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Polyol Concentrations in the Fluid Compartments of the Human Conceptus during the First Trimester of Pregnancy: Maintenance of Redox Potential in a Low Oxygen Environment

Eric Jauniaux, Joanne Hempstock, Cecilia Teng, Frederick C. Battaglia, and Graham J. Burton

Academic Department of Obstetrics and Gynecology, Royal Free and University College London Medical School (E.J.), London, United Kingdom WC1E 6HX; Department of Pediatrics, University of Colorado School of Medicine (F.C.B., C.T.), Aurora, Colorado 80045-0508; and Department of Anatomy, University of Cambridge (J.H., G.J.B.), Cambridge, United Kingdom CB2 3DY

Polyols are sugar alcohols formed by the reduction of aldoses and ketoses. Production is favored under conditions of low oxygenation, when it may provide an alternative means to production of lactate for regulating the oxidation-reduction balance of pyridine nucleotides. Polyols also act as important organic osmolytes and as precursors of cell membrane components. We measured free sugar and polyol concentrations in matched samples of maternal serum, intervillous fluid, coelomic fluid, and amniotic fluid from normal human pregnancies at 5–12 wk gestational age. The concentrations of fructose, inositol, sorbitol, erythritol, and ribitol were significantly higher in coelomic and amniotic fluids than in maternal serum, but the reverse was the case for glucose and glycerol. Intervillous fluid concentrations of inositol, mannitol, and sorbitol were also significantly higher than those in maternal serum. These results demonstrate that the polyol pathway, considered vestigial in adult tissues, is highly active in the human conceptus during early pregnancy. The pathway may serve to maintain ATP concentrations and cellular redox potential while the embryo develops in a low oxygen environment. Polyols may also play important physiological roles in development of the human conceptus, possibly drawing water and solutes across the placenta and expanding the gestational sac. (*J Clin Endocrinol Metab* 90: 1171–1175, 2005)

THE RECENT REALIZATION that development of the human fetoplacental unit during the first trimester of pregnancy takes place in a low oxygen environment (1, 2) prompted us to investigate carbohydrate metabolism within the conceptus. Polyols are sugar alcohols formed by the reduction of aldoses and ketoses, and their precursors are essential substrates of the glycolytic and pentose phosphate pathways. The polyol pathway is frequently encountered in lower forms of life, such as species of *Candida* and filamentous fungi, but its presence is restricted in mammals to a few specialized tissues, notably the seminal vesicles, lens, and neural tissue (3). It has been suggested that polyols represent the primary source of carbohydrates for early life, because it is unlikely that oxidizing molecules, such as aldoses and ketoses, could have existed in significant concentrations under the heavily reducing conditions that initially prevailed during evolution (3). The presence of the polyol pathway in embryonic tissues and in the tissues of the human umbilical cord might therefore be considered vestigial, antedating the more sophisticated pathways using phosphorylated sugars (4). Alternatively, the pathway may play a more positive and important role in regulating the oxidation-reduction balance of pyridine nucleotides (Fig. 1) (4). This function could be particularly significant under conditions of low oxygenation, when opportunities for oxidation via the respiratory chain are limited. It would provide an alternative to the production of lactate and thus avoid excessive metabolic acidosis.

It is now recognized that during the first trimester of human pregnancy, plugs of invading trophoblast cells occlude the tips of the maternal spiral arteries underlying the placenta (5–7). Consequently, there is little, if any, maternal blood flow into the placenta, and the intervillous space is filled with a clear fluid (8). This may represent maternal plasma percolating through the network of intercellular channels within the plugs (8), but may also be derived from the secretions of the uterine glands (9, 10). The absence of an oxygen carrier in this fluid means that the oxygen supply within the fetoplacental unit is low during this period, with measurements *in vivo* indicating a tension of less than 20 mm Hg before 10 wk gestation (1, 2).

During the first trimester, the placental villi arise from the entire surface of the chorionic sac, although they are beginning to regress over the superficial pole (Fig. 2). The amniotic sac surrounding the embryo is still relatively small and floats within the exocoelomic cavity. Amino acid concentrations within the coelomic fluid are approximately one third of those in maternal serum, suggesting that the fluid is an ultrafiltrate of the latter, with the addition of placental and yolk sac proteins (11). Morphologically, the mesenchyme of the placental villi is continuous with that lining the inner surface of the chorionic sac; thus, it is likely that there is free interchange between the placental tissue fluid and the coe-

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Abbreviations: AF, Amniotic fluid; CF, coelomic fluid; IF, intervillous fluid; MS, maternal serum.

