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ORIGINAL ARTICLE

Maternal L-glutamine supplementation prevents prenatal alcohol exposure-induced fetal growth restriction in an ovine model

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Abstract Prenatal alcohol exposure is known to cause fetal growth restriction and disturbances in amino acid bioavailability. Alterations in these parameters can persist into adulthood and low birth weight can lead to altered fetal programming. Glutamine has been associated with the synthesis of other amino acids, an increase in protein synthesis and it is used clinically as a nutrient supplement for low birth weight infants. The aim of this study was to explore the effect of repeated maternal alcohol exposure and L-glutamine supplementation on fetal growth and amino acid bioavailability during the third trimester-equivalent period in an ovine model. Pregnant sheep were randomly assigned to four groups, saline control, alcohol (1.75–2.5 g/kg), glutamine (100 mg/kg, three times daily) or alcohol + glutamine. In this study, a weekend binge drinking model was followed where treatment was done 3 days per week in succession from gestational day (GD) 109–132 (normal term ~147). Maternal alcohol exposure significantly reduced fetal body weight, height, length, thoracic girth and brain weight, and resulted in decreased amino acid bioavailability in fetal plasma and placental fluids. Maternal glutamine supplementation successfully mitigated alcohol-induced fetal growth restriction and improved the bioavailability of glutamine and glutamine-related amino acids such as glycine, arginine, and asparagine in the fetal compartment. All together, these findings show that L-glutamine supplementation enhances amino acid availability in the fetus and prevents alcohol-induced fetal growth restriction.

Keywords Glutamine · IUGR · FASD · Alcohol · Fetal growth

Abbreviations

FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorders
GD	Gestational day
IUGR	Intra uterine growth restriction

Introduction

Fetal alcohol spectrum disorders (FASD) is an umbrella term encompassing the full range of effects that can occur in an individual whose mother consumed alcohol during pregnancy. These include effects on physical, behavioral or cognitive development that can persist as lifelong disabilities, with the most severe end of the spectrum being fetal alcohol syndrome (FAS) (Warren et al. 2001). Facial abnormalities, growth deficits, and central nervous system abnormalities are the primary defining diagnostic features of FAS (Riley et al. 2011). In spite of efforts to educate women about the teratogenic effect of alcohol, the prevalence of alcohol consumption in women of child-bearing age remains essentially the same (Caetano et al. 2006).

In children, prenatal alcohol exposure has been associated with fetal growth deficits (Spohr et al. 1993; Ouellette et al. 1977; Rosett et al. 1983) and widespread work by day and colleagues has shown that growth deficits arising from prenatal alcohol exposure can persist into adolescence (Day et al. 1999, 2002). Animal studies, particularly

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using the rodent and sheep model have shown that alcohol exposure can produce growth deficits and impaired skeletal development in the offspring (Probyn et al. 2012; Abel and Dintcheff 1978; Ramadoss et al. 2006; Sawant et al. 2013b). As per the Barker hypothesis, low birth weight can lead to altered development and programming by increasing the risk of cardiovascular disorders later in life (Barker 1994). Although fetal growth deficits as a diagnostic feature has received minimal attention compared to the facial and CNS abnormalities, more attention should be given to the alcohol-induced fetal growth deficits since it has been associated with other prenatal and neonatal anomalies. For example, low birth weight has been associated with childhood mortality and morbidity (Tagare et al. 2013), lower I.Q. and learning disabilities (Hutchinson et al. 2013), sleep disturbances (Als et al. 1976), hyperactivity (Scott et al. 2012), delayed reflex and motor disturbances (Vohr et al. 2000), hypertension (Woodall et al. 1996), metabolic diseases (Hales and Ozanne 2003), osteoporosis (Cooper et al. 2009), respiratory issues (Lucas et al. 2004) and mental disorders such as schizophrenia (Susser et al. 1996).

The fetus depends on a steady supply of nutrients for growth and development, and disturbances in this supply can lead to impaired fetal development and growth restriction (Wu 2014). Studies using a rodent model have shown that gestational alcohol exposure reduces a number of amino acids in the maternal and fetal compartments (Padmanabhan et al. 2002; Schenker et al. 1990; Marquis et al. 1984a; Karl et al. 1995). An earlier report using a third trimester-equivalent ovine model demonstrated that alcohol exposure results in decreases in maternal glutamine and glutamine-related amino acid levels (Ramadoss et al. 2008). Glutamine is a conditionally essential amino acid and it is involved in many vital cellular processes (Kwon et al. 2003; Wu et al. 2004b; Mates et al. 2002a). Glutamine supplementation in many animal studies and in clinical cases has been shown to improve growth, function, and efficacy of amino acids (Wang et al. 2008, 2010; Wu et al. 1996; Ehrenkranz et al. 2011; Poindexter et al. 2003; van den Berg et al. 2007). Even a single acute alcohol exposure during the third trimester-equivalent period resulted in a simultaneous decrease in maternal as well as a fetal glutamine and glutamine-related amino acids, and administration of single aqueous bolus of L-glutamine improved the amino acids profile in both the maternal and fetal compartments (Washburn et al. 2013). However, the effect of repeated alcohol exposure and concurrent maternal L-glutamine supplementation during the third trimester-equivalent period on fetal growth and amino acid bioavailability has not been studied before. Therefore, the aim of this study was to explore the effect of repeated third trimester-equivalent alcohol exposure and maternal L-glutamine supplementation on fetal growth and bioavailability of amino acids.

