Hypocalcemia and Pregnancy-Induced Hypertension Produced by Low-Calcium Diet

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Abstract Recent studies from our laboratory in fasting pregnant ewes with twin gestation have implicated low serum calcium concentration in the etiology of hypertension in pregnancy. We hypothesized that the reduction in serum calcium concentration produced by feeding of a calcium-deficient diet in twin gestation would lead to a significant increase in maternal arterial blood pressure, vascular resistance, and protein in the urine and decreased uterine blood flow. Twenty-five instrumented ewes were used in the present study. After surgery a calcium-deficient diet and deionized water (calcium ion free) were provided ad libitum to 19 animals. Blood pressure, cardiac output, heart rate, and uterine blood flow were monitored every other day. Six control animals were provided with standard Rumilab diet and tap water (group 1). Animals on a low-calcium diet (group 2) were subdivided according to the blood ionized calcium response to low dietary calcium intake. Nonhypocalcemic animals were assigned to group 2a (n=10), and hypocalcemic animals (calcium concentration below two standard deviations from the control group) were assigned to group 2b (n=9). In group 2b calcium concentration decreased from 1.03±0.04 mmol/L on day 110 of gestation to 0.77±0.03 mmol/L by day 125 of gestation. Arterial blood pressure increased significantly from 74±2 to 91±2 mm Hg and uterine blood flow decreased from 950±53 to 579±48 mL/min. Urinary protein increased from 1.7±0.3 to 10.5±1.2 g/L. Despite the fact that all animals in group 2 had the same low dietary calcium intake, group 2a did not develop hypocalcemia (by definition) or an increase in arterial blood pressure. The control group (n=6) showed no significant changes in the parameters studied. From these data we suggest that calcium plays a significant role in regulating systemic arterial blood pressure and uteroplacental blood flow in twin pregnant ewes. (Hypertension. 1994;23[part 1]:695-702.)

Key Words • calcium • diet • hypocalcemia • twins • hypertension, pregnancy-induced

Several studies in animals and humans link low serum ionized calcium (iCa) concentration with the development of pregnancy-induced hypertension (PIH).1,2 Furthermore, during pregnancy calcium intake may be inversely correlated with blood pressure (BP) in humans and animals,3-6 and dietary calcium supplementation may reduce diastolic blood pressure in humans.7 In human epidemiologic studies, dietary calcium intake appears to be an important variable in determining the incidence of PIH.8-10

In humans PIH is a hypertensive disorder peculiar to pregnancy that usually develops between the 30th week of pregnancy and the end of the first week postpartum. If associated with albuminuria, edema, or both, the disorder is termed preeclampsia. The etiology of PIH is still unknown; it develops in 10% to 15% of pregnant women and characteristically occurs in young primigravidas,11,12 women with twin gestation,13 and women with preexisting hypertension or vascular disease.14

In recent studies from our laboratory in pregnant ewes with twin gestation, we have demonstrated that fasting results in maternal hypocalcemia in half the animals studied. These animals developed significant increases in arterial BP and a significant decrease in uteroplacental blood flow (UBF).2 The fetus requires a considerable amount of calcium for its skeletal development, and fetal calcium requirements increase sharply at approximately the 30th week in the human fetus. By term, the fetus has accumulated as much as 28 g calcium, 80% of this during the third trimester.15-17 Theoretically, calcium imbalance may occur in twin pregnancy where increased calcium demands and limited dietary calcium intake situations coexist. We designed the present study to investigate the cardiovascular effects of a calcium-deficient diet in twin pregnant ewes and to evaluate the effects of these changes on parameters of fetal well-being such as BP, heart rate, blood iCa concentration, and oxygenation.

