Effects of Ethanol and Folic Acid Consumption During Pregnancy and Lactation on Basal Enzymatic Secretion in the Duodenal Juice of Offspring Rats

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OBJECTIVE: Studies on duodenal juice enzyme activities were carried out on suckling Wistar rats born to dams given ethanol during gestation and suckling. The results were compared with offspring of dams given diets containing no ethanol. Comparisons were also made with offspring of dams given ethanol and folic acid supplementation to observe whether a folate supplement could sufficiently reverse the negative effect of ethanol consumption.

METHODS: The dams were fed increased amounts of ethanol (5% to 20%, vol/vol) in tap water for 4 wk. The maximum quantity, 20% ethanol, was given to the dams during pregnancy and lactation. Offspring animals were randomized into three groups: control (CG), ethanol treated (EG), and ethanol plus folic acid (EFG).

RESULTS: Body weight at birth and at 21 d after birth and pancreatic weight were lower in offspring after ethanol treatment. Folic acid supplement increased these parameters in the EFG. Under basal conditions, decreases in amylase, lipase, and chymotrypsin activities in the duodenal juice after ethanol treatment were detected. Serum and urine amylase activities also decreased in the EG and EFG. These changes were different in the ethanol-treated progenitors. In these progenitors, ethanol treatment increased serum amylase levels. In the offspring, amylase activities in the EFG decreased with respect to the CG; however, an increase in the EG was observed. In dams the folic acid supplement did not significantly alter the serum amylase activities. Lipase and chymotrypsin activities in the EFG were similar to those in the EG. An increase of serum and urine amylase in the EFG with respect to the EG was found.

CONCLUSION: Our findings indicated that, under basal conditions, ethanol treatment during gestation and lactation negatively affects the digestive function in offspring. The effects of ethanol were slightly attenuated in rats supplemented with folic acid for amylase activities. Although extrapolation from animal studies can be tenuous, the present findings may explain the use of folic acid in the prevention of damage induced by ethanol to increase the amylase levels to physiologic concentrations. *Nutrition* 2003;19: 778–783. ©Elsevier Inc. 2003

KEY WORDS: offspring, ethanol treatment, duodenal juice, folic acid

INTRODUCTION

Alcohol abuse is widely recognized as an etiologic factor in pancreatic injury. The mechanism of pancreatic toxicity to alcohol is not completely understood, although there are some hypotheses explaining how alcohol affects the pancreas. Recent studies have reported the production of free radicals as an initiator of pancreatic damage.[1,2] Exocrine pancreas is extremely vulnerable to damage from ethanol metabolites.[3,4] Further, there is an increasing body of evidence that reactive oxygen species play a role in acute and chronic pancreatitis. In the liver, cytochrome P4502E1, the inducible ethanol-metabolizing enzyme, is one of the proposed pathways by which ethanol induces oxidative stress.[5] Norton et al.[5] reported that cytochrome P4502E1 is present in the rat pancreas and is inducible by chronic ethanol administration. The metabolism of ethanol via cytochrome P4502E1 may contribute to oxidative stress in the pancreas during chronic ethanol consumption.

In addition, we recently reported that ethanol consumption during pregnancy and lactation induces oxidative stress in the pancreas of offspring and dams,[6] the oxidative stress being a key step in the pathogenesis of ethanol-induced damage.[7–10]

In this work we studied the enzymatic content in duodenal juice, under basal conditions, in suckling rats born to dams given ethanol during pregnancy and lactation. We also investigated whether folic acid supplementation during gestation and lactation could reverse the negative effects of ethanol treatment. The reasons for using folic acid were: 1) alcohol abuse produces a folate deficiency,[11,12] 2) ethanol interferes with folate absorption and metabolism,[13,14] 3) folate deficiency is common among alcoholics,[15] and 4) the need for folate rises considerably during pregnancy, whenever cells are multiplying.[16,17]

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Folate deficiency can produce a few pancreatic zymogene granules per cell and intermediate-size granules in the pancreas.[18] Previous studies have suggested that the metabolism of methyl groups is an important factor in the function of the exocrine pancreas. Because de novo synthesis of methyl groups requires the participation of folate coenzymes, Balaghi and Warner[18] investigated the effect of folate deficiency on pancreatic exocrine function and concluded that severe folate deficiency impairs pancreatic exocrine function.

