Reproductive Sciences
19(2) 173-180

DOI: 10.1177/1933719111415545
http://rs.sagepub.com
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Myocardial Performance and its Acute Response to Angiotensin II Infusion in Fetal Sheep Adapted to Chronic Anemia

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Abstract

Fetal chronic anemia causes lengthening of cardiomyocytes. In adults, severe left ventricular overload may lead to irreversible ventricular dysfunction. We hypothesized that in sheep fetuses with chronic anemia, remodeled myocardium would less successfully respond to angiotensin II (AT II) infusion than in fetuses without anemia. A total of 14 ewes with twin pregnancy underwent surgery at 113 $\pm$ 1 days of gestation. After a recovery period, anemia was induced by isovolumic hemorrhage in 1 fetus of each pair. At 126 $\pm$ 1 days of gestation, longitudinal myocardial velocities of the right (RV) and left (LV) ventricles were assessed at the level of the atrioventricular valve annuli via tissue Doppler imaging. Cardiac outputs were calculated by pulsed Doppler ultrasound. All measurements were performed at baseline and during fetal AT II infusion. Fetal serum cardiac natriuretic peptide (*N*-terminal peptide of proatrial natriuretic peptide [NT-proANP] and B-type natriuretic peptide [BNP]) concentrations were determined. Nine ewes successfully completed the experiment. At baseline, ventricular free wall thicknesses, cardiac outputs, and NT-proANP levels were significantly greater in the anemic fetuses than in the controls. The LV isovolumic contraction velocity (IVCV) acceleration and isovolumic relaxation velocity (IVRV) deceleration were lower ($P < .05$) in the anemic fetuses than in the controls. In the anemic fetuses, there was a positive correlation ($R = .93$, $P < .01$) between RV IVRV deceleration and NT-proANP concentration. Angiotensin II infusion increased ($P < .05$) LV IVCV acceleration in the anemic fetuses. We conclude that in anemic sheep fetuses, myocardial adaptation is associated with impaired LV early contraction and relaxation. However, the LV can improve its contractility with an inotropic stimulus, even in the presence of increased afterload.

Keywords

heart, remodeling, cardiac function, pregnancy, ultrasound, Doppler

Introduction

Experimental studies on sheep have shown that chronic fetal anemia increases both right (RVCO) and left ventricular cardiac outputs (LVCO), coronary artery blood flow and conductance, and concomitantly decreases fetal systemic arterial blood pressure and vascular resistance.[1–4] These hemodynamic changes are related to myocardial remodeling that occurs within several days in response to hypoxemic volume loading. This myocardial remodeling includes cardiomyocyte mass expansion by a balanced combination of cellular enlargement, increased terminal differentiation, and accelerated proliferation.[5] In chronic fetal anemia, circulating atrial natriuretic peptide (ANP) concentrations are elevated.[6] Cardiac natriuretic peptides possess multiple properties to protect the heart. They can affect the cardiac loading conditions by decreasing both preload and afterload. In addition, natriuretic peptides have antihypertrophic and antifibrotic actions.[7]

Tissue Doppler imaging (TDI) is an ultrasound modality that derives its measures from the frequency of movement of the myocardium rather than blood flow. The TDI is effective in evaluating long-axis function and can detect earlier and or milder forms of myocardial dysfunction in adults, compared to standard echocardiography.[8] In adults, tissue velocities can change rapidly during ischemic insult and are useful not only in the detection of pathology but also in the prediction of outcome. In addition, experimental studies on fetal sheep have shown that during acute fetal acidemia, myocardial

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velocities become abnormal while fetal cardiac output is still maintained.[9]

Angiotensin II (AT II), in addition to its peripheral vasoconstrictive effect, has positive inotropic and chronotropic effects on the heart independent of arterial blood pressure.[10] However, the inotropic response seems to vary, being greater in the healthy myocardium than in the failing muscle.[11] In fact, in adult dogs with pacing-induced heart failure AT II caused a direct depression in the LV contraction and relaxation and exacerbated the reduced myocyte contractile performance.[12] In addition, myocardial tissue preparations have shown altered responses to AT II after acute myocardial infarction.[13]

