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A PILOT STUDY OF ALCOHOL EXPOSURE AND PHARMACOKINETICS IN WOMEN WITH OR WITHOUT CHILDREN WITH FETAL ALCOHOL SYNDROME

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Abstract — **Aims:** To determine the alcohol exposure and pharmacokinetics of alcohol in a group of women who had given birth to children with FAS, compared with women who had not given birth to FAS children. **Methods:** 10 women who had given birth to FAS children (FAS mothers) and 20 Controls were studied to determine how they metabolize alcohol in a single limited-access quasi-experimental session of voluntary consumption of alcohol. They had free choice in the consumption of any amount of their favourite beverage for ~2.5 h, but their drinking was terminated if the breath alcohol concentrations (BrAC) exceeded 150 mg%. BrACs was measured during ethanol consumption and for several hours after, for estimation of alcohol exposure and pharmacokinetics. **Results:** FAS mothers consumed significantly larger amounts of alcohol, and achieved significantly higher peak BrAC levels than Controls. The rate of decline of alcohol from the circulation (β-60) showed a 2-fold variation across subjects but there was no significant difference between the two groups. **Conclusions:** This study did not show any difference in alcohol pharmacokinetics in free-choice drinking by non-pregnant women, who either have given or have never given birth to FAS children. However, mothers of FAS children tend to consume more alcohol per session. Future studies in larger samples will be needed to confirm these findings and to examine drinking patterns and other factors that may increase the risk of FAS in children of women who drink alcohol during pregnancy.

INTRODUCTION

Mothers who drink heavily during pregnancy have an increased risk of bearing children with fetal alcohol syndrome (FAS) or other alcohol-related birth defects (Jones *et al*., 1973; Clarren and Smith, 1978; Warren and Foudin, 2001). Characteristic features of FAS include facial anomalies, growth retardation and neurodevelopmental abnormalities. The prevalence of FAS was recently estimated to range from 0.2 to 2.0 per 1000 live births in the US and other countries in the western world (May and Gossage, 2001). However, not every woman who drinks alcohol during pregnancy will give birth to children with FAS or other alcohol-related problems. Therefore, the factors that increase the risk for pregnant women who drink of having children with FAS is a key issue. Epidemiological studies consistently reveal that factors contributing to increased risk include older maternal age, lower socio-economic status, African-American ethnicity, high parity, poor nutrition as well as genetic factors, maternal drinking patterns and maternal alcohol metabolism (Sokol *et al*., 1986; Ernhart *et al*., 1987; West *et al*., 1990; Abel *et al*., 1995; Maier and West, 2001; Warren and Foudin, 2001).

With regard to drinking patterns, it is the high number of drinks consumed during binge-drinking sessions, producing a high peak alcohol concentration, that appears to be a greater risk factor for prenatal injury from alcohol, rather than average daily quantitities. Early estimates indicated that consumption of 42 standard drinks per week (one standard drink contains 15 g ethanol) around conception was the threshold for FAS in humans (Sokol *et al*., 1988) even though some children exposed to these levels *in utero* did not exhibit FAS features (Sokol *et al*., 1986). More recently, Jacobson *et al*. (1998) showed that drinking, expressed as standard drinks per drinking occasion, was more informative than standard drinks per week, and found deficits in infant performance at maternal drinking levels of five standard drinks per occasion at least once per week. Other long-term studies have confirmed that children of binge-drinking mothers exhibit severe cognitive and behavioral deficits (Streissguth *et al*., 1990; 1994*a*,*b*; Maier and West, 2001).

The other major factor that determines the peak blood alcohol exposure to the fetus is the metabolic activity of the mother. Women who have children with FAS may metabolize alcohol at different rates than control mothers, which could help explain, at least in part, differences in alcohol exposure and ultimately risk of FAS. Studies by McCarver (1997, 2001) indicate that women who have faster rates of metabolism, as a result of a polymorphism in the gene for the ADH enzyme, have children with a lower prevalence of alcohol-related birth defects. There is however, a 2–4-fold variability in alcohol metabolism among individuals, and there are several genetic and environmental factors that underlie these differences, including genetic polymorphisms of the alcohol metabolizing enzymes, gender, age, sex hormones, body mass, liver size and food intake (Ramchandani *et al*., 2001a). Additional confounding factors include those that influence the absorption of alcohol, such as the rate of drinking, fed or fasted state, gastric emptying and first-pass metabolism. These factors determine the alcohol levels achieved *in vivo*, and thus the rate of alcohol metabolism (Gentry, 2000; Kalant, 2000).

