Original Research

Chronic placental ischemia alters amniotic fluid milieu and results in impaired glucose tolerance, insulin resistance and hyperleptinemia in young rats

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Abstract

Although small size at birth is associated with hypertension and associated co-morbidities such as insulin resistance and type II diabetes mellitus, many of the animal models employed to simulate this phenomenon do not closely mimic the ontogeny of growth restriction observed clinically. While intrauterine growth restriction (IUGR) is often detected near mid-pregnancy in women and persists until term, most rodent models of IUGR employ ligation of uterine arteries for a brief period during late gestation (days 19–21 of pregnancy). We hypothesized that IUGR associated with chronic reduction in uteroplacental perfusion (RUPP) and placental ischemia during the third trimester of pregnancy in the rat alters the amniotic fluid (AF) environment and results in hypertensive offspring presenting with metabolic abnormalities such as glucose intolerance and insulin resistance. Insulin-like growth factor-1 (IGF-1), IGF-2, Na^+ concentration and oxidative stress in the AF were increased, while K^+ concentration was decreased in the RUPP compared with normal pregnant (NP) fetuses. RUPP-offspring (RUPP-O) were smaller (6.1 ± 0.2 versus 6.7 ± 0.2 g; $P < 0.05$) at birth compared with NP-offspring (NP-O) groups. At nine weeks of age, mean arterial pressure (121 ± 3 versus 107 ± 5 mmHg; $P < 0.05$), fasting insulin (0.71 ± 0.014 versus 0.30 ± 0.08 ng/mL; $P < 0.05$), glucose (4.4 ± 0.2 versus 3.1 ± 0.3 mmol/L; $P < 0.05$), leptin (3.8 ± 0.5 versus 2.3 ± 0.3 ng/mL; $P < 0.05$) and the homeostasis model assessment of insulin resistance index was greater (2.9 ± 0.6 versus 1.0 ± 0.3; $P < 0.05$) in the RUPP-O compared with the NP-O rats. These data indicate that chronic placental ischemia results in numerous alterations to the fetal environment that contributes to the development of impaired glucose metabolism, insulin resistance and hyperleptinemia in young offspring.

Keywords: pregnancy, hypertension, diabetes, fetal programming, pre-eclampsia, IGF-2

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Introduction

Although small size at birth is associated with hypertension and associated co-morbidities such as insulin resistance and type II diabetes mellitus, many of the animal models employed to simulate this phenomenon do not closely mimic the ontogeny of growth restriction observed clinically. Whereas intrauterine growth restriction (IUGR) is often detected near mid-pregnancy in women and persists until term,[1] most rodent models of IUGR employ ligation of uterine arteries for a brief period during late (days 19–21 of pregnancy) gestation.[2–10] The contemporary literature is replete with studies indicating the existence of different 'critical windows' of developmental programming;[11–15] hence, the timing and extent of placental insufficiency is likely to have a profound impact on the phenotype of the offspring.

Placental insufficiency and early-onset pre-eclampsia are both major causes of IUGR.[16,17] Numerous epidemiological studies have identified a strong association between restricted growth during early development and an increased risk of developing chronic diseases, such as hypertension and type 2 diabetes mellitus in later life.[14,16,18–21] Although most of the early studies characterizing these observations were performed in adults, several studies have reported similar results in adolescents and young adults, suggesting that glucose intolerance and insulin resistance may be one of the earliest manifestations of metabolic abnormality in growth-restricted offspring.[22–24]

The results of these studies have led authors to postulate that an altered trajectory of early growth has persistent effects on cardiovascular and endocrine function in later life.[6]

The factors that are thought to be responsible for impaired fetal growth and development are numerous. Gene deletion studies have shown that the insulin-like growth factors (IGFs) and, in particular, IGF-2 are important for fetal growth and development.[25] Further, IGF-2 is produced in the placenta and contributes significantly to fetal growth.[25,26] While genetic models have demonstrated roles for the IGF system in fetal development,[25] the role of IGF-2 in growth restriction associated with placental ischemia and placental insufficiency has remained nebulous.[27,28] Interestingly, a recent study has shown that chronic hypoxia appears to increase IGF-2, while acute hypoxia does not alter IGF-2 expression in the human placenta.[27] In contrast, chronic placental insufficiency in the guinea pig reported decreased IGF-2 mRNA expression in the placenta.[29] In light of these conflicting reports, further studies are clearly needed to determine the role of the IGF system in different models of growth restriction.

Because of the escalating occurrence of metabolic syndrome in modern societies and the recognized relationships between conditions such as hypertension and insulin resistance, there is a need for experimental models that spontaneously present with these manifestations in a manner similar to that observed in humans.[30] Considering the significant body of evidence that indicates small size at birth is associated with hypertension and associated co-morbidities such as insulin resistance, identifying the mechanisms operating in models that spontaneously present with a predisposition towards metabolic dysfunction remains a practical objective.

The purpose of the present study was to test the hypothesis that IUGR due to chronic placental ischemia would increase amniotic fluid (AF) IGF-2 levels and result in nine-week-old adolescent offspring presenting with manifestations of metabolic abnormality, namely hypertension, glucose intolerance, insulin resistance and hyperleptinemia.

Methods and apparatus

Animals

Studies were performed in timed pregnant Sprague–Dawley rats purchased from Charles River Laboratories Inc. (Wilmington, MA, USA). 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 at the University of Minnesota. On day 14 of gestation, rat dams were randomly assigned to one of two experimental groups: (1) normal pregnant (NP, $n = 16$) or (2) reduced uterine perfusion pressure (RUPP, $n = 16$).

