	10.9 ± 0.2	6.3 ± 0.1*	7.2 ± 0.1*
Triacylglycerol (mg/g)	3.3 ± 0.1	1.8 ± 0.2*	5.0 ± 0.3*†
Parametrial adipose tissue			
Weight (g)	0.96 ± 0.08	0.05 ± 0.02*	0.48 ± 0.12*†
Plasma			
Glucose (mg/dl)	105 ± 6	107 ± 6	110 ± 5
Triacylglycerols (mg/dl)	92.7 ± 14.1	49.3 ± 5.1*	58.1 ± 7.0*
Glycerol (μM)	308 ± 32	173 ± 8*	188 ± 13*
Fatty acids (μM)	515 ± 76	226 ± 49*	225 ± 50*
β-OH-Butyrate (μM)	86 ± 8	281 ± 23*	283 ± 31*
Acetoacetate (μM)	46.6 ± 4.5	61.6 ± 8.8	42.6 ± 4.8

Values are the mean ± SEM for 6–12 rats per group.
*Statistical comparison between control and ethanol groups: $p < 0.05$.
†Significantly different from pair-fed at $p < 0.05$.

min × 5), and consisted of a mixture of 4 mg phosphatidylcholine, 20 mg triolein, 100 μCi glycerol tri[1-^{14}]oleate (59 Ci/mol; Radiochemical Center, Amersham, UK), and 5 ml saline (NaCl 0.9% w/v), of which each animal received 1 ml. Animals were killed by decapitation 4 h after the treatment and samples (about 200–300 mg) of parametrial adipose tissue, liver, and mammary glands were excised and used for lipid extraction (8). To allow intergroup comparisons and to unify the amount of radioactivity given to each animal, values of radioactivity were corrected by considering one million of DPM in the [^{14}]triolein emulsion administered to each rat and per 100 g body weight.

Statistical Analysis

Results were expressed as the mean ± SEM, and statistical comparison among groups was made by a two-way analysis of variance (ANOVA). Differences between means were tested for significance by Duncan's multiple range test.

RESULTS

Food Intake, Body, and Organ Weights and Triglyceride Concentration and Circulating Metabolites

As shown in Fig. 1, during the 12 days of the experiment, daily intake of food-derived calories intensely decreased in the AL rats compared to C rats. In the AL group the ethanol-derived calories were 35.4% of the total daily caloric intake, which is equivalent to about 11.06 ± 1.15 g of pure ethanol ingested/kg body weight/day. The food-derived calories ingested by the AL and PF animals were approximately 52% less than the ad lib controls. This was reflected in the significant reduction in body weight of AL and PF animals, whose value was, however, similar in these two groups (Fig. 2). The mean maternal blood alcohol levels in the AL rats on the 12th day of lactation was 90 ± 3.8 mg/dl.

As shown in Table 1, mammary gland weight and triglyceride concentration in AL rats were lower than in C animals but higher than in PF rats. Liver weight was similar in AL and PF

TABLE 2

EFFECT OF ETHANOL INGESTION (20%) DURING LACTATION ON TISSUE LIPOGENESIS

	Control (C)	Pair-Fed (PF)	Ethanol (AL)
Mammary glands	51.2 ± 4.8 (13)	115.0 ± 11.7* (6)	75.6 ± 9.7*† (9)
Liver	10.0 ± 0.7 (13)	25.3 ± 5.8* (6)	7.1 ± 0.8† (9)
Parametrial adipose tissue	0.6 ± 0.05 (13)	4.4 ± 1.3* (6)	0.7 ± 0.12† (9)

For experimental details see the text. The results are the mean ± SEM, with the number of rats shown in parentheses. Values are expressed as μmol ^3H_2O/g fresh tissue/hour.
*$p < 0.05$ versus control group.
†$p < 0.05$ versus pair-fed group.

