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MARKERS OF OXIDATIVE STRESS AND SYSTEMIC VASOCONSTRICTION IN PREGNANT WOMEN DRINKING ≥ 48 GRAMS OF ALCOHOL PER DAY

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Abstract

Background—The precise pathway by which alcohol causes the characteristic features of fetal alcohol spectrum disorders (FASD) is unknown. Proposed mechanisms for fetal injury from maternal alcohol use include cellular damage from oxidative stress and impaired fetal oxygenation related to maternal systemic vasoconstriction. Our objective was to compare levels of urinary markers of oxidative stress and systemic vasoconstriction between women consuming large amounts of alcohol during pregnancy and women who did not drink alcohol during pregnancy.

Methods—Pregnant women consuming ≥ 48g alcohol/day (n=29) on average and pregnant women who abstained from alcohol use (n=39) were identified using detailed interviews and home visits. Random maternal urine specimens were collected. Urinary levels of the oxidative stress marker, 8-isoprostane F2α, and of the vasoactive prostaglandin metabolites, 2,3-dinor-6-keto-prostaglandin F1α (a vasodilator) and 11-dehydro-thromboxane B2 (a vasoconstrictor), were measured using mass spectrometric methods. All analyte levels were corrected for urinary creatinine.

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Results—In crude analyses, there was no significant difference in 8-isoprostane F2α between pregnant drinkers and nondrinkers (2.16 vs. 2.08 ng/mg creatinine respectively, P=.87). There were no significant differences between the drinking and non-drinking groups in levels of 2,3-dinor-6-keto-prostaglandin F1α (1.03 vs. 1.17 ng/mg creatinine repectively, P=.50), 11-dehydro-thromboxane B2 (0.72 vs. 0.59 ng/mg creatinine respectively, P=.21), or the ratio of vasodilatory metabolite to vasoconstrictive metabolite (1.73 vs. 2.72 respectively, P=.14). Adjusting for maternal age, marital status, smoking, and gestational age at sampling did not substantially alter the results.

Conclusion—Our results show no difference in levels of urinary eicosanoid markers of oxidative stress and systemic vasoconstriction between pregnant women who drink heavily and pregnant women who abstain. These findings speak against a role for maternal oxidative stress or systemic vasoconstriction in the pathogenesis of alcohol damage to the fetus.

Keywords

Alcohol; Pregnancy; Isoprostanes; Prostacyclin; Thromboxane; Fetal Alcohol Spectrum Disorders

INTRODUCTION

The fetal alcohol spectrum disorders (FASD) comprise a range of developmental abnormalities among children exposed to alcohol in utero. Fetal alcohol syndrome (FAS) is characterized by pre- or postnatal growth restriction, facial dysmorphology, and neurodevelopmental disability (Centers for Disease Control, 2002a; Centers for Disease Control, 2002b; Committee on Substance Abuse and Committee on Children With Disabilities, 2000). While the precise mechanism by which alcohol causes the characteristic features of FAS is unknown, a number of potential pathways have been proposed (West, 1994).

Ethanol consumption promotes systemic oxidative stress in humans, and some authors suggest that alcohol-related fetal brain injury may be a result of cellular damage from oxidative stress and reactive oxygen species (Cohen-Kerem and Koren, 2003; Guerri et al., 1994). Others theorize that abnormal brain development and intrauterine growth restriction may arise from impaired placental oxygen and nutrient delivery due to ethanol-induced vasoconstriction in the umbilical vessels and placental bed (Siler-Khodr et al., 2000; West, 1994).

The F2-isoprostanes are a class of prostaglandin-like compounds that are considered the best markers of oxidative stress in vivo (Roberts and Morrow, 2000b). These compounds are excellent candidates for exploring mechanisms of alcohol-related fetal injury because they both reflect levels of oxidative stress and have potent intrinsic vasoconstrictive effects (Morrow, 2006). Thus, increased isoprostane levels may reflect oxidative injury to the fetal brain and may directly impair fetal growth via uteroplacental vasoconstriction. Isoprostane levels have not been measured in pregnant drinkers.