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.[31–33] In brief, all rats underwent surgical procedures under isoflurane anesthesia (Webster, Sterling, MA, USA) delivered by an anesthesia apparatus (vaporizer for isoflurane anesthetic, Surgivet, Waukesha, WI, USA). Pregnant rats entering the RUPP groups underwent the following clipping procedure at 14 d of gestational age. After a mid-line incision, the lower abdominal aorta was isolated and a silver clip (0.203 mm ID) was placed around the aorta above the iliac bifurcation. Silver clips (0.100 mm ID) were also placed on branches of both the right and left ovarian arteries that supply the uterus. Because pilot studies in our laboratory as well as previous work of others suggest that sham operation during pregnancy produces an intermediate phenotype,[3] only offspring from NP dams were included in the analysis.

AF collection, fetoplacental and birth weight, and somatic growth

One group of rat dams ($n = 8$, RUPP; $n = 8$ NP) was allowed to deliver naturally, while a second cohort of rat dams ($n = 8$, RUPP; $n = 8$ NP) provided tissue samples collected via C-section on day 19 of pregnancy. AF, placentas and fetuses were collected as described previously.[34] Birth weight was recorded within 12 h of birth. Offspring were weighed weekly until weaning, then bi-weekly afterward until the day of the experiment.

Measurement of mean arterial pressure in chronically instrumented conscious rats

Animals were instrumented and arterial pressure was determined in both groups of rats at nine weeks of age as described previously.[31] Briefly, two days before measurement of mean arterial pressure (MAP) rats were instrumented with carotid and jugular catheters of V-3 tubing (SCI, Lake Havasu City, AZ, USA) while under isoflurane anesthesia delivered by an anesthesia apparatus (vaporizer for isoflurane anesthetic). Catheters were tunneled to the back of the neck and exteriorized after implantation. On the day of experimentation, rats were placed in individual restraining cages for arterial pressure measurements using a pressure transducer (Cobe III Transducer CDX Sema, Birmingham, AL, USA). MAP was recorded continuously for a two-hour period after one-hour of stabilization.

Intravenous glucose tolerance test

At nine weeks of age an intravenous glucose tolerance test (IVGTT) was performed as described previously.[35] Briefly, fasting baseline glucose was measured in whole blood using the Accu-Check® Advantage glucose meter (Roche Diagnostics, Indianapolis, IN, USA) at time (t) = 0 min. A sterile glucose solution (1 g/kg) was infused immediately following the $t = 0$ min blood sample and repeated blood glucose measurements were made from whole blood samples (~10 μL) at $t = 5$, 10, 15, 30, 60, 90 and 120 min using the glucose meter described above. Catheters were flushed with sterile saline following each sample collection.

Plasma and AF assays

AF was collected by aspiration into a syringe with a 27-gauge needle as described previously.[34] Blood was collected for subsequent assays into Corvac® sterile serum separator tubes (Sherwood Davis, St Louis, MO, USA) and plasma into BD Vacutainer® tubes containing EDTA. After separation samples were snap frozen in liquid nitrogen and stored at -80°C until further analyses were performed. Circulating and AF IGF-1 (R & D Systems Minneapolis, MN, USA), AF IGF-2, leptin and insulin (Millipore, Billerica, MA, USA) concentrations were measured using commercial kits as described previously.[34,36]

A thiobarbituric acid reactive substances assay was performed to assess oxidative stress via production of malondialdehyde as described previously.[37] Trolox-equivalent antioxidant capacity of the AF was assessed with a total antioxidant assay (Cayman Chemical, Ann Arbor, MI, USA) as described previously.[35] Triglycerides and cholesterol were measured using kits (Cayman Chemical) as described previously.[35] AF electrolyte concentrations were determined by flame photometry (IL-943, Instrumentation Laboratory, Bedford, MA, USA). Glucose was measured as described above.

Statistical analysis and calculations

All data are presented as mean $\pm$ standard error of mean and statistical significance was accepted when $P < 0.05$. Area under the curve for plasma glucose was calculated by the trapezoidal rule. A Grubb's test was applied to identify statistical outliers. Comparisons between two groups were made with a *t*-test for independent samples and a Welch's correction for unequal variances was applied when indicated. Statistical calculations were made with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Conceptus and birth weights, and somatic growth to nine weeks of age

Fetal (2.3 ± 0.03 versus 2.5 ± 0.6 g; $P < 0.05$) and placental weights (0.44 ± 0.02 versus 0.49 ± 0.02 g; $P < 0.05$) were decreased in the RUPP group compared with the NP group. As shown in Figure 1a, RUPP-offspring (RUPP-O) pups were smaller at birth than NP-offspring (NP-O) pups. After weaning at three weeks of age, the RUPP-O group continued to weigh less than the NP-O group (Figure 1b). The RUPP-O group exhibited catch-up growth after weaning and were not reduced in weight compared with the NP-O at nine weeks of age (254 ± 11 versus 251 ± 13 g).

AF environment in late gestation

Figure 2a illustrates that AF IGF-1 concentration was increased in RUPP compared with NP control rats (0.24 ± 0.03 versus 0.16 ± 0.02 pg/mL; $P < 0.05$), while panel b shows that IGF-2 was also increased at day 19 of pregnancy (62699 ± 8244 versus 41960 ± 5500 pg/mL; $P < 0.05$).

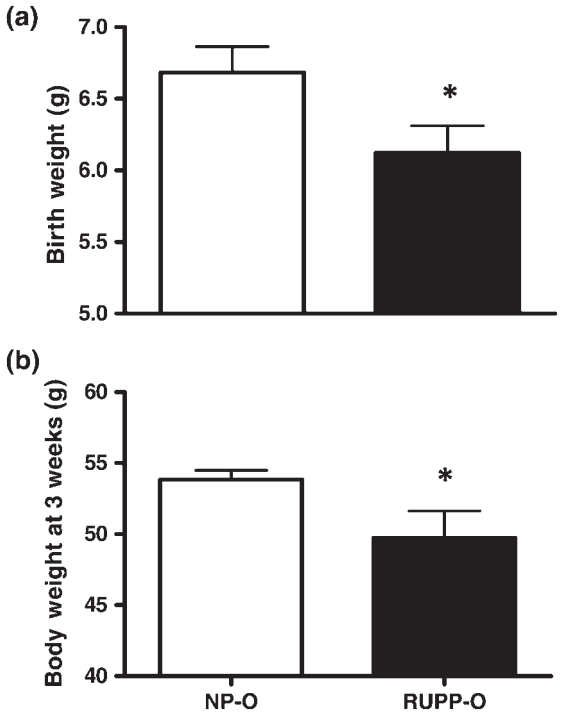

Figure 1 Birth weight (a) and body weight at three weeks of age (b) was decreased ($P < 0.05$) in the reduced uterine perfusion pressure (RUPP) offspring compared with normal pregnant (NP) offspring