Materials and methods

Animals

All aspects of the experimental protocols were approved by the Texas A&M University Institutional Animal Care and Use Committee. Suffolk ewes aged 2–5 years were obtained from a commercial supplier. Upon arrival at the animal facility, each ewe received an intramuscular injection of Covexin® 8 (Merck Animal Health, Summit, NJ, USA) and an oral bolus of Valbazen® (Zoetis, Kalamazoo, MI, USA). Ewes received progesterone impregnated vaginal implants (EAZI-BREED™, CIDR®, Zoetis, Kalamazoo, MI, USA). Implants were removed 11 days after placement at which time prostaglandin $F_{2\alpha}$ (20 mg; LUTALYSE®, Zoetis, Kalamazoo, MI, USA) was intramuscularly administered. The following day, ewes were placed with a ram fitted with a marking harness for a period of 24 h. Marked ewes were presumed pregnant until confirmed pregnant ultrasonographically on gestation days (GD) 25 and 92 (Ramadoss et al. 2006).

Upon confirmation of pregnancy, ewes were housed individually where they were able to have visual contact with herd mates in adjacent pens at all times. Conditions of constant temperature (22 °C) and fixed light/dark cycle (12 h:12 h) were maintained. During the entire pregnancy, ewes were fed a custom ration (Nutrena, Cargill Animal Nutrition, Minneapolis, MN, USA) twice daily in the amount of 15 g of feed/kg body weight/day. Feed composition was the same as described earlier (Lassala et al. 2011). Ewes were allowed free access to drinking water. Daily feed consumption was monitored and ewes consumed all of the food offered.

Treatment groups

Four treatment groups were used in this study: (a) a saline control group that received 0.9 % saline; (b) an alcohol group that received alcohol at a dosage of 1.75–2.5 g/kg body weight [40 % (w/v) diluted in 0.9 % saline]; (c) a glutamine group that received 0.9 % saline and 100 mg L-glutamine/kg body weight 3 times a day (i.e., 300 mg L-glutamine/kg body weight/day); and (d) an alcohol + glutamine group that received alcohol at a dosage of 1.75–2.5 g/kg body weight [40 % (w/v) diluted in 0.9 % saline] and 100 mg L-glutamine/kg body weight 3 times a day. Detailed description of alcohol and L-glutamine dosing paradigms is given in the subsequent section.

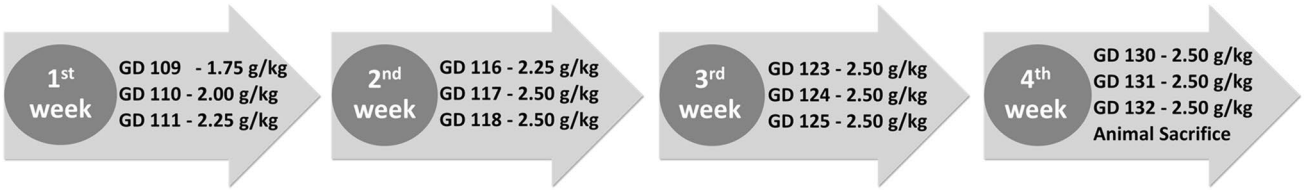

Fig. 1 Model for chronic alcohol binging paradigm. Description of weekend binge alcohol drinking paradigm followed from gestation day (GD) 109–132 using the third trimester-equivalent sheep model. Maternal L-glutamine supplementation was done on the same days with a dose of 100 mg/kg of body weight three times per day. Average gestation period of sheep is 147 days

