Effect of Reduced Inspired Oxygen on Fetal Growth and Maternal Glucose Metabolism in Rat Pregnancy

Firas Saker, Deepak M. Voora, Supriya D. Mahajan, İlknur Kiliç, Faramarz Ismail-Beigi, and Satish C. Kalhan

The effect of prolonged exposure to a reduced fraction of inspired oxygen ([FiO_2] 0.17 for 3 days) on maternal glucose kinetics, placental glucose transporters GLUT1 and GLUT3, and fetal growth was examined in rat pregnancy. Arterial and venous catheters were placed 3 days before the study. [3-^{3}H]glucose tracer and deuterium labeling of water were used to measure the rates of glucose turnover and gluconeogenesis (GNG), respectively. Glucose uptake by maternal tissues was measured using [^{14}C]2-deoxyglucose. Exposure to a reduced FiO_2 resulted in a significant decrease (mean ± SE) in fetal weight (room air, 4.02 ± 0.04 g; 0.17 FiO_2, 3.27 ± 0.6 g, $P < .02$). There was a significant increase in the maternal-fetal glucose gradient (maternal-fetal glucose ratio: room air, 1.48 ± 0.11; 0.17 FiO_2, 2.26 ± 0.24, $P < .05$), but there was no change in the maternal or fetal blood lactate concentration. No significant change in maternal blood pH was observed; however, a significant decrease in the blood partial pressure of O_2 (PO_2) occurred (room air, 97 ± 0.5 torr; 0.17 FiO_2, 81 ± 1.8) on day 3. There was no change in the rate of turnover of glucose or GNG in the maternal compartment, nor was there any effect on glucose uptake by the maternal tissues. Placental GLUT1 and GLUT3 mRNA were not different in the control or experimental animals. We conclude that a mild reduction in the FiO_2 for 3 days in rat pregnancy results in a significant fetal growth restriction that is not related to any observed alteration in maternal glucose metabolism. The lower glucose concentration in the fetal blood may be the consequence of an increase in fetal glucose metabolism, thereby resulting in an increased maternal-fetal gradient of glucose.

PERSISTENT MATERNAL or fetal hypoxemia due to either decreased environmental oxygen or decreased uterine or placental perfusion has been shown to cause decreased fetal weight and intrauterine growth restriction.[1-5] Even a mild reduction in environmental oxygen such as at high altitude has been related to low birth weight in human studies.[6] The mechanism of the hypoxemia-induced fetal growth restriction is not clear. While decreased uterine or placental perfusion results in decreased delivery of both oxygen and nutrients to the fetus, the fetal growth restriction induced by reduced environmental oxygen is the consequence of both reduced oxygen delivery and the associated adaptive circulatory and metabolic responses in the mother and fetus. Furthermore, while acute hypoxemia may trigger a transient hormonal and metabolic response that may affect maternal and fetal metabolism, most of these acute changes tend to disappear with prolonged hypoxemia.[7-9] The metabolic effects of prolonged hypoxemia have been studied in both in vitro and in vivo models. Persistent hypoxemia, depending on the severity, may result in a lower metabolic rate, an alteration in body temperature and ventilation, expression of a number of genes involved in regulation of substrate metabolism, increased utilization of glucose via the nonoxidative pathway, and stimulation of glucose transport in skeletal muscle via non–insulin-mediated mechanisms.[9-12] Whether any of these mechanisms are involved in hypoxemia-induced fetal growth restriction has not been examined.

Maternal adaptations to the growing fetus during pregnancy have been characterized to include, among others, an increase in the rate of glucose production and utilization to meet the increasing demands of the fetus.[13,14] In addition, a decreased uptake of glucose by skeletal muscle during pregnancy has been shown both in vitro in animal studies and in vivo in humans using a hyperinsulinemic glucose clamp.[15-17] Development of pregnancy-induced insulin resistance has been suggested to be an important contributor to fetal growth, possibly by diverting nutrients, particularly glucose, to the fetus.[16] Since hypoxemia has been shown to counteract insulin resistance by increasing non–insulin-mediated glucose uptake,[10] we hypothesized that fetal growth restriction induced by environmental hypoxemia may be related to alterations in maternal glucose metabolism.

In the present study, we have examined the effect of prolonged exposure to reduced environmental oxygen during pregnancy on the rate of glucose production, gluconeogenesis (GNG), glucose uptake, and fetal growth. Our data show that a prolonged (3 days) exposure to lower environmental oxygen (fraction of inspired O_2 [FiO_2] 17%) during rat pregnancy induces fetal growth restriction without any significant change in maternal glucose metabolism or placental glucose transporters.

MATERIALS AND METHODS

Deuterium oxide (99.9 atom% excess ^{2}H) was purchased from Isotec (Miamisburg, OH). [3-^{3}H]glucose (specific activity [SA] 10.4 Ci/mmol) and [U-^{14}C]2-deoxyglucose (SA 294 Ci/mmol) were obtained from NEN Research Products (Boston, MA). PE50 polyethylene nontoxic tubing (ID 0.58 mm and OD 0.965 mm) was obtained from Becton Dickinson (Sparks, MD).

Nonpregnant and timed-pregnant Sprague-Dawley rats were obtained from Zivic Miller (Zelienople, PA). The animals were kept in a controlled environment as stipulated by the American Association for the Accreditation of Laboratory Animals. They had free access to Purina (St Louis, MO) rat chow and water. The animals were kept in individual cages in a uniform 12-hour day/night cycle. The protocol was approved by the Institutional Animal Care and Use Committee.