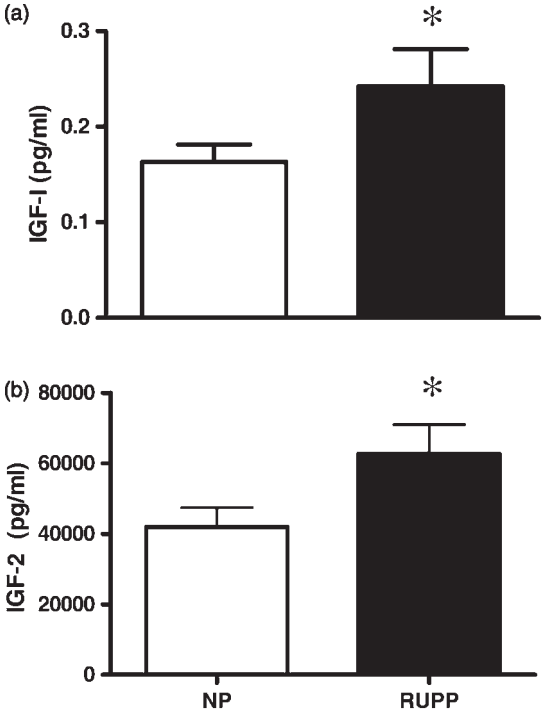

Figure 2 Panel a illustrates that insulin-like growth factor (IGF)-1 was increased in the amniotic fluid of reduced uterine perfusion pressure (RUPP) compared with normal pregnant (NP) control rats (0.24 ± 0.03 versus 0.16 ± 0.02 pg/mL; $P < 0.05$), while panel b shows that IGF-2 was also increased at day 19 of pregnancy (62699 ± 8244 versus 41960 ± 5500 pg/mL; $P < 0.05$)

In addition, Figure 3 illustrates that concentrations of Na^+ were higher (panel a: 140.0 ± 3.6 versus 127.9 ± 2.3 mEq; $P < 0.05$) while concentrations of K^+ were lower (panel b: 5.96 ± 0.27 versus 7.43 ± 0.47 mEq; $P < 0.05$) in the RUPP compared with NP fetuses. AF glucose concentrations were not different between groups (5.8 ± 0.91 versus 5.72 ± 1.15 mmol/L).

Increased oxidative stress was evident in the AF as malondialdehyde was elevated (4.3 ± 0.7 versus 2.6 ± 0.3 μmol/L; $P < 0.05$; Figure 4a) in the AF of the RUPP compared with the NP dams at day 19 of pregnancy. Further, the antioxidant capacity of the AF was decreased (0.72 ± 0.33 versus 1.45 ± 0.27 mmol/L; $P < 0.05$) in the RUPP compared with the NP pregnancies (Figure 4b).

Arterial pressure in late gestation and at nine weeks of age in the offspring

Figure 5a illustrates that MAP was increased in RUPP compared with NP dams at day 19 of pregnancy. Figure 5b shows that MAP was increased in RUPP-O compared with NP-O control rats (121 ± 3 versus 107 ± 5 mmHg; $P < 0.05$) at nine weeks of age.

Fasting glucose, triglycerides, cholesterol and metabolic hormones at nine weeks of age

Figure 6a shows that fasting glucose (4.4 ± 0.2 versus 3.1 ± 0.3 mmol/L; $P < 0.05$) and fasting insulin (0.71 ± 0.014 versus 0.30 ± 0.08 ng/mL; $P < 0.05$; panel b) were

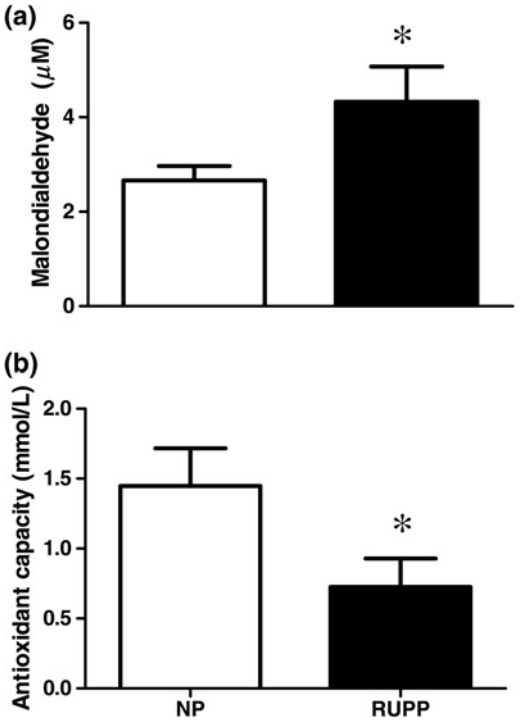

Figure 4 At day 19 of gestation, increased oxidative stress was evident in the amniotic fluid. This is shown in panel a as elevated malondialdehyde levels in the amniotic fluid of the reduced uterine perfusion pressure (RUPP) compared with the normal pregnant (NP) dams at day 19 of pregnancy. Panel b shows that the total antioxidant capacity of the amniotic fluid was decreased in the RUPP compared with the NP pregnancies. Data are presented as mean ± standard error of mean, $^*P < 0.05$

