Abstract. Hyperglycemia has been shown to induce arterial hypoxemia in the chronically catheterized fetal sheep. To investigate the mechanism behind this glucose-induced hypoxemia, eight pregnant ewes and their fetuses were studied. Fetal glucose infusion (11.9±0.6 mg glucose/kg per min) was associated with a doubling of the fetal plasma glucose concentration with concomitant elevation of the umbilical vein-distal arterial O2 content difference by 24 h of infusion (P < 0.01). Calculated fetal O2 consumption increased from 8.1±0.4 ml/kg per min in the control period to a maximum value of 10.6±0.3 ml/kg per min by third infusion day (P < 0.01), which is an increase of ~30%. The degree of stimulation of fetal O2 consumption was related to the degree of fetal hyperglycemia but not to the degree of fetal hyperinsulinemia. The increase in fetal O2 consumption was accompanied by a significant increase in fetal O2 extraction with no change in either fetal O2 delivery or fetal blood O2 affinity. In addition, fetal hypercapnea with a mild fetal respiratory acidosis was induced by fetal hyperglycemia. The increase in fetal arterial Pco2 was linearly related (P < 0.001) to the magnitude of increase in fetal O2 consumption. These studies suggest that chronic fetal hyperglycemia induces a state of accelerated fetal oxidative metabolism and may be important in explaining the etiology behind certain unusual findings in human infants of diabetic mothers.

Effects of Chronic Fetal Hyperglycemia upon Oxygen Consumption in the Ovine Uterus and Conceptus

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Introduction

The classical findings of macrosomia and postnatal hypoglycemia in newborn infants born to women with diabetes mellitus are thought to be due to the excesses in substrate uptake and insulin secretion of the fetus (1, 2). However, certain clinical and pathologic findings suggest that other disorders seen in these infants, such as polycythemia, hyperbilirubinemia, and late fetal demise (2, 3), may be related to occult in utero hypoxemia. Using a chronically catheterized fetal lamb preparation, it has recently been demonstrated that long-term fetal glucose infusion induces a relative fetal arterial hypoxemia (4), and that the degree of this hypoxemia is related to the magnitude of the hyperglycemia. It has been unclear whether or not this glucose-induced hypoxemia is secondary to abnormalities in uterus and/or fetal oxidative metabolism or is simply due to altered placental gas transport. The purpose of the current study was to investigate each of these possible etiologies using the fetal lamb model with chronic defined fetal glucose infusions.

Methods

Surgical preparation. Eight pregnant ewes were studied between 120 and 140-d gestation (term gestation in the sheep is ~150 d). Five had singleton pregnancies. Preoperatively, all ewes were anesthetized with intravenous sodium pentobarbital and intrathecal pancocaine. At hysterotomy, polyvinyl chloride catheters were implanted into the fetal distal aorta (post ductal) and inferior vena cava using a pedral approach. Of the three gestations, only one fetal twin from each was catheterized. Umbilical venous catheters were inserted directly into the common umbilical vein (5) or into a cotyledonary branch and advanced proximally until the catheter tip was in the common umbilical vein. Maternal catheters were placed in the distal aorta via a femoral insertion and into the common uterine vein of the pregnant uterine horn (5). An amniotic space catheter was inserted for pressure monitoring and instillation of antibiotics. All catheters were then tunnelled subcutaneously to a pouch on the ewe’s flank. Catheter tip placement was confirmed at autopsy in the majority of preparations and was adequate in all. A 4–5-d postoperative recovery period was allowed prior to experimentation. Postoperative management was performed as previously described (6). All animals were housed in individual carts for the duration of the experimental protocol and allowed food and water ad libitum.

Experimental protocol. During a control period of 2 d, fetal and maternal arterial blood samples were obtained for plasma concentrations

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of glucose and insulin as well as arterial blood gases (pH, Pco₂, and Po₂), hematocrit, and serum solids. Twice daily, samples were drawn simultaneously from the uterine vein and maternal aorta, and the umbilical vein and fetal distal aorta to assess venoarterial blood oxygen consumption (Qo₂) across the uterine and umbilical circulations. Uterine and umbilical blood flows were also measured twice daily using the antipyrine steady state diffusion technique (7). Uterine and fetal O₂ consumptions (Qo₂) were calculated from simultaneously measured Po₂, Po₂ measurements. Uteroplacental circulations were measured using a Corning 178 Blood Gas Analyzer (Corning Medical Products, Medfield, MA). Po₂ measurements were performed at 37°C and utilized a tonometric method (IL237 tonometer; Instrumentation Laboratory, Inc., Lexington, MA). Fetal erythrocyte 2,3-DPG concentrations were measured using the enzymatic method of Keitt (11). Plasma insulin concentrations were determined using a modified insulin radioimmunoassay (6) with ovine insulin standards (kindly supplied by Dr. M. A. Root, Eli Lilly Laboratories, Indianapolis, IN). Statistical methods. All results are expressed as mean±1 SEM. Statistical significance was assessed using the paired Student’s t test and two-way analyses of variance. Linear regression analyses were performed using the least squares method.