MATERIALS AND METHODS

Animals

Male and female Wistar rats, weighing 150 to 200 g, were randomized into three groups (10 rats/group): control (CG), ethanol (EG), and ethanol plus folic (EFG).

Ethanol was administered in tap water by a previously described method.[11] Ethanol-treated rats received increasing amounts of ethanol in the drinking fluid for 3 wk (5.5 ± 0.2, 7.8 ± 0.4, and 8.9 ± 0.4 g of ethanol/kg of body weight per day) ad libitum. A consumption of 16.6 ± 2.1 g of ethanol/kg of body weight per day was given for another 4 wk and maintained during the entire gestation and suckling periods (21 d). This proportion, used in previous works by our group,[12,19] was used to avoid adverse effects during reproduction.

The protocol of ethanol administration to dams used in our experiments produced the following serum ethanol concentrations in dams and offspring: EG dams, 51 000 ± 10 000; EG offspring: 2000 ± 500 ($P < 0.0001$); EFG dams, 47 000 ± 11 000; EFG offspring, 1000 ± 100. Results (μM) are the mean ± standard error of the mean of eight animals.

Under these conditions the postnatal mortality rates in newborns were 16.80% in the EG, 10.12% in the EFG, and 7.14% in the CG. The EFG received the same ethanol treatment and were fed a diet supplemented with folic acid during gestation and lactation. Drinking water with or without ethanol and the diet with or without supplementation were given ad libitum.

The EG and EFG were mated to obtain the first offspring. Pregnant rats were housed individually in plastic cages and fed a rat diet ad libitum. They were kept on 20% ethanol in the drinking water during the entire gestation and suckling periods.

The CG received water only and a non-supplemented diet during pregnancy and lactation. Control rats were handled in the same way as the other experimental groups.

The day of parturition was designated as day 1 of lactation and day 21 as the end of the lactation period. Experiments were performed on the offspring of all three groups 21 d postpartum.

The rats were maintained under an automatically controlled temperature (22°C to 23°C) and a 12-h light-and-dark cycle. Animal care complied with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996).

Diets

Diets were prepared according to the Council of the Institute of Laboratory Animal Resources.[20] Diet ingredients were mixed and homogenized in a double-cone blender (Rest, Haan, Germany). The CG and EG received an average of 60 μg/d of folic acid during pregnancy and lactation. In the EFG, the amount of folic acid was 152 μg/d for the same period.

Collection of Pancreatic Secretion

After 21 d postpartum, animals from each group were anesthetized with a subcutaneous injection of 125 mg of urethane per gram of body weight after an overnight fast. The collection of pancreatic secretion was performed according to a previously described method.[21] Briefly, the abdominal cavity was opened and the bile duct was ligated. The inflow cannula was tied at the beginning of the duodenum and the perfusate was collected above the Treitz ligament. After cannulation, the loops were replaced inside the body wall. The duodenum was irrigated with saline at 37°C at a constant rate of 0.7 mL/min. The duodenum was washed with saline before the start of the first collection to remove any residual secretion.

The perfusate was collected in tubes over a 10-min period. Tubes were weighed before and after collection to calculate the volume of perfusate. Volume, pH, protein content, and pancreatic enzymatic activities were assayed in the duodenal juice. The gathered volume was the sum of saline solution perfused plus the pancreatic duodenal secretion.

Blood Samples

Blood samples were taken from the fasted and anesthetized rats by cardiac puncture through the thorax and collected into tubes. Serum was prepared with low-speed centrifugation. Amylase levels were obtained by the method described for the pancreatic enzyme assay.

Urine and Feces Samples

At the end of the experimental period (21 d postpartum), the animals were housed individually in metabolic cages and deprived of food for 12 h to collect urine and feces. We measured urine volume and the amount of feces excreted within 12 h. We determined amylase in urine and chymotrypsin in feces to be the enzymatic elimination routes.

Pancreatic Enzyme Assay

Amylase content was assayed by an enzymatic colorimetric test using *p*-nitrophenol-α-D-maltoheptaoside as the substrate.[22] Chymotrypsin was determined according to the method of Delmar et al.[23] Lipase activities were determined by the colorimetric method described by Neuman and Ziegenhorn.[24] Protein concentration was measured according to the method of Lowry et al.,[25] using bovine serum albumin as the standard.

Statistical Methods

Data are expressed as mean ± standard error of the mean and were analyzed by analysis of variance. If analysis of variance indicated significant treatment effects, the significance of differences between individual means were determined with the Tukey-Kramer parametric test or Kruskal-Wallis non-parametric test followed by Dunn's multiple comparison tests. $P < 0.05$ was considered statistically significant.