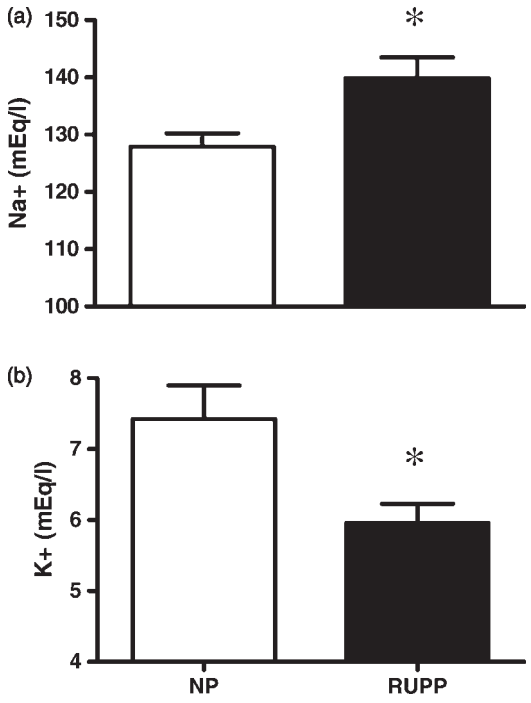

Figure 3 At day 19 of gestation amniotic fluid concentrations of Na^+ were higher (panel a; $P < 0.05$), while concentrations of K^+ were lower (panel b; $P < 0.05$) in the reduced uterine perfusion pressure (RUPP) compared with normal pregnant (NP) fetuses. Data are presented as mean ± standard error of mean, $^*P < 0.05$

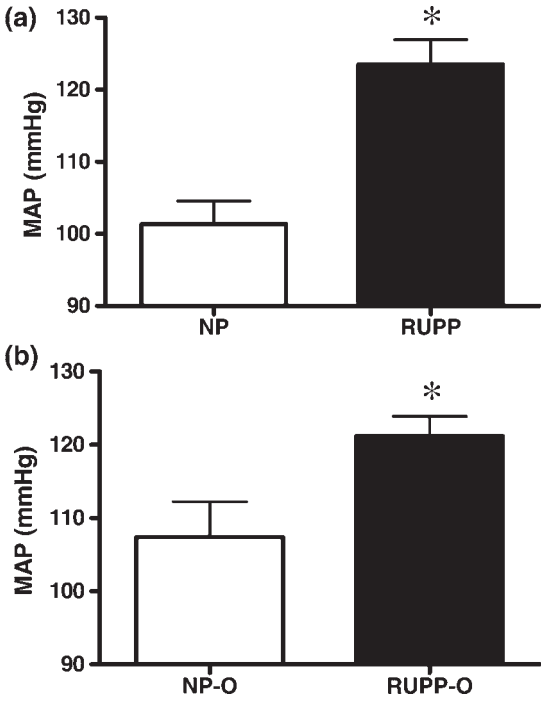

Figure 5 Panel a illustrates that mean arterial pressure (MAP) was increased in reduced uterine perfusion pressure (RUPP) compared with normal pregnant (NP) dams at day 19 of pregnancy. Panel b shows that MAP was increased in RUPP offspring compared with NP offspring control rats at nine weeks of age. Data are presented as mean ± standard error of mean, $^*P < 0.05$

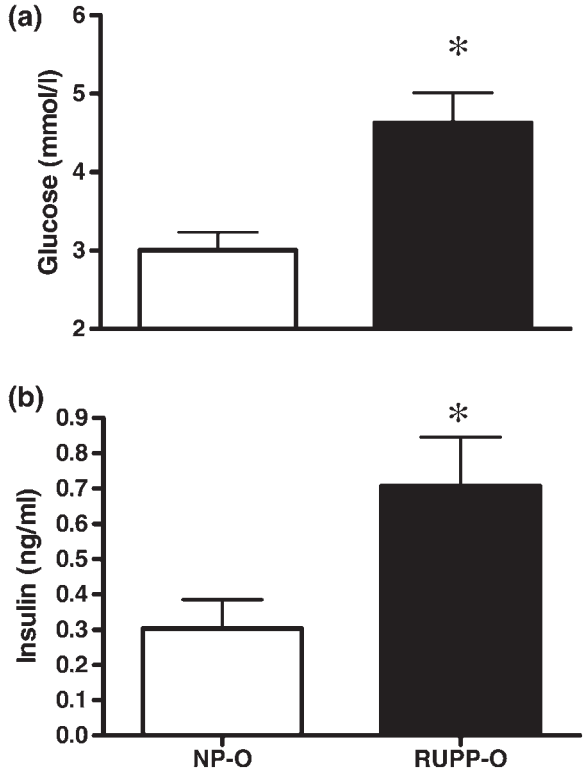

Figure 6 At nine weeks of age fasting glucose (a) and insulin (b) were increased ($P < 0.05$) in the reduced uterine perfusion pressure offspring compared with normal pregnant offspring rats. Data are presented as mean $\pm$ standard error of mean, $^{*}P < 0.05$

