Brazilian Journal of Medical and Biological Research (1998) 31: 929-932
ISSN 0100-879X Short Communication

Effects of ethanol intake on retinol concentration in the milk of lactating rats

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Abstract

The effect of the consumption of ethanol (5%) on retinol concentration in milk was studied in the rat on day 12 after delivery, together with the evolution of dam body weight and pup growth rate. Female Wistar rats receiving alcohol (5%) in drinking water during lactation (N = 7) were compared to normal controls fed *ad libitum* (N = 6). The mean maternal alcohol intake was 3.96 ± 0.23 g/kg body weight per day. To determine retinol levels in milk we used the Bessey and Lowry method, modified by Araújo and Flores ((1978) *Clinical Chemistry*, 24: 386-392). The pups were separated from dams for a 2-4-h period, after which the dams were injected intraperitoneally with anesthetic and oxytocin. The concentration of retinol in milk was 162.88 ± 10.60 µg/dl in the control group and 60.02 ± 8.22 µg/dl in the ethanol group ($P<0.05$). The ethanol group consumed less food than the controls and lost a significant amount of weight during lactation. On days 8, 10 and 12, the body weight of the pups from rats given ethanol (13.46 ± 0.43, 16.12 ± 0.48 and 18.60 ± 0.91 g, respectively) were significantly lower ($P<0.05$) than the weight of pups from controls (15.2 ± 0.44, 18.36 ± 0.54, 20.77 ± 0.81 g). These data show that ethanol intake during the suckling period, even at low concentrations, decreases the amount of retinol in milk and, therefore, the amount available to the pups.

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Research supported by Fundação José Bonifácio (FUJB).

Received May 6, 1997
Accepted March 31, 1998

Key words
- Lactation
- Ethanol
- Retinol
- Milk
- Rat

Vitamin A is an essential nutrient for fetal growth and neonatal development. Breast milk is the only source of vitamin A (retinol) during the neonatal period for exclusively breast-fed infants. On the other hand, there are two determining factors for the neonates to achieve their vitamin A requirements: the concentration of this vitamin in the milk and the milk volume consumed, both influenced by maternal vitamin A status and dietary intake (1,2).

Chronic and acute alcohol consumption has been shown to impair vitamin A metabolism and to induce vitamin A deficiency (3-5). However, a low concentration of alcohol has been traditionally recommended to lactating mothers as an auxiliary factor for lactation since it provides additional calories and fluids (6). It is assumed that intake of moderate alcohol concentrations, particularly beer, immediately before breast-feeding increases milk production and facilitates the ejection reflex (7). It has not yet been determined whether the vitamin A content of milk

undergoes any change due to maternal alcohol ingestion during lactation.

In the present study we investigated the effect of the intake of a low ethanol concentration (5%) during the first two weeks of lactation on the retinol levels of the milk of lactating rats, and on the evolution of dam body weight and postnatal growth of the offspring.

Female Wistar rats were mated with mature males of the same strain upon reaching the weight of 150-180 g. The pregnant rats were housed in individual cages under controlled temperature (24 ± 1ºC) and light (12-h light/dark cycles) conditions, and fed Purina chow pellets and water *ad libitum*. The day of parturition was considered to be day 0 of lactation and the litters were restricted to 8 pups per dam. On the first postpartum day, lactating dams were divided into two groups: 1) ethanol-treated rats (ET) (N = 7), which received ethanol diluted in drinking water (5%) and food *ad libitum* until sacrifice, and 2) control rats (C) (N = 6), which received both solid diet and tap water *ad libitum*, being handled in the same way as the ethanol-treated rats. Daily food and liquid intake and body weight (mothers and pups) were measured throughout the treatment period. The mean maternal alcohol intake was 3.96 ± 0.23 (g/kg body weight per day). All animals were sacrificed on day 12 of lactation. We ended our studies on day 12 of lactation to prevent direct consumption of the maternal diet by the young pups. Dams were separated from their litters and 2-4 h later were injected intraperitoneally with 20 mg of a muscle relaxant, ketamine (Ketalar, Parke Davis, Morris Plains, NJ), and 5 units of oxytocin (5 IU, Sigma Chemical Co., St. Louis, MO). After this procedure, 1-2 ml of milk was obtained by gentle squeezing the teats. Total retinol in milk was determined by the method of Bessey and Lowry, modified by Araújo and Flores (8). The reliability of the Bessey and Lowry method for vitamin A analysis in milk has been established (9).

