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Decreased Ethanol Consumption as a Function of Pregnancy and Lactation in C57BL Mice[1,2]

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RANDALL, C. L., E. A. LOCHRY, S. S. HUGHES AND W. O. BOGGAN. *Decreased ethanol consumption as a function of pregnancy and lactation in C57BL mice.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 149–153, 1980.—The effects of pregnancy and lactation on alcohol consumption were examined in the C57BL mouse. Pregnant mice were given a two-bottle choice between water and a 10% w/v alcohol solution from Day 5 of pregnancy, through two weeks of lactation, and an additional four weeks following the removal of nursing pups. Alcohol consumption was expressed in terms of g absolute ethanol per kg body weight and as an alcohol preference ratio (ml alcohol/ml total fluid). Alcohol consumption (g ethanol per kg body weight) fell below control values during pregnancy and lactation but increased to control levels during postlactation. Similarly, alcohol preference ratio was found to decline during pregnancy, and to further decrease during lactation, a trend that was reversed during postlactation. The results support the findings of decreased alcohol consumption during pregnancy in alcoholic as well as nonalcoholic women.

Pregnancy Alcohol consumption C57BL mice Postlactation Lactation Alcohol self-selection Alcohol preference

WITHIN the last decade, clinical reports have emphasized a relationship between maternal alcohol abuse and abnormal fetal growth and development [3, 10, 11]. These provocative findings soon generated a widespread concern about the problem of maternal alcohol abuse during pregnancy. Recently, interest has generalized to the more global topic of moderate alcohol consumption and pregnancy [6, 13, 16].

One interesting and novel finding generated by this research was that non-alcoholic women showed a tendency to markedly reduce their consumption of alcohol during pregnancy [18]. When questioned about the reasons for this decline, over half of the women reported a distaste for alcohol or aversion to the post-ingestive consequences associated with drinking. It should be noted that the reported decrease in alcohol intake was directly proportional to the level of pre-pregnancy drinking. That is, women who consumed the most alcohol prior to pregnancy, in spite of reducing their intake during pregnancy, still exhibited a higher intake relative to those who consumed less alcohol to begin with. These findings have been confirmed by more recent investigations [8,19], leading to the postulation of an inborn "feto-protective mechanism which guards against noxious substances" [7, 8, 17, 19].

While animal models could provide a convenient tool to investigate this "feto-protective" hypothesis, a suitable model must first be developed. Whether decreased voluntary consumption during pregnancy is a phenomenon specific to humans has yet to be systematically studied. Only two brief communications in the literature have appeared to suggest that the same effect may occur in animals [2,4].

The present study was designed to investigate alcohol self-selection in pregnant mice, while attempting to identify a suitable animal model for examining the "feto-protective" effect postulated to occur in human females. Voluntary alcohol consumption was monitored beyond pregnancy to include lactation as well as postlactation, since there was reason to believe that in animals, at least, alcohol intake might be reduced during nursing. One investigation designed to study crossfostering effects noted that inbred C57BL mice, who typically prefer an alcohol solution over water [20], appeared to have lowered their alcohol intake during lactation [22], while a similar trend was suggested in the alcohol consumption of lactating hamsters [2]. It remained to be determined, however, whether animals given a choice between water and alcohol during gestation, as well as lactation, would exhibit a similar decrease in alcohol consumption and, if so, whether intake would return to control values.

METHOD

C57BL/6J mice obtained from Jackson Laboratories were utilized as subjects, because it is well-documented that this inbred strain voluntarily consumes a large percentage of its daily fluid intake as alcohol when presented with a choice

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between 10% w/v alcohol solution and water [20,21]. It was reasoned that decreases in voluntary alcohol consumption during pregnancy would be relatively more visible in this strain than in those that normally avoid alcohol and thus, would preclude a floor effect. All mice were approximately 14 weeks old at the time the experiment began.

