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Ethanol Elimination in Males and Females: Relationship to Menstrual Cycle and Body Composition

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Ethanol pharmacokinetics were determined following oral ethanol, 0.5 gm per kg, in nine normal women and 10 normal men, and related to total body water measured by ³H-water dilution and body fat determined anthropometrically. Ethanol pharmacokinetics were similar in the females throughout the menstrual cycle. No variation was seen in mean peak blood ethanol concentration or elimination rate in the midfollicular (Days 8 to 10) and midluteal (Days 22 to 24) phases. Mean peak blood ethanol values were significantly higher in females (88 ± 3 mg per 100 ml) than in males (75 ± 4 mg per 100 ml) ($p < 0.05$), and the mean area under the ethanol concentration-time curve was significantly greater in females (241 ± 12 mg·hr per 100 ml) than in males (177 ± 11 mg·hr per 100 ml) ($p < 0.001$). There was no significant sex difference in mean ethanol elimination rates. The mean apparent volume of distribution of ethanol in female (0.59 ± 0.02 liter per kg) was less than in males (0.73 ± 0.02 liter per kg) ($p < 0.001$). Both apparent volume of distribution of ethanol and area under curve were significantly correlated with total body water suggesting that the sex differences in ethanol pharmacokinetics were due to sex differences in body water content. The sex differences in ethanol pharmacokinetics may partly explain reports of male-female differences in the natural history of certain ethanol-related disorders.

Female alcoholics show significantly greater morbidity and mortality than do male alcoholics (1, 2). These differences are largely unexplained but may relate, at least in part, to sex differences in ethanol pharmacokinetics. It is known that women develop higher blood ethanol levels than men after the same dose of ethanol even when the dose is adjusted for body weight (3-5). This finding has been explained on the basis of sex differences in body composition, but this assumption is unproven. Changes occurring in ethanol pharmacokinetics during the menstrual cycle are also important as they might influence male-female comparisons. In one study, ethanol absorption and peak blood ethanol levels increased premenstrually (5), whereas another study reported lower peak blood ethanol concentrations and slower ethanol elimination premenstrually (6). In the present study, we have compared ethanol elimination in normal men and women and have related differences to

measurements of body composition. We have also sought evidence of menstrual variation in ethanol pharmacokinetics.

SUBJECTS AND METHODS

The study population comprised nine women with regular menstrual cycles of mean age 30 years (range 18 to 41 years) and 10 males of mean age 28 years (range 21 to 40 years). None of the subjects was on regular medication, in particular none of the women were taking oral contraceptives. All subjects drank socially, but their average daily ethanol intake was less than 10 gm per day. No ethanol was consumed for 72 hr before each study.

Female subjects were studied on two occasions, once in the midfollicular phase of their menstrual cycle (Days 8 to 10) and once in the midluteal phase following ovulation (Days 22 to 24). Male subjects were studied on one occasion only, as ethanol pharmacokinetics in men have been shown to be reproducible (5).

ETHANOL PHARMACOKINETICS

Following an overnight fast, subjects were given a light standard breakfast, followed 2 hr later by a 25% v/v

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solution of ethanol, 0.5 gm per kg body weight, in orange juice which was drunk over 1 min. Blood ethanol concentrations were then estimated every 15 min by breath analysis using a fuel cell alcometer (Model AE-D1, Lion Laboratories, Barry, Wales). High correlation between breath ethanol analysis using this instrument and blood ethanol measurement by gas-liquid chromatography ($r > 0.98$) has been reported (7). Measurements continued until the blood ethanol level had fallen to approximately 10 mg per 100 ml. Subjects fasted throughout the study and remained semi-recumbent.