Experimental animal studies involving mice, rats and monkeys have demonstrated that frequency, duration and level

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of exposure, as measured by blood alcohol concentration (BAC), are important determinants of the extent of damage (Webster, 1989; Driscoll *et al*., 1990; West *et al*., 1990). Animal studies have also found that binge-like drinking patterns, in which the fetus is exposed to high blood alcohol concentrations over relatively short periods of time, are particularly harmful—even if the amount of alcohol consumed overall is less than those of more continuous drinking patterns (Maier and West, 2001). In rats, it has been estimated that BAC levels > 100–150 mg%, increase the likelihood of brain damage and microencephaly (Samson and Grant, 1984). Subhuman primate models of FAS have also been developed (Altshuler and Shippenberg, 1981; Clarren and Bowden, 1982), and show that BACs of 200 mg% or more produced abortions in adult rhesus monkeys and levels below 150 mg% seemed to be compatible with these animals carrying their pregnancies to term or near-term. In humans, no such data have ever been obtained.

The objective of this study was to evaluate the pharmacokinetics of alcohol following free-choice consumption of alcohol in women who have had children with FAS and in controls (women who have had no children with FAS) from the mixed ancestry (so-called 'Coloured') population of the Western Cape Province of South Africa. This population has been noted for a particularly high prevalence of FAS, with estimates ranging from 40.5 to 46.4 per 1000 children aged 5 to 9 years (May *et al*., 2000), and this study provided a unique opportunity to study women from this population who have and who have not had FAS offspring, to examine the hypothesis that differences in ethanol pharmacokinetics may be a contributing factor. To our knowledge, this is the first time that the pharmacokinetics of alcohol have been determined during free-choice drinking sessions in non-pregnant women who have given birth to FAS children and in control mothers who have never given birth to FAS children.

METHODS

Subjects

Ten women aged 21 to 40 years who had given birth to FAS children, and had continued to drink in a quantity, frequency and context similar to that during pregnancy, were recruited into the experimental group (FAS mothers). Twenty women, matched to the above in age group (range: 20–45 years), health status and drinking pattern (quantity, frequency and context) by history, but who had never given birth to FAS children, served as a control group (Control mothers).

The study was conducted in the Western Cape Peninsula, mainly on wine-producing farms in the Boland/Overberg region, South Africa, in the residences of the recruited women or in their usual drinking venues, mostly over a weekend (Friday or Saturday). The FAS mothers were recruited from a cohort of women whose children were diagnosed with FAS during an epidemiological study conducted in the Boland/Overberg region, South Africa, during 1997 and 1999. The ages of the index children were 7 to 13 years. Control mothers were recruited from the cohort of controls in the above mentioned epidemiological study. Women from both groups volunteered to participate in the study and provided informed consent (as described below), after which they underwent a medical evaluation to ensure that they qualified for inclusion in the study. Exclusion criteria were by medical history: cardiovascular and renal diseases including hypertension, asthma and other pulmonary disorders, liver disease, alcohol dependence by DSM III R criteria (American Psychiatric Association, 1987) and illicit drug use or abuse of any other medication. Cigarette smoking was not an exclusion criterion; all subjects were smokers.

Informed consent

Informed consent was obtained from all the women after recruitment to the study, including permission to obtain BrAC measurements and blood specimens that would be taken during the study. The Research Ethics Committee of the University of Cape Town Medical School approved the protocol and informed consent.

Subjects who were illiterate or who had received a rudimentary education had the protocol and informed consent explained to them in their home language, and then provided informed consent. If the prospective subjects wanted to have the consent form further examined by literate family members, they were afforded the opportunity to do so. To minimize interviewer bias and interpersonal differences, every aspect of the study was explained by one of the investigators (N.C.O.K.) in a language preferred by the subjects (English or Afrikaans, the commonly spoken languages in the geographical area of interest).

Experimental drinking sessions

Prior to the experimental session, all subjects underwent familiarization with the research team, consisting of the investigator (N.C.O.K.) and a trained nurse or medical student, as well as the study procedures including the use of the breathalyzer. They were acclimatized to the conditions of the study, i.e. free-choice drinking in the presence of the research team. The researchers were aware of which subjects were FAS mothers and which subjects were controls.

The subjects were requested to stop drinking alcohol 24 h before the day of the scheduled experiment. Prior to the start of the scheduled drinking session, a pregnancy test was performed for all subjects using the 'Precise Pregnancy Test' (Apotex S.A. Pharmaceutical Innovation, Meadowdale, South Africa). If any subject was found to be pregnant, she was excluded from the study after being counseled about the hazards of drinking during pregnancy. A baseline breath alcohol concentration (BrAC) was then obtained.

