Maternal and Fetal Blood Levels of Glucose, Lactate, Fructose, and Insulin in the Conscious Pig

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ABSTRACT: To study nutrition and metabolism in the fetal pig, a chronic catheterization method was developed that allows blood sampling in arteries and veins, at both the umbilical and uterine sources, in the conscious, unstressed animal. A catheter was inserted in the fetal aorta through a femoral artery, and another one was introduced in the umbilical vein. A catheter was put in a femoral artery of the sow so that its end was in the abdominal aorta. A fourth catheter was placed in a uterine vein draining the fetoplacental unit studied. This procedure was applied to 18 Large White primiparous sows at 99 d of gestation. Blood samples were drawn simultaneously using the four catheters before a meal at 103 d of pregnancy, and glucose, insulin, lactate, and fructose were determined. Glycemia was 2.5 times higher in the sow than in the fetus. The extraction coefficient of glucose by the fetus amounted to 14% of the umbilical supply. The insulin level in the fetal pig was very low (< 5 \mu U/mL). Lactate and fructose seemed to originate from the placenta. Blood lactate was 2.6 times lower in the sow than in the fetus, and its extraction coefficient by the fetus amounted to 8%. Fructose in the fetal blood was 2.3 times higher than that of glucose. Fructose was not utilized by the pig fetus. The present results obtained in the fetal pig are comparable to the conclusions drawn from studies with other species.

Key Words: Sows, Pigs, Fetus, Catheters, Carbohydrates, Insulin


Introduction

The study of the influence of maternal metabolism on growth and development of fetuses is needed to improve our knowledge of fetal metabolism. In other words, the metabolic demand of the fetus must be quantified through the determination of substrate concentrations in the umbilical vein and arteries and the measurement of umbilical blood flow. Due to technical difficulties, these studies are usually undertaken in species whose females are less prone to abortion, and with fetuses of larger size: cow (Comline and Silver, 1976) and sheep (Battaglia and Meschia, 1978, 1986). The research related to materno-fetal exchanges of nutrients in the pig are based on limited numbers of blood samples obtained under general anesthesia (Comline et al., 1979; Elphick et al., 1980; Reynolds et al., 1985; Thulin et al., 1989), or substrate balances across the fetoplacental unit that allow the study of the metabolism of the uterus and uterine contents, but no fetal metabolism alone (Dueé et al., 1987). This paper describes an intravascular catheterization technique developed in the fetal pig and sow to chronically measure nutrient exchange in conscious and unstressed animals. In addition, the first results obtained using this method are described.

Materials and Methods

Animals and Diet

Eighteen Large White gilts were inseminated at 254 ± 20 d of age and 147 ± 16 kg live weight (mean ± SD) with semen from Large White boars. They were kept in individual stalls and fed daily 2.5 kg of a diet containing corn (28%), barley (26%), wheat (23%), wheat bran (10%), and soybean oil meal (7%). The diet provided 3,000 kcal of DE/kg, 13% CP, and .6% lysine. Feed was given each morning at 0900 as a single meal. At approximately 95 d of pregnancy and 210 ± 15 kg live weight, the gilts were transferred to farrowing crates in which they stayed until the end of the experiment. The gilts were deprived of feed and water for 24 h before surgery, which was performed at 2994.
Figure 1. Schematic representation of the fetal and maternal catheters.

99 ± 1 d of gestation. They were fed as previously described on the following days.

Catheters and Surgery

Four catheters were placed to collect blood supplying or draining both the uterus and a fetus. Fetal catheters were made of polyvinyl tubing (Bolab Inc., Lake Havasu City, AZ). They consisted of an extravascular segment (2 m length, .99 mm o.d., .58 mm i.d.) into which an intravascular segment (3 to 4 cm length, .64 mm o.d., .28 mm i.d.) was partly inserted and bonded after the tip of the extravascular segment was dilated with a needle. To stabilize sutures to the catheter, two polyvinyl rings (2 cm apart) were bonded to the extravascular catheter at the junction with the smaller catheter (Figure 1). Maternal catheters were made of silicone tubing (Silastic, 2 m length, 1.65 mm o.d., .76 mm i.d.; Dow Corning Medical, 06561 Valbonne, France). Two rings 3 cm apart were made with silicone glue near the vascular end of the catheters. The catheters were sterilized with ethylene oxide. All the catheters used were filled with sterile saline containing 250 IU of heparin/mL just before being inserted into blood vessels.