TABLE 3

EFFECT OF ETHANOL INGESTION (20%) DURING LACTATION ON TISSUE ACCUMULATION OF ^{14}C LIPIDS AFTER [^{14}C]TRIOLEIN INTRAGASTRIC ADMINISTRATION

	Control (C)	Pair-Fed (PF)	Ethanol (AL)
Mammary glands	327233 ± 29609 (6)	146881 ± 22521* (8)	63418 ± 14679*† (6)
Liver	25901 ± 3742 (6)	14629 ± 2904* (8)	11898 ± 1670* (6)
Parametrial adipose tissue	157 ± 31 (6)	120 ± 32 (8)	46 ± 11* (6)

For experimental details see the text. The results are the means ± SEM, with the number of rats shown in parentheses. Values are expressed as dpm/total tissue and corrected as 1×10^6/dpm administered/100 g body weight.

*$p < 0.05$ versus control group.

†$p < 0.05$ versus pair-fed group.

animals but lower than in C rats, whereas triglyceride concentration was higher in rats receiving the ethanol treatment than in any of the other two groups and this parameter was lower in PF rats than in C animals. The weight of parametrial adipose tissue was lower in AL than in C, and values in the PF were practically negligible, being much lower than in any of the other two groups (Table 1).

As also shown in Table 1, plasma glucose and acetoacetate levels did not differ among the three groups. However, plasma triglycerides, glycerol, and free fatty acid concentration were lower in both AL and PF rats than in C animals, whereas plasma β-hydroxybutyrate levels were higher in the former two groups with no difference between them in any of these parameters (Table 1).

Tissue Lipogenesis

As shown in Table 2, the rate of lipogenesis in the mammary gland of AL rats appeared higher than in C rats but lower than in PF animals. In both liver and adipose tissue, the rate of lipogenesis did not differ between AL and C rats but was higher in PF rats than in any of the other two groups (Table 2).

Tissue Appearance of ^{14}C Lipid Accumulation in Tissues

The appearance of ^{14}C lipids 4 h after the oral administration of [^{14}C]trioleine is shown in Table 3. In mammary glands of AL rats the appearance of ^{14}C lipids was lower than in any of the other two groups, and the value in PF animals was also significantly lower than in the C rats. The ^{14}C lipids that appeared in the liver were also lower in the AL and PF animals than in C, with no difference between the two former groups. The amount of ^{14}C lipids in parametrial adipose tissue was much lower than the one found in the other tissues studied, and it was lower in the AL rats than in any of the other two groups, whose values were similar (Table 3).

LPL and HSL Activities

As shown in Table 4, both mammary gland LPL and HSL activities were lower in AL and PF rats than in C animals, whereas no difference could be found between the values in the two former groups. In parametrial adipose tissue of PF rats there was no possibility of determining the enzyme activities due to the insufficient amount of sample available. As shown in Table 4, LPL activity did not differ in adipose tissue

TABLE 4

EFFECTS OF ETHANOL INTAKE ON LIPOPROTEIN LIPASE (LPL) AND HORMONE-SENSITIVE LIPASE (HSL) ACTIVITY IN MAMMARY GLAND AND ADIPOSE TISSUE IN LACTATING RATS

	Control (C)	Pair-Fed (PF)	Ethanol (AL)
Mammary glands			
LPL	6191 ± 229 (6)	1919 ± 199* (8)	2498 ± 348* (6)
HSL	1661 ± 112 (6)	305 ± 21* (6)	583 ± 77*† (6)
Adipose tissue			
LPL	4.7 ± 0.5 (7)	—	4.9 ± 0.5 (6)
HSL	137 ± 10 (7)	—	86 ± 18* (4)

For experimental details see the text. The results are the mean ± SEM, with the number of rats shown in parentheses. Values are expressed as μmol of fatty acid released/total tissue protein.

*Values that are significantly different from those for control rats are shown by $p < 0.05$ versus control group.

between AL and C rats. Nevertheless, HSL activity appeared significantly lower in the former group.

