Metabolic Response to Starvation at Late Gestation in Chronically Ethanol-Treated and Pair-Fed Undernourished Rats

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To study the role of undernourishment in the negative effects of ethanol during pregnancy and to determine whether maternal ethanol intake modifies metabolic response to starvation at late gestation, female rats receiving ethanol in their drinking water before and during pregnancy (ethanol group) were compared with animals that received the same amount of solid diet as the ethanol group rats (pair-fed group) and with normal rats fed ad libitum (control group). All animals were killed on the 21st day of gestation, either in the fed state or after 24-hours fasting. The body weight of ethanol rats was lower than that of controls but higher than that of pair-fed rats. When compared with controls, ethanol and pair-fed rats had reduced fetal body weights, whereas fetal body length was reduced only in the former. In the fed state, blood glucose concentration was lower in the ethanol and pair-fed rats and fetuses than in controls. Twenty-four-hour starvation caused a reduction in this parameter only in control and ethanol mothers. In the fed state, maternal liver glycogen concentration was lower in ethanol and higher in pair-fed mothers than in controls. Blood β-hydroxybutyrate levels were higher in ethanol-treated mothers than in the others, and 24-hour starvation increased this parameter in ethanol and control rats to a greater extent than in the pair-fed ones. Liver triacylglyceride concentration was higher in ethanol-treated mothers than in the other two groups, and starvation caused this concentration to increase in ethanol and control groups but not in the pair-fed group. Maternal liver lipoprotein lipase activity did not differ among the groups when fed, but increased significantly with starvation in controls and ethanol-treated rats. Individual values of lipoprotein lipase activity in all the starved groups correlated with liver triacylglyceride concentrations. Results indicate that maternal undernourishment in ethanol-treated rats contributes to the negative effects of ethanol on fetal development in addition to certain other changes, such as the increase in maternal redox state and in circulating and liver triglycerides, directly related to ethanol oxidation. The intense maternal hypoglycemia observed in rats from the ethanol group could impair glucose availability to the fetus, and this situation would worsen with starvation. The higher hepatic glycogen levels in pair-fed mothers and their fetuses in the fed state as compared with the ethanol group, in spite of their similar hypoglycemia, seems to indicate a different metabolic adaptation in the two groups. A relationship is proposed in the starved condition between maternal liver lipoprotein lipase activity and changes in liver triacylglyceride and circulating ketone bodies in the three groups studied. The findings seem to indicate that, in spite of the metabolic disturbances produced by ethanol intake in the mother, the response to starvation in ethanol rats was similar to the one observed in controls, and different from the one in the pair-fed animals.

IT IS WELL KNOWN that maternal alcohol ingestion during pregnancy causes negative alterations in offspring development both in humans[1,2] and experimental animals.[3] The alterations are called *Fetal Alcohol Syndrome* (FAS) and range from behavioral abnormalities to physical malformations and death.[1,2,4] The mechanisms by which these disturbing effects are accomplished are not yet known. The calories provided by ethanol oxidation are called empty calories, and it has been proposed that malnourishment associated with ethanol ingestion may play an important role in the presence of FAS.[5] The deletereous effect of maternal undernutrition upon fetal development could be a direct consequence of reduced food intake, which is due to altered intestinal digestion and absorption by the mother,[6] and/or to impaired placental transport.[6,7] Excess of redox potential occurring after ethanol consumption, due to its oxidation, is responsible for several of the alterations reported in the lipid and carbohydrate metabolism in pregnant and nonpregnant animals.[8,9] It is, however, not known whether these maternal changes contribute to the development of FAS or not. Although ethanol crosses the placenta freely,[10] fetal capacity to metabolize it is very low both in humans and rats,[11,12] indicating that an important endogenous generation of redox potential does not seem to take place. Using an experimental model based on chronic ethanol adminstration in the drinking fluid of pregnant rats previously published by us[13] we reported morphological,[13-15] behavioral,[15] neuronal,[16,17] and metabolic alterations[14,18] in mothers and offspring. Following the same experimental model,[13] we study here the metabolic response to 24-hour starvation in chronically ethanol-treated pregnant rats and their fetuses. In addition, we have used another group that was pair fed to the solid intake of the ethanol-treated rats in order to test if the response to starvation in these rats was influenced by the malnutrition concomitant to ethanol administration or if it differed from that observed in chronically undernourished animals. Besides its specific information in regard to a better understanding of FAS physiopathology, the subject can give a more general insight into the differences in metabolic response to acute ethanol intake between fed and fasted subjects. In fed virgin rats, acute ethanol administration produces hyperglycemia,[19] whereas in starvation it causes hypoglycemia[20]; we recently reported that pregnancy in the rat does not modify the