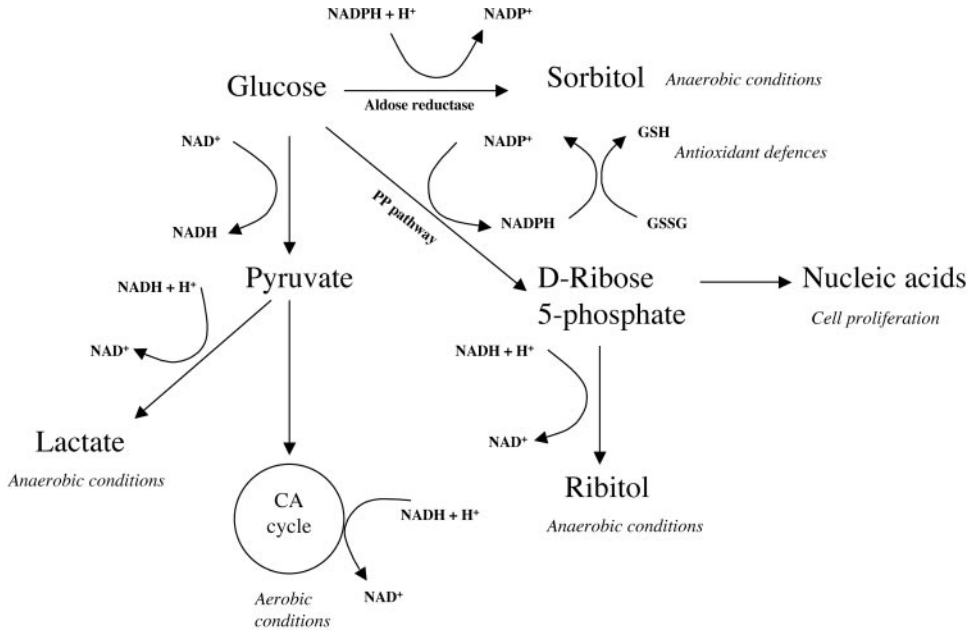

FIG. 1. Summary flow chart illustrating the possible involvement of the polyol pathway in regeneration of NAD^+ and $NADP^+$ under anaerobic conditions.

lomic fluid. This is supported by the fact that the concentrations of human chorionic gonadotropin, estradiol, estriol, and progesterone are higher in coelomic fluid than in maternal serum, strongly suggesting the presence of a direct pathway between the trophoblast and the exocoelomic cavity. We might assume, therefore, that metabolites within coelomic fluid will reflect the transport and/or metabolism of placental tissues.

By contrast, the concentrations of most molecules, including amino acids, are very low in amniotic fluid (11), indicating that the amniotic epithelium represents a relatively impermeable barrier. Because amniotic fluid surrounds the developing embryo, its composition will reflect the development and maturation of fetal organs such as kidneys, lungs, digestive system, and skin.

To investigate metabolism within the conceptus during the first trimester we sampled from intervillous space, exocoelomic cavity, and amniotic cavity and compared concentrations of free sugars and polyols with those in maternal serum. There are a number of studies that strongly suggest that the concentrations of free sugars and polyols could be very important in early development. Thus, a study comparing fetal and maternal plasma concentrations of the nonglucose carbohydrates and polyols reported much higher concentrations of most alcohols in ovine fetal blood (12). Freinkel *et al.* (13–15) reported that changes in the concentrations of some of the sugars and polyols were associated with an increased incidence of congenital anomalies in cultured rat embryos. More recently, reduced maternal myoinositol concentrations in human pregnancies have been found to be associated with an increased incidence of spina bifida (16). Thus, the roles of free sugars and polyols in early development need to be defined. The present study represents a first step toward that goal.

Materials and Methods

Fluid samples

We studied matched samples of intervillous fluid (IF), coelomic fluid (CF), amniotic fluid (AF), and maternal serum (MS) at 5–12 wk gestation. The samples were collected before elective surgical termination of pregnancy under general anesthesia. Gestational age was determined from