Data are reported as means ± SEM. The Student *t*-test was used to evaluate the statistical significance of differences between groups and $P<0.05$ was taken as the level of significance.

Table 1 shows that the rats which received ethanol (5% in drinking water) during lactation consumed an amount of food kilocalories virtually equal to the control group, except on day 8 and 12 of lactation, when the values presented significant differences. However, when the food kilocalories were added to the alcohol-originated kilocalories, the total daily kilocalorie intake of the ET animals was consistently higher than that of the control animals, with ethanol providing more than 25% of the total calo-

Table 1 - Daily energy intake (g/100 body weight per day) of food and ethanol by rats treated with ethanol in the drinking water (alcohol group) or receiving tap water *ad libitum* (controls) during lactation.

Data are reported as means ± SEM of two experiments. Number of animals per group: 6-7. *P<0.05 compared to controls.

Lactation (days)	Control	5% Ethanol-treated		
	Total kcal intake	Food kcal intake	Ethanol kcal intake	Total kcal intake
0	55.03 ± 3.26	54.91 ± 3.00	–	54.91 ± 3.00
2	39.24 ± 1.64	35.84 ± 3.00	18.70 ± 1.52	54.54 ± 4.52*
4	53.04 ± 2.56	46.80 ± 2.48	24.10 ± 1.58	70.90 ± 4.06*
6	61.64 ± 1.76	57.20 ± 2.56	23.80 ± 1.28	81.00 ± 3.84*
8	68.04 ± 2.48	60.56 ± 2.44*	27.00 ± 1.37	87.56 ± 3.81*
12	77.88 ± 4.12	67.52 ± 3.00*	27.90 ± 1.16	97.42 ± 4.16*

ries ingested by the dams during lactation (Table 1). We also note that ET rats, although showing a higher total kilocalorie intake than C rats, had a significantly lower body weight from day 2 to the end of the study period (Figure 1), which may partially be explained by an inefficient utilization of ethanol-derived calories (10). During peak lactation (10-14 days in the rat) the energy required to assure optimal milk synthesis and production is so great that, possibly, the different quality of the ethanol-derived calories makes it more difficult to make up for the decrease in food-derived calories ingested by the ET animals.

Studies have demonstrated that chronic ethanol consumption (25% in drinking water) by lactating rats during both pregnancy and lactation may alter the composition of milk, which was found to be higher in lipid and lower in lactose content when compared to the milk of control animals (11,12). The present results show that 5% ethanol (in drinking water) treatment during lactation resulted in a significant difference in retinol concentration in the milk of the treated animals when compared to controls (means ± SEM), i.e., 162.88 ± 10.60 µg/dl for C (N = 6) and 60.02 ± 8.22 µg/dl for ET (N = 7) ($P<0.05$). These data are interesting because they indicate that treatment with ethanol during lactation, even at low concentrations, affects milk composition and, therefore, the levels of vitamin A offered to the pups.

As shown in Figures 1 and 2, the changes in vitamin A level in the milk and in the nutritional status of the dams as a consequence of ethanol administration during lactation may affect pup growth: pups nursed by ethanol-treated dams had a lower body weight from the 8th to the 12th day of lactation than controls. One possible explanation would be a decrease in milk production by the lactating rat resulting from an alcohol-induced inhibition of oxytocin (13,14) and prolactin (15), as demonstrated in previous studies.