Methods

Animal Preparation

Twenty-five normal twin pregnant ewes (determined by ultrasound examination) of mixed breed were obtained from Morris & Co. Thirty minutes before surgery animals (88 to 92 days of gestation and weighing between 45 and 55 kg) were sedated with diazepam (10 mg IV; Hoffmann-La Roche Inc) and then anesthetized with sodium pentobarbital (15 mg/kg IV; Steris Laboratories). When necessary, additional sodium pentobarbital was administered to maintain anesthesia. Animals were instrumented with a Doppler flow probe (Transonic Systems) on the pulmonary artery for monitoring of cardiac output. On day 105±2 of gestation with animals under spinal anesthesia (15 mg of 1% tetracaine hydrochloride, Winthrop Pharmaceutical), all 25 ewes were instrumented with polyvinyl catheters in the maternal and fetal femoral arteries and veins. Catheters were advanced to the level of the distal aorta and inferior vena cava, respectively. Electromagnetic flow probes (Dienco) of appropriate size were placed on the left and right middle uterine artery through a 15-cm sterile lower abdominal incision. Ultrasound examination revealed twin pregnancy.
incision for subsequent monitoring of blood flow to the uterus. Flow probe cables and catheters were exteriorized through the midline incision, placed subcutaneously to the ewe's left flank, placed in a cloth pouch, and secured to the ewe's side.

Antibiotics (6 mL IM) (Combiotics, GC Hanford Manufacturing Co) were administered on the day of and three days after surgery. All animals were housed in individual portable stainless steel cages. At the time of arrival, 6 animals chosen at random were provided with standard laboratory diet (Rumilab, Ralston Purina) consisting of 0.71% calcium and tap water ad libitum and were used as controls. Nineteen others were provided with a calcium-deficient diet consisting of 0.21% calcium (Rumilab chow 5508-2) and deionized water (calcium-free) ad libitum. All animals were allowed an adequate recovery period before physiological recordings were made and were studied 110±2 days of gestation to term. Animals were not exposed to any experiments preceding the study. Maternal catheters were flushed daily with 1000 USP units/mL heparin sodium (Elkins-Sinn Inc) and fetal catheters with 500 USP units/mL heparin sodium for maintenance of patency. All surgical and experimental procedures were performed in accordance with the Institutional Animal Care and Use Committee guidelines of the University of Cincinnati.

Maternal and Fetal Measurements

Maternal cardiac output was measured by use of a Doppler flow probe (Transonic Systems Inc), and maternal and fetal systemic arterial BPs were measured via the appropriate catheters with a model MP-15 BP transducer (Micron Instruments) anchored at the level of the sternum of the mother and the fetal heart, respectively. Fetal BPs were corrected for amniotic fluid pressure. Heart rate, blood glucose concentration, and hematocrit were determined with a cardiofetocentimeter (SensorMedics) triggered by an arterial pressure pulse. Arterial UBF was measured by use of a square-wave electromagnetic flowmeter (model RF 1000, Dienco). Electromagnetic flow probes (Dienco) were calibrated with saline and were linear over the flow measured. BP, heart rate, and blood flows were recorded continuously for 60 minutes on a pen writing dynograph physiological recorder (Dynograph R612, SensorMedics).

Maternal and fetal arterial blood samples for blood iCa were collected anaerobically into nonheparinized syringes and immediately determined by use of an ionized calcium analyzer (model ICA1, Radiometer). Blood iCa was adjusted to pH 7.4. Samples for maternal and fetal arterial blood gas values (pH, PaO₂, and PaCO₂) were collected anaerobically in heparinized syringes and immediately determined with a blood gas analyzer (model BMS3 MK2 Micro System, Radiometer) operating at 39°C (ewes' body temperature). Oxygen content was determined with a model LEX-O2, CON TL oxygen content analyzer (Cavitron). Blood glucose concentrations were determined with a glucose analyzer (model 27, Yellow Springs Instrument Co). Urine samples were collected every other day (by clean catch) for determination of maternal urinary protein concentrations with the use of a protein assay reagent (BCA protein assay reagent, Pierce).

