Original Research

The tissue and plasma concentration of polyols and sugars in sheep intrauterine growth retardation

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Abstract

In an ovine model of placental insufficiency-induced intrauterine growth retardation (PI-IUGR), characterized by hypoxia, hypoglycemia and a significant reduction in fetal weight, we assessed alterations in fetal and placental polyols. Arterial maternal–fetal concentration differences of glucose and mannose were greater in the PI-IUGR fetus; glucose: C ($n = 7$), 2.68 ± 0.14 mmol/L versus PI-IUGR ($n = 9$), 3.18 ± 0.16 mmol/L ($P < 0.02$) and mannose: C, 42.9 ± 8.1 μmol/L versus PI-IUGR, 68.5 ± 19.1 μmol/L ($P < 0.001$). For PI-IUGR fetuses, fetal arterial plasma *myo*-inositol concentrations were significantly increased ($P < 0.001$). The concentrations of sorbitol, glucose and fructose were significantly reduced ($P < 0.03$, 0.01, 0.02, respectively). The cotyledons of IUGR placentas had a significantly increased concentration of *myo*-inositol ($P < 0.003$) and decreased concentrations of sorbitol, fructose and glycerol ($P < 0.01$, 0.02, 0.01, respectively). Fetal hepatic concentrations of sorbitol ($P < 0.001$) and fructose ($P < 0.03$) were also significantly reduced. These profound changes in both placental and fetal concentrations of polyols and sugars in sheep PI-IUGR pregnancies support the conclusion that within the PI-IUGR placenta there is an increased flux through the glucose 6-P:inositol 1-P cyclase system and decreased flux through the polyol dehydrogenase system, leading to increased placental *myo*-inositol production and decreased sorbitol production. The decreased placental supply of sorbitol to the fetal liver may lead to decreased fetal hepatic fructose production. These observations highlight that, in association with hypoxic and hypoglycemic PI-IUGR fetuses, there are major placental and fetal alterations in polyol production. The manner in which these alterations in fetoplacental carbohydrate metabolism contribute to the pathophysiology of PI-IUGR is currently unknown.

Keywords: pregnancy, IUGR, fetal and placental polyols and sugars, umbilical carbohydrate uptake, *myo*-inositol, mannose, sorbitol, fructose

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Introduction

Polyols, or sugar alcohols including sorbitol, fructose and *myo*-inositol, are formed through the reduction of aldoses and ketoses, through the polyol pathway. Aldose reductase (AR) is the first and rate-limiting enzyme of the polyol pathway, which catalyzes the reduction of glucose to the corresponding sugar alcohol, sorbitol, which is then converted to fructose by sorbitol dehydrogenase.[1] The activity of AR requires the presence of its co-factor, nicotinamide adenine dinucleotide phosphate (NADPH), and appears to be regulated by glucose concentrations and osmolality. The human and sheep placenta both display a functional AR pathway, promoting the production of sorbitol from glucose.[2–4] The umbilical uptake of sorbitol in these two species reflects placental production since there is no uptake into the placenta from the uterine circulation.[3,4] Fructose, the end product of sorbitol metabolism, is the major carbohydrate in fetal blood for all Ungulate and Cetacea[5,6] and, in adults, is a major precursor for hepatic glucose production.[7] In the fetus, fructose concentrations are greatly elevated compared with the placenta, consistent with the enzymatic studies that demonstrate an active fetal

hepatic sorbitol dehydrogenase pathway in conjunction with relatively low placental sorbitol dehydrogenase activity, although there is high AR activity.[8]

Overall placental glucose flux to sorbitol is dependent on adequate AR activity and adequate NADPH concentrations. It is also competing with the *myo*-inositol production pathway through glucose 6-P:inositol 1-P cyclase activity. Glucose 6-P:inositol 1-P cyclase activity has been documented in the brain, liver and kidney as well as in placental and fetal preparations,[9–12] and results in the production of *myo*-inositol from glucose-6-P. *Myo*-inositol is an important constituent of living cells, serving as precursor to phosphatidylinositol[13] and deficiencies are associated with alterations in cell growth and abnormal phosphoinositide metabolism.[14] *Myo*-inositol concentrations increase during fetal development and decrease postnatally.[12,15,16] Although glucose-6-P cyclase is found in placenta,[11] previous human and sheep studies have not demonstrated an umbilical *myo*-inositol uptake, despite significantly elevated fetal concentrations,[3,4] suggesting that fetal *myo*-inositol requirements are met through fetal production from circulating fetal glucose.