RESULTS

General Parameters

Table I lists the body weights of offspring at birth and at the end of the lactation period. Ethanol treatment decreased the body weight at birth and at 21 d postpartum in the EG compared with the CG and EFG. Body weight at the end of the lactation period in the EFG differed significantly from the EG.

Similar results are observed in pancreatic weights. The pancreatic protein concentration in the EG or EFG did not differ from that in the CG.

TABLE I.

EFFECT OF ETHANOL CONSUMPTION DURING PREGNANCY AND LACTATION ON OFFSPRING*

	CG	EG	EFG
Body weight (g) at birth	6.7 ± 0.11 (15)	5.89 ± 0.2 (15)†	6.53 ± 0.1 (15)‡
Body weight (g) 21 d after birth	35.27 ± 0.5 (15)	21.65 ± 0.3§ (15)	29.2 ± 0.7‖¶ (15)
Pancreatic weight (g)	0.111 ± 0.007 (15)	0.059 ± 0.004# (15)	0.134 ± 0.013** (15)
Pancreatic protein concentration (mg/g pancreas)	100.76 ± 4.26 (6)	102.45 ± 4.98 (10)	98.80 ± 2.83 (9)

* Values are mean ± standard error of the mean (number of animals/group).
† Significantly different from levels in CG (KW = 15.420, $P < 0.001$).
‡ Significantly different from levels in EG (KW = 15.420, $P < 0.05$).
§ Significantly different from levels in CG (KW = 59.057, $P < 0.001$).
‖ Significantly different from levels in CG (KW = 59.057, $P < 0.01$).
¶ Significantly different from levels in EG (KW = 59.057, $P < 0.001$).
# Significantly different from levels in CG (KW = 21.030, $P < 0.01$).
** Significantly different from levels in EG (KW = 21.030, $P < 0.001$).
CG, control group; EG, ethanol group; EFG, ethanol plus folic acid group; KW, Kruskal-Wallis

Basal Conditions

As shown in Figure 1, the volume of duodenal juice (basal conditions) was slightly, although not significantly, smaller in the EG than in the CG and EFG. There were no differences between the EG and EFG, although secretion was slightly less in the EG.

A significant reduction of pH in the duodenal juice was observed in the EG and EFG in relation to the CG. In the EFG, pH values were lower than in the EG, although these differences were not significant (Fig. 2).

In the EG and EFG, no significant difference in protein concentration from the duodenal juice was found (Fig. 3).

Enzymatic activity was measured as an index of digestive capacity. The duodenum was perfused for 10-min collection periods to establish the baseline secretion amount. The enzymatic activity (U/mg of protein) in offspring at 21 d, obtained in basal conditions, is shown in Figure 4 and Table II.

The EG and EFG showed decreases in amylase, lipase, and chymotrypsin activities when compared with the CG. The reduction in amylase was greater than 85% in the EG and 51% in the EFG. Amylase activity was significantly increased in EFG as compared with the EG. However, folic acid intake did not correct the values of amylase content (Table II). Chymotrypsin and lipase in duodenal juice (Fig. 4) was lower in the EG than in the CG. A similar effect was observed in the EFG in relation to the CG.

When pancreatic secretion was expressed as units per 10 min, we found similar results: amylase secretion decreased in the EG when compared with the CG and EFG. Although secretion was higher in the EFG than in the EG, this value was significantly lower than that in the CG. Chymotrypsin and lipase secretions decreased in the EG and EFG with respect to the CG. (Table II).

Serum Measurements

As can be seen in Table II, ethanol treatment increased amylase activities measured in the sera of EG and EFG dams (36% and 19%, respectively), although no significant differences between the EFG and CG were found.

In offspring, ethanol treatment diminished amylase activity (>40% and 42% in the CG and EFG, respectively). However, there were no modifications in the EFG.

Metabolic Parameters

Ethanol treatment reduced the amount of tap water (CG, 4.30 ± 0.24; EG, 3.11 ± 0.34; EFG, 3.25 ± 0.26; $P < 0.05$), urine (CG, 1.05 ± 0.08; EG, 0.146 ± 0.012; EFG, 0.200 ± 0.010; $P < 0.001$), and feces (CG, 0.176 ± 0.016; EG, 0.100 ± 0.030; EFG, 0.083 ± 0.025; $P < 0.001$) excreted in the treated rats.