Experimental Protocol

We used 25 normotensive ewes with twin gestation (90±2 days of gestation) to study the effects of low dietary calcium intake during pregnancy. BP, heart rate, cardiac output, and UBF of the mother and BP and heart rate of the fetus were measured once every other day between 9 and 11 AM for 60 minutes between days 110 and 135 of gestation. After thoracotomy, tap water and regular Rumilab chow were provided ad libitum between 9 AM and 5 PM to 6 animals that were used as controls. Deionized water (calcium free) and a calcium-deficient diet were provided ad libitum between 9 AM and 5 PM to 19 animals for the duration of the pregnancy. Blood samples were taken once every other day in the morning (9 to 11 AM) for determination of maternal and fetal glucose and lactate, PaO₂, PaCO₂, O₂ content, pH, hematocrit, and iCa. Maternal urine samples (100 to 200 mL) were collected every other day in the morning (by clean catch) for determination of urinary protein.

Histopathology of the Kidney

Kidney tissue specimens obtained from animals at the time of death were collected and fixed in 10% neutral buffered formalin solution (Sigma Chemical Co), Mossman's fixative solution (Surgipath Medical Industries), and Zeiss tissue fixative (Zeiss Scientific, Inc) and were examined by light and transmission electron microscopy at the Department of Surgical Pathology, University of Cincinnati. The examiner was blinded to the treatment groups.

Calculations and Statistical Analysis

All results are expressed as mean±SEM. For statistical analyses, animals receiving a low-calcium diet (group 2) were subdivided according to the blood iCa concentration response to the low dietary calcium intake (normocalcemics to Group 2a and hypocalcemics to Group 2b); an animal was considered to be hypocalcemic and assigned to group 2b if the ionized calcium concentration decreased below two standard deviations from control, group 1. All animals on a low-calcium diet that did not meet the criterion were considered to be normocalcemic and were assigned to group 2a. Cardiovascular and biochemical responses of the two groups (2a and 2b) were compared with each other and with the control group by multiple ANOVA. The statistical significance of differences between means was tested by Newman-Keuls test, and a value of P<0.05 was considered significant.

Results

Animals in group 2 started a low-calcium diet at 90±2 days of gestation. Although all animals on the low-calcium diet demonstrated lower blood iCa concentration after 20 days of low dietary calcium intake (110 days of gestation), only 9 animals (47%, group 2b) developed hypocalcemia and hypertension, and 10 others (53%, group 2a) remained normocalcemic and normotensive.

Effect of Low-Calcium Diet on Maternal Cardiovascular and Blood Gas Values

The six control animals (group 1) demonstrated no significant changes in mean arterial BP (Fig 1, top) or blood iCa concentration (Fig 1, middle). UBF increased with the progression of gestation (916±74 to 1152±58 mL/min) (Fig 1, bottom). Cardiac output also increased with the progression of gestation (5.94±0.42 L/min at day 110 of gestation to 7.16±0.34 L/min by day 135) (Fig 2, top), and systemic vascular resistance decreased (14±1 to 11±1 mm Hg/L per minute) (Fig 2, middle). Urinary protein concentration increased slightly but not significantly (1.5±0.2 g/L on day 110 of gestation to 2.5±0.5 g/L on day 135) (Fig 2, bottom). Maternal heart rate, blood glucose concentration, and hematocrit did not change significantly (data not shown).