Gilts were premedicated with atropine (20 mg/kg live weight given i.v.), and anesthesia was then induced with sodium thiopenthal (10 mg/kg live weight given i.v.). After intubation of the gilt with an endotracheal tube, anesthesia was maintained with 2 to 5% halothane (Fluothane, Pitman-Moore, 77100 Meaux, France) in oxygen (2 to 3 L/min). The gilt was placed supine, and the abdomen, flanks, and internal faces of the hindlegs were shaved, washed, and disinfected with β-iodine. An adhesive plastic surgical film (Wuhrlin-Soplamed, 77124 Villenoy, France) was then applied on the abdomen in order to maintain asepsis during surgery and avoid bacterial migration. A 20-cm midline incision was made through the abdomen, and a fetoplacental unit (the part of the uterus corresponding to one fetus and its placenta) was gently exteriorized. The fetus was rotated to bring its inguinal region near the antimesometrial surface of the uterus, and a 2-cm incision was made through the uterus and the membranes keeping the fetus within the uterus (Randall, 1977). The medial aspect of a fetal thigh was incised (1 to 2 cm), and the uterus, fetal membranes, and the lips of the fetal incision were joined with atraumatic Allis forceps to minimize loss of amniotic fluid. Because of their small diameter, umbilical arteries cannot be catheterized without modification of blood flow. It was therefore decided to sample blood in the abdominal aorta, near the junction with the umbilical arteries (Figure 2). After dissociation of muscle fibers of the thigh, the femoral artery of the fetus was exposed and cleaned for approximately 1 cm and clamped and a small incision in its wall was made. A fetal catheter was then inserted and advanced so that its tip was in the aorta, above the origin of the internal iliac arteries. The catheter was fixed in the femoral artery by tying the vessel on each side of the polyvinyl rings with decimal 2 plaited polyester (Ethnor, 92523 Neuilly, France), and the clamp was removed. After confirmation of its patency, the catheter was closed by knotting its external end. This was also done for the catheters that were fixed afterward. The fetal skin was then stitched with decimal 2 polypropylene (Ethnor). The fetus was rotated to bring its abdomen near the uterus opening. A small incision was made in its abdomen, near the insertion of the umbilical cord. A fetal catheter (Figure 3) was introduced into the umbilical vein by using a divisible
needle (.8 mm diameter). Bleeding was rarely encountered because of the constriction of the loose connective tissue of the umbilical cord. The catheter was then sutured to the abdomen with decimal 2 plaited polyester (Ethnor) on each side of the polyvinyl rings. It was then buried under the skin at the level of the navel before the abdomen incision was sutured. Antibiotic (4 mg of gentamycin, Vetoquinol, 70204 Lure, France) was injected into the amniotic fluid. Fetal membranes and uterus were then sutured separately with single swaged polypropylene suture.

A maternal catheter was introduced into a small collateral vein of the uterus corresponding to the catheterized fetus (Figure 3). It was advanced until its end lay in the main vein draining that fetoplacental unit. The catheter was fixed in the collateral vein by tying the vessel on each side of the silicone rings with decimal 3 plaited polyester (Ethnor). The peritoneum was then sutured with plaited decimal 3 polyester (Braun, 92107 Boulogne, France) and oxytetracyclin (5 mg/kg live weight, Pfizer, 91407 Orsay, France) was injected in the abdominal cavity. Decimal 8 plaited polyester (Braun) was used to suture the opening in the muscles (cross stitches).