Dosing paradigm

Alcohol or saline infusions were given intravenously (IV) through a jugular vein catheter over 1 h from GD 109–132, 3 consecutive days per week to mimic a weekend binge drinking pattern, a pattern common in women who use alcohol during pregnancy. In an ovine model, all three trimester-equivalents of human pregnancy occur prenatally (Washburn et al. 2014) and GD 109–132 overlaps with the human third trimester-equivalent brain growth spurt (Dobbing and Sands 1979). On gestational day 109, an intravenous catheter (16 gauge, 3.00 in Extended Use Catheter, Jorgensen, Loveland, CO, USA) was placed percutaneously into the jugular vein. On the days of infusions, ewes were connected to the infusion pump by 0830 h and alcohol or saline was infused continuously over 1 h. Infusion solutions were delivered intravenously by infusion pump (VetFlo® 7701B IV Vet Infusion Pump, Grady Medical, Temecula, CA, USA). The first four doses of alcohol were 1.75, 2, 2.25 and 2.25 g/kg, respectively and thereafter were 2.5 g/kg (Fig. 1). The alcohol solution was prepared under aseptic conditions as described earlier (Sawant et al. 2013a). The saline control and glutamine groups received a dose of 0.9 % saline that was isovolumetric to the alcohol groups. L-Glutamine powder (Sigma Aldrich) was completely dissolved in sterile water at a concentration of 4.5 % w/v and passed through a 0.2 μm bacteriostatic filter. The solution was kept at room temperature and prepared no sooner than 1–2 h prior to administration. A 100 mg/kg dose of glutamine was administered IV as a 4.5 % w/v aqueous bolus three times a day on 3 consecutive days per week. No adverse effects or safety concerns of IV or oral glutamine supplementation were observed in newborns or in adult humans for glutamine doses in the range of 400–860 mg/kg/day (Garlick 2001). In these studies, safety assessments were done by evaluating standard clinical chemistry, mental status, vital signs, temperature and clinical and subjective evidence of toxicity (Garlick 2001). Due to the short half-life of glutamine (Coster et al. 2004) and for the ease of administration, glutamine supplementation was done 3 times per day (Fig. 1). On GD 132 animals received either saline or alcohol infusion as described earlier. Animals from the glutamine and alcohol + glutamine groups received a single bolus of glutamine (4.5 % w/v, 100 mg/kg) just before the start of the final infusion. Maternal blood alcohol concentration (BAC) at the end of infusion on GD 132 was estimated using an enzymatic assay kit (Quantichrom® ethanol assay kit; BioAssay Systems, Hayward, CA, USA). At the end of 60 min, the ewes were euthanized using an IV injection of sodium pentobarbitone (75 mg/kg). The uterus was removed from the ewe and the fetus was exteriorized. The fetus was removed after carefully collecting fetal amniotic and allantoic samples. Fetal blood was collected quickly before measuring fetal body weight, abdominal and thoracic girth, crown-rump length, height, head length, head width and head circumference.

Amino acid analysis

Fetal plasma, amniotic and allantoic fluid samples (50 μL) were acidified with 50 μL of 1.5 mM $HClO_4$ and then neutralized with 20 μL of 2 mM K_2CO_3. 900 μL of water was added to this solution and samples were centrifuged at 10,000 rpm for 5 min. The supernatant fluid was used for amino acid analysis by HPLC, as described previously (Washburn et al. 2013). Concentrations of amino acids in samples were quantified on the basis of authentic standards from Sigma Chemicals (St. Louis, MO, USA) using the Waters Millenium-32 workstation (Waters Corporation, Milford, MA, USA), as described earlier (Rezaei et al. 2013).

Statistical analysis

Two-way mixed ANOVA was performed for the analysis of fetal growth parameters with treatment group and number of fetuses (single, twin or triplet) as independent factors (Assaad et al. 2015). One-way ANOVA was performed for the analysis of amino acid levels among treatment groups (Assaad et al. 2014). Further pairwise comparisons were performed when appropriate using Fisher's protected least significant difference. Level of significance was established at $P < 0.05$ and $0.05 < P < 0.1$ was considered trends.