Eleven pregnant females bred to DBA/2J males and 13 non-pregnant control females were individually housed in polypropylene cages with zinc-plated wire lids and Beta-chip bedding. Animals were maintained on a 12 hr light-dark cycle in a temperature controlled room (23°C) and were allowed free access to Wayne Breeder Blox and water.

On Day 5 of gestation (the day of plug identification was designated as Day 1), water bottles were removed from the cages and replaced with two glass 40-ml graduated centrifuge tubes capped with white rubber stoppers and straight sipper tubes, each equipped with a ball-bearing to retard spillage. One of the bottles was filled with tap water and the other with a 10% w/v alcohol and water solution prepared with 95% ethanol. To circumvent bias introduced by a position habit, the original position of the tubes was alternated daily when fluid consumption and body weight were recorded. The tubes were refilled each day as needed, although animals were given fresh alcohol solutions and water every fourth day.

Body weight, alcohol consumption, and water intake were measured daily to calculate each animal's alcohol preference (ml alcohol/total fluid intake), relative alcohol intake (g ethanol per kg body weight), and relative fluid intake (ml fluid per g body weight). Baseline data was collected from the 13 control animals for one 2-week period, while data was obtained from the 11 pregnant mice for a total of four 2-week periods: pregnancy, lactation, postlactation-I, and postlactation-II. The use of a separate non-pregnant control group to provide normative alcohol intake data was necessary because in our breeding colony only 50% of the C57BL females placed with males become plugged, and of those, not all become pregnant. For example, two of the plugged females in the present study were not pregnant, which accounts for the unequal number of mice in the experimental and control groups. Fluid self-selection in the nonpregnant control group was measured for only two weeks since previous work in our laboratory with C57BL female mice measuring voluntary alcohol consumption in a two-bottle choice situation over an eight week period indicated highly stable levels of fluid consumption after the second week of testing (unpublished observation).

Newborn litters were counted and culled to six pups when necessary. No cases of cannibalization were observed. To prevent the pups from eventually drinking from the sipper tubes, the offspring were removed from the cages at 14 days of age. Daily alcohol and water intake measures continued to be obtained on the mothers for two additional two week periods.

RESULTS

The average weekly alcohol preference, consumption (g ethanol per kg body weight), and total fluid intake (ml per g body weight) were subjected to a one-way analysis of variance with Maternal Condition (pregnancy, lactation, postlactation-I, and postlactation-II) and Week (first, second) as within-subject factors. Multiple comparisons utilizing a Newman-Keuls test [12] were then made to compare the alcohol preference, consumption, and total fluid intake

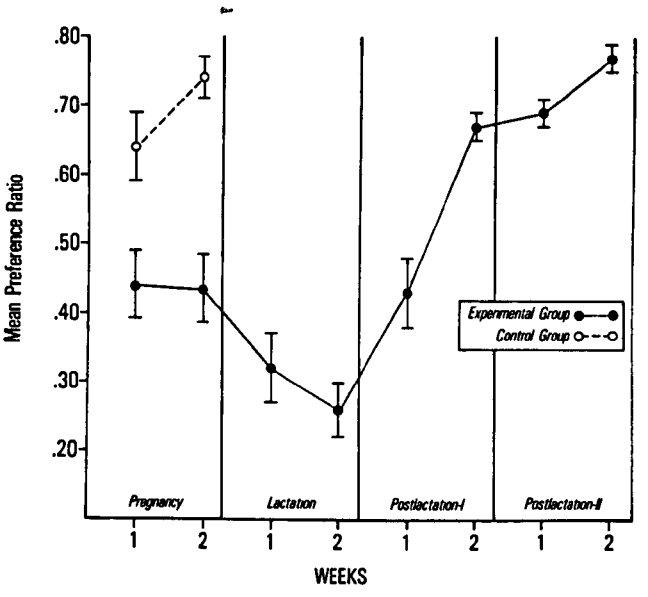

FIG. 1. The average (±SE) preference ratio (ml 10% w/v alcohol/ml total fluid intake) as a function of time during pregnancy, lactation, postlactation-I, and postlactation-II, relative to nonpregnant control values.

of: pregnancy to lactation, pregnancy to postlactation-I, pregnancy to postlactation-II, lactation to postlactation-I, lactation to postlactation-II, and finally the two postlactation periods to each other. Because the non-pregnant control group consisted of an entirely different group of females, individual ANOVAs were performed to compare the baseline data of this group to that of the experimental animals for the three dependent measures.