The reproductive tract, placenta and fetus are sites of production of polyols,[3,4,17,18] and the production is favored under relative low oxygen tension, which characterizes the placental and fetal environment during gestation.[18,19] The polyol pathway is an important pathway for the regeneration of the oxidized form of nicotinamide adenine dinucleotide ($NADP^+$), from its reduced form, NADPH. Alterations in the nutrient and oxygen supply to the fetoplacental unit could alter the balance of these polyol and sugar pathways. For instance, elevated glucose concentrations during rat embryonic culture result in an activation of the AR pathway with an accumulation of sorbitol and a corresponding decline in *myo*-inositol.[20] These changes have been associated with growth restriction and dysmorphogenesis in rat embryos, although the exact role of *myo*-inositol remains to be evaluated.

In placental insufficiency-induced intrauterine growth retardation (PI-IUGR) pregnancies, the glucose gradient across the placenta is significantly increased,[21,22] often in association with a degree of fetal hypoxia as observed in human IUGR.[23] These factors suggest that in PI-IUGR, changes in polyol metabolism and concentration may also exist. Given the important role polyols play in cell metabolism, the present study had as a goal to investigate maternal and fetal carbohydrate concentrations in the situation of PI-IUGR, with the aim of highlighting possible alterations in the metabolic pathways associated with polyol and sugar metabolism in PI-IUGR

Materials and methods

Animal treatments and surgery

Seventeen 2–3-year-old Columbia–Rambouillet ewes pregnant with a single fetus were used for these studies. Animal care was in strict compliance with National Institutes of Health guidelines within an American Association for Accreditation of Laboratory Animal Care certified facility. The study was approved by the University of Colorado Health Sciences Center Animal Care and Use Committee. The naturally occurring model of hyperthermia-induced IUGR was utilized for this study.[24] This model of PI-IUGR has highlighted some of the important metabolic and organ-specific adaptations, such as placental,[22] in fetal pancreas,[25] heart,[26] nutrient transport systems[27] and glucose/insulin interactions,[28] similar to those found in human IUGR pregnancies.[29–32]

At approximately 39 d gestational age (dGA; term = 147 ± 3 dGA), ewes were randomly allocated to either the control thermoneutral or hyperthermic treatment. A total of seven ewes were maintained in the control regimen; a further 10 ewes were moved to individual pens in a hyperthermic chamber (Bainbridge, Marcellus, MI, USA). The specific hyperthermic treatment and associated feeding regimens of animals undergoing this treatment has been described previously.[21,22,33,34] Ewes were maintained in the hyperthermic environment until 120 ± 1 dGA, at which time they were moved to the control themoneutral environment room where feeding continued *ad libitum*. At 127 ± 1 dGA, ewes underwent surgery for placement of fetal and maternal catheters for blood gas, flow and carbohydrate and polyol concentration determination. The surgery and blood flow determinations have been described elsewhere.[22] Animals were allowed to recover from laparotomy for at least five days before study.

Blood flow determination, plasma and tissue collection and calculations

All animal core body temperatures were 39.1°C at study. Ethanol was used to determine umbilical blood flow by the steady-state diffusion method.[35] At ethanol steady state, four sets of blood samples were drawn at approximately 20-min intervals from the maternal femoral artery, uterine vein and umbilical vein and artery for analysis of metabolites (polyols, sugars and lactate), maternal and fetal blood gases and umbilical ethanol concentration (steady-state period), the latter for blood flow determination.[22]

Hematocrit (Hct) was determined as packed cell volume. Umbilical plasma flows were calculated using umbilical flow multiplied by (1 − fractional maternal or fetal Hct, respectively). Ethanol clearance rates, umbilical oxygen and polyol and carbohydrate uptakes were calculated using the Fick principle, as described previously.[35,36] Oxygen uptake was expressed as mmol/min/kg_{fetus}, while polyol and sugar uptake were expressed as μmol/min/kg_{fetus} and nmol/min/kg_{fetus}.

The blood samples for polyol and sugar alcohol measurement were centrifuged at 4°C and stored as plasma in a −80°C freezer until high-performance liquid chromatography (HPLC) analysis. At the time of necropsy, the animals were anesthetized, the uterus removed and dissected into placental and fetal components. Then, the animals were injected with 12 mL Sleepaway (Fort Dodge Animal Health, Fort Dodge, IA, USA). At necropsy, catheter locations were confirmed and fetal and placental weights were obtained. Fetal liver and brain were removed and

weighed and the fetal brain/liver ratio (brain weight [g]/liver weight [g]) calculated as a measure of asymmetrical growth restriction.[33,37] Placentomes were trimmed of endometrium and fetal membranes, and weighed, with placental weight being recorded as the sum of the placentomes. A total of 10 placentomes (Types B and C)[38] per placenta were collected and rinsed in ice-cold sterile saline, carefully dissected into maternal (caruncle) and fetal (cotyledon) components. These placentomes were selected as they represent the placentomes that optimize transplacental exchange efficiency.[38,39] The cotyledon was snap frozen in liquid nitrogen and stored at -80°C. Fetal liver was also collected, divided into right and left lobes and a section of the left lobe snap frozen and stored at -80°C. Left lobe selection is carried out in these studies as fetal human and sheep livers share similar vessel arrangement, and in IUGR, distribution of blood flow to the left lobe is unaltered while to the right it is significantly reduced.[40] This would allow comparisons of tissue responses independent of flow considerations.