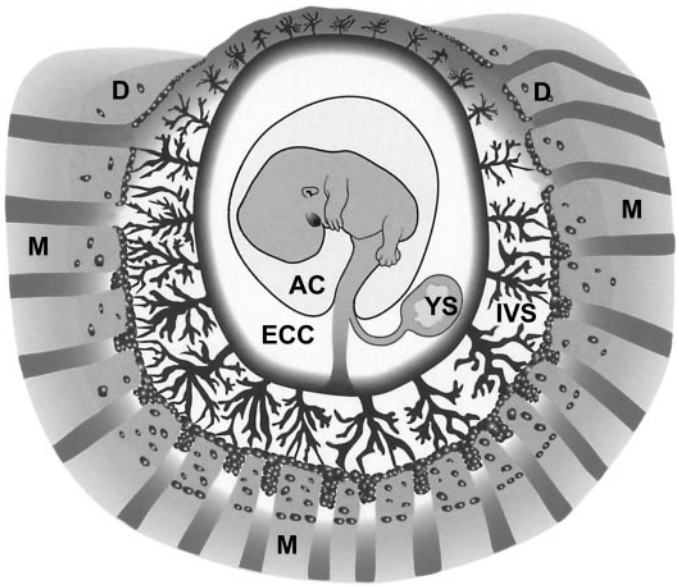

FIG. 2. Diagram of the gestational sac at 8–9 wk of pregnancy, illustrating the fetal fluid compartments. AF was withdrawn from the amniotic sac (AC), CF was withdrawn from the exocoelomic cavity (ECC), and IF was withdrawn from the intervillous space (IVS). Note that plugs of trophoblast block the tips of the spiral arteries throughout most of the placenta at this stage of development, preventing the inflow of maternal blood. D, Decidua; M, myometrium; YS, yolk sac. Modified from Ref. 28.

the first day of the last menstrual period and was confirmed by ultrasound measurement of fetal crown-rump length. Written consent was obtained from each woman after she had received complete information about the procedure. The study included only uncomplicated pregnancies and was approved by University College London Hospitals committee on the ethics of human research.

CF (n = 16) and AF (n = 12) samples were obtained by transvaginal puncture under ultrasonographic guidance. AF samples could not be obtained before 8 wk gestation because the sac is too small. The first 0.2 ml of each fluid sample was discarded to decrease the risk of contamination by maternal blood. For collection of intervillous fluid (n = 7), 1.0 ml sterile saline had to be injected into the intervillous space before any fluid could be withdrawn, because otherwise the placental villi occluded the needle. There was, therefore, an inevitable potential dilution of the intervillous contents, which could not be calculated or adjusted for. Consequently, only results where the concentration in the IF exceeded that in the corresponding samples were considered significant. In all cases (n = 17), maternal venous blood was obtained during the surgical procedure, and samples of MS and fetal fluids were stored at −80 C until assayed.

Assay technique

The frozen samples were thawed quickly; 0.1 ml 0.3 N zinc sulfate containing 30 mg/ml xylitol as an internal standard was added to 0.1 ml plasma. After mixing, another 0.1 ml 0.3 N barium hydroxide was added. The mixture was centrifuged at 14,000 × g for 10 min, and the supernatant was filtered through a 0.45-μm pore size filter before loading on a refrigerated autosampler for HPLC analysis.

A Dionex HPLC analyzer equipped with a CarboPac MA1 anion exchange column was used for the separation of the hexoses and polyols (Dionex, Sunnyvale, CA) from a 20-μl sample of supernatant. The analysis was run isocratically with 500 mM sodium hydroxide for 25 min, followed by a step change to 400 mM sodium hydroxide for 20 min at ambient temperature. The flow rate was 0.4 ml/h.

For analysis of galactosamine and glucosamine, a 20-μl sample of supernatant was loaded on a CarboPac PA 10 column in a separate run. The system was run isocratically with 18 mM sodium hydroxide at ambient temperature. The flow rate was 0.6 ml/h. All peaks were quantified by using a pulse amperometric detector (Dionex ED40 electrochemical detector) with a gold electrode. The Dionex PeakNet software was used for instrument operation and data analysis.

Results

The concentrations of the sugars at relatively high concentrations within the four fluids are plotted in Fig. 3. Figure 4 shows those sugars at relatively low concentration in the four fluids. The concentrations of the polyols inositol, sorbitol, erythritol, and ribitol were significantly higher in CF and AF than in MS (Table 1). The concentrations of fructose were also significantly higher in CF and AF than in MS, but the reverse was the case for glucose and glycerol. The concentrations of inositol, sorbitol, erythritol, ribitol, mannitol, galactose, galactosamine, and glucosamine were significantly higher in the CF than in the AF (Figs. 3 and 4 and Table 1). With regard to IF, the concentrations of inositol, sorbitol, and mannitol were significantly higher than those in MS (Figs. 3 and 4 and Table 1).