BODY COMPOSITION

Total body water was measured within 24 hr of each pharmacokinetic study using a ^3H -water dilution method. One hundred microCuries of ^3H -water was drunk in 150 ml of water and a single blood sample taken 4 hr later. The water was extracted from 1 ml plasma by cold distillation, added to 10 ml of a toluene-based scintillant, and counted with an external standard in an LKB β -counter. The ^3H -water in the external standard was prepared from the same stock solution as the dose given to the subject. The volume of total body water was calculated from the ratio of radioactivity in the external standard to that in the subject's sample. The coefficient of variation of this estimation of body water performed on six occasions from one blood sample was 1.4%.

Body fat content was determined anthropometrically by the technique of Durnin and Womersley (8) which involved measurement of skinfold thickness at triceps, biceps, subscapular, and suprailiac sites. The accuracy of this method is to within $\pm 5\%$ of body weight as fat for subjects of average build.

CALCULATIONS AND STATISTICAL ANALYSIS

The ethanol concentration-time curves were analysed using zero order kinetic analysis (9). Ethanol elimination is best described by Michaelis-Menten kinetics (10-12), but either method of analysis give comparable results (12). The slope of ethanol elimination was measured by linear least-squares regression analysis of the linear portion of the elimination profile. The data points in the distribution phase of the ethanol concentration-time profile were not included in the regression analysis. The first point used for analysis varied according to the shape of the curve but in all subjects appeared 60 to 120 min after the time of peak ethanol concentration. The derived ethanol concentration at the start of ethanol administration (C_0) was determined from the y-intercept of the regression line, the apparent volume of distribution of ethanol (V_D) by dividing the total dose by C_0 and the ethanol elimination rate from the product of the slope and V_D . The area under the concentration-time curve (AUC) was calculated using the trapezoidal method measuring from the moment of ethanol administration to the time at which breath ethanol reached 9 to 12 mg per 100 ml. The AUC beyond this point represents less than 5% of the total AUC.

Statistical comparisons of variables between males and females and in females within the menstrual cycle were made by unpaired and paired Student's *t* tests, respec-

tively. In the females, 95% confidence limits of the mean differences between values for each pharmacokinetic parameter in each half on the menstrual cycle were determined using the Minitab Statistical Package. Relationships between pharmacokinetic parameters and body composition were determined by linear regression analysis with partial correlation methods (13) being used to assess the relative importance of the body composition variables. Analysis of covariance (13) was used to compare these relationships in males and females.

RESULTS

All subjects had normal hematological and biochemical values. Serum progesterone values were elevated (>20 nmoles per liter) in the second half of the menstrual cycle in all women, indicating that ovulation had occurred. Mean (\pm S.E.) body weight in the women increased insignificantly from 58.9 ± 2.2 kg in the follicular phase to 59.2 ± 2.2 kg in the luteal phase.

INFLUENCE OF MENSTRUAL CYCLE ON ETHANOL PHARMACOKINETICS

The mean ethanol concentration-time profiles in both halves of the menstrual cycle were similar (Figure 1), and the mean pharmacokinetic parameters did not differ significantly, (Table 1). Similarly, no significant differences were observed in mean total body water during the follicular phase (30.6 ± 1.0 liters) or luteal phase (29.9 ± 1.0 liters) of the menstrual cycle.