HPLC analysis

Plasma samples were thawed and de-proteinized as follows: 0.1 mL 0.6 N zinc sulfate containing 30 mg% xylitol as internal standard was added to 0.1 mL plasma, the mixture was mixed well and 0.1 mL of 0.3 N barium hydroxide was added. The suspension was centrifuged at 14,000 *g* for 10 min and the supernatant filtered through a 0.45 μm filter before loading onto a refrigerated autosampler for HPLC analysis. Carbohydrate analysis was also conducted on the collected cotyledons and fetal liver samples. Following powdering of 6–10 g of collected tissue under liquid nitrogen, approximately one gram of tissue was thawed, homogenized and sonicated in distilled water at 4°C. After centrifuging, the tissue supernatant was de-proteinized and analyzed as for plasma. On tissue samples, a separate analysis without the addition of xylitol as internal standard was performed to quantify the xylitol concentration in tissue.[4]

A Dionex HPLC analyzer equipped with a CarboPac MA1 anion-exchange column (Dionex, Sunnyvale, CA, USA) was used for the separation of the hexoses and polyols (inositol [*myo*-inositol], glycerol, erythritol, arabitol, sorbitol, ribitol, mannitol, mannose, glucose and fructose). The analysis was run isocratically with 500 mmol/L sodium hydroxide for 25 min, followed by a step change to 400 mmol/L sodium hydroxide for 20 min at ambient temperature. The flow rate was 0.4 mL/h. The sodium hydroxide solution was prepared with de-gassed, de-ionized water. All the peaks were quantified using a pulse amperometric detector with a gold working electrode.[41] The Dionex PeakNet software was used for instrument operation and data analysis. The concentrations of each sugar were calculated by using the integrated area under the peak. The internal xylitol standard was used to correct for instrument variances with the equation:

$$\text{Concentration } (\mu\text{mol/L} = (A_u/A_s)\,K\,D\,X_s/X_u)$$

where A_u and A_s are peak areas for the sugar in the unknown and standard, X_u and X_s are the xylitol area in the unknown and standard, K is the standard, A is the concentration in μmol/L and D the dilution factor.

Statistics

All data were expressed as means $\pm$ standard error of mean. Plasma and tissue concentrations were analyzed using unpaired Student's *t*-test. The umbilical uptake data were analyzed for significance from zero. Differences between control and PI-IUGR were tested with an unpaired Student's *t*-test after testing for a normal distribution. Two-tailed values were considered significant at $P < 0.05$.

Results

Animal physiological data

At approximately 133 dGA (90% term), PI-IUGR placental and fetal weights were significantly reduced ($P < 0.001$ and $P < 0.001$; Supplementary data S1). PI-UGR liver and brain weights were reduced in absolute terms ($P < 0.001$ and $P < 0.02$; Supplementary data S1), but were increased when expressed as a percentage of fetal body weight ($P < 0.003$ and $P < 0.01$; Supplementary data S1). The fetal/placenta ratio was unchanged between the two treatments, but the brain/liver ratio was significantly increased ($P < 0.03$; Supplementary data S1), indicative of asymmetric growth restriction in PI-IUGR.

In PI-IUGR, umbilical blood flow was significantly decreased, both in absolute and relative terms ($P < 0.001$ and $P < 0.02$; Supplementary data S2). Both control and PI-IUGR animals had a similar arterial blood pH despite the growth restriction. In the PI-IUGR fetuses, Hct was elevated ($P < 0.07$) and O_2 content, saturation and partial pressure were all significantly reduced ($P < 0.003$, $P < 0.001$ and $P < 0.001$; Supplementary data S2). Fetal arterial glucose in PI-IUGR was significantly reduced ($P < 0.001$) and lactate concentrations were slightly increased ($P < 0.07$). Both umbilical uptake of oxygen and glucose per kilogram of fetus were not significantly different in the PI-IUGR animals when compared with the control group (Supplementary data S2).