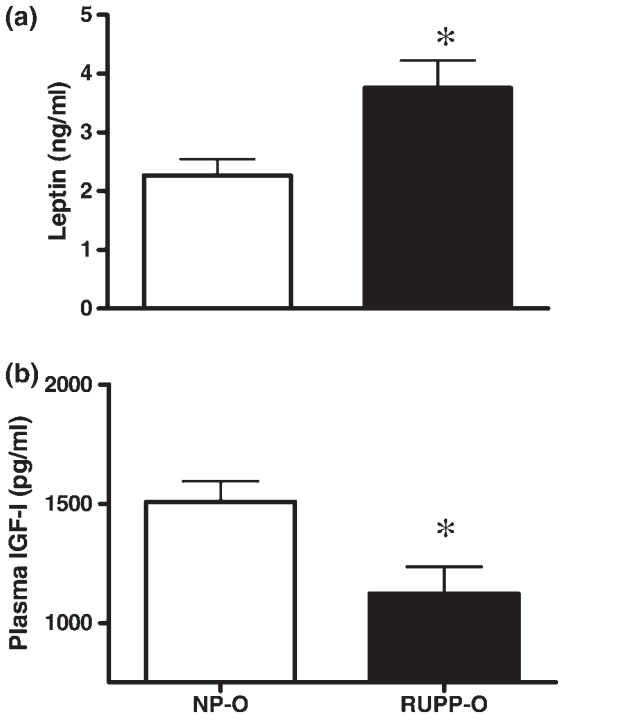

Figure 7 Panel a shows that leptin (3.8 ± 0.5 versus 2.3 ± 0.3 ng/mL; $P < 0.05$) was increased in the reduced uterine perfusion pressure offspring (RUPP-O) compared with the normal pregnant offspring (NP-O) rats at nine weeks of age. Panel b illustrates that circulating insulin-like growth factor-1 (IGF-1) (1123 ± 112 versus 1508 ± 86 pg/mL; $P < 0.05$) was decreased in the RUPP-O compared with the NP-O rats at nine weeks of age. Data are presented as mean $\pm$ standard error of mean, $^{*}P < 0.05$

increased in the IUGR RUPP-O rats compared with the NP-O controls. Figure 7a shows that leptin (3.8 ± 0.5 versus 2.3 ± 0.3 ng/mL; $P < 0.05$) was increased, while circulating IGF-1 (1123 ± 112 versus 1508 ± 86 ng/mL; $P < 0.05$) was decreased in the RUPP-O compared with the NP-O.

The homeostasis model assessment of insulin resistance index was greater in the IUGR RUPP-O rats than the NP-O rats (2.9 ± 0.6 versus 1.0 ± 0.3; $P < 0.05$). Further, the homeostatic model assessment index findings correlated strongly ($r = 0.94$, $P < 0.001$) with fasting insulin measurements ($y = 0.2253x + 0.1331$; $R^2 = 0.881$) in both groups of animals.

Fasting cholesterol levels (41.2 ± 3.6 versus 38.7 ± 4.4 mg/dL) were not changed between the RUPP-O and NP-O groups. Likewise, fasting triglyceride levels (88.1 ± 13.7 versus 137.6 ± 29.9 mg/dL; $P = 0.10$) were not significantly different between the RUPP-O and NP-O rats.

Glucose intolerance at nine weeks of age

Glucose tolerance in response to an intravenous glucose tolerance test was impaired ($P < 0.05$) in the IUGR RUPP-O group when compared with NP-O controls (Figure 8, RUPP-O, •; NP-O, □). Area under the glucose clearance curve was greater (26.8 ± 1.5 versus 23.0 ± 0.8 mmol/L/min; $P = 0.05$) in the RUPP-O than in the NP-O control offspring (Figure 8, inset).

Discussion

Clear and robust associations have been reported with regard to impaired fetal growth and later onset of cardiometabolic disease.[18–21] These associations are evident in a variety of populations and have been found in numerous experimental models.[3,7,14–16,18–21] Although a variety of experimental reports have indicated that placental

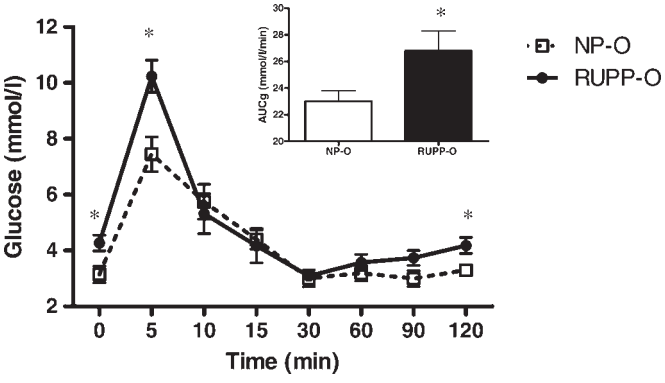

Figure 8 At nine weeks of age, the glucose tolerance following an intravenous glucose tolerance test was impaired ($P < 0.05$) in the reduced uterine perfusion pressure offspring (RUPP-O) compared with normal pregnant offspring (NP-O) rats as evidenced by elevated plasma glucose concentrations at five minutes after the infusion and by an increase in the area under the curve (inset). Data are presented as mean $\pm$ standard error of mean, $^{*}P < 0.05$

insufficiency results in manifestations of the metabolic syndrome (hypertension, glucose intolerance, etc.), a limitation shared by many previous studies is the short duration (2–4 d) of uteroplacental blood flow in late pregnancy.[2–10] With this in mind the present study sought to determine if chronic reductions of uteroplacental perfusion during the entire third trimester of pregnancy would alter the fetal environment in late gestation and result in dysregulated metabolic function in adolescent offspring.

In the present study, we report for the first time that placental insufficiency during the entire third trimester in the pregnant rat results in increased IGF-2 concentrations and increased oxidative stress in the AF in late gestation. Further, we show that chronic placental ischemia results in the development of several key features of the metabolic syndrome in the offspring.

Foremost, we report several significant alterations in the AF of rats with chronic placental insufficiency. AF is a medium in which the fetus develops and is the initial fluid that the kidneys begin processing during the early periods of organogenesis. Hence, the kidney as well as the rest of the fetus is constantly exposed to the growth factors and nutrients that comprise the AF. Taken together, the AF may represent an important avenue for the regulation of fetal development. Our present finding that IGF-2 levels are increased in the AF of RUPP rats is in accord with recent data reported from IUGR human pregnancies.[27,28] Viewed in concert, these data suggest that increased IGF-2 may be a compensatory mechanism to maintain fetal growth. Previous work has indicated that IGF-2 may be an important factor in the pathological overexpression of hypoxia-inducible factor-1α (HIF-1α). Hence, it is possible that the increased IGF-2 observed in the present study may also contribute to the persistent increases in HIF1-α and HIF-responsive genes such as soluble fms-like tyrosine kinase-1 (Flt-1) that we have reported previously in the placentas from the RUPP rats.[33,38]