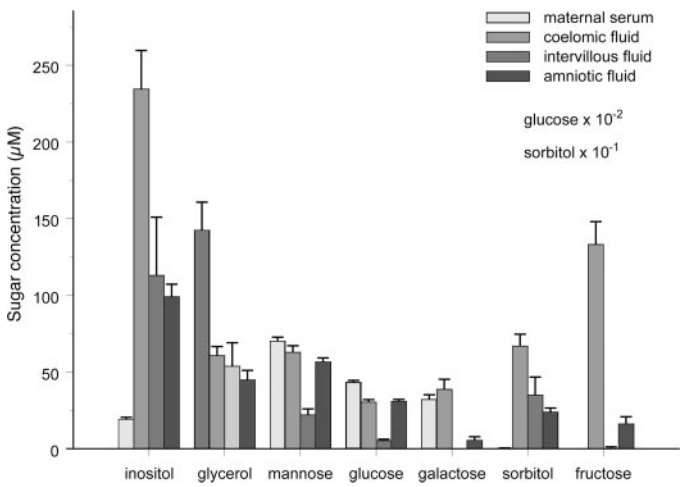

FIG. 3. Bar chart showing the higher concentrations of sugars in the four fluid samples.

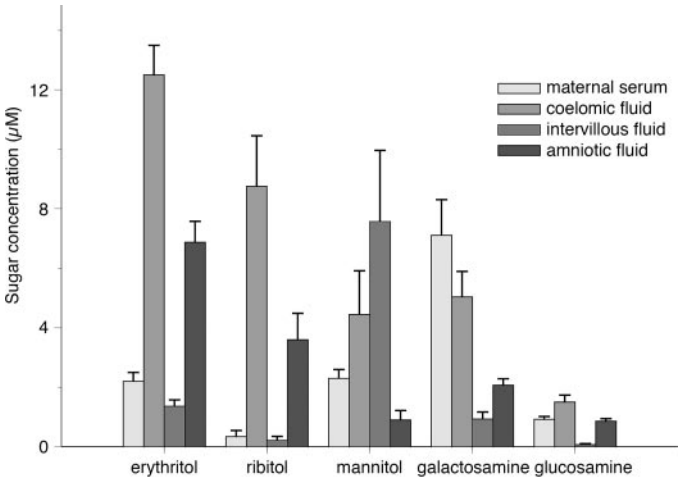

FIG. 4. Bar chart showing the lower concentrations of sugars in the four fluid samples.

Discussion

These data indicate that the polyol pathway is highly active in the human fetoplacental unit during the first trimester of pregnancy. The high concentrations of sorbitol and inositol in the CF suggest that placental and fetal tissues are the most likely sources of these sugar alcohols, and equivalent data have recently been reported for sheep (12). The aldose reductase enzyme that converts glucose to sorbitol was first identified in seminal vesicles and placenta and has subsequently been purified, cloned, and sequenced (17–19). The sorbitol pathway has also been identified in tissue homogenates derived from human umbilical cord tissues (4). Under normal conditions in adult tissues, this pathway plays a minor role in the metabolism of glucose. During episodes of hyperglycemia, it may become more significant, however, and much attention has recently focused on the role of polyols in causing the characteristic lesions associated with diabetes (20).

The high activity of the polyol pathway in the fetoplacental unit may be significant for several reasons. Firstly, it may reflect a reliance on phylogenetically older metabolic pathways during the earliest stages of development, a case, perhaps, of ontogeny recapitulating phylogeny (3). Thus, the enzymes involved display many features of primitive enzymes, such as a relatively low specificity and affinity for their substrates. Secondly, and somewhat interlinked, the pathway may provide an important mechanism for the reoxidation of pyridine nucleotides under conditions of low oxygenation. For glycolysis to continue and provide essential

TABLE 1. The *P* values for comparisons of the sugar concentrations in the different fluid samples

	Inositol	Glycerol	Erythritol	Sorbitol	Ribitol	Mannitol	Mannose	Glucose	Galactose	Fructose	Galactosamine	Glucosamine
CF/MS	<0.001	<0.001	<0.001	<0.001	<0.001	NS	NS	<0.001	NS	<0.001	NS	<0.050
IF/MS	<0.050	<0.005	<0.050	<0.050	NS	NS	<0.001	<0.001	NS	NS	<0.001	<0.001
AF/MS	<0.001	<0.001	<0.001	<0.001	<0.005	<0.010	<0.005	<0.001	<0.001	<0.001	<0.001	NS
IF/AF	NS	NS	<0.001	NS	<0.005	<0.050	<0.001	<0.001	NS	<0.001	<0.005	<0.001
IF/CF	<0.050	NS	<0.001	<0.050	<0.001	NS	<0.001	<0.001	NS	<0.001	<0.001	<0.001
AF/CF	<0.001	NS	<0.001	<0.001	<0.050	<0.050	NS	NS	<0.001	<0.005	<0.005	<0.050

NS, Not significant.