In adults, severe LV overload caused by primary chronic mitral regurgitation may lead to irreversible mechanical dysfunction of the ventricle.[14] In the anemic fetal heart, the combination of increased ventricular volume load and decreased systemic vascular resistance leads to remodeling of the myocardium that includes lengthening of cardiomyocytes.[5] Angiotensin II allows us to investigate the myocardial contractile response and whether this response is indicative of adaptive or maladaptive myocardial remodeling. In this experimental study on fetal sheep with chronic anemia, we hypothesized that remodeled myocardium would less successfully respond to an inotropic stimulus with simultaneously increased afterload as compared to control fetuses that were not anemic. We asked the following questions: (1) Does remodeling of fetal myocardium affect longitudinal myocardial tissue Doppler velocities at baseline conditions? (2) Does the remodeled myocardium respond differently to AT II infusion? and (3) Are circulating cardiac natriuretic peptide concentrations related to myocardial tissue Doppler indices?

Methods

All care and procedures in this study were reviewed and approved by the Oregon Health and Sciences University Animal use and Care Committee. Mixed Western breed ewes were time bred and obtained from a commercial supplier. The ewes were acclimatized to the research facility for several days.

Surgical Preparation and Instrumentation

Surgery was performed on 14 ewes (twin pregnancy) at 113 $\pm$ 1 day (term 145 days) after overnight fasting. General anesthesia was induced with a diazepam (10 mg) ketamine (400 mg) mixture and the ewe was intubated. Anesthesia was maintained with 1.5% to 2.5% isoflurane and 25% nitrous oxide/balance oxygen. A midline abdominal incision was made to access the uterus. Separate incisions were made in the uterus to gain access to each twin. The upper body of each twin was delivered. Polyvinyl catheters were inserted into the carotid artery and internal jugular vein of each fetus placing the catheter tips in the ascending aorta and superior vena cava, respectively. A 3-lead, 28-gauge silver-coated copper electrocardiogram (ECG) wire (New England Wire Tech., Lisbon, New Hampshire) was placed subcutaneously on the fetal chest and a separate polyvinyl catheter was placed in 1 amniotic cavity of each twin pair to monitor intra-amniotic pressure. All incisions were closed and each fetus received an intra-amniotic injection of penicillin G (1 million units). All catheters and wires were marked to designate twin specificity. These lines and wires were then tunneled to a pouch on the ewe's flank. Postoperative pain was controlled with buprenex (0.3 mg).

Induction of Anemia

After a 3- to 4-day recovery period, 1 fetus of each pair was randomly chosen as the control and 1 chosen to become anemic. On the first day, the anemic fetus of each pair had 60 to 120 mL of blood removed over 1 to 10 minutes from the carotid artery catheter with immediate infusion of the same volume of normal saline over 1 to 2 minutes. On subsequent days, 0 to 120 mL were removed to achieve an arterial oxygen content of 2 mL/dL based on chronic fetal anemia models as previously described.[2,15] Arterial blood samples for blood gases (Radiometer ABL 700, Copenhagen, Denmark) were collected from the anemic fetuses daily to guide anemia induction and every other day from the control fetuses. All catheters were flushed intermittently to maintain patency.