Circulating maternal and fetal carbohydrate concentrations

Maternal concentrations

Mannose, fructose and glucose were the major carbohydrates in maternal circulation. Maternal arterial mannose concentrations were significantly increased in PI-IUGR pregnancies compared with controls ($P < 0.001$; Table 1), while *myo*-inositol tended to be increased ($P < 0.06$). Concentrations of erythritol were significantly decreased by almost 50% ($P < 0.004$; Table 1). In the uterine vein, concentration changes of *myo*-inositol and erythritol were similar to the maternal artery ($P < 0.02$ and $P < 0.005$, respectively; Table 1) and glycerol was significantly increased ($P < 0.04$; Table 1). Concentrations of

Table 1 Maternal steady-state polyol and sugar concentrations (μmol/L) in control ($n = 7$) pregnant ewes and ewes pregnant with a PI-IUGR ($n = 10$) fetus*

	C artery	PI-IUGR artery	P value	C uterine vein	PI-IUGR uterine vein	P value
Myo-inositol	17.61 ± 3.48	26.39 ± 2.81	0.06	14.94 ± 2.15	26.54 ± 3.32	0.02
Glycerol	23.02 ± 5.64	32.06 ± 3.77	NS	20.57 ± 3.23	31.97 ± 3.92	0.04
Erythritol	9.61 ± 1.2	5.42 ± 0.59	0.01	9.47 ± 1.21	5.65 ± .048	0.01
Arabitol	24.49 ± 3.07	31.72 ± 6.22	NS	23.40 ± 1.38	35.06 ± 5.89	NS
Sorbitol	5.99 ± 0.28	5.15 ± 0.92	NS	9.53 ± 2.01	5.50 ± 0.65	NS
Mannitol	7.56 ± 1.48	6.13 ± 1.27	NS	10.78 ± 3.46	5.71 ± 0.97	NS
Mannose	71.63 ± 2.25	100.21 ± 4.37	0.001	71.02 ± 1.80	97.58 ± 4.76	0.001
Glucose	3618.9 ± 100.5	3700.8 ± 135.1	NS	3324.2 ± 107.7	3563.2 ± 147.9	NS
Fructose	121.95 ± 35.82	77.70 ± 30.57	NS	159.25 ± 39.09	39.60 ± 7.75	0.01

PI-IUGR, placental insufficiency-induced intrauterine growth retardation
*Data are means ± standard error of mean. NS, not significant ($P > 0.05$)

mannose were significantly increased in the maternal PI-IUGR uterine vein ($P < 0.001$; Table 1) and fructose concentrations were significantly depressed ($P < 0.01$; Table 1).

Fetal concentrations

Concentrations of the polyols in the fetal circulation were much higher than in the maternal circulation (Tables 1 and 2). Fructose and glucose were the major carbohydrates in fetal circulation. In PI-IUGR fetuses, arterial plasma concentrations were significantly increased for *myo*-inositol ($P < 0.001$; Table 2), while the concentrations of sorbitol, erythritol, glucose and fructose were significantly reduced ($P < 0.03$, $P < 0.03$ and $P < 0.01$ and $P < 0.02$, respectively; Table 2). These changes were mirrored in the fetal umbilical vein of the PI-IUGR fetus; *myo*-inositol concentrations were increased ($P < 0.001$; Table 2) and sorbitol ($P < 0.05$), glucose ($P < 0.03$), fructose ($P < 0.02$) and glycerol ($P < 0.04$) were all significantly decreased.

The arterial maternal–fetal concentration differences were greater in the PI-IUGR pregnancy for both glucose (C, 2.51 ± 0.11 versus PI-IUGR, 3.00 ± 0.11 mmol/L, $P < 0.02$; Figure 1a) and mannose (C, 43.30 ± 2.58 versus PI-IUGR, 73.86 ± 4.19 μmol/L, $P < 0.001$; Figure 1b).

Umbilical carbohydrate uptakes

There was a significant uptake of mannose into the umbilical circulation in both the control and PI-IUGR group, but the uptakes were not significantly different between control and PI-IUGR (Figure 2). Sorbitol and ribitol also displayed significant umbilical uptakes from zero in control pregnancies (Figure 2). Glycerol uptake was significantly reduced in IUGR pregnancies ($P < 0.04$; Figure 2), while umbilical sorbitol uptake in PI-IUGR was reduced ($P < 0.07$; Figure 2). There was a significant uptake of glucose in both control and PI-IUGR groups (C: 22834.09 ± 4208.18 nmol/min/kg and IUGR: 21581.52 ± 2852.94 nmol/min/kg; $P < 0.001$ for both).