Urine amylase decreased significantly with ethanol treatment in the EG and EFG versus the CG (CG, 6.15 ± 0.63; EG, 0.767 ± 0.05; EFG, 1.34 ± 0.31; $P < 0.001$). However, a significant increase in urine amylase from EFG compared with EG was found ($P < 0.001$). In feces, no significant difference across groups with

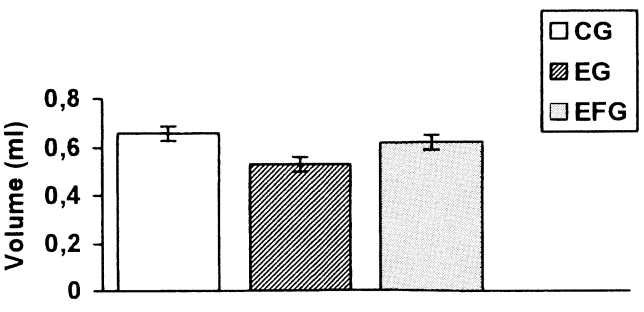

FIG. 1. Effect of ethanol and folic acid on duodenal juice volume (mL/10 min) secreted under basal condition in CG, EG, and EFG offspring 21 d after birth. Results are mean ± standard of the mean of eight animals. CG, control group; EG, ethanol group; EFG, ethanol-folic group.

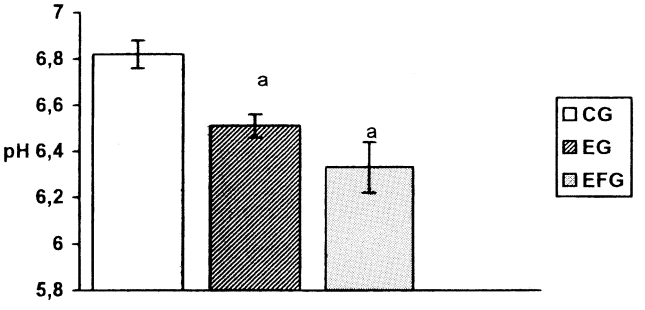

FIG. 2. Effect of ethanol and folic acid on the pH of duodenal content in CG, EG, and EFG offspring 21 d after birth. Results are mean ± standard error of the mean of eight animals. [a]Significantly different from levels in CG (Kruskal-Wallis = 9.954, $P < 0.05$). CG, control group; EG, ethanol group; EFG, ethanol-folic group.

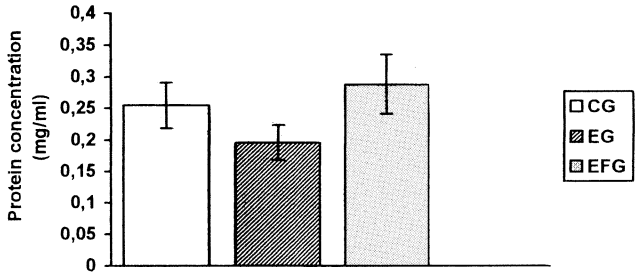

FIG. 3. Effect of ethanol and folic acid on protein concentration in the duodenal juice of CG, EG, and EFG offspring 21 d after birth. Results are mean ± standard error of the mean of eight animals. CG, control group; EG, ethanol group; EFG, ethanol-folic group.

respect to chymotrypsin activity was found (CG, 0.548 ± 0.07; EG, 0.380 ± 0.08; EFG, 0.536 ± 0.09).

DISCUSSION

The aim of this study was to investigate effects of maternal ethanol consumption, during pregnancy and lactation, on the basal enzymatic activities of the duodenal juice, in offspring, and the protective effect of folic acid on the enzymatic capacity of the offspring.

TABLE II.