Ethanol may affect fetal development by a second eicosanoid pathway as well. It promotes vasoconstriction by reducing prostacyclin, a vasodilator, and by increasing thromboxane A2, a vasoconstrictor (Nanji et al., 1994; Siler-Khodr et al., 2000; Yokoyama et al., 2005). Ylikorkala and colleagues (Ylikorkala et al., 1988) measured urinary levels of prostacyclin and thromboxane metabolites in pregnant women who drank heavily during pregnancy. They reported a relative excess of thromboxane B2, suggesting that a vasoconstricted state is present in pregnant women who consume alcohol. However, the metabolites measured in their study are derived mainly from the kidney, and it is not clear that they are a good measure of systemic balance between prostacyclin and thromboxane.

We measured levels of urinary eicosanoids in a group of pregnant women known to be consuming ≥ 48 g alcohol/day and in a group of non-drinking pregnant women. Specifically, we examined levels of 8-isoprostane F2α, the most widely studied isoprostane, to assess systemic levels of oxidative stress. In addition, we measured urinary metabolites of systemic vasoactive prostaglandins: 2,3-dinor-6-keto-prostaglandin F1α, a vasodilator, and 11-dehydro-thromboxane B2, a vasoconstrictor,. We hypothesized that if oxidative stress and prostanoid-related vasoconstriction were important mediators of alcohol-induced fetal injury, we would detect increased isoprostanes and a relative excess of vasoconstrictive prostaglandin metabolites among women who drink heavily in pregnancy.

MATERIALS AND METHODS

Study subjects are a subset of a cohort of women enrolled in a study of outcomes in children exposed to large amounts of alcohol *in utero*. Detailed methods for identifying the full cohort of study participants are described elsewhere (Aros et al., 2006). Briefly, women (n = 9,628) presenting for prenatal care at the Maipú Clinic in Santiago, Chile, between August 1995 and July 2000, were questioned about alcohol use. There were 887 women who gave answers suggestive of risky alcohol use (see Aros et al. for details) who we further evaluated with home visits. Using information from the home visits, we identified 101 pregnant women who were consuming ≥48g alcohol/day on average during pregnancy. We used the same method to identify a control group of non-drinking pregnant women (n = 101) matched for age (± 2 years) and parity (0 or ≥1). None of the women included in this study had a history of chronic hypertension, pregestational or gestational diabetes, gestational hypertension, or preeclampsia.

The larger study described above was underway when sample collection for the current investigation began. Random urine specimens were collected during pregnancy from 26 of the matched drinking and non-drinking pairs. In addition, unpaired urine specimens were collected from 3 drinking gravidas and 13 non-drinking gravidas. Thus, samples were available for a total of 29 drinkers and 39 non-drinkers. Urine samples were stored at −70° C until analysis.

Subjects were classified as FAS if they demonstrated evidence of growth deficiency, characteristic facial dysmorphology, and neurodevelopmental abnormalities as described by Jones in 1973 (Jones et al., 1973). Growth deficiency was defined as weight or length ≤ 10^{th} percentile at any one or more assessments in infancy or childhood. Facial dysmorphology was determined by serial examinations by a geneticist. Specific features required for diagnosis were short palpebral fissure length, thin vermilion border, and flat philtrum. Neurodevelopmental impairment was based on evidence of microcephaly, results of developmental and cognitive testing (Bayley Scales of Infant Development II, Wechsler Preschool and Primary Scale of Intelligence, and Wechsler Intelligence Scale for Children), serial examinations by pediatric neurologists, and evaluation by a pediatric psychologist. We considered a child to have partial FAS if he exhibited abnormalities in two of the above three domains.

Free 8-isoprostane F2α (8-iso-P), 2,3-dinor-6-keto-prostaglandin F1α (2,3-dinor), and 11-dehydro-thromboxane B2 (11-dehydro-TxB2) were quantified as previously described using highly accurate and precise mass spectrometric methods (Daniel et al., 1994; Morrow and Minton, 1993; Morrow and Roberts, 1999). Precision of each of the three assays was +/− 4%, +/− 5%, and +/− 7%, respectively. Intra- and inter-day variabilities were < 10%. All results were corrected for intersubject differences in renal function and are expressed in ng/mg urinary creatinine. All analyses were conducted by personnel blinded to the alcohol exposure status of the subjects.