Results

Nine separate glucose infusions were performed in the fetal lambs. Glucose infusion rates were 11.9±0.6 mg glucose/kg per min when corrected for known fetal delivery weights. As shown in Fig. 1, fetal glucose infusion resulted in a sustained increase in fetal plasma arterial glucose concentration from 26.0±1.9 to 58.8±3.7 mg/dl during the 72-h infusion period (P < 0.001). Umbilical venous O₂ content fell slightly from 10.7±0.6 to 9.2±0.9 mg/dl, but the change was not statistically significant. In contrast, fetal distal aortic O₂ content fell from the basal value of 7.3±0.5 to 5.7±0.4 ml/dl by 24 h of infusion (P < 0.01) and reached a nadir of 4.9±0.7 ml/dl (2.2±0.4 ml/dl below base line) by the third day of glucose infusion (P < 0.01). Consequently, fetal hyperglycemia induced a rise in the umbilical venoarterial O₂ content difference by 24 h of infusion (from 3.5±0.2 to 4.3±0.2 ml/dl, P < 0.01). The elevation in umbilical (venoarterial) O₂ content was maintained throughout the experimental period (Fig. 1).

Glucose infusion produced no alteration in umbilical blood flow from the basal value of 232±7 ml/kg per min (828±25 ml/min). Control fetal Qo₂, as calculated from the Fick equation was 8.1±0.4 ml/kg per min. During fetal hyperglycemia, fetal Qo₂ rose to 9.4±0.3 ml/kg per min by day 1 of infusion and to a maximum value of 10.6±0.3 ml/kg per min by day 3 (ΔQo₂ = +2.6±1.6 ml/kg per min, P < 0.01). When pooled individual values for the fetal Qo₂ were compared with their paired plasma arterial glucose concentrations (Fig. 2), a significant relationship was apparent between the degree of fetal hyperglycemia and stimulation of fetal oxygen consumption.

The calculations of fetal O₂ delivery and extraction during fetal hyperglycemia in the experimental animals are presented in Table 1. Although a fall in fetal O₂ delivery was noted at 24 h of glucose infusion, this change amounted to a < 7% fall from base line and was statistically insignificant. In contrast, fetal O₂ consumption increased by 25% from 26.0±1.9 to 31.6±1.9 mg/min at the end of the infusion period (P < 0.01). The increased consumption was maintained throughout the experimental period (Fig. 1).
Table 1. Comparison between Fetal Oxygen Delivery and Oxygen Extraction before and during Fetal Glucose Infusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal O₂ delivery (ml/kg per min)</td>
<td>24.8±1.6</td>
<td>22.6±1.7</td>
<td>21.4±1.7</td>
<td>21.7±1.8</td>
</tr>
<tr>
<td>Fetal O₂ extraction (%)</td>
<td>33.8±2.5</td>
<td>42.7±3.0**</td>
<td>46.4±2.6**</td>
<td>51.4±4.5**</td>
</tr>
</tbody>
</table>

** P < 0.01, difference from control.

In contrast, fetal oxygen consumption in the four control fetuses increased from 23.8±2.2 to 24.4±2.8 ml/min (8.5±0.8 to 8.7±0.9 ml/kg per min) over a 5-d period similar to that between initiation and completion of each infusion experiment. This elevation amounted to a 3% increase in Qo₂ due to advancing gestational age.

In seven of the glucose infusion experiments, simultaneous sampling from the uterine as well as the umbilical circulation was performed through the majority of the experimental period. Although a trend was evident, no changes in uterine venous or arterial O₂ content nor in uterine blood flow were noted from baseline. As shown in Fig. 3, although the calculated uterine Qo₂ rose from 50.4±4.9 ml/min to a maximum value of 57.8±7.5 ml/min by day 3 of infusion, this change was not statistically significant. Umbilical Qo₂ (not corrected for fetal weight) rose

![Figure 1. Fetal plasma glucose concentrations, umbilical blood flow (umb. bld. flow), and fetal O₂ contents and consumptions (Qo₂) before and during nine glucose infusions in eight fetal lambs. * P < 0.05; ** P < 0.01.](image1)