As also shown in Fig 1 (top), group 2a (n=10 twins) demonstrated no significant changes in arterial BP (from 80±2 mm Hg at day 110 of gestation to 75±1 mm Hg on day 135). In this group, blood iCa concentration remained stable throughout the experimental period (1.04±0.02 mmol/L at day 110 of gestation and 1.07±0.02 mmol/L by day 135) (Fig 1, middle). UBF showed initial increases with the progression of gestation (800±40 mL/min at day 110 of gestation to 889±38
In contrast, in group 2b (n=9, 47%) mean arterial BP increased significantly by 20%. The peak values were seen between 120 and 125 days of gestation in eight animals and 115 days of gestation in another one (Fig 1, top). Blood iCa concentration decreased with the progression of gestation, starting at 1.03±0.04 and falling to 0.77±0.03 mmol/L by day 125 of gestation (Fig 1, middle). UBF demonstrated gestational increases from 110 to 120 days of gestation, being 758±38 and 950±53 mL/min, respectively. However, after day 120 we observed a significant reduction of UBF from 950±53 to 579±48 mL/min (Fig 1, bottom). We observed a reduction in cardiac output from 6.33±0.29 to 5.78±0.45 L/min by day 120 of gestation and a significant (P=.05) increase in systemic vascular resistance from 12.01±0.75 at 120 days of gestation to 14.35±0.73 mm Hg/L per mL/min by day 120). However, UBF did not continue to increase as in control animals. By 125 and 130 days of gestation significant differences (P=.01) were established between control and group 2a (Fig 1, bottom). Cardiac output and systemic vascular resistance changes were similar to those observed in the control group (Fig 2, top and middle). Urinary protein was found in all 10 animals at the beginning of the experiment (2.3±0.2 g/L) and remained constant throughout gestation, ending at 2.4±0.4 g/L by day 135 of gestation (Fig 2, bottom). As shown in Table 1, maternal heart rate, blood glucose, and hematocrit values did not change significantly during the study. These animals remained on a low-calcium diet until death (average, 138±2 days) and showed stable blood concentrations of iCa and no significant changes in cardiovascular or blood gas parameters.
minute by day 125 compared with control (Fig 2, top and middle). A negative correlation ($r=-.477$, $P=.001$) was found between blood iCa concentration and arterial BP (Fig 3). Maternal heart rate, blood glucose concentration, and hematocrit remained constant during the experimental period (Table 2).

Urinary protein concentration began to rise before the appearance of increased systemic arterial BP, from 1.7±0.3 to 10.5±1.2 g/L, and this continued until the animals were killed (Fig 2, bottom). After the BP increase and the UBF decrease, premature labor occurred within 96 hours, and the fetuses were aborted. This occurred in 1 animal on day 115, 3 animals on day 127, and 4 animals on day 129, and a mother and its fetus were found dead on day 131 of gestation, for an average gestational length of 127±2 days. After abortion, there was an abrupt (48 hours) return of maternal BP and iCa toward normal.

**Histopathology of the Kidney**

Kidney tissue specimens were obtained from seven animals at the time of death and were examined by light and transmission electron microscopy. All control animals studied (n=3) demonstrated no significant histological changes (Fig 4). However, all animals receiving the low-calcium diet (n=4, groups 2a and 2b) demonstrated significant histopathologic tissue alterations characterized by glomerular swelling, segments of foot process effacement along the basal lamina of the glomerular capillaries (group 2a, Fig 5), and electron-dense paramesangial deposits that probably represent protein deposits and mesangial hypercellularity (group 2b, Fig 6).

**Fetal Changes**

In group 1 fetuses demonstrated no significant changes in fetal blood iCa when compared with the baseline period (1.50±0.03 to 1.43±0.03 mmol/L, Fig 7, top). No significant changes in fetal BP, pH, PaCO$_2$, PaO$_2$, or O$_2$ occurred during the experimental period when compared with baseline (data not shown). Heart rate decreased significantly with the progression of gestation (183±3 to 157±6 beats per minute by day 135 of gestation, $P=.05$).

In contrast, in group 2 fetuses demonstrated reductions in blood iCa concentrations (1.29±0.05 mmol/L at day 135 of gestation for group 2a and 1.31±0.04 mmol/L at day 125 for group 2b; $P=.01$, groups 2a and 2b versus group 1; Fig 7, top). In group 2b fetal BP remained constant (Table 2) despite maternal hypertension, but PaO$_2$, pH, and O$_2$ content decreased with the decrease in maternal UBF and reached significance by day 120 of gestation ($P=.01$, Fig 7, bottom, and Table 2).