Visual inspection of the data demonstrated an outlying (>3.2 standard deviations above the mean) 8-iso-P value in a control subject; this value was deleted from our final analyses.

Baseline characteristics of the drinking and non-drinking subjects were compared using the Wilcoxon Rank Sum Test for continuous variables, and Fisher's Exact Test for categorical variables. Multiple linear regression was used to compare transformed values of eicosanoid levels after adjusting for maternal age, marital status, number of cigarettes smoked per day, and gestational age at sampling. To express the result in more meaningful units, the eicosanoid values were first log-transformed until approximately normally distributed, and then standardized by the control group mean and standard deviation. Regression analyses were conducted on both the set of matched pairs, and on the larger, unmatched study sample. Because the results of the matched and unmatched analyses did not differ substantially, only the unmatched analyses are presented. All data were analyzed using SAS v. 9.0 (SAS System, Cary, NC). A P value of < 0.05 was considered statistically significant.

Written informed consent was obtained from all study participants. This study was approved by the Institutional Review Boards of both the National Institute of Child Health and Human Development and the San Borja Arriaran Hospital, which is affiliated with the University of Chile School of Medicine.

RESULTS

Characteristics of the study population are presented in Table 1. There were no significant differences in age, parity, educational level, marital status, or gestational age at urine sample collection between drinking and non-drinking subjects. A significantly higher proportion of drinkers smoked during the index pregnancy.

As shown in Table 2, there were no significant differences in 8-iso-P, 2,3-dinor, 11-dehydro-TxB2, or the ratio of 2,3-dinor/11-dehydro-TxB2 between pregnant women drinking ≥ 48 g alcohol/day and non-drinking pregnant women. Results of multiple linear regression analyses adjusted for maternal age, marital status, number of cigarettes smoked per day, and gestational age at sampling indicated no significant differences in urinary eicosanoids between drinking and non-drinking gravidas in either the matched or unmatched analyses. (unmatched analyses, Table 3).

To investigate whether eicosanoid levels differed between women drinking most heavily at the time of sampling and nondrinkers, we conducted a subgroup analysis limiting the drinking group to those who reported heavy alcohol use through the second and third trimesters (N=19/29, 66%). Of these 19 women, a substantial majority were binge drinkers; only 2/19 drank daily. On average, these women reported drinking 3.2 +/− 1.8 days/week, and consumed 147 +/− 142 grams of ethanol per drinking day. There were no significant differences in 8-iso-P, 2,3-dinor, 11-dehydro-TxB2, or the ratio of 2,3-dinor/11-dehydro-TxB2 between this subgroup of highest quantity and frequency drinkers and nondrinkers (data not shown).

We compared maternal urinary eicosanoid concentrations between the subset of drinking pregnant women whose children were later diagnosed with fetal alcohol spectrum disorders (FAS, n=1; partial FAS, n=7) and concentrations in non-drinking pregnant women (n=39), and found no significant differences (data not shown). Though statistically significant differences would not be expected with only one case, we hypothesized that isoprostane and vasoactive prostanoid levels would be most altered in the mother of the child with the most severe phenotype, i.e. FAS. Results for maternal 8-Iso-P (1.19 ng/mg creatinine), 2,3-dinor (1.14 ng/mg creatinine), 11-dehydro-TxB2 (0.95 ng/mg creatinine), and the 2,3-dinor/11-dehydro-TxB2 ratio (1.19) were not markedly different in the mother of the FAS case from

the mean or median levels of these analytes in either the drinking or non-drinking mothers (Table 2).

DISCUSSION

Exposure to alcohol in utero is a leading cause of disability among children. Several mechanisms by which alcohol may exert its teratogenic effect have been proposed, including lipid peroxidation resulting from oxidative stress and impaired placental function resulting from alterations in vasoactive prostaglandins. We assessed each of these processes in women who drank heavily during pregnancy and women who abstained from alcohol during pregnancy by testing maternal urine for isoprostanes and stable prostaglandin metabolites. We found no difference in urine levels of 8-isoprostane F2α, 2,3-dinor-6-keto-prostaglandin F1α, or 11-dehydro-thromboxane B2 between pregnant women drinking ≥ 48g alcohol/day and non-drinking pregnant controls.