From the Center for Metabolism & Nutrition and Departments of Pediatrics and Medicine, Metrohealth Medical Center, Case Western Reserve University School of Medicine, Cleveland, OH.

Submitted July 3, 1998; accepted November 24, 1998.

Supported by Grants No. HD11089 and DK45945 from the National Institutes of Health, and in part by a Metabolism Training Grant (DK-07319) from the National Institutes of Health (F.S.) and a grant from the Scientific and Technical Research Council of Turkey (I.K.).

Address reprint requests to Satish C. Kalhan, MD, Center for Metabolism & Nutrition, MetroHealth Medical Center, Bell Greve Building, Room G-775, 2500 MetroHealth Dr, Cleveland, OH 44109-1998.

0026-0495/99/4806-0011$10.00/0

Indwelling arterial and venous catheters were placed 4 days before the tracer study. The animals were anesthetized with an intramuscular injection (0.1 mL/100 g) of ketamine acepromazine mixture (90 mg ketamine and 1 mg acepromazine per 1 mL). PE50 catheters were inserted in the right external jugular vein and left carotid artery under sterile conditions. The catheters were filled with an anticoagulant mixture of polyvinylpyrolidone and heparin in isotonic saline (polyvinylpyrolidone 0.75 g and heparin 25 U in 1 mL isotonic saline). This mixture allows catheter patency for almost 1 week. The catheters were tunneled subcutaneously, and the distal end of each catheter was sutured to the dorsum of the neck. The free ends of the catheters were sealed, and the rat was placed in a Harvard rodent jacket sling (Harvard Apparatus, Holliston, MA), thus allowing the animal to move freely during the postoperative period. At the end of the surgery, the rats received intramuscular penicillin G 50,000 U. The rats were housed in individual cages and had free access to a standard diet (Purina rat chow) and water. Experimental animals were placed in the hypoxemia chamber 24 hours after surgery. Total food intake was not different for hypoxemia-exposed rats versus control rats (hypoxemia, 28.3 ± 1.1 g/d, n = 4; control, 29.7 ± 1.3 g/d, n = 4). On the day of tracer isotope infusion, the anticoagulant was aspirated and the catheter was connected to the infusion pump.

A Plexiglas box was used as the environmental hypoxemia chamber. The oxygen concentration in the box was decreased by blending air with nitrogen. Oxygen and carbon dioxide concentrations in the chamber were monitored using a gas analyzer (MGA-1100; Perkin-Elmer, Pamona, CA). The rate of airflow was adjusted to maintain CO_2 levels in the chamber below 0.1%. The animals, in their cages, were placed in the chamber for either (1) 2 hours following a basal study period (acute effects) or (2) 3 days prior to the tracer study (prolonged effects). The animals were studied while in the chamber. Pregnant animals were placed in the chamber on day 17 of gestation.

On the day of the study, food was removed at 5 AM. Deuterium oxide 0.3 g/100 g body weight was administered intraperitoneally at 8 AM to quantify GNG. The rate of appearance (Ra) of glucose was quantified using [3-^{3}H]glucose tracer. The tracer dissolved in isotonic saline solution was administered as a primed-constant infusion beginning at 11 AM. The priming dose was 1.5 to 2.0 μCi and the rate of infusion 0.05 to 0.07 μCi/kg body weight/min. Four arterial samples were obtained at 10-minute intervals between 90 and 120 minutes. Two hours after the start of [3-^{3}H]glucose infusion, a bolus dose (20 μCi) of [U-^{14}C]2-deoxyglucose was administered. Blood samples (0.2 mL) were obtained at 1, 3, 5, 7, 10, 15, 20, 30, and 50 minutes. The total volume of blood drawn from each animal was 2.8 mL and was replaced with fresh blood from a donor rat. At the end of the study, the animals were anesthetized using intravenous pentobarbital and samples of maternal tissues were rapidly obtained.

Studies were performed as follows: (1) control nonpregnant animals in room air, (2) control and pregnant rats in room air for 2 hours followed by 0.17 FiO_2 (acute effect), and (3) pregnant rats in 0.17 FiO_2 for 3 days (prolonged effect). Control nonpregnant animals were also studied to examine the effect of pregnancy, if any, on the observed maternal responses, since pregnant animals, due to the increased intraabdominal content, may not adapt as well to decreased FiO_2 as nonpregnant animals.

Studies of glucose and lactate gradients and placental glucose transporters were performed in experimental and normoxic pregnant rats without tracer infusion. Pregnant rats without indwelling catheters were placed in 0.17 FiO_2 on day 17 of gestation, as already described. Pregnant rats in room air were studied for comparison. Daily food intake and weight gain were monitored. On day 20 of gestation, the fetuses were rapidly removed under anesthesia (pentobarbital 60 mg/kg intraperitoneally). Fetal blood samples were obtained in a heparinized capillary tube by cutting the right axillary artery while still attached to the placenta. A maternal blood sample was obtained simultaneously from the abdominal aorta. The blood samples were deproteinized with an equal volume of 10% perchloric acid and frozen at −70°C for later analysis. Fetal liver, maternal liver, and placentae from four fetuses from the midpoint of the uterine horn were obtained for glycogen measurement and frozen instantly in liquid nitrogen. Samples of placentae were also obtained for measurement of glucose transporter GLUT1 and GLUT3 mRNA.