The alcohol preference ratio varied as a function of both Maternal Condition and Week. Figure 1 illustrates the decline in preference during pregnancy, the further decline during lactation, and the subsequent gradual rise to control levels during the postlactation period. There was a significant effect of Maternal Condition on alcohol preference, $F(3,30)=59.70$, $p<0.001$. A Newman-Keuls comparison indicated that the alcohol preference ratio during pregnancy, lactation, and both postlactation periods were all significantly different from each other. Additionally, there was a significant effect of Week on preference, $F(1,10)=14.81$, $p<0.01$, as well as an interaction of Maternal Condition with Week, $F(3,30)=20.42$, $p<0.001$, reflecting the initial decrease and subsequent increase in preference across both time and maternal condition. Relative to control values, the alcohol preference ratio was significantly lower during pregnancy, lactation, and postlactation-I, F's$(1,22)=20.05$, 60.26, and 8.88, p's<0.01. However, there was no difference between the preference ratio of the nonpregnant control group and that of the postlactation-II period ($F<1$), indicating that alcohol preference had returned to normal levels by this time. A significant Week effect was evident only in the individual ANOVAs comparing the preference ratios of the nonpregnant controls to that of the postlactation-I and II periods, $F(1,22)=29.04$, $p<0.001$ and $F(1,22)=4.77$, $p<0.05$, reflecting the relative increases in the alcohol preference of

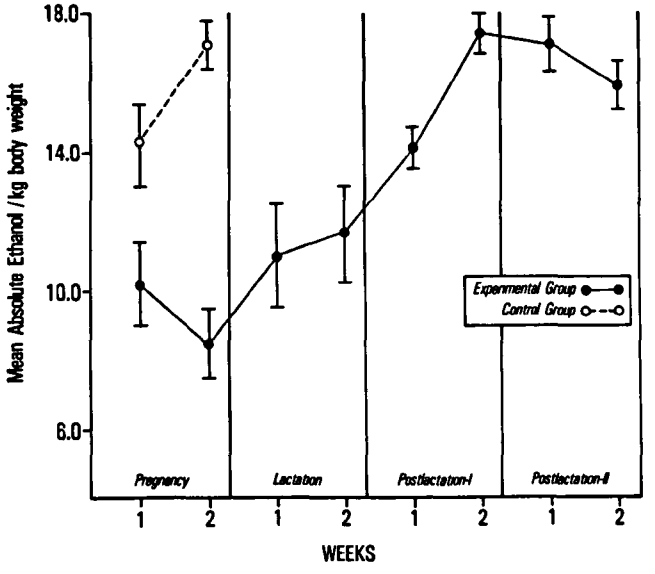

FIG. 2. The average (±SE) alcohol consumption (g EtOH per kg body weight) during pregnancy, lactation, postlactation-I, and postlactation-II, relative to nonpregnant control values.