![Figure 2. Relationship between fetal plasma glucose concentrations and Qo₂ during control and experimental periods. Each symbol represents a different animal preparation. (n = 59, r = 0.46, y = 0.05 x + 7.17, P < 0.001).](image2)

![Figure 3. Changes in fetal plasma glucose concentration and Qo₂ consumption of uterus, fetus (umbilical), and utero placental mass occurring during fetal glucose infusion. (*P < 0.05).](image3)
to a similar degree as the uterine QO2 (from 29.4±3 to 38.5±4.1 ml/min by day 3 of glucose infusion, *P < 0.05*), and thus, no change in the calculated uteroplacental QO2 was noted from the basal value of 21.6±2.6 ml/min.

Fetal hyperglycemia induced no alterations in fetal heart rate nor in fetal or maternal blood pressures (Table II). In addition, no changes were seen in uterine or umbilical vascular resistances. No changes in fetal hematocrit nor in total serum solids were detected (Table III). The 50% hemoglobin-O2 saturation point (P50) of fetal distal arterial blood was determined during five of the infusions (Table III). Hyperglycemia did not change the basal P50 of 15.8±0.6 torr. In four experiments, fetal erythrocyte 2,3-DPG concentrations were determined as well and were unaltered by chronic hyperglycemia.

Fetal arterial blood gases assessed daily showed significant changes during hyperglycemia (Table III). As expected, arterial PO2 fell significantly (*P < 0.01*) by 48 h of glucose infusion. Arterial PCO2 rose significantly by 24 h of glucose infusion and remained elevated throughout the experimental period, although this elevation was significant on days 1 and 2 only. This hypercapnea was accompanied by a significant fall in pH on day 1 with a subsequent increase in calculated buffer base noted on day 2. The sum total of events indicated an early respiratory acidosis induced by hyperglycemia, with later metabolic compensation and an ongoing arterial hypoxemia. No changes in maternal pH or PO2 were noted during the experimental period from control. Maternal arterial PCO2 during the control period was 32.6±1.5 torr and did not change significantly during days 1 and 2 of fetal glucose infusion. A small but statistically significant decrease in PCO2 to 30.3±1.2 torr (*P < 0.02*) was noted on day 3 of infusion. When the fetal PCO2 data were related to changes in fetal QO2, no relationship was apparent because of interanimal variability in both parameters. However, when fetal QO2's were expressed as percent from control and fetal PCO2's expressed as fetomaternal ratios to decrease this variability, a relationship is apparent (Fig. 4). One unusual animal (open circles) responded to hyperglycemia with a large increase in QO2 but little change in fetal arterial PCO2. It is unclear whether or not the observed changes in this animal were a result of experimental error or a true lack of response. Without the addition of this animal to the data, a highly significant relationship (*P < 0.001*) is apparent between fetal QO2 and fetal PCO2.

Fetal hyperglycemia induced significant elevations in fetal plasma arterial insulin concentration from a control value of 12.5±1.8 μU/ml to a peak concentration of 28.9±8.3 μU/ml by day 2 of infusion (*P < 0.01*). Overall insulin response, however, was quite variable between animals. One fetus (+) appeared not to respond to glucose infusion with any rise in insulin concentration. Use of weighted means or averaging data from each animal neither altered the regression equation nor the correlation

**Table II. Vascular Parameters in Uterine and Umbilical Circulations before and during Fetal Hyperglycemia**

<table>
<thead>
<tr>
<th>Blood flow (F) (ml/kg/min)</th>
<th>Fetal heart rate</th>
<th>A-V Pressure (P) difference (torr)</th>
<th>Vascular resistance (R) (torr/ml/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>395±36 (7)*</td>
<td>186±7 (8)</td>
<td>69±3 (8)</td>
</tr>
<tr>
<td>Day 1</td>
<td>394±34 (7)</td>
<td>203±11 (8)</td>
<td>70±5 (7)</td>
</tr>
<tr>
<td>Day 2</td>
<td>396±37 (7)</td>
<td>197±10 (8)</td>
<td>67±4 (7)</td>
</tr>
<tr>
<td>Day 3</td>
<td>416±67 (5)</td>
<td>197±12 (7)</td>
<td>71±4 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table III. Comparison of Changes in Fetal Arterial Blood Gases and Hemoglobin-oxygen Affinity before and during Fetal Glucose Infusion**