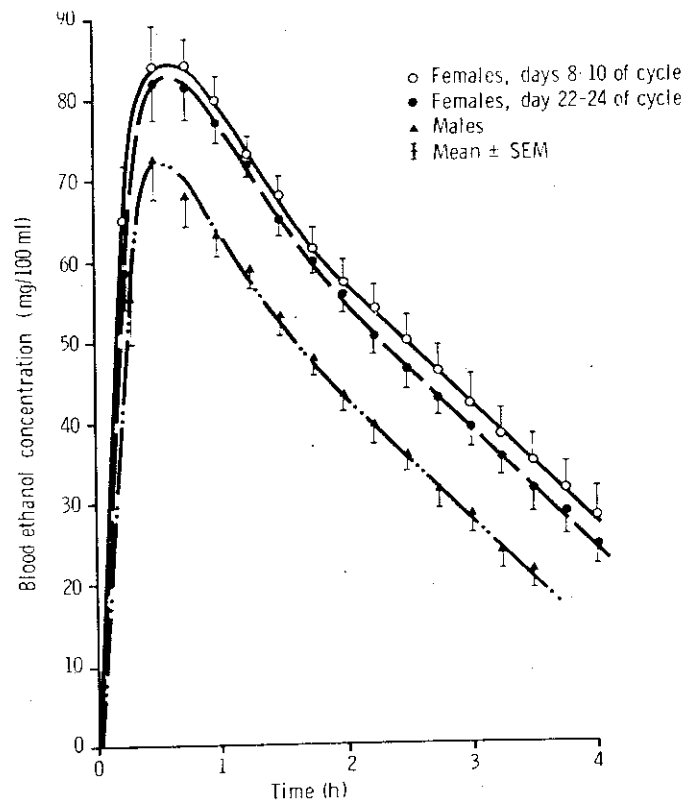


FIG. 1. Mean ethanol concentration-time profiles in females and males after 0.5 gm per kg oral ethanol.

MALE-FEMALE DIFFERENCES IN ETHANOL PHARMACOKINETICS

The data from the two female studies were averaged before comparison with the males. The mean ethanol concentration-time curves in males and females showed significant differences (Figure 2). The mean time to reach peak ethanol concentration and mean ethanol elimination rates were not significantly different between the sexes, but the mean peak ethanol concentration and the mean AUC were significantly greater in the females (Table 2). The mean V_D was significantly less in the females than in the males (Table 2). The correlation

coefficient for the slope calculation of ethanol concentration vs. time in each study was always greater than 0.99.

BODY COMPOSITION IN MALES AND FEMALES

In the males, mean body water content expressed as a percentage of body weight ($65 \pm 2\%$) was significantly greater than in the females ($51 \pm 2\%$) ($p < 0.001$). Conversely, mean body fat expressed as a per cent of body weight was significantly less in the males ($14 \pm 2\%$) than in the females ($25 \pm 1\%$) ($p < 0.001$).

A significant correlation existed between V_D and total body water for the group as a whole (Figure 2) and for males ($r = 0.99$) and females ($r = 0.86$) analyzed separately. The slopes of the correlations were not signifi-

TABLE 1. PHARMACOKINETIC DATA (MEAN \pm S.E.) IN NINE NORMAL WOMEN AFTER ORAL ETHANOL (0.5 GM/KG)

	Phase of menstrual cycle		95% CL ^a of mean difference between each parameter
	Days 8-10	Days 22-24	
Time to peak ethanol (min)	48 \pm 5	41 \pm 6	-7, 24
Peak ethanol (mg/100 ml)	90 \pm 4	86 \pm 3	-7, 15
Elimination rate (mg/hr/kg)	84 \pm 4	89 \pm 4	-11, 2
V_D^b (liter/kg)	0.59 \pm 0.02	0.60 \pm 0.01	-0.04, 0.04
AUC ^c (mg·hr/100 ml)	251 \pm 13	240 \pm 12	-0.50, 21.00

^a CL, confidence limits.

^b V_D , apparent volume of distribution of ethanol.

^c AUC, area under the ethanol concentration-time curve.

TABLE 2. PHARMACOKINETIC DATA (MEAN \pm S.E.) IN MEN AND WOMEN AFTER ORAL ETHANOL (0.5 GM/KG)^a

	Females (n = 9)	Males (n = 10)
Time to peak ethanol (min)	44 \pm 4	38 \pm 6
Peak ethanol (mg/100 ml)	88 \pm 3	75 \pm 4*
Elimination rate (mg/hr/kg)	87 \pm 4	97 \pm 5
V_D^b (liter/kg)	0.59 \pm 0.01	0.73 \pm 0.02**
AUC ^c (mg·hr/100 ml)	241 \pm 12	177 \pm 11**

^a Male-female differences; * $p < 0.05$; ** $p < 0.001$.