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direction of these changes.[21] Far less is known about the metabolic situation of rats treated chronically with ethanol, especially in the pregnant state. In this paper, we have attempted to contribute to improved knowledge of metabolic derangements induced by chronic ethanol intake during pregnancy.

MATERIALS AND METHODS

Animals

Adult female Wistar rats from our own colony were maintained under automatically controlled temperature (23 ± 1°C) and 12-hour light-dark cycles. Groups of three animals were kept in plastic wire-topped cages and given a Purina chow rat diet ad libitum (UAR-Panlab, Barcelona, Spain).

Animals were divided into three groups: (1) Ethanol group, rats given 10% ethanol (wt/vol) in 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 one nontreated male was put into each cage during the 12-hour dark periods until spermatozoids appeared in vaginal smears of the females (day 0 of gestation). Rats that had not mated after five days were removed from the experiment. Pregnant rats were maintained on 25% ethanol in the drinking fluid until death. (2) Pair-fed group, rats given the same amount of solid diet per day and per 100 g body weight as was consumed by the alcohol-treated animals during the previous day and free access to water without ethanol as drinking fluid. To avoid immediate consumption of the diet, it was given intermittently during the dark periods by means of an automatic mechanical device. (3) Control group, rats receiving no treatment and with free access to food and water.

With this experimental design, the caloric intake of the control group during pregnancy did not differ from that of the ethanol group, who derived more than 35% of their total calories from the ethanol ingested.[13]

Mothers and their fetuses were decapitated on the 21st day of gestation, either in the fed condition or after 24-hours food deprivation. Blood was collected from the neck into heparinized containers, and aliquots were directly used for deproteinization. Plasma was separated from another blood aliquot and kept at −70°C until processing. Livers were excised immediately after death and placed into liquid N_2.

Circulating Metabolites

Whole blood was deproteinized with $Ba(OH)_2$-$ZnSO_4$,[22] and supernatants were analyzed for glucose,[23] acetoacetate and β-hydroxybutyrate[24] using enzymatic methods. Aliquots of plasma were used for the determination of triacylglycerides (TAG)[25] and free fatty acids (FFA).[26]

Liver Metabolites

Liver glycogen was purified with ethanol after alkali digestion[27] and hydrolyzed with 5 N H_2SO_4 at 100°C for two hours, after which it was neutralized for glucose evaluation.[22] Lipids were extracted[28] from an aliquot of frozen liver, and after phospholipid separation with activated silicic acid treatment as previously described,[29] neutral glycerides were determined as for plasma.[25] Lipoprotein lipase (LPL) activity was measured in acetone/ether-dried powders of

Table 1. Effect of Maternal Chronic Ethanol Intake and 24-Hour Starvation on Body and Liver Weight, Blood Glucose, and Liver Glycogen Concentrations in the 21-Day Pregnant Rat