The subject(s) began drinking in their usual drinking context and environment; either at home or in a social drinking venue. The subjects had a free choice in the consumption of any amount of their favorite beverage for ~2.5 h, but their drinking was terminated if the BrAC exceeded 150 mg%. During the session, subjects were encouraged to continue their normal activities with the only requirement being a breath alcohol sample every 20–30 min. Food consumption was permitted *ad lib*.

BrAC measurements were obtained at 15 to 30 min intervals, using four identically calibrated Alco-Sensor IV breathalyzers (Intoximeter Inc., St Louis, MO). Subjects rinsed their mouths three times with carbonated mineral water before every individual BrAC measurement. Blood was drawn when BrACs had reached the maximum (the time at which the BrAC

decreased for the first time since drinking had stopped) and again after BrAC had reached 20 mg% or less. The experiment was terminated at this time. If subjects were unable to provide BrAC measurements at regular intervals, owing to sedation, attempts were made to wake them for a breath alcohol measurement at 30 to 40 min intervals. Plasma alcohol measurements were performed using a commercial kit (Abbott AXSYM System REA Ethanol) in the Pharmacology Department of the University of Cape Town Medical School. An additional sample of blood was drawn at the end of the study for a complete blood count and liver function profile: serum total protein and albumin, gamma glutamyl transferase (GGT), lactate dehydrogenase (sLD), aspartate transaminase (sAST) and alanine transaminase (sALT). A private pathology group (Penman Pathologists) in Cape Town performed complete blood count and liver function profile serum analyses.

Data analysis

The total volume and alcohol content of the beverages consumed by each subject during the drinking session were used to determine the total dose (in grams) of alcohol for each subject. The peak observed BrAC was also recorded. β-60 (in mg%/h) was estimated as the slope of the linear part of the descending limb of the BrAC vs time curve for each subject. The alcohol elimination rate was calculated as the product of β-60 and the volume of distribution of ethanol, which was assumed to be equal to total body water (TBW). TBW was estimated by the method of Watson *et al*. (1980). The pharmacokinetic measures were compared between the two groups using *t*-tests, with the level of significance set at 0.05. A power calculation was not performed in this pilot study.

RESULTS

A total of 10 FAS mothers and 20 Control mothers completed the study. The subject characteristics are summarized in Table 1. FAS mothers weighed less ($P = 0.03$) than the Control mothers. Nine of the 10 FAS mothers had one child with FAS and one had two children with FAS. The FAS mothers have between two and eight children, while the control mothers had between one and five children. Among the 10 FAS mothers, four had increased mean corpuscular volume (MCV) indices, three had decreased hemoglobin, two had increased GGT, two had increased sAST and five had increased sLD. Among the 20 Control mothers, two had decreased MCV, four had decreased hemoglobin, two had increased GGT and one had increased sLD. None of the subjects reported any concomitant medication use.

Pharmacokinetic results

The average duration of consumption was 1.5 h (range: 0.7–2.8 h) for FAS mothers and 1.2 h (range 0.5–2.6 h) for Controls. Table 2 shows the mean pharmacokinetic measures for the two groups. Complete data on dose and peak BrAC were available only for 8 FAS and 19 Control mothers. The other three subjects (two FAS mothers and one Control mother) had elevated BrAC levels (156–214 mg%) at the start of the experimental sessions. For these three subjects, no further drinking was allowed and the β-60 was estimated from the descending limb of their BrAC vs time curves.

As Table 2 indicates, FAS mothers voluntarily consumed significantly larger amounts of alcohol during the drinking session (mean ± SE: 54.3 ± 3.8 g; Controls: 42.8 ± 1.8 g; $P = 0.005$). FAS mothers showed a greater range in the dose and in the choice of beverage (four subjects drank wine, five drank beer, and one subject drank both). All but one of the control mothers drank beer. FAS mothers also showed higher peak BrACs compared to Control mothers (mean ± SE: 125 ± 8 mg%; Controls: 92 ± 5 mg%; $P = 0.0014$).

Comparison of disappearance rates (β-60) did not reveal any significant differences (mean ± SE: FAS mothers: 21.4 ± 1.3 mg%/h; Controls: 20.8 ± 0.8 mg%/h). Comparison of AER estimates also did not reveal significant differences (mean ± SE: FAS mothers: 5.5 ± 0.2 g/h; Controls: 6.0 ± 0.3 g/h).