Table 1 Fetal body growth parameters and maternal weight on GD 132

	Saline control	Alcohol	Glutamine	Alcohol + glutamine
Number of ewes	15	17	16	17
Singleton pregnancies	8	10	8	9
Twin pregnancies	6	5	7	8
Triplet pregnancies	1	2	1	–
Fetal body weight (kg)	4.7 ± 0.2^{a}	4.0 ± 0.1^{b}	4.7 ± 0.1^{a}	4.7 ± 0.1^{a}
Fetal height (cm)	41.3 ± 0.6^{a}	38.7 ± 0.7^{b}	41.3 ± 0.5^{a}	41.2 ± 0.5^{a}
Fetal crown-rump length (cm)	51.1 ± 0.8^{a}	47.9 ± 0.6^{c}	$49.4 \pm 0.7^{b,c}$	50.5 ± 0.7^{b}
Fetal thoracic girth (cm)	34.5 ± 0.5^{a}	32.7 ± 0.4^{b}	34.5 ± 0.4^{a}	34.3 ± 0.3^{a}
Fetal abdominal girth (cm)	33.5 ± 0.7^{a}	32.2 ± 0.6^{b}	33.9 ± 0.5^{a}	33.6 ± 0.5^{a}
Fetal head width (cm)	8.2 ± 0.2^{a}	7.4 ± 0.1^{c}	$7.9 \pm 0.2^{a,b}$	7.7 ± 0.1^{b}
Fetal head length (cm)	13.0 ± 0.4	12.3 ± 0.3	12.6 ± 0.2	12.7 ± 0.3
Fetal head circumference (cm)	20.7 ± 0.3	19.8 ± 0.4	20.5 ± 0.3	20.5 ± 0.4
Fetal kidney weight (g)	12.2 ± 0.6	11.5 ± 0.5	11.7 ± 0.5	12.1 ± 0.5
Fetal brain weight (g)	55.7 ± 1.2^{a}	51.4 ± 0.8^{b}	55.7 ± 1.4^{a}	54.5 ± 1.1^{a}
Fetal cerebellum weight (g)	5.1 ± 0.2^{a}	4.7 ± 0.1^{b}	4.8 ± 0.1^{b}	5.1 ± 0.1^{a}
Maternal weight (kg)	84.8 ± 3.1	86.3 ± 2.6	88.9 ± 3.3	87.5 ± 2.4

Values are mean ± SEM

Within a row, groups not sharing the same superscript are statistically different ($P < 0.05$)

Results

Blood alcohol concentration (BAC)

No statistical significant difference was observed between the alcohol and alcohol + glutamine groups BACs. Maternal BACs at the end of final alcohol infusion (60 min; the time point when BACs are known to peak) on GD 132 were 314 ± 16 and 309 ± 13 mg/dL in the alcohol and alcohol + glutamine groups, respectively.

Fetal growth parameters

Fetuses from the alcohol group had significantly lower body weight, height and thoracic girth compared to the saline control, glutamine and alcohol + glutamine groups ($P < 0.05$) (Table 1). Fetal body weight ($P < 0.001$), height ($P = 0.002$), crown-rump length ($P = 0.006$) and thoracic girth ($P = 0.004$) were significantly improved in the glutamine supplemented alcohol group compared to the alcohol group, indicating that maternal glutamine supplementation attenuated alcohol-induced fetal growth deficits. Fetal brain and cerebellum weights were significantly reduced in the alcohol group compared to the control groups ($P < 0.05$) and L-glutamine supplementation showed a protective trend on these parameters (Table 1). Statistical analysis revealed no significant interaction between treatment group and number of fetuses for any of the dependent parameters, except for fetal head width ($P = 0.005$). It is important to note that alcohol-induced fetal growth restriction was observed in the absence of maternal growth deficits and maternal weights on GD 132 was not significantly different among groups. Fetal growth parameters on GD 132 details about statistically significant differences are tabulated in Table 1.

Fetal plasma amino acid concentrations

Amino acid concentrations in fetal plasma were significantly altered among groups for asparagine, glutamine, histidine and threonine. Concentrations of asparagine and histidine in fetal plasma were significantly decreased in the alcohol group compared to the saline control ($P = 0.021$ and $P = 0.008$, respectively) and glutamine ($P = 0.002$ and $P = 0.016$, respectively) groups. Concentration of glutamine in fetal plasma was significantly decreased in the alcohol groups compared to the saline control ($P = 0.026$), glutamine ($P < 0.001$) and alcohol + glutamine ($P = 0.014$) groups. Concentration of threonine in fetal plasma was significantly decreased in the alcohol and alcohol + glutamine groups compared to the saline control group ($P = 0.003$ and $P = 0.037$, respectively). Fetal plasma amino acid levels on GD 132 and details about statistically significant differences are tabulated in Table 2.

Fetal amniotic fluid amino acid concentrations

Amino acid concentrations in fetal amniotic fluid were significantly altered among groups for asparagine, serine, glutamine, threonine, citrulline, tyrosine and leucine. The concentration of asparagine in fetal amniotic fluid was significantly decreased in the alcohol group compared to the saline control ($P = 0.011$) and alcohol + glutamine ($P = 0.018$) groups and showed a decreasing trend compared to the glutamine group ($P = 0.094$). Serine concentration in fetal amniotic fluid was significantly decreased in