Analysis

Arterial blood gases were measured in the clinical laboratory using a commercial blood gas analyzer (Radiometer, Copenhagen, Denmark). Plasma or whole-blood glucose and lactate concentrations were measured on neutralized deproteinized specimen by an enzymatic method on a Yellow Springs 2300 glucose lactate analyzer (Yellow Springs, OH). The deuterium enrichment of hydrogens on carbon-6 of glucose was measured as described previously.[18] Briefly, carbon-6 of glucose was cleaved using periodic acid, and the formaldehyde produced was condensed with ammonium hydroxide to form hexamethylenetetramine (HMT). The deuterium enrichment of HMT was measured using a HP5970 gas chromatograph–mass spectrometer (Hewlett Packard, Sunnyvale, CA). A neutral column (AT-1, 30 m, ID 0.53 mm; Alltech, Deerfield, IL) was used, and ions m/z 140 and m/z 141 were monitored using the selected-ion monitoring software. Standard glucose solutions of known deuterium enrichment were also analyzed, and the data were normalized using the standard curve. The deuterium enrichment of plasma water was measured using the zinc-reduction method on an isotope ratio mass spectrometer (Dr Catherine Leitch, Department of Pediatrics, Indiana University, Indianapolis, IN).

For measurement of the SA of glucose, plasma samples were deproteinized with 0.3 mol/L barium hydroxide and 0.3 mol/L zinc sulfate. The neutralized supernatant was evaporated to dryness to remove any radioactive water formed during glycolysis. The dry samples were reconstituted with water and passed through a mixed-bed ion-exchange column as previously described.[19] The neutral fraction was used to measure the glucose concentration and radioactivity. Radioactivity was measured by adding 10 mL scintillation fluid (Scintisafe Econo-1; Fisher Scientific, Springfield, NJ) on a scintillation counter (Packard Instrument, Downers Grove, IL). Radioactive 2-deoxyglucose-6-phosphate (dpm per milligram) in various tissues was measured as described by Sokoloff et al[20] and Ferré et al.[21] A weighed amount of tissue was homogenized in 1 mol/L sodium hydroxide and heated at 60°C for 2 hours or until total digestion was obtained. The hydrolyzed homogenate was neutralized with 1 mL 1-mol/L HCl. One aliquot of neutralized homogenate was added to 6% $HClO_4$ (total radioactivity) and another aliquot to Somogyi reagent-$Ba(OH)_2/ZnSO_4$ (2-deoxyglucose radioactivity). The radioactivity of 2-deoxyglucose-6-phosphate was calculated from the difference between total radioactivity and radioactivity due to deoxyglucose.

Tissue glycogen content was measured using a modified micromethod described by Lo et al.[22] The tissue (40 to 50 mg) was incubated with 0.5 mL 30% potassium hydroxide at 100°C for 2 hours or until total digestion. The mixture was cooled to room temperature, and 5 mL 95% ethyl alcohol and 10 mg LiCl were added. The tubes were kept at 0°C for 30 minutes and then centrifuged at 3,000 rpm for 30 minutes. The pellets were hydrolyzed with 0.5 mL 4N HCl at 100°C for 2 hours and then neutralized with 0.5 mL 4-mol/L potassium carbonate. The glucose level was measured in the neutralized hydrolysate using a Yellow Springs glucose lactate analyzer.

Total RNA from rat placenta was extracted by the procedure of Chirgwin et al.[23] RNA concentrations were determined by absorbance at 260 nm on a spectrophotometer. All samples had a 260/280 ratio of at least 1.5. For Northern blot analysis, a fixed amount of total RNA was denatured in a solution containing 7.6% formaldehyde, 12× morpholinepropanesulfonic acid, 61.1% formamide, 2.4% glycerol, 1.2% EDTA, and 0.015% bromophenol blue by incubation at 68°C for 10

minutes. RNA samples were electrophoresed on a 1% agarose-formaldehyde gel. RNA integrity was determined by examining the ethidium bromide–stained 28S and 18S ribosomal subunits by UV light. RNAs were then transferred to nitrocellulose paper by capillarity and cross-linked using UV light. These blots were either hybridized to rat GLUT1 cDNA[24] or human GLUT3 cDNA probe.[25] Hybridization was performed using $\alpha[^{32}P]$dCTP-labeled cDNA probes for 2 hours at 68°C in Quickhyb Hybridization Solution (Stratagene, La Jolla, CA). Blots hybridized to GLUT1 cDNA were washed three times at 65°C using .1X SSC (1X SSC is 0.15 mol/L NaCl, plus 0.015 mol/L sodium/citrate)/ sodium dodecyl sulfate (SDS) solution and then exposed at −80°C for 2 to 24 hours. Blots hybridized to GLUT3 cDNA were washed twice at 45°C using a .5X SSC/.5% SDS solution and then once at 45°C also using a .5X SSC/.5% SDS solution and then exposed at −80°C for 6 to 7 days. Relative GLUT mRNA levels were determined by laser scanning densitometry. GLUT1 and GLUT3 mRNA levels were normalized to ribosomal 28S RNA estimated by measurement of ethidium bromide fluorescence on the blots. To compare the relative abundance of GLUT mRNAs in the placenta of the different groups, the average ratio of the densitometric reading of GLUT isoform to ribosomal 28S RNA in control rats was calculated and normalized to 1.0. The ratios obtained from the experimental group (0.17 FiO_2) were then normalized against the mean of the control group.