It is known that the retinol level in the milk depends on the nutritional status of the mother, as well as on the dietary intake of this vitamin by the mother (16). It has been suggested that several kinds of malnutrition may affect lactational performance by decreasing milk production (17,18). On the other hand, the present study shows that malnutrition cannot be the only reason for the depletion of milk vitamin A. Even when

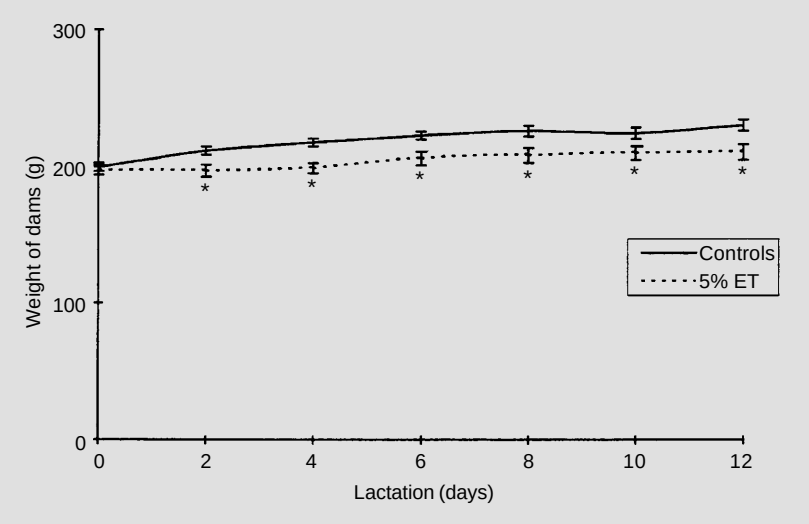

Figure 1 - Effect of ethanol (ET) intake on dam body weight. *P<0.05 compared to controls.

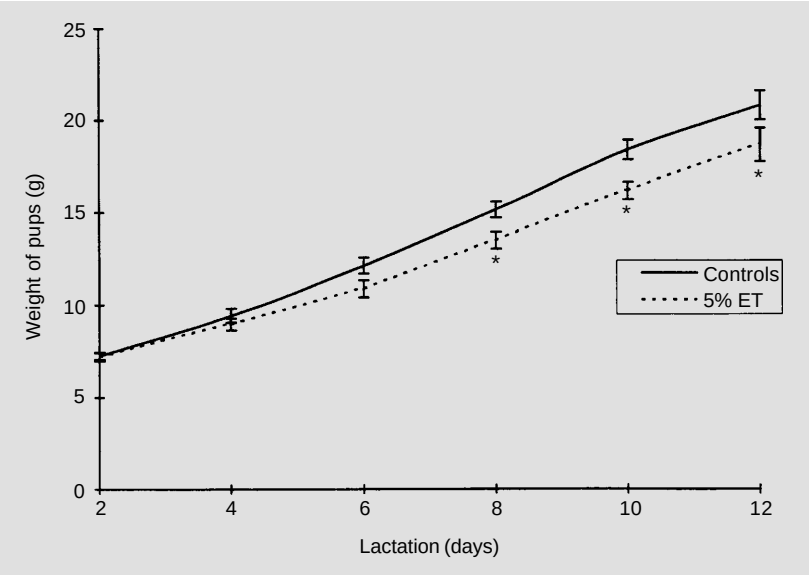

Figure 2 - Effects of ethanol (ET) intake on pup body weight. *P<0.05 compared to controls.

ethanol consumption reduced food intake by the dams, the difference in vitamin A levels in milk between ethanol-fed rats and controls was much greater than could be accounted for by the total vitamin A intake. Thus, a possible direct effect of alcohol consumption during lactation on vitamin A absorption and metabolism should be considered.

The present study suggests that alcohol intake, even at low concentrations during lactation, affects the mother's nutritional status, as it decreases the vitamin A levels in the milk of lactating rats. Therefore, the amount of this vitamin offered to the neonate decreases, with consequences to the normal development of the pups.

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