**Discussion**

In the present study we have demonstrated that when pregnant ewes with twin gestation are placed on a calcium-deficient diet, hypocalcemia occurs in approximately one half of the animals between days 120 and 125 of gestation. This is the equivalent of 32 weeks of human gestation, a time when significant fetal bone calcification is occurring. The inability of these ewes to maintain blood calcium concentrations indicates that they were unable to mobilize adequate calcium to meet the needs of both the mother and the twin fetuses. In this study hypocalcemia was associated with significant increases in arterial BP, systemic and uterine vascular resistance, proteinuria, and significant reductions in cardiac output and UBF. These hemodynamic and renal changes are similar to those reported in human PIH.18

Once animals became hypocalcemic, systemic arterial BP increased and UBF decreased, resulting in fetal hypoxia. Mothers of hypoxic fetuses went into premature labor and aborted within 48 to 96 hours, for an average gestation of 127±2 days. In fetal sheep, hypoxia
TABLE 2. Cardiovascular and Blood Measurements In Group 2b (Hypocalcemic)

<table>
<thead>
<tr>
<th>Day 110</th>
<th>Day 115</th>
<th>Day 120</th>
<th>Day 125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>96±2</td>
<td>93±2</td>
<td>92±2</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>3.96±0.27</td>
<td>3.60±0.11</td>
<td>3.01±0.16</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.29±0.01</td>
<td>0.29±0.01</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>Fetal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>44±2</td>
<td>45±2</td>
<td>47±1</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>176±4</td>
<td>179±2</td>
<td>178±6</td>
</tr>
<tr>
<td>pH</td>
<td>7.392±0.017</td>
<td>7.376±0.010</td>
<td>7.379±0.012</td>
</tr>
<tr>
<td>Paco₂, mm Hg</td>
<td>34.2±3.1</td>
<td>37.8±1.5</td>
<td>40.6±3.2</td>
</tr>
<tr>
<td>O₂ content</td>
<td>0.055±0.007</td>
<td>0.060±0.004</td>
<td>0.054±0.006*</td>
</tr>
</tbody>
</table>

bpm indicates beats per minute; MBP, mean blood pressure. Values are mean±SEM, n=9.

*P=.01 by ANOVA (group 1 vs group 2b).

is known to lead to significant increases in corticotropin and cortisol,19 which are intimately involved in the initiation of labor.20 Once sheep aborted, maternal BP and blood iCa concentration returned to normal. In contrast, animals that managed to maintain stable blood iCa concentration and BP had an average gestational length of 138±2 days, when they were killed (term, 144±2 days).

Pregnancy is associated with substantial changes in calcium metabolism that include increased urinary calcium excretion and significant placental transfer and fetal uptake of calcium.21,22 It has been reported that in humans the transport of calcium from mother to fetus increases from approximately 50 mg/24 h at 20 weeks of gestation to a maximum of approximately 300 mg/24 h at 35 weeks of gestation.15-17 This increased maternal calcium requirement theoretically may be met by enhanced intestinal absorption. However, in a situation in which calcium intake is seriously decreased (ie, low-calcium diet and deionized water, as in the present study), parathyroid hormone (PTH) would be released in response to a falling serum iCa concentration. PTH would act on bone cells to increase the mobilization rate of calcium ions out of bone to a point at which calcium from bone is no longer available, thus causing an undue disturbance to maternal calcium homeostasis. It is unclear why some animals on a low-calcium diet developed low blood iCa concentrations whereas others did not. The initial bone mineral reserves and the differences between animals in the PTH response to a falling blood

![Fig 4](https://example.com/fig4.png)

**Fig 4.** Electron micrograph shows small part of a glomerulus from group 1 (control). Part of a glomerular capillary is seen, with the basal lamina (common to the capillary and podocyte) that possesses numerous foot processes of podocytes on its outer surface. No significant histological alterations were found (magnification ×3000).