ATP, there is a need to constantly regenerate NAD^+ from NADH (Fig. 1). Under aerobic conditions, this takes place via electron transport to the enzymes of the mitochondrial respiratory chain, but in the absence of oxygen, this pathway is blocked. In the short term it is possible to ferment pyruvate to lactate, but over longer periods this may lead to unacceptably high acidity. The pH of the CF reflects a degree of metabolic acidosis, and the concentration of lactate is higher in both CF and AF (0.6 and 0.9 mmol/liter, respectively) than in MS (0.3 mmol/liter) (21). The concentration increases in the CF with advancing gestational age from 7–12 wk, but involvement of the polyol pathway in NAD^+ regeneration may prevent it from rising excessively.

Finally, the high concentrations of ribitol and erythritol in CF and AF indicate a considerable level of activity of the pentose phosphate pathway, which provides the sugar precursors. This pathway is important in rapidly proliferating tissues, because it generates the pentose phosphates necessary for the synthesis of nucleic acids. In addition, during the first step of the pathway, the enzyme glucose-6-phosphate dehydrogenase generates NADPH from $NADP^+$. NADPH is the primary reductant used in anabolic pathways and is also essential for the regeneration of reduced glutathione, the cofactor for the antioxidant enzyme glutathione peroxidase. The importance of this pathway to the protection of the embryo from free radical-mediated teratogenesis is illustrated by the fact that reducing the activity of glucose-6-phosphate dehydrogenase leads to an increased incidence of major congenital abnormalities (22).

The resultant polyols may play important roles in the homeostasis of the early conceptus. They represent an important category of organic cellular osmolytes and thus have been implicated in the regulation of fluid balance (23, 24). Their high concentration in fetal and placental tissues may help maintain cell volume without perturbing macromolecular protein structure or enzyme function, as can happen with inorganic ions (23). Equally, their presence in CF and AF may assist in the expansion of these sacs, drawing water and nutrients across the placenta before an effective fetal circulation is established. Because they cross cell membranes far more slowly than the sugars from which they were derived (*e.g.* sorbitol *vs.* glucose), they may have much higher reflection coefficients, which would amplify their role as idiosmoles.

Myoinositol is the most predominant stereoisomer of inositol in cells. It has previously been reported that its concentration in human fetal serum is 5 times that in MS at midgestation, although this differential decreases toward term (25, 26). Free myoinositol is at very high concentration in early fetal development, particularly in those tissues that have slow rates of cell division in later life, such as central nervous system and skeletal and cardiac muscle (25). Myoinositol is an important precursor of membrane phospholipids and thus plays an important role in development. It is of particular significance in the formation of the neural system, and low maternal concentrations of myoinositol have recently been linked to an increased risk of spina bifida (16). Maintaining high concentrations within the fetoplacental unit may therefore be beneficial to normal embryonic development.

As mentioned in *Materials and Methods*, the data for IF must be interpreted with considerable caution because of the difficulties in sampling from the intervillous space. This is evidenced by the greater variance in the IF data. For example, mannitol concentrations in IF are approximately 3 times higher than those in MS, yet the difference is not statistically significant due to the large variance in the IF data. The fact that the concentrations of some polyols are so much higher in IF than in MS despite the possibility of dilution during sampling suggests that there must be a slow circulation and turnover of the fluid in the intervillous space. This is consistent with the observation that Doppler signals indicative of significant fluid flow cannot be detected in the intervillous space before 10–12 wk of pregnancy (27) and with the general concept of plugging of the maternal arteries supplying the placenta early in pregnancy (6, 7).

In conclusion, this study of the early human conceptus, taken together with the data obtained in the ovine fetus, suggest that the developing tissues are exposed to rather high polyol concentrations, the meaning of which will require additional study.

Acknowledgments

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Address all correspondence and requests for reprints to: Dr. Graham J. Burton, Department of Anatomy, University of Cambridge, Downing Street, Cambridge, United Kingdom CB2 3DY. E-mail: gjb2@cam.ac.uk.

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