Isoprostanes, a class of prostaglandin-like compounds that are generated from free-radical peroxidation of arachidonic acid (Basu, 2004), are widely held to be the most accurate markers for oxidative stress *in vivo* (Kadiiska et al., 2005; Roberts and Morrow, 2000a; Griffiths et al., 2002; Montuschi et al., 2007; Tsimikas, 2006). Plasma isoprostanes have a short half-life, making urine the specimen of choice for measuring effects of non-acute exposures (Griffiths et al., 2002; Milne et al., 2007). These compounds are very stable in urine (Cracowski et al., 2002), and day-to-day fluctuations of urinary isoprostane concentrations in both health and disease are limited (Bachi et al., 1996; Meagher et al., 1999).

Isoprostanes not only reflect oxidative injury, but also have potent inherent biological activity, and may themselves mediate oxidative injury (Morrow et al., 1999; Morrow, 2006; Morrow and Roberts, 1997). 8-isoprostane F2α is a potent vasoconstrictor (Morrow, 2006). Evidence suggests that 8-isoprostane F2α may cause vasoconstriction in the placenta and thereby promote uteroplacental insufficiency and growth restriction. Exposure to 8-isoprostane F2α reduces trophoblast invasion *in vitro* (Staff et al., 2000), and elevated isoprostane levels have been demonstrated in placentas of women with preeclampsia (Staff et al., 1999; Walsh et al., 2000), a condition known to be associated with impaired uteroplacental circulation and fetal growth restriction (Papageorghiou et al., 2004; Sibai et al., 2005). Alcohol exposure has been shown to increase markers of oxidative stress in placental villi *in vitro* (Kay et al., 2006). Ours is the first investigation of isoprostane levels in drinking and non-drinking pregnant women.

The vasodilator, prostacyclin, and the vasoconstrictor, thromboxane A2, are rapidly metabolized in vivo. Levels of these metabolites may be indicators of general uteroplacental dysfunction. A number of authors (Chavarria et al., 2003; de Jong et al., 2000; Malatyalioglu et al., 2000; Trudinger et al., 1989; Walsh et al., 1993) have reported that alterations in levels of renally-derived prostacyclin and thromboxane A2 metabolites, 6-keto-prostaglandin F1α (PGF1α) and thromboxane B2 (TxB2), are associated with vasoconstriction and impaired fetoplacental blood flow, though others have found no such association (Sorem and Siler-Khodr, 1995; Ylikorkala et al., 1983; Ylikorkala et al., 1984), and none of these studies specifically assessed the effects of alcohol exposure on prostanoid concentrations. The compounds tested in the current study, 2,3-dinor-6-keto-prostaglandin F1α and 11-dehydro-thromboxane B2, are stable metabolites of PGF1α and TxB2, respectively, and are produced systemically. Interestingly, alterations in urinary levels of these metabolites are associated with placental insufficiency in the form of preeclampsia (Mills et al., 1999).

There are fewer data on the effect of ethanol on umbilical vessel and placental tissue prostanoid production, and results are conflicting. In *in vitro* studies, ethanol exposure has been associated with increased (Siler-Khodr et al., 2000), decreased (Ylikorkala et al., 1987), or unchanged (Randall and Saulnier, 1995) levels of vasoconstrictive prostanoids in human umbilical vessels and placenta. Very few measurements of prostanoid metabolites in pregnant drinkers have been reported. One previous study of heavily drinking (140 – 840 g ethanol/week during the first half of pregnancy or beyond) pregnant women, showed that urinary levels of renally-derived prostanoid metabolites were altered favoring vasoconstriction (Ylikorkala et al., 1988). However, it is unlikely that urinary concentrations of these compounds (6-keto-prostaglandin F1α and thromboxane B2) reflect systemic effects capable of affecting placental blood flow (Daniel et al., 1994; Morrow and Minton, 1993). The vasodilator (2,3-dinor) and vasoconstrictor (11-dehydro-TxB2) tested in the current study more accurately represent systemic effects; we found no difference between drinkers and non-drinkers in urinary levels of these compounds, or in their ratio.