Data Acquisition

Eight days after the beginning of anemia induction (gestational age 126 $\pm$ 1 day), fetal ultrasound data were acquired. Ewes were studied under general anesthesia. Prior to induction of anesthesia, blood gases were evaluated for each fetus. Each ewe was prehydrated with 1 L of lactated Ringer to prevent general anesthesia-associated hypotension. Thereafter, Ringer solution was infused at a fixed rate of 200 mL/h. Each ewe was also given midazolam (0.2 mg/kg) to assist with minimizing general anesthesia. Anesthesia was induced with propofol (4-7 mg/kg) and the ewe was intubated. Anesthesia was maintained with 1% to 2% isoflurane mixed with room air. Anesthesia was titrated to keep the ewe's heart rate and blood pressure normal and allow for ultrasound imaging without discomfort while minimizing the physiologic alterations associated with its use. Propofol was also given as a bolus as needed to maintain anesthesia. A maternal peripheral arterial line was placed in the femoral artery to allow for blood gas and blood pressure monitoring. The sheep were positioned supine with a left lateral tilt. The animals were allowed to stabilize for 30 to 60 minutes prior to collecting baseline ultrasound measurements.

During the study of each fetus, its respective ECG leads were connected to the ultrasound equipment. Fetal and maternal blood pressures were monitored continuously with pressure transducers calibrated with a mercury manometer and digitally stored using commercial hardware and software (Powerlab, ADI, Castle Hill, Australia). The recordings were later analyzed in 1-minute periods and the median value per variable was chosen to represent a particular minute. The means of the last 5 minutes of each phase were used in the analyses.

Fetal blood pressure was calculated as the mean arterial pressure minus intra-amniotic pressure. Heart rate was determined from the pressure waveform. The fetus, anemic or control, chosen to be studied first was done in an alternating fashion. Ultrasonographic examinations were performed by a single investigator (J. R.). After baseline ultrasonographic data were collected, AT II (Bachem, Torrance, California) in a concentration of 2 μg/mL was then infused intravenously with a mean rate of 1.5 mL/min into each fetus to increase blood pressure by 15 mm Hg. The ultrasonographic examination identical to baseline study was then done during AT II infusion.

Ultrasonographic examination was done using a Vivid 7 Dimension ultrasound system (GE Vingmed Ultrasound, Horten, Norway) with a 10-MHz phased-array transducer. Pulsed Doppler was used to obtain ventricular outflow blood velocity waveforms. The angle of insonation was maintained at <15°. From aortic valve (AoV) and pulmonary valve (PV) blood velocity waveforms, time–velocity integral (TVI) was obtained by planimetry of the area underneath the Doppler spectrum.[16] The diameters of PV and AoV were measured during systole using the leading edge method to calculate their cross-sectional areas (CSAs). Volumetric blood flows (Q) across the PV (Q_{PV}) and AoV (Q_{AoV}) were calculated ($Q = CSA \times TVI \times$ fetal heart rate [FHR]). Right ventricular cardiac output RVCO equals the Q_{PV}, and left ventricular cardiac output LVCO equals the Q_{AoV}.[16,17] Right (RVFS) and left (LVFS) ventricular fractional shortenings were calculated from M-mode recordings using the following formula[18]: ventricular fractional shortening (%) = ([inner diastolic diameter − inner systolic diameter]/inner diastolic diameter) × 100. In addition, the velocity of circumferential fiber shortening (VCFS) was calculated for both ventricles by dividing FS by ejection time.[18]

The longitudinal velocities of the right (RV) and left ventricular (LV) free wall during the cardiac cycle were assessed using pulsed-wave TDI, with the sample volume (1-1.5 mm) placed at the level of the atrioventricular valve annuli and aligned as parallel as possible to the myocardial wall (<15° angle of insonation). Myocardial velocities were recorded during 3 to 6 cardiac cycles at a sweep speed of 100 mm/s. The frame rate was maximized. Peak myocardial velocities were measured during isovolumic relaxation velocity (IVRV), early ventricular filling (E′), atrial contraction (A′), isovolumic contraction velocity (IVCV), and ventricular systole (S′). The isovolumic myocardial acceleration and deceleration were calculated by dividing the peak IVCV and IVRV by the time intervals from the onset to the peak of these velocity waveforms.[9] Each ultrasonographic data collection took about 15 to 20 minutes. Intraobserver variabilities of tissue Doppler-derived parameters were analyzed in 5 fetuses. The analyses were performed during baseline and AT II infusion.