Table 2 Fetal plasma amino acid levels on GD 132

	Saline control	Alcohol	Glutamine	Alcohol + glutamine
Aspartate	24 ± 3	26 ± 2	30 ± 3	29 ± 2
Glutamate	121 ± 22	126 ± 19	145 ± 15	180 ± 23
Asparagine	47 ± 5[a]	31 ± 3[c]	50 ± 5[a]	36 ± 3[b]
Serine	430 ± 60	435 ± 49	494 ± 50	513 ± 48
Glutamine	333 ± 35[b]	203 ± 19[c]	430 ± 41[a]	332 ± 38[b]
Histidine	54 ± 6[a]	34 ± 3[c]	50 ± 6[a]	42 ± 4[b]
Glycine	421 ± 67	426 ± 58	485 ± 57	450 ± 53
Threonine	216 ± 34[a]	106 ± 12[c]	199 ± 22[a]	144 ± 23[b]
Citrulline	177 ± 23	153 ± 22	145 ± 17	154 ± 20
Arginine	124 ± 31	147 ± 38	133 ± 23	128 ± 24
β-Alanine	172 ± 30	160 ± 21	159 ± 18	151 ± 17
Taurine	113 ± 18	81 ± 11	111 ± 17	105 ± 18
Alanine	242 ± 32	228 ± 26	285 ± 26	268 ± 28
Tyrosine	105 ± 18	82 ± 9	96 ± 9	88 ± 10
Tryptophan	43 ± 7	46 ± 8	51 ± 8	49 ± 9
Methionine	46 ± 11	33 ± 7	48 ± 12	44 ± 8
Valine	145 ± 23	148 ± 18	141 ± 17	178 ± 26
Phenylalanine	91 ± 15	85 ± 12	102 ± 12	96 ± 13
Isoleucine	51 ± 6	61 ± 7	46 ± 4	47 ± 4
Leucine	117 ± 16	124 ± 14	110 ± 10	131 ± 20
Ornithine	121 ± 23	146 ± 30	174 ± 27	167 ± 27
Lysine	116 ± 20	128 ± 18	140 ± 18	158 ± 42
Branched-chain AA	312 ± 43	334 ± 37	297 ± 30	354 ± 45

Values, expressed as nmol/mL, are mean ± SEM

AA Amino acids

Within a row, groups not sharing the same superscript are statistically different ($P < 0.05$)

the alcohol group compared to the glutamine ($P = 0.015$) and alcohol + glutamine ($P = 0.011$) groups. Glutamine concentration in fetal amniotic fluid was significantly decreased in the alcohol group compared to the saline control ($P = 0.016$) and glutamine ($P = 0.017$) groups and showed a decreasing trend compared to the alcohol + glutamine group ($P = 0.092$). Glycine concentration in fetal amniotic fluid was significantly decreased in the alcohol group compared to the saline control ($P = 0.025$) and alcohol + glutamine ($P = 0.033$) groups. Threonine concentration in fetal amniotic fluid was significantly decreased in the alcohol group compared to the saline control group ($P = 0.001$). Citrulline and leucine concentrations in fetal amniotic fluid were significantly decreased in the alcohol and glutamine groups compared to the saline control group (all $P < 0.05$). Alanine concentration in fetal amniotic fluid was significantly decreased in the alcohol group compared to the saline control ($P = 0.034$) and glutamine ($P = 0.017$) groups and showed a decreasing trend compared to the alcohol + glutamine group ($P = 0.071$). Tyrosine concentration in fetal amniotic fluid was significantly increased in the alcohol + glutamine group compared to the alcohol ($P = 0.019$) and glutamine ($P = 0.006$) groups. Fetal amniotic fluid amino acid levels on GD 132 and details about statistically significant differences are tabulated in Table 3.

Fetal allantoic fluid amino acid concentrations

Amino acid concentrations in fetal amniotic fluid were significantly altered among groups for asparagine, threonine, taurine, tyrosine and tryptophan. The concentration of asparagine in fetal allantoic fluid was significantly decreased in the alcohol group compared to the saline control ($P = 0.013$) and glutamine ($P = 0.012$) groups. Histidine concentration in fetal allantoic fluid was significantly decreased in the alcohol group compared to the saline control group ($P = 0.020$) and exhibited a decreasing trend compared to the glutamine ($P = 0.078$) group. Threonine concentration in fetal allantoic fluid was significantly decreased in the alcohol and alcohol + glutamine group compared to the saline control group ($P < 0.001$ and $=0.003$, respectively). Arginine concentration in fetal allantoic fluid was significantly decreased in the alcohol and alcohol + glutamine groups compared to the glutamine group ($P = 0.020$ and $P = 0.038$, respectively). Taurine concentration in fetal allantoic fluid was significantly decreased in the alcohol group compared to the saline control ($P = 0.008$) and glutamine ($P = 0.028$) groups, and it was significantly decreased in the alcohol + glutamine group compared to the saline control group ($P = 0.031$). Tyrosine