Placental and liver carbohydrate concentrations

The cotyledons (fetal placenta) had a significantly increased concentration of *myo*-inositol ($P < 0.003$) in PI-IUGR and decreased concentrations of sorbitol, fructose and glycerol ($P < 0.01$, $P < 0.02$ and $P < 0.01$; Figure 3). Fetal hepatic concentrations of sorbitol and mannitol were significantly reduced ($P < 0.001$ and $P < 0.02$; Figure 4a), while those of arabitol and ribitol were significantly increased in the PI-IUGR ($P < 0.01$ and $P < 0.02$; Figure 4a). Hepatic glucose and *myo*-inositol concentrations were not significantly different between the two groups, whereas hepatic fructose concentration in PI-IUGR was reduced ($P < 0.03$; Figure 4b), and mannose concentration tended to be elevated in PI-IUGR ($P < 0.06$; Figure 4b).

Discussion

The placenta and fetus are sites of production and transport of a variety of polyols and sugar alcohols, and in normal ovine pregnancy, there is a high activity of the polyol pathway in the fetoplacental unit.[4] In a model of PI-IUGR

Table 2 Fetal steady-state polyol and sugar concentrations (μmol/L) in control ($n = 7$) and PI-IUGR ($n = 9$) fetuses*

	C artery	PI-IUGR artery	P value	C umbilical vein	PI-IUGR umbilical vein	P value
Myo-inositol	540.74 ± 166.49	1748.8 ± 222.0	0.001	570.44 ± 183.25	1800.4 ± 221.65	0.001
Glycerol	25.79 ± 2.86	20.11 ± 4.64	NS	37.66 ± 4.99	21.09 ± 5.36	0.04
Erythritol	323.92 ± 27.02	252.98 ± 14.49	0.03	327.84 ± 26.42	273.96 ± 19.42	NS
Arabitol	77.34 ± 12.94	72.50 ± 6.96	NS	87.63 ± 10.77	86.03 ± 9.66	NS
Sorbitol	108.02 ± 9.65	75.15 ± 9.09	0.03	120.59 ± 7.32	82.61 ± 8.37	0.005
Ribitol	139.04 ± 21.11	171.21 ± 18.79	NS	153.40 ± 21.35	187.38 ± 15.62	NS
Mannitol	66.48 ± 11.54	51.75 ± 8.20	NS	77.75 ± 15.34	58.67 ± 8.86	NS
Mannose	28.32 ± 1.33	28.03 ± 4.62	NS	31.05 ± 1.79	33.37 ± 4.50	NS
Glucose	1105.1 ± 34.4	730.78 ± 93.21	0.01	1264.3 ± 34.9	975.50 ± 104.53	0.03
Fructose	6479.3 ± 881.5	3378.6 ± 765.9	0.02	6680.3 ± 954.1	3552.8 ± 775.5	0.02

PI-IUGR, placental insufficiency-induced intrauterine growth retardation
*Data are means ± standard error of mean. One PI-IUGR fetus was not measured due to umbilical vein catheter failure. NS, not significant ($P > 0.05$)

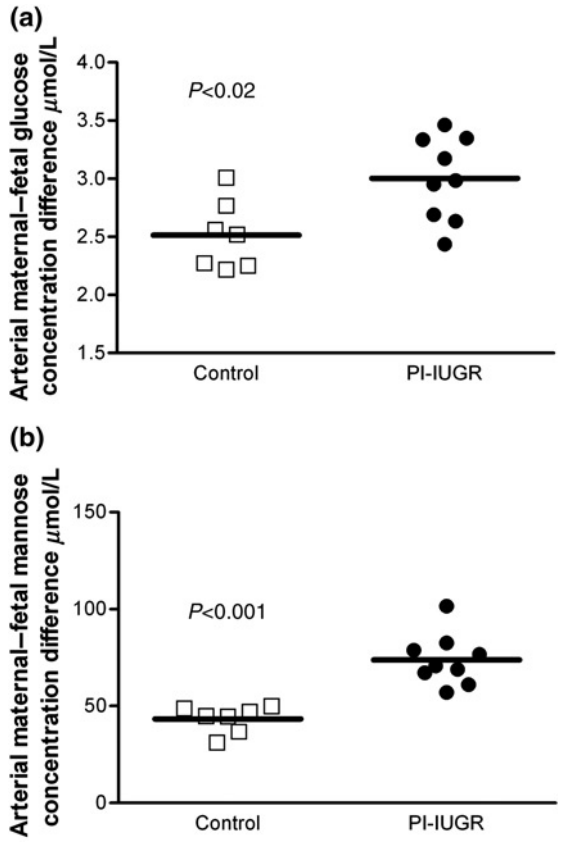

Figure 1 Arterial maternal–fetal glucose (a) and mannose (b) concentration differences in control ($n = 7$) and placental insufficiency-induced intrauterine growth retardation ($n = 9$) pregnancies near term. Data scatter plot with mean. PI-IUGR, placental insufficiency-induced intrauterine growth retardation

(Supplementary data S1 and S2), there appears to be major changes in this activity. Overall, both the plasma and tissue concentration changes reported here support the conclusion that, in conjunction with an increased maternal–fetal glucose gradient, within the PI-IUGR placenta there is an increased flux of glucose through the glucose 6-P:inositol 1-P cyclase system. This change is accompanied by a decreased flux through the AR polyol dehydrogenase system leading to increased placental *myo*-inositol production and decreased placental sorbitol production. The decreased sorbitol production likely leads to decreased fetal hepatic fructose production and a lower fetal fructose plasma concentration in PI-IUGR.