	Body Weight (g)	Conceptus-free Body Weight (g)	Body Length (cm)	Liver Weight (g)	Blood Glucose (mg/dL)	Liver Glycogen (%)
Mothers						
Controls						
Fed	356 ± 13	273 ± 11	—	12.5 ± 0.7	105.4 ± 5.2	3.84 ± 0.40
24-h starved	320 ± 18	243 ± 12	—	8.5 ± 0.4	65.5 ± 1.7	0.61 ± 0.02
P	NS	NS		<.001	<.001	<.001
Ethanol						
Fed	261 ± 11*	210 ± 12†	—	8.0 ± 0.2*	58.3 ± 6.1*	1.95 ± 0.22†
24-h starved	258 ± 9*	205 ± 9‡	—	7.3 ± 0.4	44.3 ± 1.7*	0.31 ± 0.01
P	NS	NS		NS	<.05	<.001
Pair-fed						
Fed	239 ± 6§	182 ± 11*	—	8.0 ± 0.3*	60.1 ± 4.5*	6.15 ± 0.37†¶
24-h starved	221 ± 17†§	170 ± 10*§	—	6.5 ± 0.8‡	74.4 ± 2.3¶	0.15 ± 0.01
P	NS	NS		NS	NS	<.001
Fetuses						
Controls						
Fed	5.61 ± 0.12	—	4.89 ± 0.06	0.35 ± 0.02	59.5 ± 3.5	6.37 ± 0.62
24-h starved	5.02 ± 0.07	—	4.91 ± 0.07	0.25 ± 0.01	52.3 ± 3.2	3.13 ± 0.17
P	<.01		NS	<.01	NS	<.01
Ethanol						
Fed	4.54 ± 0.12*	—	4.59 ± 0.05†	0.25 ± 0.01*	32.2 ± 1.8*	4.82 ± 0.26
24-h starved	4.35 ± 0.08*	—	4.51 ± 0.05*	0.23 ± 0.01	38.2 ± 2.1†	1.75 ± 0.25‡
P	NS		NS	NS	NS	<.001
Pair-fed						
Fed	4.85 ± 0.13*	—	4.73 ± 0.04	0.28 ± 0.02‡	32.9 ± 3.7*	6.77 ± 0.25‖
24-h starved	4.22 ± 0.23†	—	4.69 ± 0.08	0.25 ± 0.01	43.7 ± 5.2	3.62 ± 1.18
P	<.05		NS	NS	NS	<.05

Values represent the mean ± SEM of five to seven rats per group. Ethanol-treated rats received progressively increasing amounts of ethanol in the drinking water, attaining a 25% (wt/vol) concentration 1 week prior to being mated, and pair-fed were given the same daily amount of diet per 100 g body weight as that consumed by the ethanol-treated rats. Statistical differences between starved and fed rats are shown by the *P* values, whereas those between each group and the controls are shwon by *, †, ‡, and those between pair-fed and ethanol rats by §, ‖, ¶.

*$P < .001$.
†$P < .01$.
‡$P < .05$.
§$P < .05$.
‖$P < .01$.
¶$P < .001$.

another liver aliquot.[30] Previously, we demonstrated that, with this experimental procedure, the lipase activity measured actually corresponds to LPL, but not to hepatic lipase.[31]

Statistics

Values are expressed as means ± SEM. Statistical comparison between the groups was done by the Student's *t* test.

RESULTS

As shown in Table 1, on the 21st day of gestation body weight of ethanol-treated rats was lower than that of controls but higher than that of pair-fed rats. These differences between groups were not modified when animals were studied after 24-hour fasting (Table 1), and they partially corresponded to the conceptus-free maternal weight. This latter parameter was significantly lower in ethanol-treated rats than in controls and slightly, but not significantly, higher than in pair-fed animals either fed or fasted (Table 1). Fetal body weight and length also were reduced in fed ethanol-treated rats as compared with controls, whereas in pair-fed animals fetal body weight, but not length, was significantly lower than in controls (Table 1). Maternal starvation reduced fetal body weight in controls and pair-fed animals but not in the ethanol-treated group, whereas it did not affect fetal length in any of the groups studied (Table 1). Maternal and fetal liver weights were reduced both in ethanol and pair-fed groups as compared with the control group; starvation reduced the weight in the three groups, but differences were significant only in the control group. Blood glucose concentration in fed animals was lower in ethanol-treated and pair-fed mothers and fetuses than in their respective controls, with no differences between the first two groups (Table 1). Starvation caused a significant reduction in blood glucose concentration in both ethanol and control mothers, and the preexisting differences from the fed state remained (Table 1). Blood glucose levels, however, were not modified by starvation in pair-fed mothers, nor in fetuses from either of the three groups studied (Table 1). Liver glycogen concentration in fed mothers was lower in ethanol than in control rats, whereas it was higher in pair-fed animals, and starvation caused a marked reduction in this parameter in all the groups studied, making the differences disappear (Table 1).

