PHARMACOKINETICS OF ETHANOL IN THE MATERNAL-FETAL UNIT

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ABSTRACT

Due to its wide range of deleterious effects on the unborn baby, knowledge on the disposition of ethanol in the maternal-fetal unit is critical. This review summarizes and updates the existing evidence on ethanol disposition in the mother, the placenta and the fetus, and relates them to their potential fetal effects.

Ethanol is widely used by women worldwide. Because of its major deleterious effects on the developing fetus, it is critical to consider its' disposition by the mother, placental and fetus. Women are different from men in ethanol disposition, pregnant women differ from non pregnant women in ethanol pharmacokinetics, and the different stages of pregnancy introduce yet more variability. Hence careful analysis of these variables is needed in order to understand fetal toxicology.

Absorption and Distribution

When alcohol-containing beverages are ingested orally, ethanol (ethyl alcohol, C_2H_5OH, Mw= 46 g/mol) is quickly absorbed through the mucosal surface by simple diffusion in the stomach (about 20% of the ingested ethanol) and in the upper portions of the small intestine (80% of the ingested ethanol).[1,2] Since ethanol absorption from the small intestine is much more rapid than from the stomach, the rate of gastric emptying is an important determinant of the rate of absorption. Thus, factors affecting gastric emptying time such as the presence and type of food, as well as other factors like the concentration of ethanol, rate of alcohol consumption, blood flow at the site of absorption, and mucosal integrity; can all influence the rate of absorption.[2,3]

Being a small, uncharged, water-soluble molecule, ethanol rapidly distributes and equilibrates throughout total body of water[4], which is approximately 50-60% of total body weight in males and 45-55% in females.[1] Thus, ethanol's volume of distribution is approximately 0.45 to 0.6 L/kg, and its distribution throughout the body is primarily dependent on the blood flow to different organs and tissues.[1] As expected, body composition (which is affected by factors like gender, age, and fitness) plays an important role in determining ethanol's volume of distribution in an individual. Typically, men, younger individuals, and leaner individuals have greater volumes of distribution compare to women, older individuals, and those with a greater percentage of body fat, respectively. Furthermore, physiological changes in body composition and total body of water that occur in pregnancy can greatly increase the volume of distribution of ethanol.[5]

Metabolism and Elimination

The vast majority of ingested ethanol is metabolized, with the remainder (2-10%) excreted unchanged in urine, breath and sweat.[1,3] Ethanol is metabolized by both oxidative and non-oxidative pathways (Figure 1), with approximately 85% of ethanol metabolized in the liver via enzymatic oxidation.[2] The bulk of oxidative ethanol metabolism involves ethanol's biotransformation to acetaldehyde (a highly reactive compound that can bind macromolecules and create adducts) by alcohol dehydrogenase (ADH), and the subsequent conversion of acetaldehyde to acetic acid by aldehyde dehydrogenase (ALDH).[1] This

process takes place primarily in the liver. During each of the two steps (i.e., ethanol to acetaldehyde, and acetaldehyde to acetate), a nicotinamide adenine dinucleotide (NAD+) is reduced.[1] The produced acetic acid enters Kreb's cycle where it is converted to water and carbon dioxide.[1] The rate-limiting step in this process is the oxidation of ethanol to acetaldehyde by cytosolic ADH, which has a low Km of 0.05-0.1 g/L.[1,4] The metabolism of ethanol follows Michaelis-Menten kinetics and exhibits zero-order elimination for a large part of the blood-concentration time course (BAC above 20 mg/dL) due to saturation of hepatic oxidative enzymes.[4] During zero-order elimination, ethanol is eliminated at a rate of approximately 7 g/hr in a 70 kg person.[2]

Multiple isoforms of ADH and ALDH with different kinetic profiles (grouped into different classes) and numerous polymorphisms lead to significant variation in human alcohol metabolism rates.[6] The variable expression of the different isoforms and polymorphisms may account for differences in tissue capacity to metabolize ethanol, differences in ethanol metabolism observed between individuals and between racial and ethnic groups, as well as, for variation in toxicities associated with drinking among different racial groups.[2,4,7,8] Class I ADH and Class II ALDH are considered the main isoforms responsible for ethanol metabolism in the liver -- the main site of oxidative ethanol metabolism.[4] These enzymes are also expressed extrahepatically but the contribution of these other sites to overall ethanol metabolism is considered minimal. Nonetheless, lower expression of gastric ADH in women is speculated to contribute to a higher ethanol bioavailability as compared to men.[2] Other gender differences include higher rates of hepatic metabolism of ethanol in females, which is speculated to be due to higher relative liver volumes.[9]