EFFECT OF ETHANOL AND FOLIC ACID ON DUODENAL JUICE AND SERUM ENZYME ACTIVITIES 21 D AFTER BIRTH*

	CG	EG	EFG
Duodenal juice			
Amylase (U/10 min)	3.75 ± 0.38	0.36 ± 0.02†	1.46 ± 0.16‡§
Amylase (U/protein)	18.04 ± 1.45	2.88 ± 0.25†	8.89 ± 0.75‡**
Lipase (U/10min)	1.21 ± 0.10	0.32 ± 0.04‖	0.40 ± 0.06¶
Chymotrypsin (U/10 min)	0.108 ± 0.011	0.029 ± 0.003#	0.046 ± 0.007#
Serum amylase (U/L)			
Dams	1044 ± 105	1424 ± 71††	1241 ± 54
Offspring	1365 ± 121	534 ± 41†	1260 ± 75**

* Values are mean ± standard error of the mean (n = 8 animals/group).
† Significantly different from levels in CG (KW = 25.087, $P < 0.001$).
‡ Significantly different from levels in CG (KW = 25.087, $P < 0.05$).
§ Significantly different from levels in EG (KW = 25.087, $P < 0.05$).
‖ Significantly different from levels in CG (KW = 14.889, $P < 0.001$).
¶ Significantly different from levels in CG (KW = 14.889, $P < 0.05$).
# Significantly different from levels in CG (KW = 50.930, $P < 0.001$).
** Significantly different from levels in EG (KW = 29.140, $P < 0.01$).
†† Significantly different from levels in CG (KW = 4.18, $P < 0.025$).
CG, control group; EG, ethanol group; EFG, ethanol plus folic acid group; KW, Kruskal-Wallis

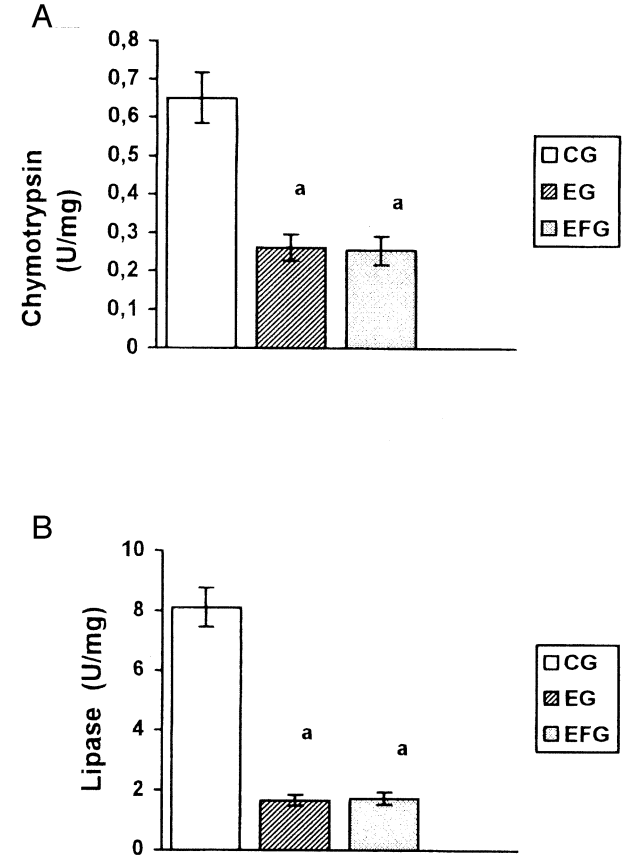

FIG. 4. Effect of ethanol and folic acid on enzymatic activities on duodenal juice in CG, EG, and EFG offspring 21 d after birth. Results are mean ± standard error of the mean of eight animals. (A) [a]Significantly different from levels in the CG (Kruskal-Wallis = 18.776, $P < 0.001$). (B) [a]Significantly different from levels in the CG (Kruskal-Wallis = 20.183, $P < 0.001$). CG, control group; EG, ethanol group; EFG, ethanol-folic group.

Ethanol had a negative effect during the suckling period, demonstrated by a retardation of body and pancreatic weight gains in the offspring, which was partly prevented by folic acid supplementation. In addition, under basal conditions, ethanol administration decreased enzymatic activities and pH of the duodenal juice in the offsping.

The pH was more alkaline in the CG than in the EG and EFG. These results were due, in part, to an increase in stomach acid secretion and/or a decrease in alkaline pancreatic secretion in the EG and EFG. However, we studied the duodenal segments, so these data might have been due to a decrease in pancreatic secretion or an alteration in the electrolyte exchange in the intestinal mucosae produced by ethanol.[26] Alcohol ingestion decreases bicarbonate output in the pancreatic juice of alcoholic patients.[27]

Under basal conditions, the decrease in the total eliminated amount of amylase (U/10 min) in the EG offspring may indicate a decrease in pancreatic enzymatic production. Our data with regard to low amylase activities in offspring agree with previously published reports on alcoholic adults rats[28,29] and represent an adaptation to the low carbohydrate content of alcoholic diets. In our case, ethanol consumption accompanied a decrease in milk uptake by the litters nurtured by ethanol-treated dams.[30] Thus, total milk nutrient levels decreased, which reduced enzymatic secretion.