Table 2. Effect of Maternal Chronic Ethanol Intake and 24-Hour Starvation on Circulating Lipidic Components in the 21-Day Pregnant Rat

	Plasma Triacylglyceride (mmol/L)	Plasma FFA (μmol/L)	Blood β-Hydroxybutyrate (μmol/L)	Blood Acetoacetate (μmol/L)	β-Hydroxybutyrate/ Acetoacetate
Mothers					
Controls					
Fed	2.9 ± 0.5	558 ± 79	29 ± 11	3.5 ± 1.1	5.9 ± 0.5
24-h starved	4.2 ± 0.3	823 ± 183	3,152 ± 339	53.5 ± 23.3	58.8 ± 19.4
P	NS	NS	<.001	NS	<.01
Ethanol					
Fed	4.3 ± 0.5‡	439 ± 181	254 ± 57‡	5.4 ± 0.4	55.8 ± 14.9†
24-h starved	4.5 ± 0.2	1,024 ± 160	3,099 ± 219	54.5 ± 12.4	66.8 ± 18.7
P	NS	NS	<.001	<.05	NS
Pair-fed					
Fed	1.9 ± 0.3¶	480 ± 91	52 ± 18‖	2.4 ± 0.3¶	16.3 ± 3.4‡§
24-h starved	2.4 ± 0.7‡‖	5,678 ± 625*‖	2,252 ± 186	68.3 ± 21.9	31.9 ± 8.8
P	NS	<.001	<.001	<.01	NS
Fetuses					
Controls					
Fed	0.63 ± 0.05	—	69 ± 12	2.5 ± 1.1	33.7 ± 18.5
24-h starved	0.51 ± 0.03	—	2,595 ± 387	40.5 ± 10.6	63.6 ± 19.4
P	NS		<.001	<.01	NS
Ethanol					
Fed	0.58 ± 0.02	—	130 ± 18‡	4.9 ± 1.4	35.8 ± 14.3
24-h starved	0.64 ± 0.04	—	2,519 ± 311	28.2 ± 5.9	90.9 ± 27.2
P	NS		<.001	<.01	NS
Pair-fed					
Fed	0.52 ± 0.03	—	91 ± 16	3.7 ± 1.9	20.7 ± 4.0
24-h starved	0.61 ± 0.02	—	1,287 ± 220‡§	30.8 ± 2.6	54.4 ± 18.5
P	<.05		<.01	<.001	NS

Values represent the mean ± SEM of five to seven rats per group. Statistical comparison between groups and experimental condition of the animals are as indicated in Table 1.

*$P < .001$.
†$P < .01$.
‡$P < .05$.
§$P < .05$.
‖$P < .01$.
¶$P < .001$.

In fed fetuses, the liver glycogen concentration was insignificantly lower in ethanol mothers than in controls, whereas in pair-fed animals values were very similar to the latter and notably higher than in the ethanol group (Table 1). Starvation caused a significant reduction in fetal liver glycogen in all the groups, and values attained by the ethanol group were significantly lower than in the controls (Table 1).