We also found that the fetal environment in the RUPP pregnancy appears to be subjected to increased oxidative stress. Increased oxidative stress is a common aspect of pre-eclamptic pregnancies[39] and we have reported previously that it is increased in the maternal compartment in the RUPP model.[37] The possible role of oxidative stress in the programming of later disease remains unclear; however, it may result in epigenetic modification such as methylation or acetylation, which in turn may alter gene expression and development.[6] Alternatively, the disruption of the angiogenic balance between soluble Flt-1(sFlt-1) and vascular endothelial growth factor (VEGF)/placental growth factors observed in this model[34] and in human IUGR[40,41] and pre-eclamptic pregnancies[42] may also play a role. Previous work has shown that reductions in bioavailable VEGF during pregnancy are implicated in developmental programming of blood pressure, postnatal growth and organogenesis of the kidney[43] and the pancreas.[44] Indeed, we have previously shown that the sFlt-1 is elevated during RUPP pregnancy and that VEGF is decreased in the maternal circulation and in the AF.[34] Taken together with the present findings, VEGF antagonism that is driven by a combination of hypoxia and increased HIF-1α and IGF-2 levels provides a possible mechanism by which intrauterine programming of the offspring in this model may occur.

Because IGFs are important regulators of placental transporters, many of which are co-transporters of Na^+ and K^+,[45] we also sought to determine if AF electrolyte levels were altered in this model. Interestingly, we found that Na^+ concentrations were increased and K^+ concentrations were decreased in the AF of the RUPP fetuses that subsequently became hypertensive. This striking observation has been reported previously in another model of hypertension, the spontaneously hypertensive rat.[46] Since the fetal kidney begins to process AF during gestation, this raises the possibility that set points for the excretion of electrolytes may be altered *in utero* and in a manner that results in hypertension after parturition. Further studies are planned to evaluate renal Na^+ excretion in these offspring.

We report for the first time using the chronic RUPP model that IUGR offspring have higher fasting insulin and glucose concentrations than offspring from NP rats. Further, we found that young RUPP offspring were glucose intolerant as determined by an IVGTT and observed that the IUGR offspring from RUPP hypertensive pregnancies were hyperleptinemic and had reduced plasma IGF-1 concentrations at nine weeks of age.

Prior studies have shown that leptin infusion results in elevated blood pressure in the rat and that this is mediated by increased adrenergic activity. It is interesting that fasting leptin concentrations are observed to be elevated in these adolescent IUGR rats with elevated blood pressure. Previous work in this model has shown that renal denervation attenuates the sustained high blood pressure in these animals.[47] Although the underlying mechanism responsible for the increased sympathetic outflow in this model remains unclear, the present finding of hyperleptinemia does provide an additional intriguing possibility that may link hypertension and metabolic function in this model. Nevertheless, this hypothesis remains speculative and further studies are required to determine if the chronic elevations in plasma leptin concentrations contribute to the increased renal sympathetic activity previously reported in this model.

Low levels of circulating IGF-1 are related to increased risk for cardiometabolic disease and recent studies have reported a relationship between reduced IGF-1 concentrations and metabolic abnormalities that may manifest during childhood.[48,49] In the present study, we found that circulating IGF-1 concentrations were decreased at eight weeks of age in the IUGR offspring of hypertensive pregnancies. Our findings are in agreement with this prior work and support the hypothesis that fetal programming of the IGF axis may play an important role in the development of metabolic abnormalities and high blood pressure in growth restricted offspring.

While insulin resistance and hypertension are observed in humans, the mechanisms linking the two remain poorly defined. Recent studies have identified several endothelial pathways by which chronic elevations may negatively impact endothelial function by enhancing endothelin-1 activity or decreasing nitric oxide (NO) bioavailability.[50,51] This is consistent with a previous report by Payne *et al.*[52]

indicating that endothelium-dependent NO-induced vasorelaxation is impaired in this model as early as four weeks of age. Low levels of IGF-1 such as found in the present study may also contribute to decreased NO bioavailability.[53] Studies are currently planned to determine if endothelial dysfunction and hypertension precedes the increased fasting glucose, insulin and leptin in this model.

We also found that the offspring in the present study exhibited catch-up growth between three and eight weeks of age. A previous study has shown that uteroplacental insufficiency beginning on day 19 of pregnancy in the rat alters mammary development and results in less milk per pup with an altered composition.[54] Similarly, we have preliminary data indicating that mammary weights are reduced on day 19 of gestation after five days of RUPP (JS Gilbert, unpublished observations) when compared with NP control rats; however, it remains unclear presently if this is associated with altered milk production or content. Viewed in concert, it appears that the catch-up growth observed in the present study occurs entirely after weaning and may be related to alterations in metabolic regulation (i.e. insulin, IGF-1, leptin) that is present in these animals.

Although relationships between low birth weight and hypertension have long been recognized in humans and in a variety of animal models, the underlying mechanisms remain unclear. This is especially true of the ontogeny of hypertension and metabolic dysregulation in this as well as other animal models. A common limitation of IUGR models in rodents is that they often only encompass a short period of gestation (e.g. days 17-term or 19-term) and do not extend for the entire third trimester as is often observed in human pregnancy. In contrast, the present study relies on data obtained from a well-characterized and robust animal model that mimics many features shared by pregnancies with pre-eclampsia and placental insufficiency such as altered angiogenic and growth factor balance, altered AF milieu, hypertension and low birth weight. Moreover, the RUPP model of placental insufficiency employed in the present study more closely mimics the growth restriction that occurs in pregnant women. Taken together, these findings shed new light on potential mechanisms underlying the development of hypertension and metabolic dysfunction in IUGR young offspring.

Author contributions: All authors participated in either the design, interpretation of the studies or analysis of the data and review of the manuscript; AH, ELBS, KNJ, AG, NB, BKJ and JSG conducted the experiments; JSG supplied critical reagents and animals; AH, JSG and AG wrote the manuscript; and ELBS, NB and KNJ contributed revisions to the final draft of the manuscript.