^b V_D , apparent volume of distribution of ethanol.

^c AUC, area under the ethanol concentration-time curve.

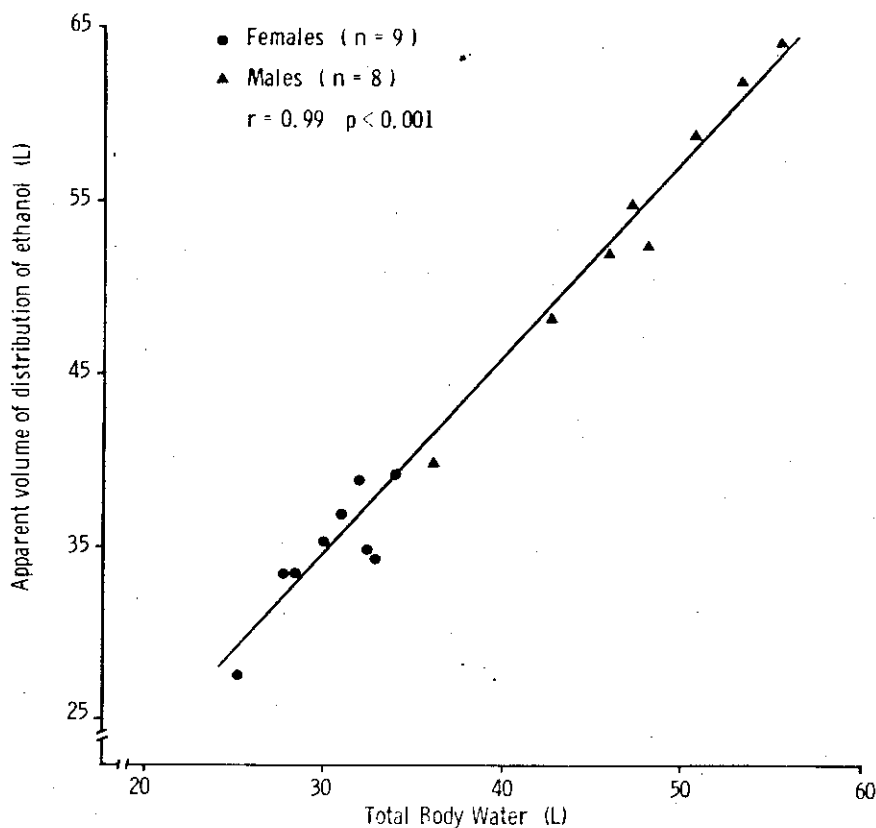


FIG. 2. Correlation of apparent volume of distribution of ethanol and total body water in females and males.

cantly different between the sexes (females: slope = 1.05; males: slope = 1.25; $F = 0.75$, $df = 1,13$, NS). There was no significant difference in mean V_D between males and females when values were adjusted for sex differences in total body water ($F = 1.0$, $df = 1,14$, NS). A significant correlation between V_D and body fat content was not observed ($r = -0.28$, $n = 19$, NS).

There was a significant negative correlation between AUC and total body water for all subjects (Figure 3). The slopes of the correlations were not significantly different between males and females (female: slope = -5.37 ; males: slope = -4.09 ; $F = 0.1$, $df = 1,13$, NS). There was no significant difference in mean AUC when adjusted for sex differences in body water ($F = 0.001$, $df = 1,14$, NS). AUC also correlated significantly with body fat content ($r = 0.69$, $n = 19$, $p < 0.01$). However, using partial correlation methods in which total body water was held constant, the correlation coefficient between AUC and body fat was close to zero. Thus, the relationship between AUC and fat content occurred only because total body water and body fat were inversely related.

These analyses suggest that the sex differences in V_D and AUC can be accounted for by differences in body water content. There were no significant correlations between other pharmacokinetic parameters and body water or body fat.