Table 3 Fetal amniotic fluid amino acid levels on GD 132

	Saline control	Alcohol	Glutamine	Alcohol + glutamine
Aspartate	36 ± 3	31 ± 3	34 ± 2	39 ± 4
Glutamate	74 ± 9	57 ± 7	71 ± 11	78 ± 9
Asparagine	58 ± 10[a]	29 ± 4[b]	46 ± 6[a]	54 ± 9[a]
Serine	646 ± 83[a]	435 ± 47[b]	701 ± 91[a]	713 ± 65[a]
Glutamine	155 ± 44[a]	46 ± 7[c]	147 ± 33[a,b]	116 ± 27[b]
Histidine	46 ± 7	29 ± 4	54 ± 13	43 ± 7
Glycine	337 ± 38[a]	243 ± 16[b]	297 ± 25[a]	329 ± 30[a]
Threonine	114 ± 21[a]	26 ± 4[c]	72 ± 18[b]	71 ± 18[b]
Citrulline	54 ± 12[a]	8 ± 2[c]	25 ± 6[b]	30 ± 12[b]
Arginine	139 ± 29	77 ± 10	110 ± 16	100 ± 14
β-Alanine	83 ± 35	60 ± 14	124 ± 35	42 ± 10
Taurine	248 ± 81	138 ± 40	162 ± 35	138 ± 32
Alanine	145 ± 28[a]	79 ± 9[b]	149 ± 27[a]	131 ± 14[a]
Tyrosine	96 ± 12[a,b]	78 ± 6[b,c]	73 ± 7[c]	107 ± 8[a]
Tryptophan	37 ± 12	44 ± 11	48 ± 20	31 ± 12
Methionine	33 ± 10	32 ± 7	44 ± 19	23 ± 10
Valine	127 ± 23	84 ± 14	87 ± 19	96 ± 20
Phenylalanine	83 ± 35	103 ± 31	77 ± 28	67 ± 18
Isoleucine	28 ± 6	18 ± 3	20 ± 3	19 ± 3
Leucine	67 ± 11[a]	39 ± 3[c]	48 ± 5[b,c]	53 ± 4[b]
Ornithine	194 ± 16	203 ± 22	248 ± 25	192 ± 26
Lysine	196 ± 41	128 ± 26	168 ± 29	188 ± 29
Branched-chain AA (BCAA)	228 ± 36	141 ± 14	217 ± 62	175 ± 24

Values, expressed as nmol/mL, are mean ± SEM

AA Amino acids

Within a row, groups not sharing the same superscript are statistically different ($P < 0.05$)

concentration in fetal allantoic fluid was significantly decreased in the alcohol group compared to the glutamine group ($P = 0.008$). Tryptophan concentration in fetal allantoic fluid was significantly decreased in the alcohol and alcohol + glutamine groups compared to the glutamine group ($P = 0.006$ and $P = 0.009$, respectively). Fetal allantoic fluid amino acid levels on GD 132 and details about statistically significant differences are tabulated in Table 4.

Discussion

Four major findings can be gleaned from this study. First, maternal alcohol exposure at these doses during the third trimester-equivalent period of human pregnancy results in intra uterine growth restriction (IUGR). Second, maternal glutamine supplementation provided concurrently with the alcohol exposure during the third trimester-equivalent period was able to prevent the alcohol-induced IUGR. Third, maternal alcohol exposure leads to significant alterations in fetal amino acid availability. Fourth, maternal glutamine supplementation during the third trimester-equivalent period was able to improve fetal amino acids bioavailability.

Alcohol exposure restricts fetal growth

Our finding shows that maternal alcohol exposure during pregnancy restricts fetal growth, and this was evident by decreases in fetal body weight, height, crown-rump length, thoracic girth and head width. This is consistent with clinical studies conducted by various investigators at different locations who report that women who consumed alcohol during pregnancy gave birth to fetuses with lower birth weight and length, smaller head and chest circumference (Cornelius et al. 1999; Smith et al. 1986; Streissguth et al. 1981). Day and colleagues evaluated the long-term effects of prenatal alcohol exposure on growth in adolescence by assessing growth at birth, at 8 and 18 months, and at 3, 6, 10 and 14 years of age. The growth deficits associated with prenatal alcohol exposure were still persistent in offspring at the age of 14 and their weight, height, head circumference and skin thickness was significantly affected (Day et al. 1990, 1999, 2002). In another study, investigators reported that children who were exposed to alcohol during pregnancy had smaller head circumferences at the age of 5–8 years (Coles et al. 1991). Animal studies in other models have also reported that developmental alcohol exposure leads to growth deficits. Chronic low to moderate maternal alcohol consumption (6 % v/v, 15 %