Fetal arterial blood samples (≥1 mL) were collected during the induction of anemia, at baseline and during AT II infusion from control and anemic fetuses in serum tubes and centrifuged. Serum samples were stored at −80°C until analyzed. Circulating *N*-terminal peptide of proatrial natriuretic peptide (NT-proANP) and B-type natriuretic peptide (BNP) concentrations were determined from SepPak C18-extracted serum samples by radioimmunoassays as described previously.[19,20] The intra-assay and interassay coefficients of variation for the NT-proANP and BNP assays were below 10% and 15%, respectively.

At the end of the experiment, the ewe and the fetuses were killed with an intravenous injection of a commercially available euthanasia solution (Somnasol, Butler, Dublin, Ohio). Fetal weights were determined.

Statistic Analysis

Data were analyzed using SPSS v 15.0 (Chicago, Illinois). Unpaired *t* tests were used to compare continuous data between anemic and control fetuses at baseline and during AT II infusion. Paired *t* tests were used to compare data between baseline and AT II period in the anemic and control fetuses, respectively. Additionally, we calculated for each parameter the difference between the AT II and baseline periods and evaluated the significance of these differences between the control and anemia groups with an unpaired *t* test. Correlations were tested using the Pearson correlation coefficient. A *P* value <.05 was considered statistically significant. All data are presented as mean ± standard deviation (SD).

Results

Of the 14 twin gestations, 9 successfully completed the study protocol. In 5 cases, fetal death or delivery occurred either during the recovery or during the anemia induction period. Hemoglobin, hematocrit, and oxygen content in anemic fetuses were significantly lower than those in controls ($P < .05$) at the baseline and during AT II infusion (Table 1). At baseline, partial pressure of oxygen (pO_2) was less in anemic fetuses when compared with the controls ($P < .05$). Angiotensin II infusion significantly increased fetal mean arterial blood pressure both in the control and in the anemic fetuses ($P < .05$; Table 1). During AT II infusion FHR was higher in anemic fetuses when compared with the controls ($P < .05$). Fetal weights did not differ statistically significantly between the anemic (3196 ± 304 g) and the control (3145 ± 410 g) groups.

In anemic fetuses, weight-indexed RVCO and LVCO were significantly greater at baseline and during AT II infusion than in the control fetuses (Table 2). Angiotensin II infusion did not significantly affect fetal cardiac outputs. Right ventricular stroke volume was greater in the anemic fetuses than in the control fetuses both at baseline and during AT II infusion. At baseline, LV stroke volume was greater in the anemia group compared with the control group. However, during AT II infusion, LV stroke volume increased significantly in the control fetuses (Table 2). At baseline, RV (3.7 ± 0.3 vs 3.2 ± 0.1 mm; $P = .02$) and LV (4.2 ± 0.4 vs 3.1 ± 0.5 mm; $P = .001$) free wall, and interventricular septal (4.2 ± 0.4 vs 3.3 ± 0.4 mm; $P = .001$) thicknesses were greater in the anemic fetuses that in the control fetuses. Both ventricular FS and VCFS did not differ significantly between the groups. During AT II infusion, RV FS and VCFS

Table 1. Fetal Hemodynamic, Blood Gas, and Metabolic Parameters at Baseline and During Angiotensin II (AT II) Infusion in the Study Groups (n = 9)[a]

