Comparative Metabolic Effects of Chronic Ethanol Intake and Undernutrition in Pregnant Rats and Their Fetuses

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Female rats receiving ethanol in the drinking water before and during gestation (ET) were compared to pair-fed animals (PF) and normal controls (C) fed ad libitum. On the 21st day of gestation the maternal body and liver weight, blood glucose, and plasma protein concentrations were lower in ET and PF animals as compared to C. In contrast to C or PF mothers, ET-fed mothers had higher circulating β-hydroxybutyrate and triacylglyceride levels and β-hydroxy-butyrate/acetoacetate ratio. Liver triacylglycerides were increased whereas liver glycogen concentration was reduced in ET-fed animals. Only fetal body and liver weights and blood glucose were lower in both ET and PF than in C. Blood β-hydroxybutyrate was increased and liver glycogen was decreased only in ET fetuses. There were no differences among the groups in fetal circulating β-hydroxy-butyrate/acetoacetate ratio, plasma proteins, and triacylglycerides or liver triacylglyceride content. Results indicate that certain changes in ET mothers are specifically produced by the ethanol intake rather than undernutrition. Further, metabolic changes occurring in the fetus are influenced by the ethanol effects in the mother and these actions may be added to those directly produced by the ethanol crossing the placenta.

MATERNAL ALCOHOL INGESTION during pregnancy can result in deleterious effects on the offspring's development. In humans it can cause a number of behavioral and physical alterations that have been named the "fetal alcohol syndrome."[1-3] Some of these alterations have been reproduced in experimental animals, particularly the rat,[4-7] but it is not yet well established which of these effects are a direct consequence of the metabolism of ethanol or an indirect consequence of concomitant undernutrition.[8] Undernutrition is usually present in animals given ethanol because of a decrease in food intake, altered food digestion and absorption, and impaired metabolism of absorbed nutrients.[9] Fetuses of mothers given ethanol have also been reported to be undernourished. This has been attributed to a decrease of both placental blood flow[10] or placental aminoacid transfer.[11] It is hypothesized that some of the negative effects

produced by ethanol intake in adults are caused indirectly by products of its metabolism, such as NADH and acetaldehyde.[12] Although ethanol crosses the placenta freely,[13] fetal capacity to metabolize ethanol is negligible both in humans[14] and rats.[15, 16] After an oral single dose of ethanol, blood acetaldehyde levels are higher in late pregnant than in virgin rats, however fetal acetaldehyde concentrations are 4-fold lower than in the mother[17] and furthermore lower than the levels reported as teratogenic "in vitro."[18] Acetaldehyde can cross the placental barrier[19] and, in consequence, the difference between maternal and fetal concentrations might be due to the known capacity of placental tissues to metabolize it[20] or to other factors. Therefore, adverse effects of maternal alcohol ingestion at late gestation may be the consequence of changes in maternal metabolism rather than due to a direct action on the fetus by ethanol. As neither this latter aspect nor the role of maternal malnutrition during alcohol feeding on fetal development have been clearly established, we used our recently reported rat model for the fetal alcohol syndrome[7] to study these two aspects.

MATERIALS AND METHODS

Female Wistar rats from our own colony weighing 150 ± 10 g at the onset of the experiment were maintained under automatically controlled temperature (22 ± 2°C) and 12-hr light-dark cycles (9.00–21.00 hr). Groups of three animals were kept in plastic wire-topped cages with wire units over the floor to avoid coprophagia. They were given ad libitum a Purina-chow diet for rat (UAR-Panlab, Barcelona, Spain) with a caloric value of 3.200 cal/kg. Animals were divided into three groups: (a) Ethanol-treated rats (ET) that were given a 10% ethanol (w/v) solution as drinking fluid for 1 week, 15% ethanol during the second, 20% ethanol during the third, and 25% ethanol during the fourth week. At the end of the fourth week, the rats were mated with nontreated males and maintained on the 25% ethanol solution until sacrifice. Day 0 of gestation was established by the appearance of spermatozoids in vaginal smears; (b) Control rats (C) receiving no treatment were handled in the same way as the alcohol treated ones; (c) Pair-fed rats (PF) given 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 and "ad libitum" water. To avoid immediate consumption of the diet, it was given intermittently during the dark periods by means of a mechanical automated device. Animals of all groups were killed by decapitation between 9:00 and 10:00 a.m. on the 21st day of gestation and blood was collected from the neck wound into ice-chilled heparinized receptacles. A piece of liver was immediately excised and frozen in liquid N_2. Fetuses were removed for decapitation and blood and liver collected. Aliquots of maternal and fetal blood were deproteinized[21] for glucose,[22] β-hydroxybutyrate, and acetoacetate[23] determinations. Another aliquote was used for "head space" gas chromatography ethanol determination as previously described,[24] using a Sigma-3 Perkin-Elmer Gas Chromatograph equipped

with a headspace injection device and a Sigma 15 integrator. The remaining blood was centrifuged for plasma collection in which both triacylglycerides[25] and protein[26] were determined. Liver glycogen was assayed by ethanol extraction, alkali digestion,[27] hydrolysis with 5 N H_2SO_4 at 100°C for 24 hr, then it was neutralized and the glucose was measured.[22] Lipids were extracted[28] from another aliquot of frozen liver and phospholipids were removed[29] prior to the analysis of triacylglycerides.[25] Results were expressed as means ± SEM of 9–12 individual data. Individual values for each parameter are the mean values of all fetuses of each litter. Statistical analysis was done with one-way ANOVA and further comparison between the groups by the Student's t test.

