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Circulating and utero-placental adaptations to chronic placental ischemia in the rat

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ABSTRACT

While utero-placental insufficiency is associated with adverse outcomes for both mother and fetus, many of the maternal-fetal adaptations during pregnancy in models of fetal compromise remain unclear. The purpose of this study was to determine if chronically reduced uterine perfusion pressure (RUPP) during days 14–19 of gestation alters feto-placental growth differentially from the cervical to ovarian ends of the uterus and generates metabolic adaptations such as increased blood lactate (BLa) concentrations and lactate transporter expression in the placenta. Fetal growth restriction was evident, placental efficiency (fetal weight/placental weight) decreased (4.7 ± 0.35 *vs.* 5.9 ± 0.30; $P < 0.05$) and fetal growth pattern within the uterus was altered in the RUPP compared to the normal pregnant (NP) rats. Blood lactate concentrations were increased (3.3 ± 0.3 *vs.* 2.1 ± 0.4 mmol/l; $P < 0.05$) in NP compared to virgin rats, and in RUPP compared to NP (5.0 ± 0.6 *vs.* 3.3 ± 0.3 mmol/l; $P < 0.05$). Lactate concentration was increased (10.0 ± 0.6 *vs.* 7.1 ± 0.8 mmol/l; $P < 0.05$) in the media from hypoxic compared to normoxic BeWo cells. No changes in expression of placental MCT1, 2, or 4 were observed between RUPP and NP rats. RUPP resulted in decreased plasma leptin (2.0 ± 0.3 *vs.* 3.1 ± 0.4; $P < 0.05$) but no change in IGF-1 compared to NP. The present data indicate chronic placental ischemia results in numerous endocrine and metabolic changes during late pregnancy in the rat and that the RUPP model has differential effects on fetal growth depending on uterine position.

1. Introduction

Placental insufficiency and preeclampsia are both major causes of intrauterine growth restriction [1–4] and numerous studies have identified strong associations between restricted growth during early development and an increased risk of developing chronic diseases in later life [1,5–9]. While there are clear indications that an altered trajectory of early growth has widespread and persistent effects on a variety of organ systems in later life [10], the exact mechanisms underlying origins of poor fetal growth and development remain enigmatic. As a consequence, a large number of experimental models have been developed to investigate what has now become termed the developmental origins of health and disease (DOHaD) [3,10,11]. Despite the proliferation of experimental models to study DOHaD, there remains much to be learned about the maternal and fetal adaptations to these experimental stimuli during gestation.

Two main sources of blood supply the utero-placental unit in the rat. The uterine arteries which derive from the iliac arteries; and the ovarian artery, whose source is the abdominal aorta on the right uterine horn and the left renal artery on the left uterine horn [12,13]. These two vessels anastomose in the mesometrium surrounding the uterine horns. While previous studies have reported different effects on fetal growth depending on the location (ovarian *vs.* uterine arteries) and the timing (early *vs.* late gestation) of the ligatures [14,15], the effects of the silver clips placed on the inferior abdominal aorta (0.203 mm) and ovarian arteries (0.100 mm) employed in the RUPP model used to study preeclampsia and programmed hypertension remains unknown.

The placenta plays a central role in facilitating transport of nutrients between the maternal and fetal systems and a delicate balance between pro- and anti-angiogenic factors such as VEGF, sFlt-1, leptin and IGF-1 appears to be important for maintenance of normal feto-placental growth and development. Although the placenta produces very little lactate during the first half of pregnancy, there are significant increases near term with much of the lactate produced by the placenta entering the fetal circulation [16] suggesting this monocarboxylate is an important substrate for fetal development. Further, lactate which comprises approximately

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15–20% of total fetal metabolism is an important carbon source in the near-term fetus [16] and in glucose-deficient states can become an important substrate for brain metabolism [17].

Although clinical studies have suggested links between elements of the metabolic syndrome such as glucose intolerance, insulin resistance and dyslipidemia [18–20] and the occurrence of preeclampsia, our previous work suggests that placental insufficiency alone is not an underlying cause of these observations [21]. Previous investigations have demonstrated that lactate production and transport is altered in placentas from pregnancies characterized by preeclampsia and intrauterine growth restriction [22–24] which suggests that alterations in the availability of this important substrate may have important implications for fetal development and long term health.

While fetal adaptations are clearly important in the developmental origins of disease, there are numerous aspects of fetal development during chronic reductions of utero-placental perfusion pressure (RUPP) and placental insufficiency that remain unstudied. Therefore, the purpose of this study was to determine the effect of RUPP on fetal and placental growth throughout the uterus and to determine if chronic placental ischemia results in alterations such as increased blood lactate (BLa) concentration, increased lactate transporter expression in the placenta and decreases in growth promoting and pro-angiogenic factors such as IGF-1 and leptin.

2. Methods and apparatus

2.1. Animals

Studies were performed in timed pregnant Sprague–Dawley rats purchased from Charles River (Wilmington, MA). Animals were housed in a temperature-controlled room (23 °C) with a 12:12 light:dark cycle. All experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care of animals. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Minnesota. On day 14 of gestation, rat dams were randomly assigned to either RUPP ($n = 15$) or normal pregnant (NP; $n = 15$) control groups. Age matched non-pregnant virgin rats served as pregnancy control for relevant measurements.

2.2. Reduced utero-placental perfusion pressure (RUPP) procedure

The RUPP procedure is a well established model for studying the link between placental ischemia and hypertension in the pregnant rat and has been described in detail previously [25–27]. In brief, silver clips were placed on the lower abdominal aorta (0.203 mm ID) above the iliac bifurcation and also on branches (0.100 mm ID) of both the right and left ovarian arteries proximal to the uterus on day 14 of pregnancy. NP rats underwent a sham surgery on the same day of pregnancy.