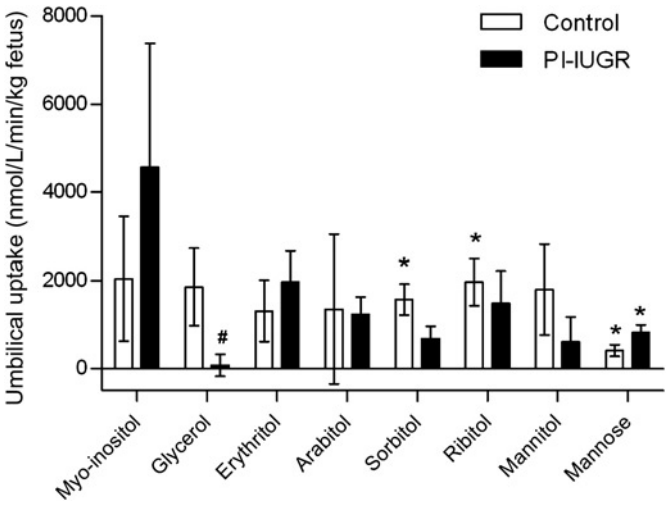

Figure 2 Umbilical uptakes of polyol and sugar (nmol/L/min/kg fetus) in control ($n = 7$) ewes and ewes carrying a PI-IUGR ($n = 9$) fetus when studied under control conditions. Data are means ± standard error of mean. *t*-test, *significant from zero ($P < 0.05$), #significant between control and PI-IUGR ($P < 0.01$). PI-IUGR, placental insufficiency-induced intrauterine growth retardation

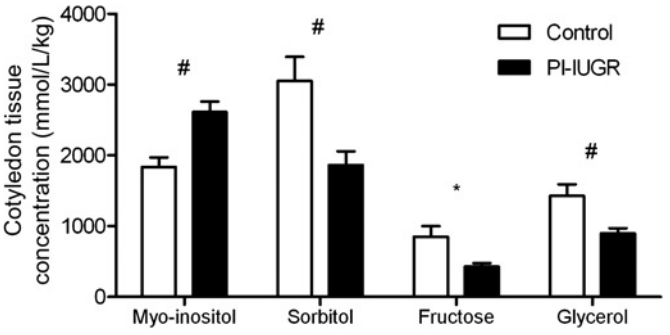

Figure 3 Placenta cotyledon (fetal placenta) tissue polyol concentrations in control ($n = 7$) and PI-IUGR ($n = 8$). Data are means ± standard error of mean. *t*-test, *significant between control and PI-IUGR ($P < 0.05$), #significant between control and PI-IUGR ($P < 0.01$). PI-IUGR, placental insufficiency-induced intrauterine growth retardation

Interestingly, in the current study, PI-IUGR is characterized by not only an increased maternal–fetal gradient for glucose, but also for mannose (Figure 3). Previous studies (normal human and sheep pregnancies) found a significant uptake of mannose into the fetal circulation from the placenta,[3,4] supporting the idea of a requirement for an exogenous supply of mannose. Given the very low maternal mannose concentration, these studies also suggest the existence of a specific placental mannose transporter. In the present study, fetal plasma mannose concentrations were correlated with maternal concentration. When umbilical uptake of mannose was expressed per kg fetus, there was a significantly higher uptake in PI-IUGR pregnancy. As mannose glycans and glycoproteins must be synthesized during fetal development, the delivery of an exogenous supply of mannose to the fetus may be important in overall fetal development and growth. While these observations for mannose parallel those found for glucose, it is surprising when one considers that the maternal mannose concentration is only 2% of the maternal glucose concentration. This is not a finding unique to sheep pregnancy as studies of uncomplicated human pregnancies at the time of elective cesarean section gave similar results both in terms of a significant uptake of mannose into the fetal circulation and in the linear relationship of fetal mannose concentration to maternal concentration.[4,42] Therefore, we postulate that an exogenous supply of mannose from the mother, through the placenta, is required to meet fetal mannose requirements, suggesting the activity of a specific high-affinity placental mannose transport system.