<table>
<thead>
<tr>
<th>Fetal Arterial Blood Values</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33.9±0.9 (9)</td>
</tr>
<tr>
<td>pH</td>
<td>7.39±0.01</td>
</tr>
<tr>
<td>PCO2 (torr)</td>
<td>44.2±2.1</td>
</tr>
<tr>
<td>PO2 (torr)</td>
<td>17.1±0.9</td>
</tr>
<tr>
<td>Base excess (meq/liter)</td>
<td>+1.6±0.9</td>
</tr>
<tr>
<td>Total serum solids (g/dl)</td>
<td>4.1±0.1</td>
</tr>
<tr>
<td>Arterial P50 (torr)</td>
<td>15.8±0.6</td>
</tr>
<tr>
<td>2,3-DPG (mol/ml erythrocyte)</td>
<td>1.49±0.20</td>
</tr>
</tbody>
</table>

Difference from control: *, *P < 0.05*; †, *P < 0.01*.
Figure 4. Relationship between $Q_O_2$ expressed as percent of control and fetal/maternal arterial $PCO_2$ ratios. One animal (o) has been deleted from regression calculations. ($n = 24$, $y = 0.01 x + 0.6$, $r = 0.69$, $P < 0.001$).

Discussion

Previous work using the chronically catheterized fetal lamb model has demonstrated that chronic fetal hyperglycemia is associated with significant distal arterial hypoxemia and a mild arterial hypercapnea (4). With pronounced hyperglycemia, progressive hypoxia, metabolic acidosis, and fetal demise were evident. Although a linear relationship was apparent between the degree of hyperglycemia and the relative fall in fetal arterial $O_2$ content, the mechanism behind the fetal hypoxemia was unclear. Major hypotheses to explain these alterations included either stimulation of fetal and/or uteroplacental $O_2$ consumption or depression of transplacental $O_2$ transport due either to decreased $O_2$ diffusion or altered fetal hemoglobin-$O_2$ affinity.

The present data may be interpreted to indicate that the development of hyperglycemia in the fetal lamb induces an acceleration of the fetal metabolic rate and that the increase in fetal $O_2$ consumption is almost wholly responsible for the observed fall in preplacental arterial $O_2$ content. Although hyperglycemia has been shown to alter hemoglobin-$O_2$ affinity in diabetic states (12), no changes in fetal hemoglobin-$O_2$ affinity or fetal erythrocyte 2,3-DPG concentrations were observed. In addition, no changes in monitored vascular parameters were noted to account for the above mentioned changes in fetal $O_2$ content.

In further studies using this model (13), concomitant increases in glucose and lactate uptake have been demonstrated during fetal hyperglycemia. It is of particular interest that significant increases in fetal glucose entry rate as well as fetal $O_2$ consumption occurred. Such accelerated oxidative metabolism would be expected to be accompanied by increased fetal $CO_2$ excretion. The small but significant elevation in preplacental arterial $PCO_2$ is suggestive evidence for accelerated $CO_2$ diffusion since it is unlikely that transplacental $CO_2$ diffusion would be
significantly altered in the face of normal \( O_2 \) diffusion and stable umbilical and uterine blood flows (14, 15). From the theoretical model of transplacental \( CO_2 \) diffusion derived by Hill et al. (15), the observed rise of 3.8 to 4.4 torr in fetal arterial \( PCO_2 \) would be associated with a 20–30% increase in femotomal \( CO_2 \) transfer. The observed change in fetal arterial \( PCO_2 \) could not be accounted for by changes in maternal \( CO_2 \) tension, which were stable to decreased during the experimental period.

Some precedent exists to suggest that the fetal metabolic rate may be altered under experimental conditions. Lorijn and Longo (16, 17) have demonstrated significant stimulation of \( O_2 \) consumption in fetal lambs using direct fetal infusions of nor-epinephrine or triiodothyronine. In addition, other recent work (18, 19) suggests that acceleration of fetal oxidative metabolism may also occur during fetal insulin infusion. The increase was effected by widening of the umbilical venastral \( O_2 \) content difference without change in umbilical blood flow although significant increases in cardiac output occurred. In the insulin infusion studies, pharmacologic doses of insulin were used and fetal insulin concentrations were generally 2–20-fold above the range seen with the endogenous hyperinsulinemia observed in the present series of experiments. Since in the fetal state, alterations in cardiac output relate more to change in heart rate than stroke volume (20), the absence of significant fetal tachycardia in the chronically hyperglycemic fetal lamb suggests that little increases in cardiac output of the type seen with exogenous insulin infusion occurred. This finding, in addition to the lack of a significant relationship between fetal plasma insulin concentration and either the degree of arterial hypoxemia (4) or the rise in fetal \( O_2 \) consumption would suggest that the glucose-induced stimulation of fetal \( O_2 \) consumption may not be mediated by insulin. However, since the degree of fetal hyperinsulinemia produced was variable, a significant relationship between endogenous hyperinsulinemia and acceleration of fetal \( O_2 \) consumption may have been obscured.