Circulating lipidic components are summarized in Table 2. Plasma triacylglyceride concentration was higher in ethanol-treated mothers than in either controls or pair-fed rats when fed. Starvation caused an increase in plasma triacylglyceride in controls, although it was insignificant. This increase was not paralleled in either the ethanol or pair-fed groups. As a consequence, the differences in the fed state between the control and ethanol group vanished with starvation, and so plasma triacylglyceride levels were higher in both groups than in pair-fed animals (Table 2). The level of plasma triacylglyceride in fetuses was always much lower than in their mothers, and no differences among groups was detected (Table 2). Maternal plasma FFA levels did not differ among groups when fed, and whereas starvation produced only a slight and insignificant increase in ethanol and control rats, this effect was very noticeable and highly significant in pair-fed animals, their values being much higher than in the other two groups. As shown also in Table 2, blood β-hydroxybutyrate levels were higher in ethanol-treated than control and Pair-fed rat mothers and their fetuses when fed. Values in pair-fed mothers were significantly higher than in controls when fed (Table 2). Starvation produced a significant increase in blood β-hydroxybutyrate levels in all the groups, although values observed in the pair-fed rats were lower than in the other groups. However, statistical comparisons between pair-fed and the control or ethanol rats were significant in fetuses but not in mothers (Table 2). Blood acetoacetate levels were similar in all groups both in mothers and fetuses, and values were higher in the fasted rather than in the fed animals (Table 2). The β-hydroxybutyrate/acetoacetate ratio, an index of redox state, was significantly higher in the ethanol group as compared with both control and pair-fed animals. As a consequence of the marked increase in β-hydroxybutyrate levels due to starvation, prior differences among groups vanished, although the ratio was lower in the pair-fed group. There were no significant differences in the fetuses in either the fed or the starved state (Table 2).

Liver triacylglyceride concentration and LPL activity are shown in Table 3. In the fed state, liver triacylglyceride concentration was higher in ethanol-treated rats than in controls and pair-fed rats, with no difference between the two latter groups. Starvation produced a significant increase in liver triacylglyceride concentration in both controls and ethanol-treated rats but not in pair-fed animals. Liver triacylglyceride concentration in fetuses was lower than in mothers and did not differ between groups when fed. Starvation produced a significant increase in this parameter in fetuses from control and ethanol-treated rats but not in those from the pair-fed ones (Table 3). Liver LPL activity did not differ among groups when the mother rats were fed. Starvation produced a significant increase in this parameter in control and ethanol-treated mothers but not in pair-fed ones whose values were significantly lower than those of the other two groups (Table 3). Due to the similarity in the change produced by starvation in liver triacylglyceride concentration and LPL activity, individual values of these two parameters from all the groups were analyzed, and a highly significant linear correlation was found in the case of the fasted animals ($r = .791$, n = 17, $P < .001$), but not in the fed ones ($r = .021$, n = 19, NS). Fetal liver LPL activity was higher than maternal LPL when animals were fed (Table 3), and values were significantly lower in ethanol-treated animals than in the control or pair-fed group. Maternal starvation did not affect fetal liver LPL activity in controls and ethanol-treated animals but did produce a significant reduction in those from pair-fed mothers (Table 3), with no significant correlation between all these values and those of liver triacylglyceride concentration ($r = .301$, n = 40, NS). However, a linear and significant correlation was found between fetal liver triacylglyceride concentration and blood total ketone bodies (β-hydroxybutyrate + acetoacetate) ($r = .592$, n = 36, $P < .001$).

Table 3. Effect of Maternal Chronic Ethanol Intake and 24-Hour Starvation on Liver Triglyceride Concentration and LPL Activity in the 21-Day Pregnant Rat