DISCUSSION

There are conflicting reports of variation in ethanol pharmacokinetics during the menstrual cycle in normal women (5, 6). In the present study, however, the various ethanol pharmacokinetic parameters did not differ significantly during the cycle.

Jones and Jones (5) reported that women absorbed ethanol more rapidly and achieved significantly higher peak ethanol levels premenstrually. These findings are not easily explained. First, gastrointestinal transit is reduced by 25% premenstrually (14) with the result that peak blood ethanol levels, which correlate with speed of

gastric emptying (15, 16), should if anything, decrease rather than increase. Second, fluid retention is common premenstrually and as blood ethanol values relate to total body water, lower rather than higher blood ethanol values might be expected at this time. Zeiner and Kegg (6), in contrast, showed that peak blood ethanol concentrations following a single oral dose of ethanol were lower premenstrually. The mean premenstrual weight gain in their subjects was only 1.4 lb which is unlikely to represent significant fluid retention. However, it is difficult to comment further on the significance or extent of fluid retention in these subjects in the absence of a direct measure of body water. In our study, female subjects did not show significant weight gain or change in total body water during the second half of the cycle; thus fluid retention was negligible and no significant change in peak ethanol concentration was observed.

Significant differences in ethanol elimination rates during the menstrual cycle were not observed in our study nor by Jones and Jones (5). Zeiner and Kegg (6), however, showed that the ethanol elimination rate significantly decreased premenstrually in normal women. This may, in part, have been artifactual as the elimination rates were determined using blood ethanol values measured during the first 2 hr after peak ethanol concentration was reached. Both distribution and elimination of ethanol are occurring during this period, so that the elimination profile is frequently nonlinear (17). Linear analysis of this part of the curve may, therefore, have biased the results.

It is difficult to make direct comparisons between the present and previous studies (5, 6) because of differences in the timing of studies within the menstrual cycle. In our study, comparisons were made in ethanol kinetics between Days 8 to 10 and Days 22 to 24 of the menstrual cycle, while Jones and Jones (5) compared kinetics on Days 1 to 3, 13 to 18, and 21 to 28 and Zeiner and Kegg (6) compared kinetics on Days 1 and 24. Nevertheless, major discrepancies exist that cannot be attributed solely to differences in the timing of studies. In all three studies, ethanol kinetics were measured around Day 24 of the cycle yet with very different results. Jones and Jones (5) reported values higher than at other times in the cycle, Zeiner and Kegg (6) values lower than at other times while we report values unchanged from the first half of the cycle.

It may be argued that because of the relatively small size of the female group in our study, significant intramenstrual variations in pharmacokinetic parameters may have been missed by chance. However, the true average effect of the menstrual cycle, assessed by 95% confidence limits for the mean difference between each parameter, lay in general between a 15% increase and a 10% decrease in the 8- to 10-day value. Any alteration observed within this range is within acceptable biological variation and is probably of little significance. It is unlikely, therefore, that the present study would have failed to detect, by chance, the mean increase of 25% in peak ethanol concentration observed premenstrually by Jones and Jones (5) or the mean decrease of 17% observed by Zeiner and Kegg (6). The AUC is a much more reliable