Stimulation of fetal \( O_2 \) extraction as seen in the current work has been demonstrated in a variety of other studies dealing with nonhormonal alterations in fetal oxygenation, particularly as a response to states of altered \( O_2 \) availability. Increases in umbilical \( O_2 \) extraction have been observed as responses to umbilical vascular ischemia (21), fetor hemorrhage (22), maternal \( O_2 \) deprivation (23), or decreases in uterine blood flow (24) without concomitant alterations in umbilical blood flows unless hypoxemia was severe. Since neither blood flow nor maternal oxygenation were altered during fetal hyperglycemia, the exact stimulus for the increase in fetal oxygen extraction remains obscure.

At present, little information is available regarding the ability of excesses in substrate delivery to directly alter fetal \( O_2 \) consumption. However, significant information does exist in in vivo experiments done in adults to indicate that the observed fetal response is not unusual. In the adult human, starvation and refeeding are associated with a fall and subsequent rise in \( O_2 \) consumption (25). In addition, ingestion of a carbohydrate meal in fasting adults resulted in increases in both \( O_2 \) consumption and \( CO_2 \) excretion (26). Several other studies in adult humans supported with parenteral nutrition have tended to confirm the observation that substrate excess induces acceleration of metabolic rate. In studies utilizing parenteral infusions of glucose and amino acids with or without supplemental fat, significant enhancement of alveolar ventilation, \( O_2 \) consumption, and \( CO_2 \) excretion were noted when compared with control (27–29). In one case, these resultant increases were so great that they were detrimental to the patient under study (30).

No data are available at present to indicate the metabolic goal of increased fetal metabolism found in the present study. Using data derived by Battaglia et al. (31), the observed increase in fetal lamb \( O_2 \) consumption would amount to an increase of \( \sim 15 \) kcal/kg per d in caloric production. Even a doubling of the short-term fetal growth rate would not be expected to increase caloric expenditure beyond \( \sim 5 \) kcal/kg per d. In this regard, it is of interest to note that Rurak et al. (32) recently observed that normal fetal respiratory movements can account for up to 17% of the fetal \( O_2 \) consumption rate. Several authors (33, 34) have documented stimulation of fetal breathing by hyperglycemia in fetal lambs and humans, and thus, giving rise to the possibility that stimulation of muscular work may be in part responsible for the observed increases in fetal \( QO_2 \). Alternatively, it has been hypothesized (33) that these increases in fetal breathing movements are due to accelerated glucose-induced fetal \( CO_2 \) production with consequent respiratory center stimulation. Other possible mechanisms for a glucose-induced increase in fetal oxidative metabolism include excessive heat production (35), acceleration of fetal growth, and stimulation of futile cycling within some fetal tissues (36).

No direct information is available from human fetuses or infants of diabetic mothers (IDM's) to support the hypothesis that fetal hyperglycemia induces changes similar to those seen in fetal lambs. MacKay (37) noted a marked umbilical arterial hypoxemia in a small number of IDM's at delivery in the only study available in which \( O_2 \) content was measured in umbilical vessels. Naeye (38) noted that postmortem hepatic specimens of IDM's contained increases in extramedullary hematopoietic elements and suggested that this finding might be explained on the basis of an erythropoietic response to chronic fetal hypoxemia. Excesses in cord blood erythropoietin have been found in a number of IDM's (39). Since several investigators have demonstrated stimulation of fetal erythropoietin secretion induced by fetal hypoxemia (40, 41), particularly during fetal hyperglycemia, it is tempting to speculate that a number of clinical features found in human IDM's (late fetal demise, neonatal polycythemia, and neonatal hyperbilirubinemia) may be the result of hyperglycemia-induced increases in fetal \( QO_2 \) and relative fetal arterial hypoxemia.

In summary, chronic glucose infusion in the fetal lamb induced a significant increase in fetal \( O_2 \) consumption within 24 h of the onset of hyperglycemia. The increase in \( O_2 \) consumption was linearly related to the degree of elevation in fetal plasma
glucose concentration. The increase in O2 consumption was due to increased fetal O2 extraction and not due to alterations in fetal blood O2 affinity or in placental O2 transport. No fetus became hypoxic as judged by the absence of significant metabolic acidosis. However, significant hypercapnea and respiratory acidosis were noted. The findings suggest that defined fetal glucose infusions induced stimulation of fetal oxidative metabolism but had little effect upon placental O2 consumption.

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