	Baseline	AT II	*P*	Δ
Heart rate, beats/min				
Control	148 ± 26	156 ± 17	.3	8 ± 22
Anemia	167 ± 32	183 ± 13	.1	16 ± 30
P	.2	.001		.5
Mean arterial BP, mm Hg				
Control	37 ± 4	52 ± 7	.001	16 ± 4
Anemia	32 ± 5	47 ± 5	.001	15 ± 7
P	.1	.2		.9
Arterial pH				
Control	7.30 ± 0.05	7.24 ± 0.08	.002	−0.06 ± 0.04
Anemia	7.26 ± 0.07	7.17 ± 0.10	.01	−0.07 ± 0.08
P	.004	.04		.4
Arterial pCO_2, torr				
Control	53 ± 5	58 ± 8	.1	4 ± 6
Anemia	54 ± 6	58 ± 8	.1	3 ± 6
P	.6	.9		.9
pO_2, torr				
Control	22 ± 4	18 ± 3	.005	−4 ± 3
Anemia	18 ± 3	17 ± 3	.5	−1 ± 3
P	.1	.6		.07
Hct, %				
Control	33.4 ± 1.8	31.2 ± 3.1	.5	0.8 ± 1.8
Anemia	12.8 ± 0.8	17.2 ± 2.9	.2	1.4 ± 1.0
P	.0001	.0001		.08
Hemoglobin, g/dL				
Control	10.8 ± 1.8	11.1 ± 1.7	.5	0.2 ± 0.6
Anemia	4.0 ± 0.8	4.4 ± 1.0	.2	0.4 ± 0.3
P	.0001	.0001		.45
Arterial O_2 content, mL/dL				
Control	7.4 ± 2.3	5.4 ± 2.8	.004	−2.0 ± 1.3
Anemia	1.9 ± 0.7	1.6 ± 0.5	.6	−0.3 ± 0.6
P	.0001	.0001		.02
Arterial glucose, mmol/L				
Control	1.5 ± 1.2	1.4 ± 1.0	.7	−0.1 ± 0.7
Anemia	1.6 ± 1.2	1.5 ± 1.0	.5	−0.1 ± 0.6
P	.6	.4		.9
Arterial lactate, mmol/L				
Control	3.5 ± 1.4	4.3 ± 1.7	.02	0.8 ± 0.7
Anemia	5.6 ± 4.1	7.9 ± 4.3	.05	2.2 ± 1.8
P	.1	.006		.05

[a] Δ indicates change from baseline to AT II for each group. Values are means ± SD.

decreased significantly in the control group, and the response to AT II was significantly different between the groups (Table 2).

At baseline, tissue Doppler-derived LV IVCV acceleration and IVRV deceleration were significantly lower in the anemic fetuses compared with the control fetuses (Table 3). In the RV, these parameters did not differ statistically significantly between the groups. The velocities of RV and LV IVCV, IVRV, E′, A′, and S′ maximum were comparable between the groups at baseline. During AT II infusion, LV IVCV acceleration increased significantly in the anemic fetuses (Table 3). In addition, LV A′ maximum velocity increased significantly in both groups during AT II infusion. The mean intraobserver variabilities of tissue Doppler-derived absolute myocardial velocities, and IVCV acceleration and IVRV deceleration were <4%.

In the anemic fetuses, serum NT-proANP concentrations were significantly higher at baseline compared with the control group (Table 4). In addition, in the anemia group there was a significant positive correlation ($R = .93$, $P < .01$) between RV IVRV deceleration and serum NT-proANP concentration (Figure 1). Serum BNP concentrations were comparable between the 2 groups at baseline. *N*-terminal-proANP and BNP concentrations were not significantly affected by AT II infusion.

Discussion

In the present study, RV and LV free wall, as well as interventricular septal thicknesses were increased in the anemic fetuses,

Table 2. Fetal Cardiac Outputs, Stroke Volumes, Ventricular Fractional Shortenings, and Velocity of Circumferential Fiber Shortenings (VCFS) at Baseline and During Angiotensin II (AT II) Infusion in the Study Groups (n = 9)[a]