A 3-cm longitudinal incision was made just proximal on the medial side of the knee, and a fourth catheter was inserted in the femoral artery of the gilt and advanced through the iliac artery so that its tip was in the abdominal aorta. The composition of the blood collected via this catheter will be the same as that of blood supplying the uterus. It was tied on each side of the silicone rings with decimal 3 plaited polyester (Braun).

The catheters were gathered together, tunneled under the skin using a stainless steel trocar 50 cm long, and externalized through the skin of the back, at the level of the lumbar vertebrae. They were put in a small tissue bag sutured to the skin. The skin was sutured with decimal 3 plaited polyester (simple stitches; Braun). Postsurgical antibiotic therapy consisted of i.m. injections of Ampicillin (10 mg/kg live weight\(^{-1}\)d\(^{-1}\), Rigaux-Galena, 49500 Segré, France) for three consecutive days. Surgery required approximately 2.5 h. Postsurgical recovery, as assessed by the gilts' appetite, required 2 to 3 d.

**Sample Collection and Analyses**

Catheters were flushed daily with normal saline solution (.9% NaCl) containing 250 IU of heparin/mL. Before the meal at 103 ± 2 d of gestation, 1-mL blood samples were drawn simultaneously through the four catheters with heparinized syringes. Handling of the catheters was performed under as sterile a condition as possible. Blood samples were immediately divided into three subsamples and kept on ice. The first one was centrifuged and plasma samples were recovered for glucose and insulin determinations. The second one was deproteinized with 0.6 mol/L perchloric acid for lactate analysis, and the third one was deproteinized by adding 10% zinc sulfate (wt/vol) and .5 N NaOH for fructose determination. All the subsamples were centrifuged at 3°C, and supernatants were stored at −20°C until they were analyzed.

Glucose, lactate, and fructose were determined by automated enzymatic methods (kits from Boehringer Mannheim, 38242 Meylan, France) adapted to a Cobas Mira apparatus (Roche, Basel, Switzerland). The sensitivity was 19, 10, and 3 mmol/L for these determinations, respectively. Insulin concentrations were measured by a validated radioimmunoassay (Prunier et al., 1993). The sensitivity was 5 µU/mL.

**Statistical Analysis**

Differences in substrate concentrations between arterial and venous blood in both gilts and fetuses were calculated. Extraction coefficients were calculated as the differences between arterial and venous substrate concentrations in percentage of the substrate concentration in the arterial blood for the sow, or in the umbilical venous blood for the fetus. Paired comparisons of the substrate concentrations in arterial and venous blood in the sow and in the fetus were performed by using the t-test of the MEANS procedure of SAS (1989).

**Results**

The main difficulty of the technique lies in the maintenance of aseptic conditions during surgery. It seems also that anesthesia should last no longer than 2.5 h to limit subsequent abortion. A total of 35 sows underwent the operation, and 11 of them were eliminated due to death of the operated fetus or abortion of the sow within the period of recovery. The
maternal catheters remained generally patent, but the fetal catheters sometimes became blocked or withdrawn before farrowing. Six other sows had one or two catheters that were not patent, because of catheter occlusion or displacement. To prevent catheter withdrawal due to movements of the fetal limbs, the umbilical venous catheter was buried under the skin of the abdomen before suturing the incision. However, autopsy showed that in three fetuses this catheter was displaced from the umbilical vein before farrowing, whereas the position of the other catheters was confirmed.

Blood concentrations of glucose, insulin, lactate, and fructose at the four sampling sites after an overnight fast are presented in Table 1. Glycemia in the pig fetus was approximately 2.5 times lower than that in the dam. The arteriovenous concentration difference across the uterus was .26 mmol/L, which represents an extraction coefficient of 5%. The blood level of glucose was higher (P < .001) in the umbilical vein than in the umbilical artery, and the extraction coefficient of glucose was approximately 14% for the fetus. Insulinemia in the gilt was approximately 16 mU/mL, and the arteriovenous concentration difference across the uterus was 1.4 mU/mL. Insulin levels in fetal plasma were lower than the sensitivity of the radioimmunoassay (5 mU/mL).