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REFERENCES

1 Smith GCS. First trimester origins of fetal growth impairment. *Semin Perinatol* 2004;**28**:41–50
2 Lane RH, Kelley DE, Gruetzmacher EM, Devaskar SU. Uteroplacental insufficiency alters hepatic fatty acid-metabolizing enzymes in juvenile and adult rats. *Am J Physiol Regul Integr Comp Physiol* 2001;**280**:R183–90
3 Nusken KD, Dotsch J, Rauh M, Rascher W, Schneider H. Uteroplacental insufficiency after bilateral uterine artery ligation in the rat: impact on postnatal glucose and lipid metabolism and evidence for metabolic programming of the offspring by sham operation. *Endocrinology* 2008;**149**:1056–63
4 Lane RH, Dvorak B, MacLennan NK, Dvorakova K, Halpern MD, Pham TD, Philipps AF. IGF alters jejunal glucose transporter expression and serum glucose levels in immature rats. *Am J Physiol Regul Integr Comp Physiol* 2002;**283**:R1450–60
5 Lane RH, Crawford SE, Flozak AS, Simmons RA. Localization and quantification of glucose transporters in liver of growth-retarded fetal and neonatal rats. *Am J Physiol Endocrinol Metab* 1999;**276**:E135–42
6 Gilbert JS, Nijland MJ. Sex differences in the developmental origins of hypertension and cardiorenal disease. *Am J Physiol Regul Integr Comp Physiol* 2008;**295**:R1941–52
7 Simmons RA, Templeton LJ, Gertz SJ. Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. *Diabetes* 2001;**50**:2279–86
8 Neitzke U, Harder T, Schellong K, Melchior K, Ziska T, Rodekamp E, Dudenhausen JW, Plagemann A. Intrauterine growth restriction in a rodent model and developmental programming of the metabolic syndrome: a critical appraisal of the experimental evidence. *Placenta* 2008;**29**:246–54
9 Boloker J, Gertz SJ, Simmons RA. Gestational diabetes leads to the development of diabetes in adulthood in the rat. *Diabetes* 2002;**51**:1499–506
10 Sadiq HF, deMello DE, Devaskar SU. The effect of intrauterine growth restriction upon fetal and postnatal hepatic glucose transporter and glucokinase proteins. *Pediatr Res* 1998;**43**:91–100
11 Gilbert JS, Lang AL, Grant AR, Nijland MJ. Maternal nutrient restriction in sheep: hypertension and decreased nephron number in offspring at 9 months of age. *J Physiol* 2005;**565**(Part 1):137–47
12 Gilbert JS, Ford SP, Lang AL, Pahl LR, Drumhiller MC, Babcock SA, Nathanielsz PW, Nijland MJ. Nutrient restriction impairs nephrogenesis in a gender-specific manner in the ovine fetus. *Pediatr Res* 2007;**61**:42–7
13 Mcmillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev* 2005;**85**:571–633
14 Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 1998;**351**:173–7
15 Zambrano E, Bautista CJ, Deás M, Martínez-Samayoa PM, González-Zamorano M, Ledesma H, Morales J, Larrea F, Nathanielsz PW. A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. *J Physiol* 2006;**571**: 221–30
16 Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *Am J Obstetr Gynecol* 2006;**195**:40–9
17 Baschat AA, Hecher K. Fetal growth restriction due to placental disease. *Semin Perinatol* 2004;**28**:67–80
18 Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation* 1996;**94**:3246–50
19 Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clark PMS. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 1993;**36**:62–7
20 Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 1991;**303**:1019–22
21 Phipps K, Barker DJ, Hales CN, Fall CH, Osmond C, Clark PM. Fetal growth and impaired glucose tolerance in men and women. *Diabetologia* 1993;**36**:225–8