RESULTS

As shown in Table 1, during the last week of the experiment, daily intake of food-derived calories were intensely decreased in the ET rats as compared to C. In the ET group the ethanol-derived calories were 35% of the total daily caloric intake. This total was 960 kjoules/kg body wt/day which does not differ from the value of the C group (Table 1) ($p > 0.05$), and therefore both groups were isocalorics. Blood ethanol levels in the ET group were 128 ± 23 mg/100 ml in the mothers and 95 ± 18 mg/100 ml in the fetus.

When compared to the C animals, maternal body and liver weights and fetal body and liver weights were significantly reduced in both ET and PF rats (Table 1). Maternal body weight was also significantly reduced in PF versus ET animals whereas no differences were found between those two groups in the other morphometric parameters studied (Table 1).

Circulating metabolites and liver glycogen and triacylglycerol concentrations in the mothers and fetuses are shown in Table 2. It is seen that the blood glucose concentration was similarly reduced in ET and PF mothers and fetuses as compared to their respective C groups with no differences between the ET and PF animals (Table 2). Maternal blood concentration of β-hydroxybutyrate was significantly greater in ET than in the C and PF rats whereas fetal β-hydroxybutyrate was only significantly augmented in ET when compared to the C group (Table 2). Similar intergroup changes were found with the blood acetoacetate concentration although the difference was only significant between ET and PF mothers. Blood β-hydroxybutyrate/acetoacetate ratio was intensely augmented in the ET mothers as compared to either C or PF animals whereas in the fetuses no difference was found

among the three groups (Table 2). Plasma protein concentrations were reduced in ET and PF mothers versus C, values in the PF mothers being also significantly lower than in ET, whereas plasma triacylglyceride levels were higher in ET mothers as compared to C and lower in PF mothers as compared to either C or ET animals (Table 2). Neither plasma proteins nor triacylglyceride concentrations in fetuses differed between any of the groups studied (Table 2). In the liver, maternal glycogen concentration was reduced in ET versus C whereas it was significantly augmented in PF versus any of the other two groups, and in fetuses it was slightly reduced in ET as compared to either C or PF animals (Table 2). Liver triacylglyceride concentration was higher in ET mothers than in C or PF animals, whereas in fetal liver there was no difference in this parameter between the groups (Table 2).

DISCUSSION

In our previously reported rat model for the study of fetal alcohol syndrome,[7] the percentage of ethanol-derived calories (35%) and the maternal and fetal blood ethanol levels were similar to those reported when alcohol was given in a liquid diet.[30–33]

By using this model, present findings show that chronic maternal alcohol intake modifies several maternal metabolic parameters in a different manner than undernourishment. The fetus passively follows maternal circulating glucose changes but neither other parameters known not to cross the placental barrier (triacylglycerides and proteins) nor the blood β-hydroxybutyrate/acetoacetate ratio were changed, indicating that there is not a specific fetal metabolic response to the ethanol crossing the placenta.

Both conditions, ethanol administration (ET group) and underfeeding (PF group), reduced fetal body and liver weights confirming previous findings[7, 10, 30, 32] but maternal metabolic changes appeared substantially different, indicating that although the two factors (ethanol intake and undernourishment) may be superimposed in the ET animals, some of the observed changes in the mothers are specifically produced by ethanol. Among these are the observed reduced glycogen and augmented liver triacylglyceride concentration and augmented circulating β-hydroxybutyrate/acetoacetate ratio and concentration of triacylglycerides in the ethanol-treated mothers. None of

Table 1. Effect of Ethanol Ingestion during Gestation on the Pregnant Rat and Its Fetuses

	Food-derived calories (kj/kg, day)	Ethanol-derived calories (kj/kg, day)	Maternal body wt (g)	Maternal liver wt (g)	Fetus body wt (g)	Fetus liver wt (g)
Control	1008 ± 25		357 ± 13	12.5 ± 0.6	5.6 ± 0.1	0.35 ± 0.02
Ethanol	607 ± 21	347 ± 8	261 ± 11	8.0 ± 0.3	4.5 ± 0.1	0.25 ± 0.02
p vs. C	<0.001		<0.001	<0.001	<0.001	<0.001
Pair fed	607		239 ± 6	7.8 ± 0.4	4.9 ± 0.1	0.28 ± 0.002
p vs. C			<0.001	<0.001	<0.001	<0.05
p vs. ET			<0.05	NS*	NS	NS

Pregnant rats were fed ethanol and control diets as described in the text. Values, means ± SEM of data from 9–12 pregnant rats in each group.
* NS, not significant.