Two minor pathways also exist that metabolize ethanol oxidatively and these involve the microsomal ethanol-oxidizing system (cytochrome P450 2E1) and catalase. Since CYP2E1 in the liver is inducible and thus more active in chronic heavy drinkers, it accounts for increased ethanol metabolism at high alcohol concentrations observed in chronic drinkers.[2] Catalase, which is ubiquitously expressed, is thought to play a small role in overall ethanol metabolism, but may contribute to localized production of acetaladehyde and thus adduct formation in various tissues.[2] Non-oxidative biotransformation is a minor pathway of ethanol metabolism and involves the enzymatic conjugation of ethanol to endogenous substrates such as fatty acids, phospholipids, sulfate, or glucuronic acid. The derivatives of non-oxidative ethanol metabolism are termed fatty acid ethyl esters (FAEEs), phosphaditylethanol (PEth), ethyl sulfate (EtS), and ethyl glucuronide (EtG), respectively.[10,11] The esterification of ethanol with fatty acids is the best studied non-oxidative pathway to date. Many mammalian tissues and organs have been shown to possess the ability to synthesize FAEE upon ethanol exposure. A generally accepted terminology of FAEE-synthetic enzymes delineates two enzyme activities; FAEE synthase (FAEES) and acyl-CoA: ethanol *O*-acyltransferase (AEAT). The term FAEES is used to designate the FAEE-synthetic activity that uses ethanol and free fatty acids as substrates, while AEAT describes the FAEE synthetic activity that uses ethanol and fatty acyl-CoA as substrates.[12] FAEES activity has been purified from several tissues and shown to be associated with enzymes with other principal physiological functions, primarily in lipid metabolism (several lipases and carboxylesterases).[3] Conversely, several known enzymes have been shown to possess FAEES activity, including pancreatic lipases, lipoprotein lipase, hepatic carboxylesterase, and cholesterol esterase.[3] Ethanol conjugation to phospholipids is mediated by the action of phospholipase D (PLD); to glucoronic acid by the action of UDP-glucuronosyltransferases (UGT); and to sulfate by the actions of sulfotransferase.[13] It should be noted that oxidative and non-oxidative pathways are metabolically linked in that there is increased production of non-oxidative metabolites in instances where oxidative metabolism is deficient or inhibited. This is due to a shift from oxidative to non-oxidative pathways by substrate

loading.[14,15] This may be of interest since ADH deficiency in chronic alcoholics may be a determining factor for the increased body burden of ethanol and its disposition via non-oxidative metabolism.[14] Non-oxidative ethanol metabolites are also of particular interest due to their prolonged half-life in the body as compared to ethanol itself and its oxidative metabolites. For this reason, they have been investigated and used as biomarkers of ethanol consumption and chronic alcohol use.