22 Hovi P, Andersson S, Eriksson JG, Jarvenpaa AL, Strang-Karlsson S, Makitie O, Kajantie E. Glucose regulation in young adults with very low birth weight. *N Engl J Med* 2007;**356**:2053–63
23 Regan FM, Cutfield WS, Jefferies C, Robinson E, Hofman PL. The impact of early nutrition in premature infants on later childhood insulin sensitivity and growth. *Pediatrics* 2006;**118**:1943–9
24 Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, Gluckman PD. Insulin resistance in short children with intrauterine growth retardation. *J Clin Endocrinol Metab* 1997;**82**:402–6
25 DeChiara TM, Efstratiadis A, Robertson EJ. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* 1990;**345**:78–80
26 Shen SJ, Wang CY, Nelson KK, Jansen M, Ilan J. Expression of insulin-like growth factor II in human placentas from normal and diabetic pregnancies. *Proc Natl Acad Sci USA* 1986;**83**:9179–82
27 Trollmann R, Klingmüller K, Schild RL, Rascher W, Dötsch J. Differential gene expression of somatotrophic and growth factors in response to in vivo hypoxia in human placenta. *Am J Obstetr Gynecol* 2007;**197**:601
28 Street ME, Seghini P, Fieni S, Ziveri MA, Volta C, Martorana D, Viani I, Gramellini D, Bernasconi S. Changes in interleukin-6 and IGF system and their relationships in placenta and cord blood in newborns with fetal growth restriction compared with controls. *Eur J Endocrinol* 2006;**155**:567–74
29 Carter AM, Kingston MJ, Han KK, Mazzuca DM, Nygard K, Han VKM. Altered expression of IGFs and IGF-binding proteins during intrauterine growth restriction in guinea pigs. *J Endocrinol* 2005;**184**:179–89
30 Aleixandre de Artinano A, Miguel Castro M. Experimental rat models to study the metabolic syndrome. *Br J Nutr* 2006;**27**:1–8
31 Gilbert JS, Dukes M, LaMarca BB, Cockrell K, Babcock SA, Granger JP. Effects of reduced uterine perfusion pressure on blood pressure and metabolic factors in pregnant rats. *Am J Hypertension* 2007;**20**:686–91
32 Granger JP, LaMarca BB, Cockrell K, Sedeek M, Balzi C, Chandler D, Bennett W. Reduced uterine perfusion pressure (RUPP) model for studying cardiovascular-renal dysfunction in response to placental ischemia. *Methods Mol Med* 2006;**122**:383–92
33 Gilbert JS, Gilbert SAB, Arany M, Granger JP. Hypertension produced by placental ischemia in pregnant rats is associated with increased soluble endoglin expression. *Hypertension* 2008;**53**:399–403
34 Gilbert JS, Babcock SA, Granger JP. Hypertension produced by reduced uterine perfusion in pregnant rats is associated with increased soluble Fms-like tyrosine Kinase-1 expression. *Hypertension* 2007;**50**:1142–7
35 Gilbert JS, Dukes M, Babcock SA, Cockrell K, LaMarca B, Granger JP. Effects of reduced uterine perfusion pressure on blood pressure and metabolic factors in the pregnant rat. *FASEB J* 2007;**21**:A894
36 Bridges JP, Gilbert JS, Colson D, Gilbert SA, Dukes MP, Ryan MJ, Granger JP. Oxidative Stress Contributes to soluble Fms-like tyrosine kinase-1 induced vascular dysfunction in pregnant rats. *Am J Hypertens* 2009;**22**:564–8
37 Sedeek M, Gilbert JS, LaMarca BB, Sholook M, Chandler DL, Wang Y, Granger JP. Role of reactive oxygen species in hypertension produced by reduced uterine perfusion in pregnant rats. *Am J Hypertens* 2008;**21**:1152–6
38 Gilbert JS, LaMarca BB, Babcock SA, Cockrell K, Granger JP. Hypertension produced by reduced uterine perfusion in pregnant rats is associated with increases in soluble flt-1 expression. *Hypertension* 2007;**50**:E108
39 Gilbert JS, Ryan MJ, LaMarca BB, Sedeek M, Murphy SR, Granger JP. Pathophysiology of hypertension during preeclampsia: linking placental ischemia with endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2008;**294**:H541–50
40 Wallner W, Sengenberger R, Strick R, Strissel PL, Meurer B, Beckmann MW, Schlembach D. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clin Sci (Lond)* 2007;**112**:51–7
41 Nevo O, Many A, Xu J, Kingdom J, Piccoli E, Zamudio S, Post M, Bocking A, Todros T, Caniggia I. Placental expression of soluble fms-like tyrosine kinase 1 is increased in singletons and twin pregnancies with intrauterine growth restriction. *J Clin Endocrinol Metab* 2008;**93**:285–92
42 Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;**111**:649–58
43 Cox LA, Nijland MJ, Gilbert JS, Schlabritz-Loutsevitch N, Hubbard GB, McDonald TJ, Shade RE, Nathanielsz PW. Effect of thirty percent maternal nutrient restriction from 0.16 to 0.5 gestation on fetal baboon kidney gene expression. *J Physiol* 2006;**572**(pt 1):67–85
44 Boujendar S, Arany E, Hill D, Remacle C, Reusens B. Taurine supplementation of a low protein diet fed to rat dams normalizes the vascularization of the fetal endocrine pancreas. *J Nutr* 2003;**133**:2820–5
45 Roos S, Lagerlof O, Wennergren M, Powell TL, Jansson T. Regulation of amino acid transporters by glucose and growth factors in cultured primary human trophoblast cells is mediated by mTOR signaling. *Am J Physiol Cell Physiol* 2009;**297**:C723–31
46 Erkadius E, Morgan TO, Di Nicolantonio R. Amniotic fluid composition and fetal and placental growth rates in genetically hypertensive and normotensive rats. *Reprod Fertil Dev* 1995;**7**:1563–7
47 Alexander BT, Hendon AE, Ferril G, Dwyer TM. Renal denervation abolishes hypertension in low-birth-weight offspring from pregnant rats with reduced uterine perfusion. *Hypertension* 2005:**1**
48 Sesso R, Franco MCP. Abnormalities in metalloproteinase pathways and IGF-I axis: a link between birth weight, hypertension, and vascular damage in childhood. *Am J Hypertens* 2009;**23**:6–11
49 Schutte AE, Huisman HW, van Rooyen JM, Malan L, Malan NT, Fourie CMT, Louw R, van der Westhuizen FH, Schutte R. A significant decline in IGF-I May predispose young africans to subsequent cardiometabolic vulnerability. *J Clin Endocrin Metab* 2010;**95**:2503–7
50 Potenza MA, Addabbo F, Montagnani M. Vascular actions of insulin with implications for endothelial dysfunction. *Am J Physiol Endocrinol Metab* 2009;**297**:E568–77
51 Potenza MA, Marasciulo FL, Chieppa DM, Brigiani GS, Formoso G, Quon MJ, Montagnani M. Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between NO and ET-1 production. *Am J Physiol Heart Circ Physiol* 2005;**289**:H813–22
52 Payne JA, Alexander BT, Khalil RA. Reduced endothelial vascular relaxation in growth-restricted offspring of pregnant rats with reduced uterine perfusion. *Hypertension* 2003;**42**:768–74
53 Perticone F, Sciacqua A, Perticone M, Laino I, Miceli S, Care' I, Galiano Leone G, Andreozzi F, Maio R, Sesti G. Low-plasma insulin-like growth factor-I levels are associated with impaired endothelium-dependent vasodilatation in a cohort of untreated, hypertensive caucasian subjects. *J Clin Endocrinol Metab* 2008;**93**:2806–10
54 O'Dowd R, Kent JC, Moseley JM, Wlodek ME. Effects of uteroplacental insufficiency and reducing litter size on maternal mammary function and postnatal offspring growth. *Am J Physiol Regul Integr Comp Physiol* 2008;**294**:R539–48

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