Calculation

The glucose Ra was calculated during isotopic steady state using a tracer dilution equation: Ra (mg/min) = (I/SA), where I is the rate of infusion of tracer isotope (dpm per minute) and SA is the SA of plasma glucose (dpm per milligram). The fractional contribution of GNG via pyruvate to glucose Ra was calculated as follows: GNG via pyruvate = 100 × (0.5 × [^{2}H] enrichment on C-6 of glucose/[^{2}H] enrichment in body water) %. The rationale for these calculations has been discussed previously.[26] Inasmuch as the equilibrium between the methyl hydrogens of pyruvate and water is not complete, this method will result in an underestimation (~15% to 20%) of the contribution of GNG. GNG (milligrams per minute) = Ra × fractional contribution of GNG to glucose Ra.

Tissue uptake of glucose was calculated as (nanograms per milligram of tissue) = [2-deoxyglucose-6-phosphate]$\tau/_0\int^{\tau}$ 2-deoxyglucose SA in plasma, where the numerator is 2-deoxyglucose-6-phosphate radioactivity in tissue (dpm per milligram). The integer of 2-deoxyglucose SA in the plasma was calculated by establishing the best-fit exponential curve for plasma SA data.[21]

Statistics

All data are presented as the mean ± SEM. Groups were compared using the Mann-Whitney *U* test for unpaired data and Wilcoxon rank-sum test for paired data. A *P* value less than .05 (two-tailed) was considered statistically significant.

RESULTS

The effects of prolonged exposure to 17% FiO_2 late in gestation on fetal weight, placental weight, and litter size are shown in Table 1. Although a prolonged reduction of FiO_2 did not cause any significant change in maternal weight or litter size, it resulted in a significant decrease in fetal weight ($P < .02$). Of significance, no change in the placenta weight was observed. The slightly lower weight of the control fetus compared with the normative data on rat pups in the literature may be the consequence of the termination of pregnancy in the present study on day 20, rather than allowing it to continue to term gestation (day 21).

The effect of an acute and a prolonged decrease in FiO_2 on arterial blood pH, partial pressure of CO_2 (PCO_2), and PO_2 was examined in nonpregnant and pregnant rats. After 90 minutes of decreased FiO_2, there was an insignificant increase in blood pH and a decrease in blood PCO_2 and PO_2 (Table 2). Between 3 and 5 hours of continuous exposure, there was a statistically significant increase in blood pH and a significant decrease in PO_2. Prolonging the hypoxemia for 3 days resulted in a significant decrease in blood PCO_2. There was a small increase in PO_2 after 3 days of reduced FiO_2 as compared with the acute study, such that PO_2 at 3 days was similar to the level in the room air. Control animals placed in room air in a similar chamber with a similar air flow rate showed no change in arterial blood gases (data not shown). In contrast, when exposed to 0.17 FiO_2, pregnant rats showed a small but significant decrease in PO_2, with no change in pH or PCO_2.

The effect of prolonged exposure to decreased FiO_2 on maternal/fetal glucose and lactate concentrations was examined in five rats. A prolonged decrease in FiO_2 had no effect on the maternal plasma glucose concentration (Table 3). However, there was a decrease in fetal glucose, resulting in a significant increase in the maternal-fetal glucose gradient. No significant change in the maternal or fetal lactate concentration was observed.

The plasma glucose concentration and glucose kinetics in controls and experimental groups are displayed in Table 4. As reported previously, plasma glucose was lower in pregnant rats compared with nonpregnant rats. However, the glucose Ra and contribution of GNG normalized for body weight were not significantly increased during pregnancy. A decrease in FiO_2 for 2 hours caused a significant increase in plasma glucose in nonpregnant animals. Although plasma glucose also increased in pregnant animals in response to acute hypoxemia, the increase was not statistically significant. The glucose Ra significantly increased during acute hypoxemia in nonpregnant animals; the increase was not significant in pregnant animals. An acute decrease in FiO_2 had no impact on the contribution of GNG to the glucose Ra in either pregnant or nonpregnant animals.

A chronic decrease in FiO_2 for 3 days resulted in a significant ($P < .05$) increase in plasma glucose in pregnant animals. There was no change in the glucose Ra or the contribution of GNG to glucose production.

2-Deoxyglucose uptake by skeletal muscle, liver, and kidney was not affected by decreased FiO_2 following 12 hours of fasting during pregnancy (Table 5). Hypoxemia did not affect

Table 1. Effect of Chronic Hypoxemia on Fetal Weight, Litter Size, and Placental Weight

Condition	Maternal Weight (g)	Litter Size (n)	Fetal Weight (g)	Placental Weight (g)
Room air (n = 9)	349.6 ± 9.7	13.5 ± 0.50	4.02 ± 0.04	0.64 ± 0.02
Hypoxemia (n = 12)	368.6 ± 13.1	14.3 ± 0.63	3.27 ± 0.06*	0.61 ± 0.03

NOTE. Mothers were placed in room air or 17% FiO_2 on day 17 of gestation; pups were delivered on day 20 of gestation. The reported fetal weight is an average of the mean for each litter (mean ± SEM).

*$P < .02$ *v* room air.