Blood lactate levels in the fetus were approximately 2.6 times greater than those in the gilt. The arteriovenous concentration difference across the uterus was not significantly different from zero. The extraction coefficient of lactate was approximately 8% for the fetus.

Fructose was not detectable in the gilts' blood, whereas its concentration in fetal blood was very high (approximately 4.6 mmol/L). The fructose arteriovenous difference in the umbilical cord was not significantly different from zero.

**Discussion**

The methodology used in the present experiment allows the measurement of arteriovenous differences of substrates across the uterus and fetus. Extraction coefficients, which represent the relative magnitude of the net metabolite uptake or release by the uterus or the fetus, can be calculated. However, to quantify the substrates taken up or produced it is necessary to associate blood flow measurements with those determinations, which was not done in this experiment.

Plasma glucose at the uterus level was similar to values found by others (3 to 6 mmol/L) at the same period of pregnancy in anesthetized (Aherne et al., 1969; Randall and L'Ecuyer, 1976; Fowden et al., 1982; Martin et al., 1984; Reynolds et al., 1985) or catheterized fasted gilts (Randall, 1977; Ford et al., 1984; Duée et al., 1987). Glucose arteriovenous difference in the uterus was in the same range as determined by Aherne et al. (1969) and Ford et al. (1984) (.22 mmol/L). It was also similar to that found in cows (Ferrell and Ford, 1980; Ferrell et al., 1983) and ewes (Christenson and Prior, 1978; Meschia et al., 1980). Slightly higher arteriovenous differences (.35 to .69 mmol/L) were recorded in sows by Reynolds et al. (1985) and Duée et al. (1987), leading to a higher glucose extraction coefficient (8 to 10%). Differences in fast duration between experiments may explain these discrepancies. However, the extraction coefficient of glucose by the pig uterus found here (5%) is similar to that in sheep (Simmons et al., 1979), cows (Comline and Silver, 1976), and guinea pigs (Block et al., 1985).

Except in the experiments of Randall (1977, 1982) and Fowden et al. (1982), in which fetal pigs were chronically catheterized, glycemia has been determined through surgery on anesthetized fetuses. Glycemia was 2 to 2.5 times lower in the pig fetus than in the dam, as already reported (Aherne et al., 1969; Randall, 1977; Fowden et al., 1982). In the pig, as in all species studied (Battaglia and Meschia, 1986), the fetal glucose concentration is lower than that in maternal blood. Values similar to those recorded here were found in blood from the carotid or the femoral artery of the pig fetus: 2.03 (Fowden et al., 1982) or 2.70 mmol/L of glucose (Randall, 1977, 1982). Plasma

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**Table 1. Substrate levels and arteriovenous differences in the uterus and the fetus at approximately 105 days of pregnancy in conscious gilts fasted overnight**

<table>
<thead>
<tr>
<th>Item</th>
<th>Gilt</th>
<th>Fetus</th>
<th>Uterine extraction b</th>
<th>Uterine extraction b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uterine artery</td>
<td>Uterine vein</td>
<td>(A_U - V_U)</td>
<td>(V_F - A_F)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.00 ± .16</td>
<td>4.74 ± .16</td>
<td>.26 ± .07***</td>
<td>2.00 ± .12</td>
</tr>
<tr>
<td>Insulin, mU/mL</td>
<td>16.4 ± 1.6</td>
<td>14.9 ± 1.4</td>
<td>1.4 ± .5**</td>
<td>ND</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>.90 ± .13</td>
<td>.88 ± .12</td>
<td>.03 ± .03NS</td>
<td>2.33 ± .29</td>
</tr>
<tr>
<td>Fructose, mmol/L</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>4.57 ± .28</td>
</tr>
</tbody>
</table>

a n = 18 for glucose and insulin, n = 11 for lactate and fructose.
bExtraction differs from zero: ***P < .001; **P < .01; NS, P > .05.
ND = not detectable.
glucose in the umbilical vein was higher than in the umbilical artery at a comparable stage of pregnancy (Randall and L’Ecuyer, 1976; Comline et al., 1979; Reynolds et al., 1985) or at term (Randall, 1987). In the pig fetus, the glucose extraction coefficient and the difference between glucose in umbilical vein and artery measured in the present experiment (14% and .31 mmol/L, respectively) are similar to the corresponding values in the literature (11 to 14% and .30 to .35 mmol/L, respectively). As in other species, glucose seems to be a major energy substrate for the fetal pig.