Chronic ethanol abuse often leads to the development of vitamin deficiencies, with folate deficiency being one of the most common.[31] Although the poor diet of the chronic alcoholic plays a major role in the etiology of folate deficiency, ethanol ingestion interferes with the absorption and metabolism of folates. Lopez et al.[32] examined the combined effects of long-term alcohol intake and low fat ingestion on rat pancreatic enzymatic activities. They concluded that malnutrition (diet with 5% of lipids) decreases lipase and amylase activities in pancreatic tissue, whereas ethanol administration (ethanol plus a diet with 5% lipids) increases lipase activity and decreases amylase activity. However, in rats and human alcoholics, basal pancreatic enzyme output was increased when compared with non-alcoholics.[33,34] In these studies, the volume of pancreatic juice was similar in control and alcoholic subjects, and the increase in basal pancreatic enzymes may indi-

cate true hypersecretion of pancreatic proteins in chronic alcoholics. In our study, the protein concentration in the duodenal juice showed no significant differences across the groups studied, although a slight decrease in the offspring of EG dams was observed. This tendency was in accordance with that reported by others.[35]

The urine amylase level was, according to the present data, in the duodenal juice. The amylase secreted in urine over 12 h decreased significantly in the EG and EFG. However, urine amylase secretion increased with folic acid supplementation.

The effect of ethanol on serum amylase activity in the dams differed from that in the offspring. Ethanol exposure in the offspring significantly decreased amylase levels. In contrast, in the dams, ethanol consumption increased serum amylase activity, likely due to an increase in the permeability of the pancreatic conducts[36] in adult rats, which likely explains how the amylase was liberated actively from the pancreas to the blood and increased its levels in the serum.

The different results found in the offspring and the dams might be explained by a direct effect of ethanol and/or its metabolite on the pancreas of offspring rats. Ethanol acts on pancreatic development and the neoformation process and may diminish enzymatic synthesis. For this reason, although the permeability of the pancreatic ducts increased, the serum amylase concentration was decreased. These results are in agreement with those found in the duodenal content.

Long-term ethanol ingestion may damage acinar cells.[37] Alcohol may stimulate protein synthesis, but protein concentration subsequently diminishes, perhaps due to acinar cell injury.[38] This finding may mean that the offspring of ethanol-treated rats, during gestation and lactation, had lower enzymatic content.

No significant difference was found between dams in the EG and EFG with regard to serum amylase levels. Ethanol consumption produces pancreatitis, so the pancreatic enzymes are released immediately from the pancreatic parenchyma into the blood. This action may have been responsible for the elevation of enzyme concentration in serum levels of amylase in the EG and EFG dams.

In previous works,[12,39] we found that milk and serum folic acid levels are lower in ethanol-fed pups, which may contribute to growth retardation in litter exposed to ethanol. However, supplementation with folic acid during pregnancy and lactation in ethanol-treated rats increased the levels of folic acid in rat milk and offspring plasma. Our results showed that the negative effect of ethanol may be due, at least in part, to a deficit in folic acid levels, because the offspring of EGF dams recovered the values of serum amylase in comparison with the EG offspring.

The eliminated amounts of chymotrypsin and lipase over 10 min and chymotrypsin and lipase activities in duodenal juice decreased in the EG and EFG. No significant differences in EG or EFG offspring were found. Similar findings with regard to lipase activity in rats fed ethanol and a diet of 5% lipids compared with rats fed ad libitum were found by others.[32] The chymotrypsin excreted in feces over 12 h did not differ significantly across the groups studied.

These results suggested that ethanol consumption may, at least in part, exert its toxic effects on basal amylase secretion by a direct effect of ethanol or by producing folate deficiency. Folic acid increases amylase activity, but folate supplementation partly corrects the enzyme activity in duodenal juice that is altered by ethanol. We previously reported[6] the protective effects of folic acid on oxidative stress in offspring caused by chronic maternal ethanol consumption during pregnancy and lactation. We concluded that a diet supplemented with folic acid administered to ethanol-exposed offspring could be used to prevent the damage induced by ethanol and to increase amylase levels to physiologic concentrations.

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(For an additional perspective, see Editorial Opinions.)