Table 2. Effect of Hypoxemia on Arterial Blood Gas and Oxygen Saturation

Group	No. of Animals	pH	PO_2 (torrs)	O_2 Saturation (%)	PCO_2 (torrs)	HCO_3 (mmol/L)
Nonpregnant						
Room air	7	7.41 ± 0.02	92 ± 4.8	97 ± 0.02	38.8 ± 1.65	24.5 ± 0.97
FiO_2 17%						
1.5 h	5	7.44 ± 0.03	75 ± 3.7*	95 ± 0.45*	36.0 ± 1.21	24.8 ± 1.67
3-5 h	4	7.48 ± 0.02*	73 ± 2.2†	95 ± 0.47*	34.5 ± 0.81	26.7 ± 1.68
3 d	6	7.47 ± 0.01*	86 ± 2.4	98 ± 0.41	30.0 ± 2.20†	21.8 ± 1.29
Pregnant						
Room air	4	7.40 ± 0.01	97 ± 0.5	98 ± 0.2	32.5 ± 1.6	24.1 ± 0.93
FiO_2 17%						
1 d	4	7.43 ± 0.01	71 ± 1.0*	93 ± 0.3*	25.3 ± 1.6	20.0 ± 1.19
2 d	4	7.40 ± 0.01	75 ± 0.7*	94 ± 0.3*	30.3 ± 2.9	20.2 ± 0.94
3 d	4	7.39 ± 0.02	81 ± 1.8*	95 ± 0.6*	27.9 ± 0.9	18.1 ± 1.08

NOTE. Results are the mean ± SEM. Nonpregnant and pregnant animals were placed in 17% FiO_2 for the indicated time. Blood samples were obtained from indwelling catheters placed in the carotid artery.

*$P < .03$ *v* room air.

†$P < .01$ *v* room air.

the placental uptake of 2-deoxyglucose (3.8 ± 0.2 *v* 3.7 ± 0.6 ng/mg · min).

The effect of a prolonged decrease in FiO_2 on GLUT1 and GLUT3 mRNA expression in the placenta was determined. Because GLUT1 mRNA is known to be highly expressed in the placenta,[27] Northern blots were performed under conditions where hybridization would occur under saturating levels of probe. Figure 1A shows the results of a Northern blot using 2 μg total RNA per lane. No change in GLUT1 mRNA content was observed in placentae obtained from rats exposed to 17% FiO_2. Figure 1B shows a Northern blot using 20 μg total RNA per lane, which was hybridized to the GLUT3 probe. No change in the relative content of GLUT3 was evident. The experiment was repeated on four sets of pregnant rats, and the effect of hypoxemia on both GLUT1 and GLUT3 mRNA was quantified (Table 6). The relative abundance of GLUT1 mRNA and GLUT3 mRNA was not changed as a result of exposure of the mothers to 17% FiO_2 for 3 days.

There was no change in the glycogen concentration in maternal and fetal liver as a result of prolonged hypoxemia. In addition, there was no significant change in the placental glycogen concentration (0.37 ± 0.02 mg/g tissue in control *v* 0.34 ± 0.05).

DISCUSSION

The significant finding of the present study is that even a mild decrease in FiO_2 (0.17) in the rat causes significant growth restriction in the fetus. Of interest, the decreased fetal growth was not associated with changes in placental weight or any measurable change in maternal glucose metabolism. Several different models have been used to induce intrauterine growth restriction.[1-5] The commonly used models of reduction in uterine or placental blood flow (by ligation of uterine artery or by placental emboli) result in reduced delivery of both oxygen and nutrients to the fetus.[3] In contrast, a decrease in FiO_2 primarily reduces the maternal/fetal oxygenation and leads to secondary effects on nutrient transport and metabolism.[7-9] Previous studies have used severe hypoxemia, an environmental O_2 concentration of 10% to 14%, to induce fetal growth restriction.[1] Such severe hypoxemia could cause changes in both maternal and fetal metabolism. In contrast, we have used a relatively milder decrease in FiO_2 that resulted in minimal change in the maternal blood PO_2, and even this condition resulted in significant fetal growth restriction. Although the observed change in blood PO_2 is considered to be of no physiological significance, this magnitude of hypoxemia is comparable to that observed at high altitude and associated with low birth weight in humans.[6]

Growth restriction was found consistently in our study of rat pregnancy, and has also been reported by our group in mice.[28] Of interest, a minimal decrease in maternal PO_2 and no change in hemoglobin oxygen saturation was found. A prolonged reduction in FiO_2 caused a low PCO_2 and a small increase in pH, probably as a result of hyperventilation in nonpregnant animals but not during pregnancy. Whether these changes resulted in increased oxygen (energy) consumption by the mother or by the fetus remains unknown. In this rat model, because of the inaccessibility of the fetus due to its size, we were not able to measure the effect of maternal hypoxemia on fetal oxygenation. However, since maternal oxygenation and hemoglobin saturation as measured by arterial blood gas analysis were minimally changed, we speculate that the fetal

Table 3. Maternal and Fetal Blood Glucose and Lactate Ratio

Condition	Glucose			Lactate		
	Mother (mmol/L)	Fetus (mmol/L)	Maternal/Fetal Ratio	Mother (mmol/L)	Fetus (mmol/L)	Maternal/Fetal Ratio
Room air (n = 4)	3.70 ± 0.42	2.49 ± 0.14	1.48 ± 0.11	1.80 ± 0.18	8.52 ± 1.28	0.24 ± 0.06
FiO_2 17% (n = 5)	4.23 ± 0.20	1.95 ± 0.23	2.26 ± 0.24*	1.81 ± 0.41	9.67 ± 0.24	0.19 ± 0.04

*$P < .05$ *v* room air.