Table 4 Fetal Allantoic fluid amino acid levels on GD 132

	Saline control	Alcohol	Glutamine	Alcohol + glutamine
Aspartate	95 ± 14	129 ± 31	135 ± 20	75 ± 13
Glutamate	379 ± 150	333 ± 81	308 ± 70	223 ± 45
Asparagine	173 ± 25[a]	76 ± 12[c]	173 ± 40[a]	116 ± 23[b]
Serine	17,745 ± 2,834	12,193 ± 2,331	21,036 ± 4,709	15223.17 ± 2,174
Glutamine	920 ± 105	479 ± 91	896 ± 241	770 ± 112
Histidine	247 ± 42[a]	112 ± 24[c]	213 ± 63[a]	156 ± 26[b]
Glycine	978 ± 172	869 ± 107	1,249 ± 278	1,064 ± 123
Threonine	928 ± 116[a]	400 ± 61[d]	662 ± 141[b]	478 ± 67[c]
Citrulline	327 ± 59	183 ± 35	278 ± 67	224 ± 34
Arginine	1,600 ± 181[b]	1,023 ± 171[c]	1,926 ± 456[a]	1,108 ± 189[c]
β-Alanine	851 ± 212	597 ± 105	628 ± 141	528 ± 106
Taurine	5,825 ± 742[a]	3,004 ± 540[b]	5,296 ± 926[a]	3,529 ± 657[b]
Alanine	1,504 ± 306	822 ± 119	1,276 ± 259	1,129 ± 172
Tyrosine	623 ± 84[a,b]	373 ± 46[c]	717 ± 123[a]	498 ± 87[b]
Tryptophan	165 ± 17[b]	100 ± 13[c]	253 ± 72[a]	104 ± 16[c]
Methionine	353 ± 58	225 ± 37	322 ± 117	243 ± 44
Valine	96 ± 21	62 ± 9	96 ± 12	86 ± 14
Phenylalanine	164 ± 74	149 ± 47	197 ± 73	89 ± 32
Isoleucine	132 ± 19	77 ± 11	116 ± 28	89 ± 9
Leucine	312 ± 35	191 ± 36	263 ± 86	239 ± 49
Ornithine	874 ± 184[a]	343 ± 46[c]	776 ± 190[a]	506 ± 122[b]
Lysine	464 ± 104	441 ± 57	452 ± 185	552 ± 103
Branched-chain AA	540 ± 39	330 ± 48	479 ± 113	414 ± 57

Values, expressed as nmol/mL, are mean ± SEM

AA Amino acids

Within a row, groups not sharing the same superscript are statistically different ($P < 0.05$)

derived calories) during pregnancy in Sprague–Dawley rats resulted in a significant decrease in fetal body weight and hind limb length on embryonic day 20 and a significant decrease in snout–rump length and crown–rump length was observed at 8 months of age compared to the control group (Probyn et al. 2012). Moderate to heavy maternal alcohol exposure (20–35 % derived calories) in the rodent model during pregnancy has been shown to decrease birth weight and size (Abel and Dintcheff 1978; Weinberg 1985; Subramanian 1992). All of these results in humans and other animal models at various times and alcohol doses support our findings that prenatal alcohol exposure results in fetal growth deficits. Recently, we and others have reported that maternal alcohol exposure hampers maternal uterine blood flow and vasculature function (Sawant et al. 2014; Subramanian et al. 2014a, b). This reduction in uterine blood flow could be directly or indirectly responsible for alcohol-induced intra uterine growth restriction. In addition, the disturbances in amino acid bioavailability discussed below likely contribute to the growth restriction.

Alcohol alters amino acid availability in the fetus

Amino acids play a crucial role in maintaining normal physiological function and the nutritional status of the body. Amino acids are known to regulate the key metabolic pathways of cell survival, growth, development, and reproduction (Wu 2009, 2010). Sufficient availability of amino acids in the fetal compartment is not only required for fetal development, but also essential to reduce the risk of chronic diseases in adult life (Wu et al. 2004a). Results from this study indicate that repeated maternal alcohol exposure during the third trimester-equivalent period in the sheep model significantly decreased the bioavailability of asparagine, glutamine, histidine and threonine in the fetal plasma and also decreased asparagine, glutamine, glycine, threonine, citrulline, alanine and leucine in fetal amniotic fluid. Levels of asparagine, histidine, threonine, taurine and ornithine were also reduced in fetal allantoic fluid. During gestation, the fetus is suspended in the amniotic fluid compartment, which is a significant source of fetal nutrients and connected to the allantoic sac via the urachus. Allantoic fluid plays a vital role in accumulation and transfer of nutrients (Kwon et al. 2003). A number of amino acids have been demonstrated to be reduced in the maternal and fetal compartments in response to gestational alcohol exposure in rodents. Acute alcohol exposure (0.03 mL/g, 25 % v/v) in the pregnant mouse model resulted in a significant reduction in plasma concentrations of threonine, serine, glutamine, glycine, alanine, and methionine (Padmanabhan et al. 2002). Chronic alcohol exposure during the first two trimester-equivalents of human brain growth (Schenker