In uncomplicated pregnancies, the fetal/maternal ratios for the majority of polyols are much greater than those

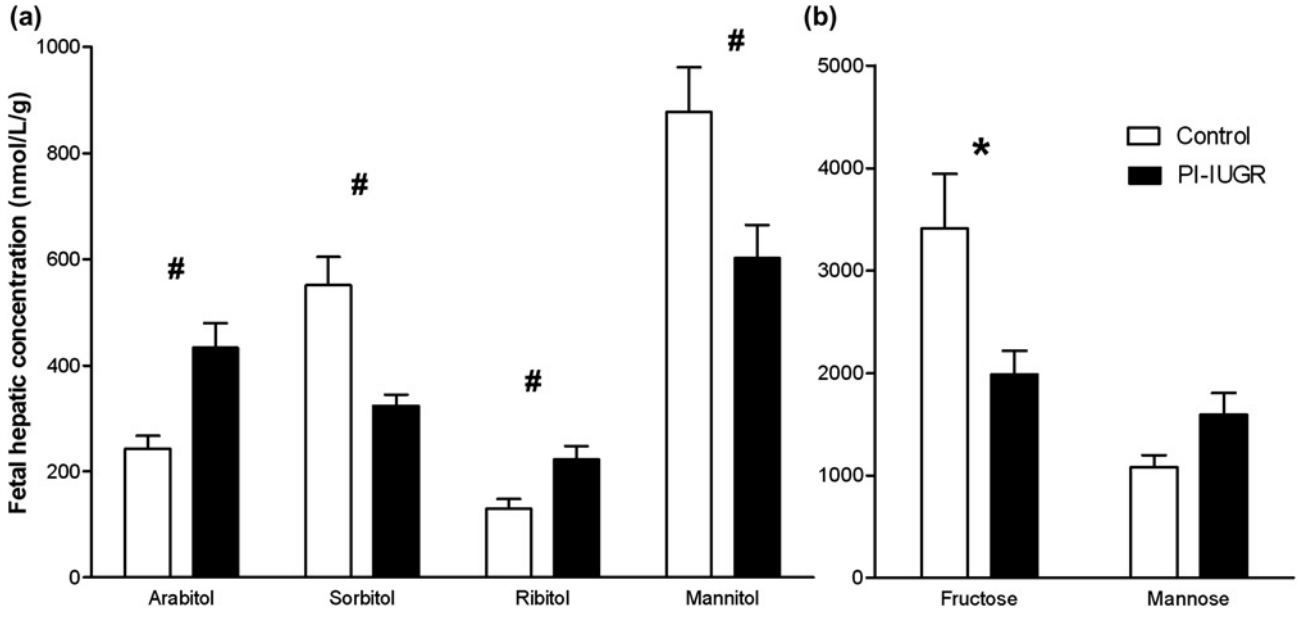

Figure 4 Fetal hepatic (a) polyol and (b) fructose and mannose concentrations in control ($n = 7$) and PI-IUGR ($n = 8$) fetuses. Data are means $\pm$ standard error of mean. *t*-test, *significant between control and PI-IUGR ($P < 0.05$), #significant between control and PI-IUGR ($P < 0.01$). PI-IUGR, placental insufficiency-induced intrauterine growth retardation

displayed for amino acids[3,4] The high concentrations of fetal polyols suggest high production rates and a low placental permeability. Of particular interest is a significant increase in placental and fetal plasma *myo*-inositol concentrations and associated changes in sorbitol. Both *myo*-inositol and sorbitol are known to be important in reproductive tissues and in early development with multiple functions in neural, lung and muscle tissue. *Myo*-inositol is at very high concentrations as the free alcohol in embryonic and fetal tissues.[43–46] In fact, in brain, skeletal and cardiac muscle, it is much higher in concentration than any other carbohydrate, including glucose,[47] suggesting that it may play an important role in fetal development of these organs.

In a model of PI-IUGR, it appears that increased placental and fetal plasma *myo*-inositol concentrations are the result of increased activation of the glucose 6-P:inositol 1-P cyclase pathway. These data strongly suggest that in PI-IUGR, a marked increase in placental glucose flux through the *myo*-inositol cyclase pathway leads to a net delivery of *myo*-inositol into the fetal circulation. *Myo*-inositol is an important constituent of living cells, serving as precursor to phosphatidylinositol. Reduced maternal *myo*-inositol has been associated with an increased incidence of spina bifida.[16] Conversely, it appears that excessive *myo*-inositol may be harmful to the fetus. Reports of elevated *myo*-inositol concentrations *in utero* have been associated with a deficient fetal lung surfactant phosphatidylglycerol (PG) production in diabetic pregnancies and reduced blood oxygenation in newborns with PG-deficient surfactant as a result of elevated *myo*-inositol concentrations *in utero*.[48]