Table 2. Blood Glucose and Ketone Bodies, Plasma Proteins and Triacylglycerides, and Liver Glycogen and Triacylglycerides in Mothers and Fetuses at 21 Days of Gestation

	Blood glucose (mg/dl)	Blood β-OH-B (μM)	Blood AcAc (μM)	Blood β-OH-B/AcAc ratio	Plasma proteins (mg/dl)	Plasma TAG (mM)	Liver glycogen (%)	Liver TAG (mmol/g)
Mothers								
Control	105 ± 5	26 ± 11	3.5 ± 1.1	6 ± 1	5.6 ± 0.2	2.9 ± 0.5	3.8 ± 0.4	4.3 ± 0.3
Ethanol	58 ± 6	254 ± 37	5.4 ± 0.4	56 ± 15	4.4 ± 0.3	4.4 ± 0.5	1.9 ± 0.2	8.3 ± 0.5
p vs. C	$p < 0.001$	$p < 0.01$	NS	$p < 0.01$	$p < 0.001$	$p < 0.05$	$p < 0.01$	$p < 0.001$
Pair-fed	60 ± 5	52 ± 18	2.4 ± 0.3	16 ± 4	3.7 ± 0.2	1.9 ± 0.3	6.1 ± 0.4	4.9 ± 0.8
p vs. C	$p < 0.001$	NS	NS	$p < 0.05$	$p < 0.001$	$p < 0.05$	$p < 0.01$	NS
p vs. ET	NS	$p < 0.01$	$p < 0.001$	$p < 0.05$	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.05$
Fetuses								
Control	59 ± 4	69 ± 12	2.9 ± 1.1	34 ± 19	1.9 ± 0.1	0.6 ± 0.1	6.4 ± 0.6	1.6 ± 0.2
Ethanol	32 ± 2	130 ± 18	4.9 ± 1.4	36 ± 15	1.7 ± 0.1	0.6 ± 0.1	4.8 ± 0.3	2.6 ± 0.7
p vs. C	$p < 0.001$	$p < 0.05$	NS	NS	NS	NS	NS	NS
Pair-fed	33 ± 4	91 ± 6	3.8 ± 1.8	16 ± 4	1.8 ± 0.1	0.5 ± 0.1	6.8 ± 0.2	1.8 ± 0.2
p vs. C	$p < 0.001$	NS	NS	NS	NS	NS	NS	NS
p vs. ET	NS	NS	NS	NS	NS	NS	$p < 0.01$	NS

Details of the protocol are given in the text. Values, means ± SEM of data from 9–12 pregnant rats in each group. β-OH-B, β-hydroxybutyrate; AcAc, acetoacetate; TAG, triacylglycerides; NS, not significant.

these alterations were found in the PF rats and all of them are known to be produced as a result of acute or chronic ethanol intake in pregnant or nonpregnant animals.[32,34–38] Enhanced glycogen liver concentration found in the PF mothers agrees with other reports showing a similar effect in animals subjected to different degrees of undernourishment,[39,40] but the mechanism through which this effect is produced is not known.

Observed metabolic changes in fetuses from ET mothers are seen to be an indirect consequence of maternal events rather than a response to metabolic effects of the ethanol crossing the placenta. Decreased fetal glycemia and enhanced circulating levels of β-hydroxybutyrate parallel the changes occurring in the ET mothers, and although through different mechanisms it is known that these two metabolites cross the placenta proportionally to maternal concentrations.[41] In fact, there was a significant relationship (regression coefficient, 0.83; significant with $p < 0.001$) between maternal and fetal glucose levels from the three experimental groups.

One specific consequence of ethanol metabolism in adults is the switch to the reducing side of the redox state causing an increase in circulating β-hydroxybutyrate/acetoacetate ratio, an effect which was clearly seen here in the ET mothers but not in their fetuses. Similarly, ethanol caused hypertriglyceridemia and liver triacylglyceride accumulation in ET mothers as it normally occurs after the ingestion of ethanol.[37,38] Triacylglycerides are known not to cross the placental barrier[41] and their unaltered concentrations observed in fetuses from ET mothers again indicate a lack of response to ethanol in the former.

The slightly lower glycogen liver levels observed in fetuses from ET mothers than in fetuses of the C group, agree with findings of other authors[32,34] and may result from the hypoglycemia of the fetuses as it is also known to occur in starvation.[42] Unchanged liver glycogen con-

centration in fetuses from PF mothers in spite of their hypoglycemia, would not agree with such a hypothesis, but this is similar to what occurs in their respective mothers. We do not know the metabolic adaptations which take place in this condition allowing for the preservation of carbohydrate stores in these animals, as commented above.

Present findings allow the conclusion that most of the observed changes in the ET mothers are specifically produced by the ethanol intake rather than being consequences of undernutrition. Furthermore, they suggest that certain metabolic changes that occur in fetuses from ET mothers are mainly the result of the quantitative and/or qualitative modification of metabolites crossing the placenta, rather than to a direct metabolic response to ethanol or its oxidation, which fits with the lower activity of ethanol oxidizing enzymes in fetal structures even at late gestation.[15,19]

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