et al. 1990) modeled in the rat has been shown to reduce maternal plasma proline and fetal plasma aspartate concentrations (Marquis et al. 1984b). In the ovine model, a chronic third trimester-equivalent alcohol exposure (1.75 g/kg) in a weekend binge drinking pattern (from GD 109–132) resulted in a decrease in glutamine and glutamate, and an increase in methionine, leucine, valine and overall branched-chain amino acids (BCAA) in maternal plasma; in this study, (Ramadoss et al. 2008) which examined an acute after chronic exposure, the authors also reported a decrease in arginine, asparagine, citrulline, threonine, tryptophan, methionine, leucine, histidine, tyrosine, valine and isoleucine levels in maternal plasma. Another study in sheep examined the effect of a single acute alcohol exposure (1.75 g/kg) during the third trimester-equivalent period and observed that it resulted in a decrease in glutamine, citrulline, branch chain amino acids, serine and asparagine in maternal plasma, and glutamine, phenylalanine, asparagine and tryptophan in fetal plasma (Washburn et al. 2013). Collectively, these reports are consistent with our findings and imply that alcohol exposure during pregnancy alters amino acid bioavailability in both the maternal and fetal compartments, which could be a mechanism for the IUGR observed.

Effect of glutamine supplementation on amino acid availability and fetal growth

Glutamine is an abundant free amino acid in the cellular as well as the extracellular compartment. It is not only a key precursor for the synthesis of many amino acids including glutamate, arginine, proline, asparagine, ornithine and citrulline (Wu et al. 2011), but it also a precursor of the brain neurotransmitter glutamate, the cellular anti-oxidant glutathione and other macromolecules (Kwon et al. 2003; Mates et al. 2002a; Wu et al. 2004b). Glutamine has also been associated with having an important role as an apoptosis suppressor (Mates et al. 2002b). In an ovine model, between GD 60–140, the bioavailability of glutamine in fetal plasma is 2–3 times greater than that of maternal plasma (Kwon et al. 2003). Results from this study demonstrate that maternal L-glutamine supplementation mitigates alcohol-induced fetal growth restriction and improves amino acid bioavailability. A single administration of an aqueous bolus of L-glutamine during the third trimester-equivalent period in sheep improved the amino acid profiles in the maternal as well as fetal compartments (Washburn et al. 2013). Glutamine supplementation in postweaning pigs prevented jejunal atrophy, increased plasma concentration of aspartate, glutamate and alanine, improved body weight gain, increased intestinal expression of genes related to cell growth and antioxidants, and suppressed expression of genes that promote oxidative stress and immune activation (Wu et al. 1996; Wang et al. 2008). Haynes and colleagues reported that glutamine administration in neonatal piglets enhanced growth performance and prevented endotoxin-induced enterocytes death by reducing intestinal expression of Toll-like receptor-4, active caspase-3 and NF-κB (Haynes et al. 2009). Glutamine supplementation in severely ill or extremely low birth weight infants has shown improvement in physical growth, neurodevelopmental outcomes, hepatic tolerance, plasma glutamine concentrations and lowered infectious morbidity (Ehrenkranz et al. 2011; Wang et al. 2010; Poindexter et al. 2003; van den Berg et al. 2007). These results from human clinical cases and animal studies support our findings that maternal glutamine supplementation mitigates alcohol-induced fetal growth deficits and improves amino acid availability. Clearly, under alcohol-induced acidic conditions, pregnant ewes have requirements for exogenous glutamine, as proposed for non-ruminants during gestation and postnatal growth (Wu et al. 2014). As a functional amino acid (Wu 2013), glutamine plays an important role in regulating fetal growth and development, and can be a key nutrient for treatment of human FASD.

In summary, we demonstrated that maternal alcohol exposure administered in a binge drinking paradigm during the human third trimester-equivalent period alters amino acid homeostasis and leads to fetal intra-uterine growth restriction (IUGR). Maternal glutamine supplementation was able to prevent alcohol-induced alterations in amino acid availability and improved fetal growth. Perturbations during gestation can have detrimental effects on the postnatal development of offspring. Nutritional disturbances during the period of development can be associated with an increased risk of early onset of various diseases in the future. Findings from this study not only demonstrate that alcohol-induced imbalances in amino acids are directly or indirectly responsible for fetal growth restriction but also create a foundation for designing nutrition-based therapeutic interventions to ameliorate alcohol-induced IUGR.

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