Because isoprostanes and other vasoactive prostanoids could not be measured directly in the placenta, we cannot rule out the possibility that ethanol induces oxidative stress or prostanoid alterations localized to the uteroplacental circulation that are not reflected in maternal urine levels. A potential limitation of our study is that our sample size might have been too small to allow us to detect significant differences between the alcohol exposed and unexposed pregnancies. However, assuming a two-sided analysis at the 5% level, our study had 80% power to detect median differences between drinkers and non-drinkers of 25–36% (relative to the control median) in each of our analytes. Thus, we have confidence that there are not major changes in these particular eicosanoids associated with heavy drinking in pregnancy. We are not concerned about an unmeasured confounding influence of antioxidant vitamin intake in our study because prenatal or other vitamins are not commonly used by pregnant women in Chile, and because it is not clear from the current literature whether antioxidant supplements affect urinary F2-isoprostane levels (DeCaterina et al., 2002; Montuschi et al., 2007; Reilly et al., 1996). Another strength of our study was the rigorous assessment of maternal alcohol use and non-use through home visitation and interviews.

In summary, our results show no difference in levels of urinary eicosanoid markers of oxidative stress and systemic vasoconstriction between women who drink heavily during pregnancy and women who abstain from alcohol during pregnancy. These findings speak against a role for maternal oxidative stress or systemic vasoconstriction in the pathogenesis of alcohol damage to the fetus.

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References

Aros S, Mills JL, Torres C, Henriquez C, Fuentes A, Capurro T, Mena M, Conley M, Cox C, Signore C, Klebanoff M, Cassorla F. Prospective identification of pregnant women drinking four or more standard drinks (> or = 48 g) of alcohol per day. Subst Use Misuse. 2006; 41:183–197. [PubMed: 16479683]

Bachi A, Zuccato E, Baraldi M, Fanelli R, Chiabrando C. Measurement of urinary 8-Epi-prostaglandin F2alpha, a novel index of lipid peroxidation in vivo, by immunoaffinity extraction/gas chromatography-mass spectrometry. Basal levels in smokers and nonsmokers. Free Radic Biol Med. 1996; 20:619–624. [PubMed: 8904305]

Basu S. Isoprostanes: novel bioactive products of lipid peroxidation. Free Radic Res. 2004; 38:105–122. [PubMed: 15104204]

Centers for Disease Control. Fetal alcohol syndrome--Alaska, Arizona, Colorado, and New York, 1995 – 1997. MMWR. 2002a; 51:433–435.

Centers for Disease Control. Alcohol use among women of childbearing age--United States, 1991 – 1999. MMWR. 2002b; 51:273–276.

Chavarria ME, Lara-Gonzalez L, Gonzalez-Gleason A, Garcia-Paleta Y, Vital-Reyes VS, Reyes A. Prostacyclin/thromboxane early changes in pregnancies that are complicated by preeclampsia. Am J Obstet Gynecol. 2003; 188:986–992. [PubMed: 12712098]

Cohen-Kerem R, Koren G. Antioxidants and fetal protection against ethanol teratogenicity. I. Review of the experimental data and implications to humans. Neurotoxicol Teratol. 2003; 25:1–9. [PubMed: 12633732]

Committee on Substance Abuse and Committee on Children With Disabilities. Fetal Alcohol Syndrome and Alcohol-Related Neurodevelopmental Disorders. Pediatrics. 2000; 106:358–361. [PubMed: 10920168]

Cracowski JL, Durand T, Bessard G. Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications. Trends Pharmacol Sci. 2002; 23:360–366. [PubMed: 12377577]

Daniel VC, Minton TA, Brown NJ, Nadeau JH, Morrow JD. Simplified assay for the quantification of 2,3-dinor-6-keto-prostaglandin F1 alpha by gas chromatography-mass spectrometry. J Chromatogr B Biomed Appl. 1994; 653:117–122. [PubMed: 8205238]