![Fig 5](https://example.com/fig5.png)

**Fig 5.** Electron micrograph shows small part of a glomerulus from group 2a (normocalcemic). Arrows show segments of foot process effacements on its outer surface. M indicates mesangial cell; E, endothelial cell (magnification ×2500).
iCa concentration (not studied in the present report) may in part explain some of the differences.

Vascular smooth muscle cells contract when intracellular free calcium concentration ([Ca$^{2+}$]) is increased, either by calcium release from intracellular stores or by an influx of [Ca$^{2+}$], from the extracellular compartment. Conversely, vascular smooth muscle relaxation results from a decrease in [Ca$^{2+}$], which may occur by efflux across the cell membrane and/or sequestration of [Ca$^{2+}$], into intracellular organelles. It has been proposed that increasing the concentration of blood iCa decreases [Ca$^{2+}$], by calcium-regulated mechanisms. Theoretically, decreasing the concentration of blood iCa increases [Ca$^{2+}$].

The physiological concentration of active free calcium ion in the blood of healthy pregnant women compared with women with PIH is currently unclear. Fogh-Ander sen and Schultz-Larsen reported that the blood concentration of free calcium ion in healthy pregnant women is significantly increased, whereas Richards et al reported no differences in the levels of blood iCa in either healthy gravid women or women with PIH. In contrast, Moodley et al reported that calculated blood iCa concentration in women with eclampsia was significantly lower than in healthy pregnant and non-pregnant women with or without hypertension. Recently, Sowers et al reported that erythrocyte [Ca$^{2+}$], is higher in patients with PIH compared with healthy pregnant women. These results were interpreted to indicate that in response to the low extracellular calcium, cells such as erythrocytes increase their [Ca$^{2+}$]. This effect appeared specific for calcium because no changes were observed in intracellular sodium, potassium, or magnesium concentrations.

The hypothesis that an increased need for calcium during pregnancy is accompanied by physiological hyperparathyroidism was first expressed by Bodansky and Duff in 1939. However, more recently reported changes in PTH concentration during pregnancy are not unanimous. Part of the differences seen in these measurements is due to the use of different PTH assay methodologies. Moreover, dietary intake of calcium and vitamin D as well as age differences could be confounding variables among studies. Similar deficits exist in our knowledge concerning PTH and calcitriol production in PIH. August et al reported increased PTH and decreased 1,25-dihydroxyvitamin D concentrations in 11 women with preeclampsia, but Lalau et al reported no changes in mild PIH, and increased PTH and calcitriol in severe PIH. Nevertheless, the reported increase in serum PTH concentration during pregnancy and PIH may be the result of a low dietary calcium intake, increased calcium needs, or increased calcium loss. It is not clear how PTH could directly contribute to the development of hypertension during pregnancy. PTH actions would include stimulation of renal reabsorption of calcium, bone resorption, and production of calcitriol (1,25-dihydroxyvitamin D), the physiologically active form of vitamin D. Calcidiol would enhance intestinal absorption of calcium. Moreover, PTH infusions to isolated vascular preparations result in vasodilatation. However, Kawashima et al has reported that PTH can induce transient increases in [Ca$^{2+}$], in vascular smooth muscle cells. Calcitriol is also a positive modulator of contractile activity of vascular
smooth muscle cells. Nevertheless, the specific role of PTH and calcitriol, if any, in the development of hypertension during pregnancy is unresolved.