Table 1. Subject characteristics

	FAS mothers ($n = 10$)	Control mothers ($n = 20$)
Age (years)	32.3 ± 5.0	30.4 ± 7.7
Height (cm)	156 ± 1.8	158 ± 1.1
Weight (kg)*	46.2 ± 2.2	55.9 ± 2.7
Total Body Water (l)**	26.0 ± 0.7	28.6 ± 0.7
Number of Smokers	10	20

FAS: Fetal Alcohol Syndrome. Age, height, weight and total body water are expressed as mean ± SD.
*FAS mothers vs Controls: $P = 0.0274$.
**FAS mothers vs Controls: $P = 0.0293$.

Table 2. Summary of pharmacokinetic results

	FAS mothers ($n = 10$)		Controls ($n = 20$)	
	Mean ± SE	Range	Mean ± SE	Range
Dose (g of ethanol)*	54.3 ± 3.8	40.8–66.0	42.8 ± 1.8	27.2–68.0
Peak BrAC (mg %)**	125 ± 8	82–161	92 ± 5	51–139
β-60 (mg %/h)	21.4 ± 1.3	16.4–30.7	20.8 ± 0.8	13.7–28.6
AER (g/h)	5.5 ± 0.2	4.5–6.6	6.0 ± 0.3	3.8–8.6

FAS: Fetal Alcohol Syndrome; BrAC: breath alcohol concentration; AER: alcohol elimination rate.
*FAS mothers ($n = 8$) vs Controls ($n = 19$): $P = 0.005$.
**FAS mothers ($n = 8$) vs Controls ($n = 19$): $P = 0.0014$. Note: Complete data on dose and peak BrAC were available for only 8 FAS and 19 Control mothers.

DISCUSSION

The objective of this study was to obtain preliminary data on the pharmacokinetics of alcohol in FAS mothers and in control mothers. Results revealed that FAS mothers voluntarily drank more alcohol over the 2.5 h experimental setting than the controls, and achieved higher peak BrACs. The β-60 did not differ significantly between groups, but there was a 2-fold variation within each of the groups. These values are higher than the values observed in previous studies for women (Mezey, 1976; Holford, 1987; Thomasson *et al*., 1995), and may be a result of the lower body weights and total body water of the subjects compared to the subjects in other studies. However, the alcohol elimination rate (AER) estimates are similar to those observed in BrAC clamp studies of Caucasian and African-American women (Kwo *et al*., 1998; Li *et al*., 2000; Li *et al*., 2001; Sato *et al*., 2001). Alcohol is predominantly metabolized by the alcohol dehydrogenase enzymes and follows Michaelis–Menten kinetics, although at the alcohol levels achieved in the women in this study, the enzymes would be essentially saturated and the elimination of alcohol would follow apparently zero-order kinetics at the maximum metabolic capacity (Vmax). The lack of differences in alcohol elimination rates at the BrAC levels achieved in this study suggest that the Vmax did not differ between the FAS mothers and controls. Differences between FAS mothers and controls in ADH Km, which would be apparent at lower alcohol levels, could not be evaluated in this study, since the last measured BrACs were higher than published estimates of Km (5–10 mg%).

This study has provided, for the first time, data on pharmacokinetic differences between FAS mothers and controls; however, it does have several limitations, including limitations of the study design, small sample size, recruitment issues and control of potential confounding factors. These are discussed below.

Most pharmacokinetic studies typically examine the absorption, distribution and elimination characteristics of the drug following a fixed dose. On the other hand, drinking studies typically evaluate the quantity and patterns of consumption during free-choice (unlimited) drinking, either in a naturalistic or a laboratory setting. Our study was a combination, and thus a compromise of both methods. In our study, we allowed a 2.5 h drinking session, with a limitation on drinking if the BrAC level exceeded 150 mg%, for ethical and safety-related reasons. As a result, the drinking pattern is not entirely 'free-choice', but we believe that if drinking were allowed without any limitations, BrAC levels much higher than the cut-off of 150 mg% would have occurred in some of the subjects. Indeed, the BrACs of the three subjects (two FAS mothers and one control) who came into the study with already elevated levels were greater than 150 mg% (156–214 mg%). An additional consideration is that the presence of the investigators and intermittent assessments might have somehow modified the quality or quantity of drinking. However, it would be reasonable to presume that any such effect would have influenced drinking in both subject groups. Moreover, all subjects were familiarized with the research team and the use of the breathalyzer, and acclimatized to their presence during the experimental sessions. While these factors do confound the interpretation of the total amount consumed, it does not detract from the finding that the FAS mothers all ingested more alcohol in the same time interval as the control mothers and achieved higher peak BrAC levels.