2.3. Measurement of mean arterial pressure (MAP) in chronically instrumented conscious rats

Animals were instrumented on day 17 of gestation and arterial pressure was determined via indwelling carotid arterial catheters in both groups of rats at day 19 of gestation as described previously [25–27].

2.4. Conceptus measurements & serum collection

After the measurement of MAP, the dams were placed under isoflourane anesthesia and a midline ventral incision was made to isolate the abdominal aorta for plasma and serum collection as reported previously [25–27]. Pups and placentas were excised and weighed and placental efficiency (fetal weight/placental weight) was calculated in each animal. Placentas were snap frozen in liquid nitrogen and stored at −80 °C until further analyses were performed.

Fetal weight, placental weight, number of resorptions, and each fetus's uterine position were recorded in the manner described previously [14]. Briefly, uterine position was determined by numbering the fetus closest to the ovary as number one and then numbered in ascending order toward the cervix. We then designated an ovarian, middle, and cervical fetus within each uterine horn in order to compare fetal weights and uterine position. The fetus closest to the ovary was also designated as the ovarian fetus and the fetus closest to the cervix was the cervical fetus. In a uterine horn with an even number of fetuses, we averaged the weight of the two fetuses in the middle as the middle fetus. In a uterine horn with an odd number of fetuses, the fetus directly in the middle was designated as the middle fetus of that horn.

2.5. Lactate measurement

Lactate was measured in freshly collected whole blood, amniotic fluid or culture media immediately after collection with a portable lactate analyzer (Lactate-Scout, Senslab, Leipzig, Germany) as described previously [28]. All lactate measurements are in mmol/l.

2.6. In vitro experiments

BeWo cells were plated at 1 x10^6 cells/mL in 6 well plates and treated with Ham's F12 medium F12-Medium with L-glutamine (Cellgro Mediatech -10-080-CV) with 10% fetal bovine serum, 1% penicillin/streptomycin. After 48 h cells were placed into modular incubator chambers (Billups-Rothenburg; del Mar, CA) and filled with premixed gas (Praxair; Danbury, CT) that was either: normoxic (20% O_2, 5% CO_2, 75% N_2), or hypoxic (1.5% O_2, 5% CO_2, 93.5% N_2) and cells were harvested at 48 h. At the end of the treatment period, cell were placed on ice and rinsed three times with cold PBS. Laemmli sample buffer w/o bromophenol blue and β-mercaptoethanol was added and cell extracts collected following scraping. Conditioned media was centrifuged at 1000 × g for 5 min and the supernatant was snap frozen and stored at −80 °C for analysis.

2.7. Protein extraction and quantitation

As described previously [27], total soluble protein was extracted from middle placentas in radioimmunoprecipitation assay (RIPA) lysis buffer containing phenylmethanesulphonylfluoride (PMSF) in dimethyl sulfoxide (DMSO), sodium orthovanadate and a protease inhibitor cocktail (Santa Cruz Biotechnology, Inc.). Total soluble cellular protein concentration was determined using the bicinchoninic acid (BCA) method (Pierce Biotechnology).

2.8. Western immunoblot

Western blots were performed as previously described [25,28] using 50 μg of total protein separated on 4–12% gradient gels (Invitrogen, Carlsbad, CA) then transferred (Genie, Idea Scientific, Minneapolis, MN) to nitrocellulose membranes and Ponceau stained to assure even transfer across each gel. Commercially available antibodies for MCT 1 (1 μg/ml; Millipore: Billerica, MA, AB3540P), MCT 2 (1 μg/ml; Millipore, AB3542), MCT 4 (1 μg/ml; Millipore, AB3314P) and β-actin (0.2 μg/ml; Abcam, ab20272) were incubated in 5% TBS-T milk overnight at 4 °C. Membranes were washed in TBS-T and incubated with appropriate horse radish peroxidase-conjugated antibodies (0.1 μg/ml; Cell Signaling: Danvers, MA, 7074) for 45 min at room temperature. Signals were detected with SuperSignal West Femto Maximum Sensitivity Substrate and visualized with a digital imaging system (Alpha Innotech FluorChem® HD2). All digitized images were quantified using Un-Scan-It gel 6.1 software (Silk Scientific, Orem UT).

2.9. Enzyme linked immuno-assay data

Leptin and IGF-1 concentrations were determined using commercially available ELISAs (Leptin, Millipore, Billerica, MA; IGF-1, R&D Systems, Minneapolis, MN) as reported previously [29].

2.10. Statistical analysis and calculations

All data are presented as mean ± SEM and statistical significance was accepted when $P < 0.05$. Western immunoblot data was calculated as the ratio of target protein to β-actin. Conceptus data were calculated as mean per pregnancy. Comparisons and relationships between groups were made with a Pearson's correlation coefficient test, *t*-test for independent samples, one-way ANOVA or two-way ANOVA as indicated. Statistical calculations were made with GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA USA).

3. Results

3.1. Cardiovascular adaptations to chronic placental ischemia

Fig. 1 (panel A) illustrates that similar to previous reports by us and others arterial pressure was increased in the RUPP compared to the NP rats. Panel B in Fig. 1 shows that a strong negative relationship ($r = -0.73$, $P < 0.001$) was present between the number of total viable pups and mean arterial pressure on day 19 of pregnancy in the rat. In the present study, HR was increased (465 ± 10 *vs.* 433 ± 10 bpm; $P < 0.05$) in RUPP compared to NP dams.