	Triglycerides (mmol/g)	LPL Activity (pkatal/g)
Mothers		
Controls		
Fed	4.3 ± 0.4	91 ± 12
24-h starved	18.5 ± 2.7	264 ± 24
P	<.001	<.001
Ethanol		
Fed	8.3 ± 0.6*	82 ± 12
24-h starved	16.2 ± 2.5	251 ± 35
P	<.01	<.01
Pair-fed		
Fed	5.0 ± 0.1§	127 ± 18
24-h starved	3.4 ± 0.6†‖	113 ± 18*‖
P	NS	NS
Fetuses		
Controls		
Fed	1.6 ± 0.2	357 ± 32
24-h starved	5.6 ± 1.7	294 ± 30
P	<.01	NS
Ethanol		
Fed	2.6 ± 0.7	220 ± 26†
24-h starved	6.6 ± 0.7	202 ± 17†
P	<.01	NS
Pair-fed		
Fed	1.8 ± 0.2	406 ± 48‖
24-h starved	3.2 ± 1.1§	246 ± 17
P	NS	<.01

Values represent the mean ± SEM of five to seven rats per group. Statistical comparison between groups and experimental condition of animals are as indicated in Table 1.

*$P < .001$.

†$P < .01$.

§$P < .05$.

‖$P < .01$.

DISCUSSION

The present findings show a reduction of maternal body weight as well as a decrease in fetal weight and maternal tissues as a consequence of ethanol intake or food restriction (pair-fed animals). In addition, maternal and fetal liver weights were lower in the latter two groups. This indicates that both conditions, ethanol intake and food restriction, reduce the mother's stores and impair her capacity to support the intense fetal draining of nutrients at late gestation. Maternal hypoglycemia found in both ethanol-treated and pair-fed rats supports this hypothesis. Placental glucose transfer is directly dependent upon circulating maternal glucose concentration,[32,33] and reductions in fetal glycemia in ethanol and pair-fed rats must be a consequence of reduced maternal-fetal glucose transfer in these two groups. Both ethanol intake and undernourishment during pregnancy are known to reduce placental blood flow[6,34] which also participates in the proposed reduction in maternal-fetal glucose transfer. The different responses to starvation found in blood glucose between ethanol-treated and pair-fed rats is noticeable. We believe that their different liver glycogen storage when fed could contribute to such a difference. Decreased liver glycogen concentrations found in the ethanol-treated rats fits well with the reported glycogenolytic action of ethanol.[35,36] In the case of the pair-fed rats, enhanced liver glycogen concentration when fed and preservation of blood glycemia with starvation coincide with results from others who used a different food restriction system than the one used here[37,38]; they also indicate that chronic undernourishment produces metabolic adaptations that maximize efficiency in energetic transformations with a reduction in the rate of certain catabolic pathways (glycogenolysis in the fed state and glucose utilization in the fasted one). Preservation of fetal glycemia in conditions of intense maternal hypoglycemia such as that found in the starved ethanol-treated rats has also been found in previous experimental situations.[39,40] It may be hypothesized that in these conditions the fetus has both an enhanced degradation of liver glycogen, as we have observed in previous studies,[40] and an increase in the utilization of alternative substrates such as ketone bodies from maternal circulation, permitting glucose preservation. The ketone body levels are greatly augmented in the fasting state, and it is well known that these substrates may be used by the fetus as preferential energetic fuels.[41]

In the fed state, mothers from the ethanol group presented an enhanced mitochondrial redox potential, as indicated by the β-hydroxybutyrate/acetoacetate ratio, and a higher liver triacylglycerol concentration as compared with both control and pair-fed animals. These alterations are characteristic of chronic ethanol intake,[9,42] and their presence is an indicator of its severity. Starvation caused a striking increase in blood β-hydroxybutyrate levels in the three groups (although lower in pair-fed animals) that canceled any previous existing differences in the β-hydroxybutyrate/acetoacetate ratio. The lower activity of ethanol-oxidizing enzymes existing in fetal liver, even at late gestation,[12] corresponds with the lack of increase in redox state observed in fetuses from ethanol rats, although their fetal blood β-hydroxybutyrate levels were higher than in fetuses from the control group, reflecting maternal concentration. The increase in NADH availability as a consequence of ethanol oxidation also favors the formation of α-glycerolphosphate from dihydroxyacetone,[43] allowing higher fatty acid sterification.