	Right Ventricle				Left Ventricle			
	Baseline	AT II	*P*	Δ	Baseline	AT II	*P*	Δ
Cardiac output, mL/min per kg								
Control	263 ± 62	261 ± 53	.9	−2 ± 63	165 ± 52	208 ± 48	0.1	42 ± 58
Anemia	384 ± 64	398 ± 50	.5	23 ± 57	240 ± 61	260 ± 60	0.4	22 ± 61
P	.0004	.002		.6	.004	.04		.4
Stroke volume, mL/kg								
Control	1.7 ± 0.3	1.7 ± 0.3	.5	−0.1 ± 0.3	1.1 ± 0.3	1.3 ± 0.4	0.03	0.2 ± 0.2
Anemia	2.3 ± 0.3	2.1 ± 0.3	.1	−1.4 ± 0.3	1.5 ± 0.4	1.4 ± 0.4	0.8	−0.1 ± 0.3
P	.001	.02		.4	.02	.9		.08
Fractional shortening, %								
Control	30.3 ± 4.9	19.1 ± 7.7	.003	−11.2 ± 8.1	28.6 ± 6.6	30.3 ± 5.1	0.5	1.7 ± 7.0
Anemia	25.8 ± 4.0	24.7 ± 13.2	.8	1.1 ± 12.0	29.5 ± 9.2	30.9 ± 7.7	0.6	3.0 ± 8.2
P	.1	.3		.05	.9	.8		.9
VCFS, circumference								
Control	1.97 ± 0.51	1.02 ± 0.41	.003	−0.96 ± 0.70	1.98 ± 0.47	1.65 ± 0.32	0.1	0.33 ± 0.46
Anemia	1.57 ± 0.29	1.44 ± 0.79	.6	−0.01 ± 0.73	1.82 ± 0.53	1.88 ± 0.51	0.7	0.12 ± 0.43
P	.1	.2		.03	.2	.2		.09

[a] Δ indicates change from baseline to AT II for each group. Values are means ± SD.

Table 3. Fetal Cardiac Tissue Doppler-Derived Parameters at Baseline and During Angiotensin II (AT II) Infusion in the Study Groups[a]

	Right Ventricle—Tricuspid Annulus				Left Ventricle—Mitral Annulus			
	Baseline	AT II	*P*	Δ	Baseline	IAT II	*P*	Δ
IVCV, cm/s								
Control	8.4 ± 3.9	7.7 ± 2.9	.5	−0.7± 3.1	7.9 ± 4.5	6.8 ± 2.1	.4	−1.0 ± 3.6
Anemia	5.7 ± 2.2	7.6 ± 3.6	.2	2.5 ± 4.3	6.0 ± 2.1	7.4 ± 3.4	.2	1.5 ± 3.0
P	.1	.9		.16	.2	.6		.13
IVCV acceleration, m/s^2								
Control	5.4 ± 1.9	6.3 ± 2.5	.1	1.0 ± 1.7	5.8 ± 2.9	5.2 ± 1.7	.5	−0.6 ± 2.5
Anemia	4.3 ± 1.9	6.2 ± 3.7	.1	2.6 ± 3.3	3.4 ± 0.9	4.8 ± 1.7	.04	1.6 ± 1.8
P	.1	.9		.4	.04	.6		.06
IVRV, cm/s								
Control	4.3 ± 1.6	3.7 ± 1.2	.1	−0.6 ± 0.8	4.1 ± 1.5	4.2 ± 1.2	.9	0.1 ± 2.0
Anemia	4.0 ± 1.2	4.0 ± 0.9	.8	0.0 ± 1.1	4.0 ± 1.4	4.0 ± 1.3	.9	−0.2 ± 1.9
P	.7	.6		.3	.9	.7		.9
IVRV deceleration, m/s								
Control	4.1 ± 1.0	5.0 ± 1.3	.2	0.8 ± 1.6	4.7 ± 1.3	4.6 ± 2.1	.8	−0.1 ± 1.1
Anemia	3.4 ± 1.2	4.5 ± 1.3	.1	1.6 ± 1.9	3.0 ± 0.9	3.7 ± 0.8	.1	0.5 ± 1.0
P	.1	.4		.8	.002	.1		.12
A′ wave, cm/s								
Control	12.9 ± 7.6	18.3 ± 6.3	.1	5.4 ± 7.7	12.3 ± 5.7	20.4 ± 5.2	.005	8.1 ± 6.2
Anemia	15.0 ± 7.4	22.2 ± 8.6	.1	9.8 ±11.2	13.3 ± 4.5	22.1 ± 5.5	.001	9.5 ± 5.2
P	.5	.2		.7	.6	.4		.8
S′ wave, cm/s								
Control	8.5 ± 4.5	7.1 ± 1.8	.2	−1.4 ± 3.3	7.9 ± 2.5	7.8 ± 1.4	.9	−0.6 ± 2.4
Anemia	9.9 ± 2.4	8.4 ± 1.7	.2	−0.9 ± 2.8	10.1 ± 2.8	9.7 ± 2.4	.7	−0.5 ± 3.4
P	.4	.1		.9	.1	.04		.8