Another major limitation of this study is the small sample size. Given the known large variation in alcohol metabolism in humans, the lack of significant differences in alcohol disappearance rates could have been due to an insufficient number of subjects. Post-hoc analysis indicates that, given the variability in β-60 observed in this study, a sample size of 34 would be needed to observe even a 20% difference in disappearance rates between the FAS mothers and controls. Future studies will attempt to study alcohol metabolism in larger samples to more definitively demonstrate differences in β-60 between the two groups.

A third limitation is the possibility of sampling bias, in that the women who volunteered to participate in the study might be somehow different from the larger cohort. However, examination of the demographic and other characteristics of the subjects in this study indicated that they were fairly representative of the larger cohort. On the other hand, the FAS mothers in our study did have a lower body weight compared to the control mothers, suggesting a lower volume of distribution for alcohol (since alcohol distributes into total body water, which is proportional to body weight), which may underlie differences in peak BrAC levels reached, as well as disappearance rates.

Other potential confounds that were not accounted for in the analysis include differences in drinking history and drinking patterns, food intake as well as differences in nutritional status between the two groups. Drinking history remains a confounding factor in this study and a potential limitation in the interpretation of the findings. Attempts to assess drinking history in these populations have been made, but with only limited success, with authors indicating that alcohol drinking was likely under-reported in this population (May *et al*., 2000; Viljoen *et al*., 2002). The assessment of drinking history in this population is extremely difficult, because the subjects drink alcohol together in group settings, routinely sharing bottles of beer or wine, and could not themselves provide accurate estimates of the quantities they consumed. Another possible factor might have been differences in drinking pattern, however, it appeared that rapid drinking was the predominant pattern of drinking in both groups. This drinking practice is pervasive in these settings as it obviates sharing the alcohol with friends from adjoining farms and others in the group, who may not have contributed to the funds to buy alcohol, at that particular drinking session. Food intake results in a decrease in rate of absorption of alcohol, resulting in delayed and lower blood alcohol levels following oral ingestion (Sedman *et al*., 1976; Rogers *et al*., 1987; Watkins *et al*., 1993), and also results in an increase in the rate of elimination of alcohol (Ramchandani *et al*., 2001b). Food intake was not controlled in the current study, mainly due to the naturalistic experimental setting, however, the consumption of meals by subjects during the study session was documented. Almost all subjects (26 of 30) consumed a meal (usually a sandwich) ~0.5–1 h after alcohol drinking ceased, by which time BrACs would be expected to reach their peak, with no consistent differences in the number of FAS mothers and controls who consumed food, or in the composition or timing of the meal between the two groups. However, the influence of food intake on differences in the pharmacokinetics of alcohol in the two groups cannot be ruled out.

Differences in nutrition could influence the weight and metabolic activity of the subjects, however, the nutritional status of the subjects, for example dietary history, was not formally determined in this study. Examination of complete blood counts and liver function profiles, including serum total protein and albumin, indicated that FAS mothers had elevated MCV, decreased hemoglobin and increased GGT, sLD and sAST, which could be related to their heavy drinking pattern.

The women in this study were from the mixed ancestry (so-called 'Coloured') population of the Western Cape Province of South Africa, which represents the large majority of workers in the wine-producing and fruit-growing farms of the Western Cape Province (Croxford and Viljoen, 1999). Heavy alcohol consumption, mostly in social group settings, is a major form of recreational activity on the farms. This population has also been noted for a particularly high prevalence of FAS, with 15% of children in a pre-school day-care center and 27% of the children in a school for the mentally handicapped having FAS (May *et al*., 2000). It has been estimated that mothers who have given birth to FAS offspring have an average intake of seven drinks per drinking session in this population, although some mothers in this community have not given birth to FAS offspring despite drinking similar average amounts of alcohol (D.L.Viljoen, personal communication). This study represents probably the first attempt at examining differences in drinking and pharmacokinetics in these FAS mothers and controls, within their natural living environment and normal drinking context.

In summary, this preliminary study suggests that in a voluntary limited access experimental drinking setting, women who have had children with FAS drank more than women who have not had FAS children, and attained higher peak BrACs. No difference in alcohol elimination kinetics and alcohol elimination rate was observed between the groups. Mean peak BrACs exceeded 125 mg% in the FAS mothers, whereas the mean peak BrACs were considerably lower in the Controls (92 mg%). This level of ethanol exposure is consistent with exposures associated with FAS development in animal models. The preliminary data from this study emphasize the need for further research in larger samples, using different approaches to examine the determinants of increased alcohol exposure, including drinking patterns and pharmacokinetic differences, that increase the risk of FAS in children of mothers who drink alcohol during pregnancy.

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