DISCUSSION

The present findings show that ethanol ingestion during lactation in the rat reduces food intake and greatly affects lipid metabolism when compared to animals fed ad lib. Although some of the effects of ethanol intake may be a consequence of its intrinsic metabolism (i.e., increased intracellular redox potential and its metabolic consequences within the liver and enhanced utilization of acetaldehyde and acetate by the liver or extrahepatic tissues), other effects may be a consequence of reduced food intake and even impaired intestinal absorption of nutrients (11). Therefore, in this study rats receiving ethanol were also compared to pair-fed rats, whose body weight declined at the same rate as the ethanol-treated rats. Actually, some of the metabolic differences between the groups disappared when ethanol-treated groups were compared to these pair-fed controls, as was the case for the reduced liver and mammary gland weights, increased plasma β-hydroxybutyrate levels, and decreased free fatty acids found in the ethanol-treated rats. These findings are in agreement with previous ones (25,26) when using lactating rats receiving ethanol for more time than the 12 days used here. In these studies quoted, no pair-fed animals were followed, but the fact that these metabolic changes were also found here in pair-fed rats indicates that they are a consequence of the reduced food intake seen in the ethanol-treated rats. However, in contrast to the intense decline in the triglyceride concentration in both mammary glands and liver found in the pair-fed rats, our results show that ethanol intake causes an accumulation of triglycerides in the liver and avoids the decline of triglycerides seen in the mammary gland in the pair-fed rats. The findings in liver triglycerides are consistent with the known tendencies to develop a fatty liver after prolonged treatment with alcohol (26). Because present results show that our ethanol-treated rats do not have an enhanced liver lipogenesis or uptake of orally administered triglycerides when compared to the pair-fed rats, such accumulation of triglycerides in liver could be a consequence of a reduced secretion of hepatic lipoproteins (2). The maintanance of mammary gland triglyceride concentration in the ethanol rats at a level close to the one found in control rats fed ad lib seems, however, to be the result of the higher lipogenic activity seen in the former. This effect could compensate for the lower uptake of circulating triglycerides present in the ethanol rats. These findings in the ethanol rats contrast with the highly decreased triglyceride content in both liver and mammary glands seen in the pair-fed rats, in spite of their enhanced lipogenic activity, which seems to be unable to compensate for their decreased uptake of orally administered triglycerides and low LPL activity. The enhanced lipogenetic activity seen in the pair-fed rats is probably caused by an overshoot effect such as that seen under conditions of starvation or restricted feeding and refeeding (4,7,19).

The activity of HSL was also lower in ethanol-treated rats than in control rats fed ad lib, and this effect may well be a consequence of the reduced food intake caused by the ethanol intake, because it was also seen in the pair-fed animals. This is the first time that the presence of HSL is described in the mammary gland, and no indication therefore exists of its functional role. We may, however, speculate that the role of this enzyme in the mammary gland differs from that present in adipose tissue, where it is known to control the breakdown of intracellular triglycerides and the subsequent release of free fatty acids into circulation. Due to the specific HSL action on triglyceride molecules, which depends on their esterified fatty acid structure (16), this enzyme in the mammary gland may control the intracellular restructuration of available triglycerides and therefore affect milk composition. Further studies would be necessary to demonstrate this hypothesis. In any event, we know that milk production is greatly reduced in ethanol-treated lactating rats compared to control rats fed ad lib (25), and the decreases in both LPL and HSL activities found here in the mammary gland of the ethanol-treated rats could contribute to such limited milk production.

The reduced uptake of orally given labelled triglycerides found here in the adipose tissue of the ethanol-treated rats contrasts with their unchanged LPL activity in this tissue, but the slower blood flow in this tissue and the rapid use of circulating triglycerides by other tissues, including the mammary gland and liver, make the possibilities of these tracers reaching the capillaries of this tissue very limited. This hypothesis is supported by the low amount of radioactivity found in this tissue after the oral administration of labelled triglycerides, as compared to those found in the mammary gland and liver. The activity of HSL also appears lower in the ethanol-treated rats than in the control rats fed ad lib, and this change is in accordance with the decreased circulating levels of both FFA and glycerol seen in these animals, which would indicate a decreased lipolytic activity in adipose tissue. This effect would also contribute to the reduced level of plasma triglycerides seen in the ethanol-treated rats, decreasing the availability of circulating triglycerides for their uptake by the mammary gland.

The present results show that ethanol intake during lactation in rats causes major alterations in lipid metabolism in the rat, some of which are an indirect consequence of the reduced food intake of the animals.

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