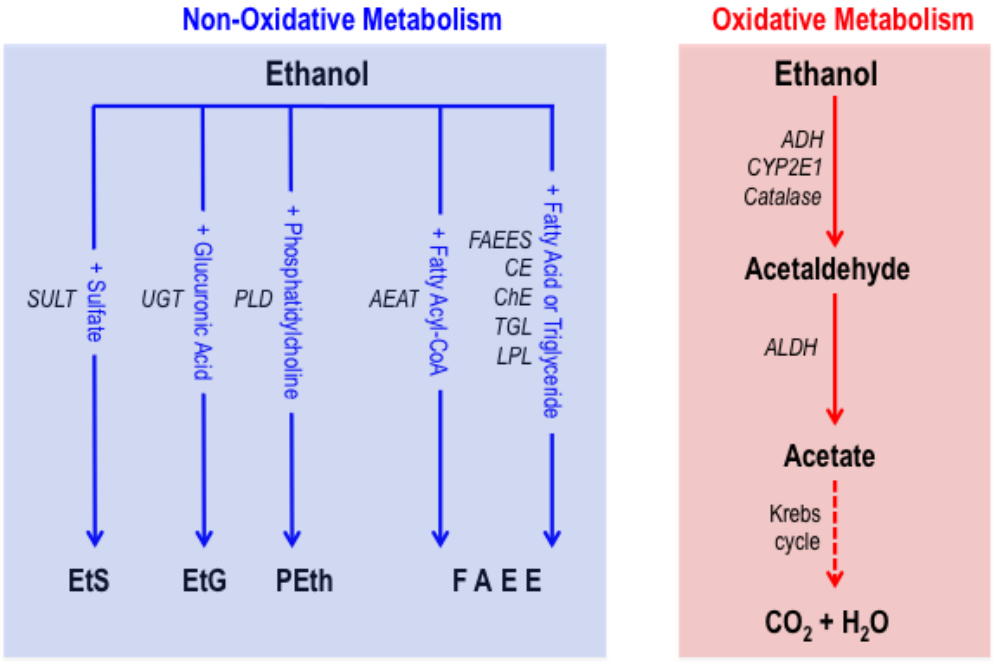

Figure 1. Oxidative and non-oxidative ethanol metabolism. FAEES and AEAT refer to enzymatic activity mediating FAEE synthesis from free fatty acids and fatty-acyl-CoA, respectively. Abbreviations: ADH, alcohol dehydrogenase; AEAT, acyl-CoA: ethanol O-acyltransferase; ALDH, aldehyde dehydrogenase; CE, carboxylesterase; ChE, cholesterol esterase or carboxylester lipase; CoA, coenzyme A; EtG, ethyl glucuronide; EtS, ethyl sulfate; FAEES, fatty acid ethyl ester synthase; FAEE, fatty acid ethyl esters; LPL, lipoprotein lipase; PEth, phosphatidylethanol; PLD, phospholipase D; SULT, sulfotransferase; TGL, triglyceride lipase; UGT, UDP-glucuronosyltransferase (Best and Laposate, 2003; Kalant and Khanna, 2007; Swift, 2003).

Fetal Exposure to Ethanol

Animal studies and clinical studies conducted in pregnant women demonstrated that ethanol readily crosses the placenta and rapidly distributes into the fetal compartment resulting in similar BACs in maternal and fetal circulations.[16-19] Of interest, Brien et al.,[16] who studied the disposition of ethanol in six healthy pregnant women at 16-18 weeks gestation, found that the maximal ethanol concentration was lower (2-fold) in the amniotic fluid than in maternal venous blood, but the rate of ethanol elimination from amniotic fluid was about half the elimination rate from maternal venous blood such that 3.5 hours later ethanol was still present in the amniotic fluid while being undetectable in maternal blood. Furthermore, the ethanol $AUC_{0-3.5\,h}$ in amniotic fluid was only 16% lower than the value for maternal blood. The authors suggested that following maternal ethanol consumption, amniotic fluid may act as reservoir

for ethanol exposing the fetus to ethanol for a longer time period.[16,20] Similar findings were reported in animal studies (in near-term ewe, guinea pigs and rats) that examined the disposition of ethanol and acetaldehyde.[18,21,22] These studies indicated that there is bidirectional placental transfer of ethanol in the maternal-fetal unit such that the ethanol concentration in maternal and fetal blood are similar, however, fetal exposure can be prolonged since the amniotic fluid serves as a reservoir for ethanol. In the last two trimesters of pregnancy, fetal exposure to ethanol in humans is further prolonged because of the recurrent cycle of fetal swallowing of the amniotic fluid, metabolism (which is low in the fetus), and elimination of urine back into the amniotic fluid.