Insulin levels in gilts were similar to the values reported by Simoes Nunes et al. (1987) and were between those determined by Martin et al. (1984) and Fowden et al. (1982) (6 and 34 μU/ml, respectively). A low arteriovenous difference for insulin was shown across the uterus in fasting conditions, although no uptake of insulin by the uterus could be detected by Fowden et al. (1982). Higher insulin levels in the sow than in the fetus were also found by Fowden et al. (1982), and by others in other species: primate (Mintz et al., 1969), sheep (Bassett and Jones, 1976), rabbit (Kervran et al., 1972), rat (Girard et al., 1974), cow (Grigsby et al., 1974), and horse (Fowden et al., 1980). Fowden et al. (1982) demonstrated that the pancreatic β cells of the fetal pig are functional during late gestation, but basal insulin concentrations are low. They were lower than 5 μU/mL in the present work, in agreement with Martin et al. (1984) (2.7 to 2.8 μU/mL), whereas Fowden et al. (1982) recorded slightly higher values (8.4 μU/mL).

The preprandial plasma levels of lactate were similar to values reported by Duée et al. (1987) and Simoes Nunes et al. (1987) in the sow and by Comline et al. (1979) in the sow and in the catheterized fetus. A much higher lactate level in the fetus than in the dam also was found in many species (Battaglia and Meschia, 1986). Lactate did not originate from the dam also was found in many species (Battaglia and Meschia, 1986). Lactate did not originate from the placenta in the pregnant sheep (Huggett et al., 1951; Alexander et al., 1970). Similar results have been obtained in the cow and mare (Silver and Comline, 1976) and sheep (Tsoulos et al., 1971), in which fructose concentrations did not differ between umbilical vein and arteries. This supports the view that the fetal pig makes little use of fructose as an energy source, as concluded by Aherne et al. (1969), Randall and L’Ecuyer (1976), and Randall (1977). It was shown that its rate of utilization by the fetal calf and lamb is extremely low (Battaglia and Meschia, 1986). In fact, fructose is not a significant substrate for fetal energy metabolism under normal conditions.

Previous data (Comline et al., 1979) showed that a 1.5- to 2-h sampling period under anesthesia after catheterization of the fetal pig led to hyperglycemia and reversal of the normal umbilical venous-arterial difference in plasma glucose and lactate, indicating glycogenolysis in the fetus. These consequences could be mimicked in an exaggerated form by the infusion of catecholamines. Such an effect was not observed here. Moreover, preprandial levels and extraction of substrates and insulin determined in the catheterized conscious sow and fetus agree with previous results obtained in anesthetized animals (Aherne et al., 1969; Randall and L’Ecuyer, 1976; Reynolds et al., 1985) and with the levels recorded in conscious unstressed pig fetuses with a carotid or femoral catheter (Randall, 1977, 1982; Comline et al., 1979; Fowden et al., 1982). This suggests that the preparation described in the present paper is valuable to study nutritional maternofetal exchanges in the pig.

Implications

The plasma concentrations of substrates measured in the sow and the fetal pig using the chronic catheterization described in this paper are similar to those previously measured by other sampling methods. Glucose extraction coefficient by the conceptus amounts to 14%. Lactate and fructose are produced by the placenta. Glucose and lactate are utilized by the fetal pig, whereas fructose utilization seems to be very low. This methodological approach allows the study of nutrition and metabolism in the conscious unstressed pig fetus.
Literature Cited