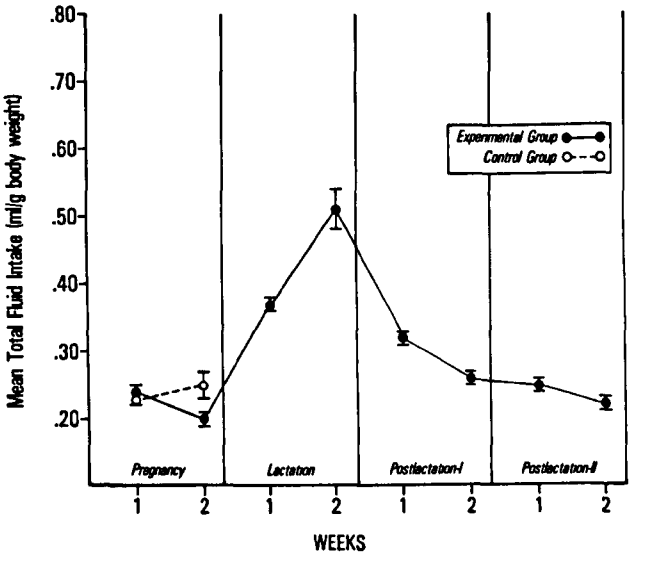

FIG. 3. The average (±SE) fluid intake (ml per kg body weight per day) as a function of time during pregnancy, lactation, postlactation-I, and postlactation-II, relative to nonpregnant control values.

these three groups from Week 1 to Week 2. As seen in Fig. 1, from Week 1 to Week 2 there was a decline in the preference ratio of the lactating mice and a sharp increase in preference during postlactation-I, compared to the moderate increase in the preference of the nonpregnant controls. These shifts in alcohol preference over time and condition accounted for the significant Maternal Condition × Week interaction evident in the individual analyses comparing these two groups to the nonpregnant control, $F(,1,22)=8.08$, $p<0.01$ and $F(1,22)=4.38$, $p<0.05$, respectively.

Alcohol consumption (g ethanol per kg) was also found to vary with Maternal Condition and Week. Figure 2 depicts the drop in alcohol intake during pregnancy followed by a gradual increase in consumption during the lactation and postlactation-I periods. Consumption was significantly affected by Maternal Condition, $F(3,30)=19.62$, $p<0.001$, as well as the interaction with Week, $F(3,30)=7.31$, $p<0.01$. A main effect of Week failed to reach significance. Subsequent comparisons across maternal condition indicated that only alcohol consumption during pregnancy and lactation were comparable, while the remaining comparisons indicated that all other conditions were significantly different from one another. As compared to the nonpregnant controls, alcohol consumption was significantly reduced during pregnancy, $F(1,22)=25.84$, $p<0.001$ and lactation, $F(1,22)=8.62$, $p<0.01$, but was equivalent during both postlactation periods ($F<1$). In each ANOVA comparing the nonpregnant controls to each maternal condition, a significant Week effect was apparent only for the alcohol consumption during lactation, $F(1,22)=6.37$, $p<0.01$, and postlactation-I, $F(1,22)=19.31$, $p<0.001$, reflecting the significant relative increases in alcohol intake from the first to the second week. The relative decrease in alcohol consumption of the pregnant and postlactation-II mice across the two week period in contrast to the relative increase of the nonpregnant group was indicated by a significant Maternal Condition × Week interaction, $F(1,22)=11.41$, $p<0.01$, and $F(1,22)=11.02$, $p<0.01$, respectively.

Total fluid consumption (ml/g fluid intake) was also significantly altered by Maternal Condition, $F(3,30)=90.11$, $p<0.001$, and the interaction of Maternal Condition with Week, $F(3,30)=32.08$, $p<0.001$. As Fig. 3 illustrates, fluid consumption was dramatically increased during the first week and particularly during the second week of lactation. Again, a main effect of Week was absent. Subsequent comparisons across Maternal Condition revealed that the total fluid consumption during pregnancy, lactation, and the two postlactation periods were significantly different from each other, with the exception of pregnancy and the postlactation-II period. As expected, total fluid consumption significantly exceeded the nonpregnant control values during lactation and the postlactation-I period, $F(1,22)=94.28$, $p<0.001$ and $F(1,22)=13.82$, $p<0.001$, respectively. Fluid intake levels of pregnancy and the postlactation-II period were equivalent to those of the nonpregnant group. The relative increase in fluid requirement of lactation from Week 1 to Week 2 accounted for the only significant Week effect among the various individual control comparison ANOVAs, $F(1,22)=26.07$, $p<0.001$, while the greater degree of this increase, relative to control increases, accounted for a significant interaction of Maternal Condition with Week, $F(1,22)=12.52$, $p<0.001$. Similarly, comparison of the relative increase in fluid consumption from Week 1 to Week 2 in the nonpregnant mice in contrast to the relative decrease in fluid intake from Week 1 to Week 2 of the pregnant, postlactation-I, and postlactaion-II mice, produced significant Mater-

nal Condition × Week interactions, F's(1,22)=8.53, 13.49, and 4.85, p's<0.01, 0.001, and 0.05, respectively.