Evidence that the AR and *myo*-inositol cyclase act in balance comes from studies associated with diabetic cataracts.[49–51] An alteration between the AR and *myo*-inositol cyclase pathways in human lens tissue promotes the flux of glucose into the sorbitol pathway rather than the *myo*-inositol pathway leading to high sorbitol concentrations in the lens associated with diabetic cataracts and to reduced *myo*-inositol concentrations.[52,53] In this case, it is well established that an increased flux through the AR pathway (with sorbitol production) results in a decreased flux through the cyclase pathway (with decreased *myo*-inositol production).

Our data show that as a result of altered glucose metabolism in this model of PI-IUGR, dramatic changes in placental *myo*-inositol, sorbitol and fructose concentrations also occur. The reason for the re-direction of placental glucose flux into *myo*-inositol production may rest, in part, upon the decreased sorbitol and fructose production. The production of sorbitol within the placenta via AR requires the availability of the co-factor NADPH. In the placenta, which lacks a pentose phosphate pathway, NADPH is provided by the oxidation of glutamate. Normally, there is a very large placental uptake of glutamate from the fetal circulation into the placenta.[54] However, our studies have shown that placental glutamate uptake is markedly reduced in the IUGR ovine pregnancy.[55] Hence, the reduction in the availability of NADPH may explain the reduced placental sorbitol production and increased flux through the cyclase pathway to *myo*-inositol. This is presented in Figure 5. This is the first time that a possible link between glutamate metabolism and carbohydrate metabolism has been proposed.

Hepatic concentrations of sorbitol and mannitol are reduced in IUGR. The reduction in sorbitol is likely a reflection of the fetal hypoglycemic status and a downregulation of the AR pathway resulting in reduced hepatic fructose as well. Interestingly, *myo*-inositol is unchanged, which might be indicative of a maintenance or elevation in the IUGR

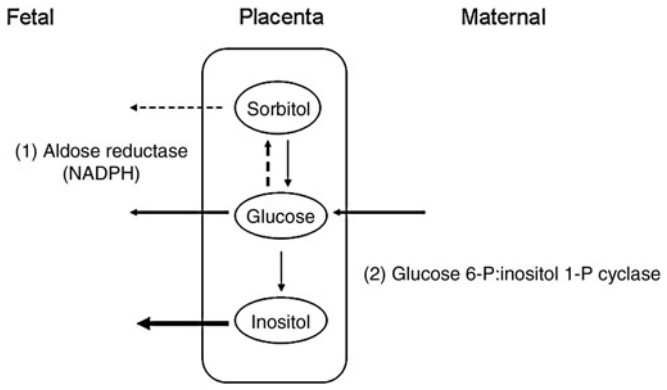

Figure 5 Possible explanation of how a reduction in the availability of nicotinamide adenine dinucleotide phosphate from glutamate oxidation may explain a reduced placental sorbitol production and increased flux through the cyclase pathway to inositol

situation of hepatic inositol cyclase activity. The polyols arabitol and ribitol, conversely, are elevated in IUGR livers. The pentitols, ribitol and arabitol elevated in PI-IUGR livers are formed from their corresponding pentoses, D-ribose and D-arabinose, likely catalyzed by a pentose reductase. Interestingly, unlike other pentitols, ribitol and arabitol appear to be able to cross-cell membranes.[56] Most likely, ribitol and arabitol are metabolic end products, since no evidence is reported for an arabitol or ribitol dehydrogenase in human studies.[56] Changes in concentrations of these products, however, have been associated with a number of clinical conditions. Deficiencies in ribose-5-phopshate isomerase, a new defect in the pentose phosphate pathway, have been associated with white matter disorders and highly elevated ribitol and arabitol levels in the brain.[57] In addition, transaldolase deficiency, another defect in the pentose phosphate pathway, has been diagnosed in patients with liver dysfunction, where increased concentrations of D-arabitol and ribitol as well as erythritol are observed.[57–59] While little is known about the roles of these polyols, limited data suggest they may play a role in a number of complications associated with the pentose phosphate pathway.

In our current studies in the PI-IUGR placenta and fetus, there appears a re-direction of glucose flux away from the AR pathway in favor for sorbitol production and a re-direction into the glucose–*myo*-inositol cyclase pathway, resulting in increased *myo*-inositol production. Furthermore, there appears to be a dysfunctional pentose phosphate pathway, in that increased ribitol and arabitol production occurs. These changes may also have additional impact through their role as potential idioosmoles.

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