Table 4. Effect of Hypoxemia on Glucose Kinetics in Nonpregnant and Pregnant Rats

Group	Weight (g)	Plasma Glucose (mmol/L)	Glucose Ra (μmol/100 g · min)	GNG	
				%	μmol/100 g · min
Nonpregnant					
Room air (n = 6)	275 ± 22	7.10 ± 0.30	5.91 ± 0.79	63 ± 9	3.85 ± 0.67
2 h FiO_2 17% (n = 6)		7.90 ± 0.55*	6.80 ± 1.04*	63 ± 8	4.37 ± 0.82
Pregnant					
Room air (n = 6)	381 ± 15†	3.70 ± 0.50†	6.30 ± 0.98	83 ± 11	5.20 ± 1.18
2 h FiO_2 17% (n = 6)		4.01 ± 0.56	7.54 ± 1.66	89 ± 10†	6.53 ± 1.99
3 d FiO_2 17% (n = 7)	371 ± 24†	4.90 ± 0.21‡	5.80 ± 0.56	80 ± 11	4.80 ± 0.27

NOTE. The response to acute hypoxemia was examined in nonpregnant (n = 6) and pregnant (n = 6) animals by placing them in a 17% FiO_2 hypoxemia chamber for 2 hours. Glucose kinetics were measured in separate group of pregnant animals (n = 7) following 3 days of hypoxemia.

*$P < .05$ *v* nonpregnant in room air.

†$P < .001$ *v* nonpregnant in room air.

‡$P < .05$ *v* pregnant in room air.

growth restriction was not related directly to changes in oxygen delivery, but was more likely related to changes in the delivery or uptake of nutrients and substrates or due to a primary change in fetal metabolism as a result of acid-base disturbance.

As reported previously, an acute decrease in FiO_2 caused an increase in blood glucose and glucose Ra in nonpregnant animals. Such a response was not evident in pregnant animals. In addition, a prolonged decrease of FiO_2 had no significant effect on the plasma glucose, glucose Ra, or contribution of GNG in the pregnant animal. As anticipated from the previous data, basal plasma glucose was lower and the glucose Ra and contribution of GNG to glucose Ra were slightly higher (not statistically significant) in pregnant versus nonpregnant animals.[14] It should be underscored that the estimation of GNG from the enrichment of ^2H on C-6 of glucose following $[^2H_2]O$ administration will underestimate the contribution of pyruvate to glucose, because of incomplete equilibrium between the methyl hydrogens of pyruvate and water; in addition, it does not measure the contribution of glycerol.[26] Therefore, if there was any change in lipolysis and GNG from glycerol, it would not be measured by this method.

A mild decrease in FiO_2 caused a small increase in maternal plasma glucose and a decrease in fetal plasma glucose such that the maternal-fetal plasma gradient for glucose was significantly increased. This may be the consequence of increased glycolysis or glucose utilization by the fetus. Lueder et al[1] also have shown increased utilization of glucose by selected fetal tissues in response to chronic hypoxemia and fetal growth restriction. However, in their study, intrauterine growth restriction (IUGR)

Table 5. Glucose Uptake (nmol/g wet weight tissue/min)

Condition	EP	GCR	GCW	Kidney	Liver
Room air (n = 6)	13.0 ± 1.3	13.4 ± 1.5	7.5 ± 1.0	3.2 ± 1.2	3.1 ± 0.8
FiO_2 17% (n = 5)	14.4 ± 1.5	16.3 ± 4.6	24.8 ± 4.9	11.4 ± 4.3	4.4 ± 1.7

NOTE. 2-Deoxyglucose uptake was measured in pregnant rats following 3 days of hypoxemia or exposure to room air during fasting (16 hours). Results are the mean ± SEM; none of the changes were significant.

Abbreviations: EP, epitrochlearis; GCR, gastrocnemius (red); GCW, gastrocnemius (white).