DISCUSSION

The results of the present investigation indicate that voluntary alcohol consumption decreased significantly during pregnancy and lactation in C57Bl mice, but returned to strain-typical high levels [21] following cessation of nursing. With regard to pregnancy, the decrease in alcohol intake observed was evident regardless of whether the data were expressed as a preference ratio or as a function of body weight. These results are in agreement with those of previous studies which have reported decreased alcohol consumption during pregnancy in humans [7, 18, 19], as well as in subhuman species [2,4].

The reduction of alcohol intake observed during the pregnancy period cannot be explained by a general reduction in fluid consumption. No difference existed between the total fluid intake of the nonpregnant controls and that of the pregnant mice. Thus, it appears that while alcohol intake declined, water intake increased proportionally. The ability of C57BL mice to regulate absolute fluid intake by shifting their "preference" from alcohol to water during pregnancy is a very interesting finding that, to our knowledge, has not been reported previously.

Alcohol consumption was significantly reduced from nonpregnant control levels during lactation, as well. These results confirm earlier reports of reduced alcohol preference in lactating hamsters [2] and mice [22]. It is interesting to note that the alcohol preference ratio during lactation was significantly lower than it was during pregnancy, but absolute levels of alcohol (i.e., g alcohol/kg) were similar for these two periods. It appears, then, that the lactating mice met the physiological fluid demand of lactation by consuming more water, which resulted in a lower preference ratio, but did not affect relative alcohol consumption. Although alcohol intake was depressed during pregnancy and lactation, the amount consumed still was higher than that reported for many strains of mice [21]. It is possible that mice, like non-alcoholic women, reduce their alcohol intake during pregnancy in direct proportion to pre-pregnancy levels [18].

That alcohol intake was restored to control levels in the postlactation period indicates that the reduction of alcohol consumption observed during pregnancy and lactation was not permanent. In fact, absolute alcohol intake returned to baseline level within the first two-week postlactation period (i.e., postlactation-I), while alcohol preference ratio was similar to controls by postlactation-II.

Taken together, the results of this study tend to support a "feto-protective" hypothesis [7, 8, 17] in that alcohol intake was reduced during the time the fetus or neonate would have been exposed to the drug. However, the mechanism underlying this phenomenon remains to be elucidated.

It is likely that hormones may mediate the decline in alcohol consumption, since pregnancy and lactation are characterized by reliable hormone fluctuations [15]. That pregnancy is accompanied by alterations in taste for various substances has been well documented in humans [7,8] as well as in animals [14]. Estrogen seems to be a reasonable candidate. Studies in human females [9] and animals [1,5] have reported an inhibitory effect of elevated estrogen levels on alcohol consumption, but prolactin may also play a role, especially during lactation. Additional studies are needed to confirm or refute these speculations.

In summary, this study revealed a pronounced depression in the alcohol consumption of pregnant and lactating C57BL mice, which returned to control values within four weeks following removal of nursing pups. These results have implications for research relying on the liquid diet method of administering alcohol to pregnant animals. Since the liquid diets are typically provided as the sole source of food and water, a decrease in caloric consumption necessarily accompanies a decrease in alcohol consumption. As such, the need to use isocaloric pairfed control groups becomes even more apparent, as well as the need to include an ad lib chow control group to assess the effects of this restricted caloric intake on the variable under study. This animal model promises to be an excellent one for future studies investigating the mechanisms underlying the "feto-protective" hypothesis.

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