DeCaterina R, Cipollone F, Filardo FP, Zimarino M, Bernini W, Lazzerini G, Bucciarelli T, Falco A, Marchesani P, Muraro R, Mezzetti A, Ciabattoni G. Low-density lipoprotein level reduction by the 3-hydroxy-3-methylglutaryl coenzyme-A inhibitor simvastatin is accompanied by a related reduction of F2-isoprostane formation in hypercholesterolemic subjects: no further effect of vitamin E. Circulation. 2002; 106:2543–2549. [PubMed: 12427649]

de Jong CL, Paarlberg KM, van Geijn HP, van Kamp GJ, van Dis H, Dekker GA. Maternal thromboxane and prostacyclin levels in relation to fetal birth weight. Eur J Obstet Gynecol Reprod Biol. 2000; 93:65–69. [PubMed: 11000507]

Griffiths HR, Moller L, Bartosz G, Bast A, Bertoni-Freddari C, Collins A, Cooke M, Coolen S, Haenen G, Hoberg AM, Loft S, Lunec J, Olinski R, Parry J, Pompella A, Poulsen H, Verhagen H, Astley SB. Biomarkers. Mol Aspects Med. 2002; 23:101–208. [PubMed: 12079771]

Guerri C, Montoliu C, Renau-Piqueras J. Involvement of free radical mechanism in the toxic effects of alcohol: implications for fetal alcohol syndrome. Adv Exp Med Biol. 1994; 366:291–305. [PubMed: 7771260]

Jones KL, Smith DW, Ulleland CN, Streissguth P. Pattern of malformation in offspring of chronic alcoholic mothers. Lancet. 1973; 1:1267–1271. [PubMed: 4126070]

Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, Nyska A, Wachsman JT, Ames BN, Basu S, Brot N, FitzGerald GA, Floyd RA, George M, Heinecke JW, Hatch GE, Hensley K, Lawson JA, Marnett LJ, Morrow JD, Murray DM, Plastaras J, Roberts LJ, Rokach J, Shigenaga MK, Sohal RS, Sun J, Tice RR, Van Thiel DH, Wellner D, Walter PB, Tomer KB, Mason RP, Barrett JC. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med. 2005; 38:698–710. [PubMed: 15721980]

Kay HH, Tsoi S, Grindle K, Magness RR. Markers of oxidative stress in placental villi exposed to ethanol. J Soc Gynecol Investig. 2006; 13:118–121.

Malatyalioglu E, Adam B, Yanik FF, Kokcu A, Alvur M. Levels of stable metabolites of prostacyclin and thromboxane A2 and their ratio in normotensive and preeclamptic pregnant women during the antepartum and postpartum periods. J Matern Fetal Med. 2000; 9:173–177. [PubMed: 10914626]

Meagher EA, Barry OP, Burke A, Lucey MR, Lawson JA, Rokach J, FitzGerald GA. Alcohol-induced generation of lipid peroxidation products in humans. J Clin Invest. 1999; 104:805–813. [PubMed: 10491416]

Mills JL, DerSimonian R, Raymond E, Morrow JD, Roberts LJ, Clemens JD, Hauth JC, Catalano P, Sibai B, Curet LB, Levine RJ. Prostacyclin and thromboxane changes predating clinical onset of preeclampsia: a multicenter prospective study. JAMA. 1999; 282:356–362. [PubMed: 10432033]

Milne GL, Yin H, Brooks JD, Sanchez S, Jackson RL, Morrow JD. Quantification of F2-isoprostanes in biological fluids and tissues as a measure of oxidant stress. Methods Enzymol. 2007; 433:113–126. [PubMed: 17954231]

Montuschi P, Barnes P, Roberts LJ. Insights into oxidative stress: the isoprostanes. Curr Med Chem. 2007; 14:703–717. [PubMed: 17346157]

Morrow JD. The isoprostanes - unique products of arachidonate peroxidation: their role as mediators of oxidant stress. Curr Pharm Des. 2006; 12:895–902. [PubMed: 16533158]

Morrow JD, Chen Y, Brame CJ, Yang J, Sanchez SC, Xu J, Zackert WE, Awad JA, Roberts LJ. The isoprostanes: unique prostaglandin-like products of free-radical-initiated lipid peroxidation. Drug Metab Rev. 1999; 31:117–139. [PubMed: 10065368]

Morrow JD, Minton TA. Improved assay for the quantification of 11-dehydrothromboxane B2 by gas chromatography-mass spectrometry. J Chromatogr. 1993; 612:179–185. [PubMed: 8468374]