The suggested role for \([\text{Ca}^{2+}]\) in the synthetic regulation of several vasoconstrictor and vasoconstrictor humoral factors (eicosanoids, nitric oxide, and endothelins) may be significant in the control of systemic and uterine hemodynamics during abnormal pregnancy. Pre-eclampsia is characterized by alterations in the equilibrium and production of prostacyclin and thromboxane \(A_2\). Although prostacyclin synthesis decreases, concentrations of the potent vasoconstrictor thromboxane \(A_2\) increase in conjunction with platelet aggregation, resulting in a shift in the ratio of prostaglandin \(I_2\) to thromboxane \(A_2\), which could increase arterial BP and reduce blood flow. Plasma concentration of thromboxane \(A_2\) seems to play an important role in ovine pregnancy-induced hypertension, as its hemodynamic effects are reversed with thromboxane synthetase inhibitors. Hassid and Oudinet, and more recently Magnness and Rosenfeld, have shown a direct relation between prostacyclin synthesis and blood calcium concentration. The vascular reactivity to angiotensin II is known to be increased in PIH, whereas prostacyclin synthesis is reduced. The reduction of prostacyclin production theoretically might be the result of low blood iCa concentration, because calcium supplementation blunts the vascular reactivity to angiotensin II. The synthetic alteration in a second potent vasoconstrictor agent, nitric oxide, may also play a major role in controlling systemic arterial BP and UBF. Yallampalli and Garfield have reported a syndrome consistent with the hemodynamics and renal alterations seen in pre-eclampsia: increased BP, proteinuria, and intrauterine growth retardation after the infusion of an inhibitor of nitric oxide synthesis during rat pregnancy.

Vascular endothelial cells also produce endothelin-1, a potent vasoconstrictor agent in a number of vascular beds whose synthesis is significantly increased by the elevation of \([\text{Ca}^{2+}]\). Endothelin-1 has been shown to be elevated in both essential hypertension and PIH. Endothelin-1 might be involved in the renal vascular alterations leading to proteinuria. Thus, increased calcium concentrations in the underlying vascular smooth muscle cell and changes in humoral factors in the endothelial cells may explain some of the hemodynamic alterations seen in PIH.

Examination of the kidneys and urine of animals receiving a low-calcium diet revealed glomerular hypercellularity, paramesangial deposits, and fusion of mesangial cell podocytes as well as increased urinary protein concentration and red blood cells in the urine of hypertensive animals. Such changes are characteristic of reductions in glomerular blood flow and cellular damage. Thatcher and Keith have also reported that after the induction of hypertension by fasting pregnant ewes for 72 hours, proteinuria was present in all animals and averaged 3.5+ (>7.75 mmol/L). Ferris et al have reported similar histological abnormalities of the glomeruli in stress-induced hypertensive pregnant ewes, with deposits of amorphous materials in the cytoplasm of the endothelial cells, which are characteristic of human pre-eclampsia. They have reported tubular necrosis and glomerular lesions secondary to ischemia, which in turn may explain the presence of increased protein in the urine. We have demonstrated paramesangial deposits, mesangial hypercellularity, and segments of foot process effacement in all animals studied that received a low-calcium diet. The histopathologic alterations were not present in animals on a normal calcium diet, suggesting that low dietary calcium intake per se is capable of producing significant glomerular alterations during twin pregnancy.

Hypertension in pregnancy and pre-eclampsia are associated with high perinatal morbidity and mortality, with an increased incidence (as high as 40%) of intrauterine growth retardation. Studies in an ovine model of intrauterine growth retardation have shown a clear relation between reduced UBF and fetal growth. In the present study we have observed a 39% decrease in UBF in hypocalcemic and hypertensive animals, which, if maintained throughout the latter part of gestation, theoretically would lead to fetal growth retardation.

In summary, from the evidence presented here as well as from previous studies from our laboratory we conclude that reductions in dietary calcium intake during ovine pregnancy with twin gestation can produce symptoms similar to human pre-eclampsia, including increases in maternal arterial BP, protein in the urine, and decreases in cardiac output and UBF. The present animal model will allow us to investigate the underlying mechanisms responsible for the hemodynamic changes observed in PIH.

Acknowledgments

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