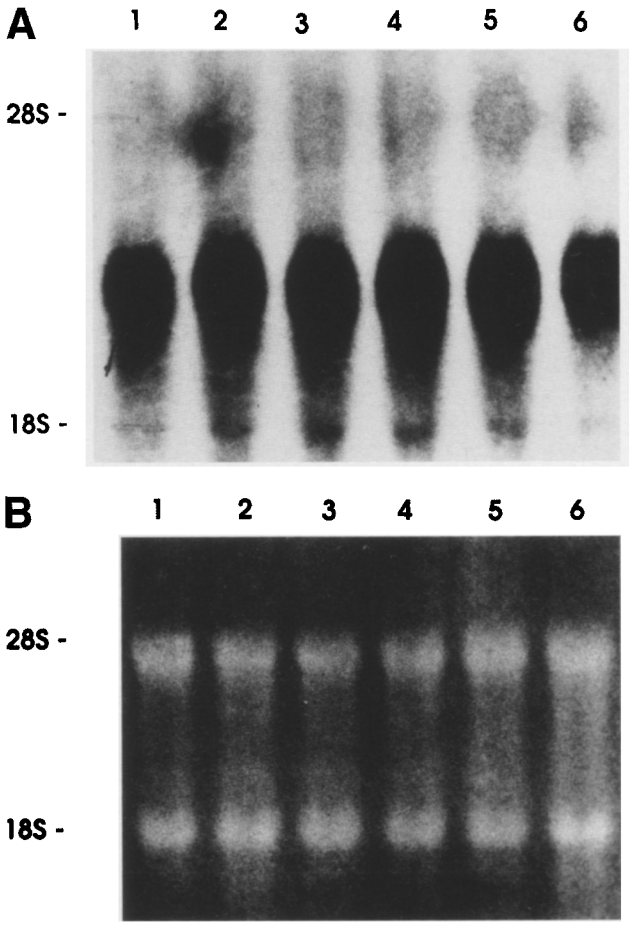

Fig 1. Effect of chronic hypoxemia on GLUT1 and GLUT3 mRNA expression in placenta. Mothers were placed in room air or 17% FiO_2 on day 17 of gestation. At delivery on day 20, placentae were removed and frozen in liquid N_2. Two (A) and 20 (B) μg total RNA were loaded per lane, and the resulting blots were probed with full-length rat GLUT1 and human GLUT3 cDNA probes, respectively. (A) GLUT1 mRNA: lanes 1, 3, and 5, placenta from a rat at room air; lanes 2, 4, and 6, placenta from a rat exposed to 17% FiO_2. Position of the 28S and 18S ribosomal band is shown. (B) GLUT3 mRNA: lanes correspond to lanes in A.

Table 6. Effect of Chronic Hypoxemia on the Relative Abundance of GLUT1 and GLUT3 mRNA in Placenta

GLUT mRNA	Control	17% FiO_2
GLUT1 (n = 9)	1.00 ± 0.03	0.89 ± 0.10
GLUT3 (n = 6)	1.00 ± 0.06	1.05 ± 0.17

NOTE. Mothers were placed in room air or 17% FiO_2 on day 17 of gestation. At the time of delivery on day 20, placentae were removed and frozen in liquid N_2. Twenty and 40 μg of total RNA were used in blots to be probed for GLUT1 and GLUT3 mRNA, respectively. Three placentae from rats at room air and 3 from hypoxemia-treated rats were analyzed on the same blot in each experiment. The intensity of GLUT mRNA bands was determined by laser densitometry. In each experiment, the relative abundance of GLUT mRNA in hypoxic rats was calculated as the ratio to the mean value derived from rats in room air. The experiment was repeated 4 times in triplicate for GLUT1 mRNA and 3 times in triplicate for GLUT3 mRNA analysis. Results are the mean ± SEM. None of the changes are statistically significant.

was induced by more severe hypoxemia (10% FiO_2) than in the present study (0.17 FiO_2). Although the fetal plasma glucose concentration was lower in animals exposed to lower FiO_2, the lactate concentration or maternal/fetal lactate ratio were not affected. This would be contrary to the argument of increased glucose utilization by the fetus,[1] or it could be due to a rapid clearance of lactate by the placenta. The relative abundance of GLUT1 mRNA encoding the predominant glucose transporter expressed in the placenta, and of GLUT3 mRNA, remained unchanged following exposure to 17% FiO_2. Hypoxemia has been reported to cause upregulation of the mRNAs (especially of GLUT1) in a variety of tissues and cell systems, including trophoblasts in culture.[29,30] The lack of change in the present study could reflect either the lack of any significant change in blood PO_2 induced by our perturbations, the absence of regulatory pathways in the tissue in vivo, or the extremely high level of expression of GLUT1 mRNA in the placenta under basal (room air) conditions. Since the expression of placental GLUT3, in particular,[31,32] appears to be regulated posttranscriptionally, it would be important to quantify the effect of hypoxemia on placental glucose transporter protein levels. These measurements were not performed and remain a limitation of the present study. However, the protein levels alone, without examining the transport function, will not address the mechanism of the decrease in fetal glucose.

Pregnancy in humans, as well as animals, is characterized as an insulin-resistant state.[15,16] Using the hyperinsulinemic clamp technique, a progressive increase in insulin resistance has been demonstrated with advancing gestation. Since hypoxemia has been shown to result in increased glucose uptake by the non–insulin-mediated pathway even in the insulin-resistant muscle,[10] we hypothesized that fetal growth restriction may be related to increased glucose uptake by the maternal muscle. However, no effect on 2-deoxyglucose uptake by either red or white muscle in pregnant animals was observed. There was also no effect on 2-deoxyglucose uptake by the placenta, liver, or kidney. Considering the large variance in the data, it is possible that there are changes masked by a type 2 error; however, the magnitude of such changes, even if significant, is not likely to explain the fetal growth restriction and changes in fetal blood glucose observed.

Thus, intrauterine fetal growth restriction induced by exposure to 17% FiO_2 is not related to alterations in maternal glucose production or skeletal muscle glucose uptake. Whether it is due to a change in maternal/fetal energy metabolism or to perturbations in protein metabolism as a result of acid-base disturbances remains to be examined.

ACKNOWLEDGMENT

The authors appreciate the secretarial assistance of Joyce Nolan. We thank Dr Dennis Super for assistance with the statistical analysis.