Morrow JD, Roberts LJ. The isoprostanes: unique bioactive products of lipid peroxidation. Prog Lipid Res. 1997; 36:1–21. [PubMed: 9373618]

Morrow JD, Roberts LJ. Mass spectrometric quantification of F2-isoprostanes in biological fluids and tissues as measure of oxidant stress. Methods Enzymol. 1999; 300:3–12. [PubMed: 9919502]

Nanji AA, Khwaja S, Sadrzadeh SM. Eicosanoid production in experimental alcoholic liver disease is related to vitamin E levels and lipid peroxidation. Mol Cell Biochem. 1994; 140:85–89. [PubMed: 7877602]

Papageorghiou AT, Yu CK, Nicolaides KH. The role of uterine artery Doppler in predicting adverse pregnancy outcome. Best Pract Res Clin Obstet Gynaecol. 2004; 18:383–396. [PubMed: 15183134]

Randall CL, Saulnier JL. Effect of ethanol on prostacyclin, thromboxane, and prostaglandin E production in human umbilical veins. Alcohol Clin Exp Res. 1995; 19:741–746. [PubMed: 7573802]

Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. Circulation. 1996; 94:19–25. [PubMed: 8964113]

Roberts LJ II, Morrow JD. Measurement of F2-isoprostanes as an index of oxidative stress in vivo. Free Radical Biology and Medicine. 2000b; 28:505–513. [PubMed: 10719231]

Roberts LJ, Morrow JD. Measurement of F2-isoprostanes as an index of oxidative stress in vivo. Free Radical Biology and Medicine. 2000a; 28:505–513. [PubMed: 10719231]

Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet. 2005; 365:785–799. [PubMed: 15733721]

Siler-Khodr TM, Yang Y, Grayson MH, Henderson GI, Lee M, Schenker S. Effect of ethanol on thromboxane and prostacyclin production in the human placenta. Alcohol. 2000; 21:169–180. [PubMed: 10963940]

Sorem KA, Siler-Khodr TM. Placental prostanoid release in severe intrauterine growth retardation. Placenta. 1995; 16:503–515. [PubMed: 8570572]

Staff AC, Halvorsen B, Ranheim T, Henriksen T. Elevated level of free 8-iso-prostaglandin F2alpha in the decidua basalis of women with preeclampsia. Am J Obstet Gynecol. 1999; 181:1211–1215. [PubMed: 10561647]

Staff AC, Ranheim T, Henriksen T, Halvorsen B. 8-Iso-prostaglandin f(2alpha) reduces trophoblast invasion and matrix metalloproteinase activity. Hypertension. 2000; 35:1307–1313. [PubMed: 10856282]

Trudinger BJ, Connelly AJ, Giles WB, Hales JR, Wilcox GR. The effects of prostacyclin and thromboxane analogue (U46619) on the fetal circulation and umbilical flow velocity waveforms. J Dev Physiol. 1989; 11:179–184. [PubMed: 2530267]

Tsimikas S. Measures of oxidative stress. Clin Lab Med. 2006; 26:571–5vi. [PubMed: 16938585]

Walsh SW, Vaughan JE, Wang Y, Roberts LJ. Placental isoprostane is significantly increased in preeclampsia. FASEB J. 2000; 14:1289–1296. [PubMed: 10877821]

Walsh SW, Wang Y, Jesse R. Peroxide induces vasoconstriction in the human placenta by stimulating thromboxane. Am J Obstet Gynecol. 1993; 169:1007–1012. [PubMed: 8238110]

West JR. Fetal alcohol syndrome: the vulnerability of the developing brain and possible mechanisms of damage. Metabolic brain disease. 1994; 9:291–322. [PubMed: 7898398]

Ylikorkala O, Halmesmaki E, Viinikka L. Urinary prostacyclin and thromboxane metabolites in drinking pregnant women and in their infants: relations to the fetal alcohol effects. Obstet Gynecol. 1988; 71:61–66. [PubMed: 3275910]

Ylikorkala O, Halmesmaki E, Viinikka L. Effect of ethanol on thromboxane and prostacyclin synthesis by fetal platelets and umbilical artery. Life Sci. 1987; 41:371–376. [PubMed: 3110528]