The smaller increases in circulating maternal and fetal ketone body levels with starvation in pair-fed rats as compared with control and ethanol-treated animals contrast with the higher maternal circulating FFA levels and may indicate a reduced liver utilization of these metabolites. We do not yet know the reason for this effect, but it could be related to diminished liver handling of FFA for both triacylglyceride synthesis and ketogenesis. In the fasting condition these two pathways are interrelated, since lipogenesis is reduced[44,45] and both triacylglyceride synthesis and ketogenesis depend upon the arrival in the liver of fatty acids derived from adipose tissue lipolysis. In the normal fasted late pregnant rat, these pathways are intensely enhanced[46,47] and this would explain the observed parallel increments in liver triacylglyceride concentration and circulating ketones in control and ethanol-treated animals. We even hypothesize that these pathways are interconnected by the increments in liver LPL activity in a way that is similar to the neonatal liver, in which there is a transient increase in LPL activity allowing direct fatty acid uptake from TAG mother's milk immediately at the beginning of the suckling period.[48,49] We previously described the presence of greater hepatic LPL activity in fasted pregnant rats in comparison with fed rats,[31] and that activity is also observed in ethanol mothers but not in pair-fed ones in this paper. Thus, the linear correlation between liver triacylglyceride concentration and LPL activity found in the fasted rats from the three studied groups supports the possibility of direct hepatic uptake of circulating triacylglyceride. The different responses in ethanol-treated and pair-fed rats may be explained by the enhanced redox state in the former, caused by ethanol metabolism which would facilitate the production of α-glycerolphosphate from gluconeogenic intermediates[43] in spite of the severe hypoglycemia. Through this mechanism, and whereas fasted ethanol-treated animals route their lipolytic products, glycerol and FFA, to liver triacylglyceride and ketone body synthesis, undernourished pair-fed animals would route glycerol to gluconeogenesis and restrain the consumption of FFA. If this mechanism was true for the pair-fed animals, it would justify the response to starvation observed in these rats: preservation of circulating glucose, lower circulating levels of triacylglyceride, lack of change in liver triacylglyceride, lower increase in circulating ketone bodies, enormous increase in plasma FFA, and lack of change in liver LPL activity. However, we have not yet identified the possible agent that initiates all these integrated changes.

In fetuses, circulating and liver triacylglyceride concentrations cannot be related to maternal circulating levels as they are known not to cross the placental barrier.[50] They must, therefore, be the result of endogenous synthesis, and linear correlation between liver triacylglyceride and circulating ketone bodies found in fetuses from mothers in the three groups, fed or fasted, would indicate a direct relationship between these two parameters, and agrees with the proposed

utilization of ketone bodies for lipid synthesis in the fetal liver.[51] Unlike the maternal liver, there does not seem to be a relationship between liver triacylglyceride and LPL activity in the fetal liver, since the values found in the fed condition were much higher than in the adult liver and thus are in agreement with previous findings.[48,49] This suggests a role for this activity in postnatal metabolic adaptation, as we have already mentioned.

In summary, present findings show that chronic ethanol intake during pregnancy reduces conceptus-free maternal weight but does not impair conceptus capacity to respond to food deprivation. It is possible that this response is forced by the mother's intense hypoglycemia, which, in turn, may be a consequence of impaired gluconeogenic activation, secondary to ethanol-induced enhancement of hepatic redox state. These observed metabolic changes differ from those found in pair-fed animals and support the notion that in the former group the maternal ethanol metabolism, as well as undernourishment, contributes to the negative effects on fetal development. The more intense malnourishment found in pair-fed rats, with a 35% lower caloric intake than control animals during pregnancy, also contributes to the different metabolic response observed in the fasted state. The difference between ethanol and pair-fed groups is further emphasized by the greater impairment of fetal development, affecting both fetal weight and length in the former group, whereas in the latter, only the fetal weight was reduced. This occurred in spite of the fact that in ethanol-treated rats total daily caloric intake is practically the same as in the controls, due to the supplementation of reduced food intake with the calories from ethanol oxidation.[13]

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