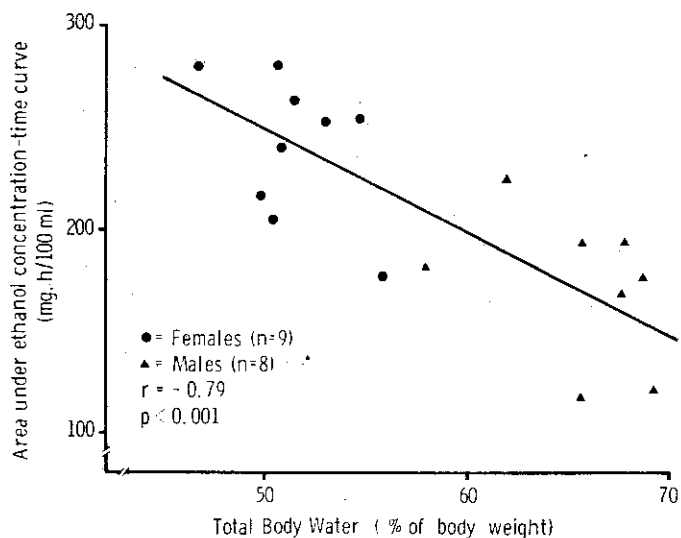


FIG. 3. Correlation of area under ethanol concentration-time curve and total body water content in females and males.

measure of systemic exposure to orally ingested alcohol than peak ethanol concentration and in our study this did not change significantly between the two halves of the menstrual cycle. Values for AUC were not reported in previous studies (5, 6).

The major sex difference in ethanol pharmacokinetics observed in the present study was that AUC, which is a measure of mean blood ethanol concentration, was 36% greater in females than in males. This implies that systemic exposure to ethanol following oral administration of a standard dose is considerably greater in females. The sex difference in AUC may have been due to male-female differences in oral bioavailability of ethanol or to differences in its volume of distribution.

Elimination of a few drugs including ethanol can be described by Michaelis-Menten Kinetics (18). For such drugs, the AUC may be related to the square of the systemic dose and inversely related to the volume of distribution (12, 18). A previous comparison of ethanol pharmacokinetics after oral and peripheral intravenous dosing in normal males has shown that oral dosing provides a reliable estimate of these two pharmacokinetic parameters. Oral bioavailability was close to 100%, and the two estimates of volume of distribution were very similar (12). In addition, the rates of absorption in the present study, as judged by the time to peak ethanol concentration were very similar in males and females. These observations indicate that the sex differences in AUC were due to male-female differences in V_D rather than to differences in bioavailability.

No significant sex difference in ethanol elimination rate was found in the present study confirming the findings of previous studies (5, 17). This is not surprising, as in humans, there are no major sex differences in the activity of the main ethanol-metabolizing enzyme, alcohol dehydrogenase (19). Studies in which women have taken oral contraceptives have shown a significant slowing of the ethanol elimination rate (6, 20), indicating that exogenous sex steroid hormones may influence ethanol pharmacokinetics. Animal studies however, show that estradiol, administered to female rats, does not alter ethanol elimination despite causing an increase in total activity of alcohol dehydrogenase (21). In contrast, the androgen dihydrotestosterone may competitively inhibit ethanol oxidation by alcohol dehydrogenase and decrease the rate of ethanol elimination in the male rat (22). Such studies only emphasize the wide interspecies variation in the elimination of xenobiotic compounds.

The present study has shown that V_D and AUC are significantly correlated with measurements of body composition. Although total body water and body fat were inversely correlated, the partial correlation analysis showed that the sex differences in V_D and AUC were significantly associated with total body water but not with body fat. The analysis of covariance also suggested that body water was the primary factor determining the sex difference in ethanol distribution.

Female alcoholics tend to drink less ethanol than do male alcoholics in absolute terms (23, 24) but the amounts taken per kilogram of body weight may be similar (23, 25). If ethanol intake adjusted for body

weight is similar in males and females, then toxic sequelae might be expected to occur earlier or with less ethanol in the female alcoholic. There is some evidence to support this suggestion particularly when liver disease is considered (1, 2, 26-28). Pequignot, for example, has shown that the risk of developing cirrhosis increases in women with ethanol intakes of 20 gm daily compared with 60 gm daily in men (28).

Thus, ethanol elimination does not vary significantly during the menstrual cycle. Male-female differences in ethanol pharmacokinetics, namely the lower mean V_D and higher mean AUC in women, are closely associated with sex differences in body water content. These differences in ethanol distribution may partly explain the reported differences in morbidity and mortality between male and female alcoholics.

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