Surprisingly little research has been done on the effects of physiologic and endocrine changes in pregnancy on maternal alcohol disposition, particularly on metabolism and clearance. Increase in the volume of distribution that occurs during pregnancy is expected to result in lower ethanol plasma concentrations after the same given dose. With respect to metabolism, in pregnant rats, increased alcohol clearance rate compared with weight-matched non-pregnant controls was observed.[23] Since the rate of alcohol clearance ultimately determines tissue ethanol concentrations, faster ethanol clearance may help minimize tissue concentrations and thus mitigate fetal alcohol effects.[24] The authors showed that the increased alcohol clearance was not due to changes in hepatic ADH1 activity and not due to changes in CYP2E1 activity, which was actually suppressed.[23] On the other hand, although hepatic ALDH enzyme activity was also not elevated, the amount of mitochondria in the liver was increased resulting in increased overall ALDH activity on a whole-liver basis. This increase in ALDH activity may increase alcohol clearance by accelerating acetaldehyde removal. Additionally, there was an up-regulation of gastric ADH4 activity in pregnant rats compare to controls suggesting that increased first-pass metabolism.[23] This upregulation may be mediated by hormonal changes in pregnancy. Little work has been published on how chronic ethanol intake during pregnancy can lead to changes in maternal metabolism.

The ability of the fetus to metabolize ethanol varies during development. At birth, the rate of ethanol elimination from neonatal blood is only about half of the elimination rate from maternal blood.[19] Studies indicate that early on, the fetus has a limited capacity for oxidative ethanol metabolism as evidenced by low hepatic ADH activity in fetuses and low expression of some ADH isozymes in first trimester fetuses.[25,26] Hepatic ADH activity appears to increase with gestational age, and studies report progressive expression of various ADH isoforms during development and tissue-specific changes in the relative amounts of expressed isozymes.[26] With regard to CYP2E1, although some groups did not find detectable expression in the fetus[27], others reported detectable CYP2E1 as early as 16 weeks gestation in fetal liver.[28] It appears that CYP2E1 expression increases with gestational age, being undetectable in first trimester liver samples, but reportedly found in 37% of second trimester, and majority (80%) of third trimester fetal liver samples tested.[29] Although this expression is relatively low in the fetus, it gradually increases after birth, reaching 30-40% of adult hepatic levels by one year of age.[25,27] Of interest, CYP2E1 expression in fetal brain was detected as early as 7-9 weeks gestation, which, given the relatively limited expression of ADH and ALDH enzymes in this tissue, may be sufficient to generate reactive intermediates that may mediate toxicity following maternal alcohol consumption.[30] In another study, CYP2E1 content in the placenta was found to be variable among heavy drinkers, suggesting that induction may be taking place.[31] The authors speculated that this inter-subject variability in induction may play a role in individual susceptibility to alcohol related defects. With respect to catalase, it has been shown that the activity of catalase in human fetal liver and kidney also increases in parallel with gestational age.[32] However, the authors found that in the fetal brain, catalase activity is relatively low and does not show any significant changes during gestation except for a decrease in activity later in

pregnancy (28 weeks and above). The significance of this finding is not clear and, overall, it is not known what role or contribution, if any, the ontogeny of catalase plays in fetal metabolism of ethanol and susceptibility to its negative effects during gestation given the minor role it plays in ethanol metabolism in adults.

With respect to non-oxidative enzymes, FAEES activity (FAEE synthesis from ethanol and free fatty acids) has been demonstrated in supernatant from tissue homogenates of human term placenta and mouse placenta (at gestational day 14), mouse embryo, primary cultures of rat fetal brain cells, fetal and postnatal rat brain, and in human fetal brain (2nd trimester) incubated with ethanol and oleic acid, demonstrating that the enzyme activity necessary for FAEE synthesis is present in fetuses and fetal brain tissue relatively early in gestation.[33,34] The ontogeny of various non-specific enzymes shown to possess FAEES activity, such as the different carboxylesterases and lipases, varies greatly depending on the enzyme, isozyme, and tissue of interest. The ontogeny of AEAT activity has not been studied and it is not known what specific enzymes possess this activity as of yet. The onset of hepatic UGT expression and activity (catalyze EtG formation) occurs after 20 weeks of gestation with significant increases in the first weeks of life[35], while some sulfotransferases (catalyze EtS production) appear to be widely expressed in the developing human, with most present at levels equivalent to or higher than the adult.[36] As with catalase, given their minimal contribution to overall ethanol metabolism in adults, it is unlikely that changes in the expression of non-oxidative metabolic enzymes during development play a significant role in overall fetal handling of ethanol. However, it is possible that the low expression of oxidative enzymes can shift ethanol metabolism towards non-oxidative pathways if they are expressed and active earlier in the gestational period, thus increasing their importance.

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