Ylikorkala O, Jouppila P, Kirkinen P, Viinikka L. Maternal prostacyclin, thromboxane, and placental blood flow. Am J Obstet Gynecol. 1983; 145:730–732. [PubMed: 6338724]

Ylikorkala O, Jouppila P, Kirkinen P, Viinikka L. Maternal thromboxane, prostacyclin, and umbilical blood flow in humans. Obstet Gynecol. 1984; 63:677–680. [PubMed: 6546976]

Yokoyama Y, Nimura Y, Nagino M, Bland KI, Chaudry IH. Role of thromboxane in producing hepatic injury during hepatic stress. Arch Surg. 2005; 140:801–807. [PubMed: 16103291]

Table 1

Characteristics of the study sample

	Drinkers (≥48 g/day) N = 29	Non-drinkers N = 39	P value
Age (years)	23.4 ± 7.5	25.1 ± 8.1	0.37
Parity			0.63
0	16 (55.2)	19 (48.7)	
≥1	13 (44.8)	20 (51.3)	
Education (years)			0.65
<8	3 (10.3)	2 (5.1)	
8	6 (20.7)	4 (10.3)	
9–11	7 (24.1)	13 (33.3)	
12	7 (24.1)	10 (25.6)	
>12	6 (20.7)	10 (25.6)	
Marital Status			0.12
Never married	23 (79.3)	23 (59.0)	
Married/divorced/Widowed	6 (20.7)	16 (41.0)	
GA at sampling (weeks)	27.5 ± 7.4	26.6 ± 6.5	0.58
Current smoker	17 (58.6)	6 (15.4)	0.0003
Cigarettes per day (among current smokers)	6.3 ± 7.8	5.5 ± 3.7	0.80

Values are expressed as mean ± standard deviation or number (percent). GA = gestational age.

Table 2

Urinary eicosanoid concentrations among drinking and non-drinking gravidas

	Drinkers	Non-drinkers	P value*
8-iso-P	N=29	N=37	
Mean +/− SD	2.16 +/− 1.11	2.08 +/− 0.87	0.87
Median	1.86	1.88	
IQR	1.35 – 3.02	1.53 – 2.35	
Range	0.76 – 5.84	0.911 – 4.72	
2,3-dinor	N=29	N=38	
Mean +/− SD	1.03 +/− 0.47	1.17 +/− 0.60	0.50
Median	0.92	1.10	
IQR	0.750 – 1.374	0.73 – 1.396	
Range	0.150 – 2.24	0.194 − 2.988	
11-dehydro-TxB2	N=28	N=38	
Mean +/− SD	0.72 +/− 0.44	0.59 +/− 0.31	0.21
Median	0.57	0.54	
IQR	0.463 – 0.823	0.379 – 0.714	
Range	0.216 – 2.030	0.061 – 1.561	
2,3-dinor/11-dehydro-TxB2 Ratio	N=28	N=38	
Mean +/− SD	1.73 +/− 0.92	2.72 +/− 3.18	0.14
Median	1.57	1.75	
IQR	1.067 – 2.438	1.322 – 2.927	
Range	0.50 – 3.47	0.64 – 19.34	

Values are expressed as ng/mg creatinine. 8-iso-P, 8-isoprostane F2a; 2,3-dinor, 2,3-dinor-6-keto-prostaglandin F1a; 11-dehydro-TxB2, 11-dehydro-thromboxane B2.

* Wilcoxon rank sum test

Table 3

Adjusted* mean urinary eicosanoid levels** in drinkers expressed as standard deviations (z-scores) from the control mean

	Parameter Estimate	SE	P Value
8-iso-P	−0.13	0.30	0.67
2,3-dinor	−0.37	0.24	0.13
11-dehydro-TxB2	0.21	0.22	0.35
2,3-dinor/11-dehydro-TxB2 Ratio	−0.43	0.22	0.06

8-iso-P, 8-isoprostane F2a; 2,3-dinor, 2,3-dinor-6-keto-prostaglandin F1a; 11-dehydro-TxB2, 11-dehydro-thromboxane B2.

*Multiple linear regression on z-score transformations, adjusted for maternal age, marital status, number of cigarettes smoked per day, and gestational age at sampling.

**Values log-transformed as needed.