REFERENCES

1. Lueder FL, Kim S-B, Buroker CA, et al: Chronic maternal hypoxia retards fetal growth and increases glucose utilization of select fetal tissues in the rat. Metabolism 44:532-537, 1995

2. Tapanainen PJ, Bang P, Wilson K, et al: Maternal hypoxia as a model for intrauterine growth retardation: Effects on insulin-like growth factors and their binding proteins. Pediatr Res 36:152-158, 1994

3. Wigglesworth JS: Experimental growth retardation in the foetal rat. J Pathol Bacteriol 88:1-13, 1964

4. Lueder FL, Ogata ES: Uterine artery ligation in the maternal rat alters fetal tissue glucose utilization. Pediatr Res 28:464-468, 1990

5. Boyle DW, Lecklitner S, Liechty EA: Effect of prolonged uterine blood flow reduction on fetal growth in sheep. Am J Physiol 270:R246-R253, 1996

6. Unger C, Weiser JK, McCullough RE, et al: Low birth weight, and infant mortality in Colorado. JAMA 259:3427-3432, 1988

7. Challis JRG, Fraher L, Oosterhuis J, et al: Fetal and maternal endocrine responses to prolonged reductions in uterine blood flow in pregnant sheep. Am J Obstet Gynecol 160:926-932, 1989

8. Hooper SB, Coulter CL, Deayton JM, et al: Fetal endocrine responses to prolonged hypoxemia in sheep. Am J Physiol 259:R703-R708, 1990

9. Hooper SB: Fetal metabolic responses to hypoxia. Reprod Fertil Dev 7:527-538, 1995

10. Azevedo JL Jr, Carey JO, Pories WJ, et al: Hypoxia stimulates glucose transport in insulin-resistant human skeletal muscle. Diabetes 44:695-698, 1995

11. Cartee GD, Douen AG, Ramlal T, et al: Stimulation of glucose transport in skeletal muscle by hypoxia. J Appl Physiol 70:1593-1600, 1991

12. Zinker BA, Namdaran K, Wilson R, et al: Acute adaptation of carbohydrate metabolism to decreased arterial PO_2. Am J Physiol 266:E921-E929, 1994

13. Assel B, Rossi K, Kalhan S: Glucose metabolism during fasting through human pregnancy: Comparison of tracer method with respiratory calorimetry. Am J Physiol 265:E351-E356, 1993

14. Ogata ES, Metzger BE, Freinkel N: Carbohydrate metabolism in pregnancy SVI: Longitudinal estimates of the effects of pregnancy on D-[6-^{3}H]glucose and D-[6-^{14}C]glucose turnover during fasting in the rat. Metabolism 30:487-492, 1981

15. Leturque A, Burnol AF, Ferré P, et al: Pregnancy-induced insulin resistance in the rat: Assessment by glucose clamp technique. Am J Physiol 246:E25-E31, 1984

16. Catalano PM, Tyzbir ED, Roman NM, et al: Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. Am J Obstet Gynecol 165:1667-1672, 1991

17. Holness MJ, Changani KK, Sugden MC: Progressive suppres-

sion of muscle glucose utilization during pregnancy. Biochem J 280:549-552, 1991

18. Kalhan SC, Trivedi R, Singh S, et al: A micromethod for the measurement of deuterium bound to carbon 6 of glucose to quantify gluconeogenesis in vivo. J Mass Spectrom 30:1588-1592, 1995

19. Kalhan SC, Gilfillan CA, Tserng K-Y, et al: Glucose-alanine relation in normal human pregnancy. Metabolism 37:152-158, 1988

20. Sokoloff L, Reivich M, Kennedy C, et al: The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897-916, 1977

21. Ferré P, Leturque A, Burnol A-F, et al: A method to quantify glucose utilization in vivo in skeletal muscle and white adipose tissue of the anesthetized rat. Biochem J 228:103-110, 1985

22. Lo S, Russell JC, Taylor AW: Determination of glycogen in small tissue samples. J Appl Physiol 28:234-236, 1970

23. Chirgwin JM, Przbyla AE, MacDonald RJ, et al: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294-5299, 1979

24. Birnbaum MJ, Haspel HC, Rosen OM: Cloning and characterization of cDNA encoding the rat brain glucose-transporter protein. Proc Natl Acad Sci USA 83:5784-5788, 1986

25. Kayano T, Fukumoto H, Eddy RL, et al: Evidence for a family of human glucose transporter like proteins: Sequence and gene localization of a protein expressed in total skeletal muscle and other tissues. J Biol Chem 263:15245-15248, 1988

26. Landau BR, Wahren J, Chandramouli V, et al: Use of $^{2}H_2O$ for estimating rates of gluconeogenesis: Application to the fasted state. J Clin Invest 95:172-178, 1995

27. Mueckler M: Family of glucose-transporter genes: Implication for glucose homeostasis and diabetes. Diabetes 39:6-11, 1990

28. Riley T, Lechner P, Hanson R, et al: Intrauterine hypoxia modulates PEPCK gene transcription via a nuclear factor-1 (NF-1) binding site in newborn transgenic mice. Pediatr Res 37:68A, 1995 (abstr)

29. Behrooz A, Ismail-Beigi F: Dual control of GLUT1 glucose transporter gene expression by hypoxia and by inhibition of oxidative phosphorylation. J Biol Chem 272:5555-5562, 1997

30. Esterman A, Greco MA, Mitani Y, et al: The effect of hypoxia on human trophoblast in culture: Morphology and glucose transport and metabolism. Placenta 18:129-136, 1997

31. Jansson T, Wennergren M, Illsley NP: Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation. J Clin Endocrinol Metab 77:1554-1562, 1993

32. Zhou J, Bondy CA: Placental glucose transporter gene expression and metabolism in the rat. J Clin Invest 91:845-852, 1993