Abbreviations: IVCV, isovolumic contraction velocity; IVRV, isovolumic relaxation velocity; A′, velocity during atrial contraction; S′, velocity during ventricular systole (n = 9).

[a] Δ indicates change from baseline to AT II for each group. Values are means ± SD.

indicating significant myocardial remodeling. In fetuses with chronic anemia, LV longitudinal IVCV acceleration and IVRV deceleration were less than those in the control fetuses. The IVCV acceleration reflects the ability of the myocardium to generate pressure sufficient to exceed systemic arterial diastolic blood pressure and is a load-independent noninvasive index

Table 4. Fetal Serum Concentrations of *N*-Terminal Peptide of Proatrial Natriuretic Peptide (NT-proANP) and B-Type Natriuretic Peptide (BNP) at Baseline and During Angiotensin II (AT II) Infusion in the Study Groups (n = 6)[a]

	Baseline	AT II	*P*	Δ
NT-proANP, pmol/L				
Control	1003 ± 294	1495 ± 757	.1	390 ± 519
Anemia	2839 ± 135	3131 ± 102	.1	380 ± 576
P	.03	.005		.9
NT-proBNP, pmol/L				
Control	114 ± 31	132 ± 44	.1	21 ± 33
Anemia	118 ± 27	176 ± 83	.2	43 ± 52
P	.8	.2		.4

[a] Δ indicates change from baseline to AT II for each group. Values are means ± SD.

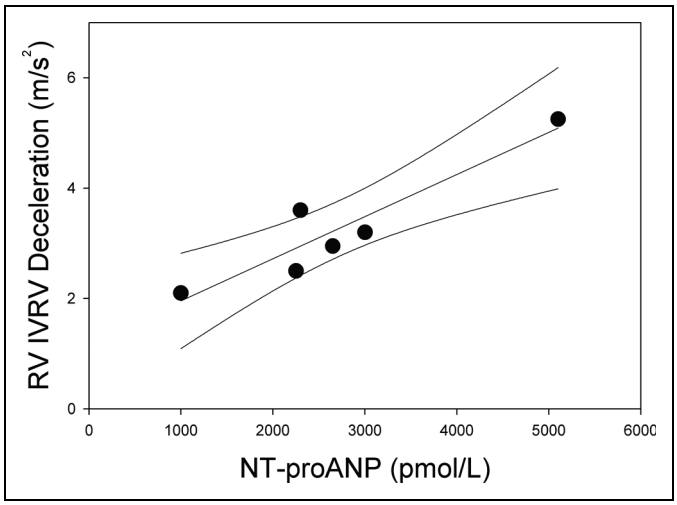

Figure 1. Correlation between serum *N*-terminal peptide of proatrial natriuretic peptide (NT-proANP) and tissue Doppler-derived right ventricular isovolumic relaxation velocity (IVRV) deceleration (RV IVRV deceleration = 0.00076 × NT-proANP + 1.2, $R = .93$, $P < .01$) in the anemic fetuses at baseline (n = 6).

of myocardial contractility as validated in adult animal experiments.[21,22] The myocardial remodeling in chronic fetal anemia includes cardiomyocyte mass expansion, increased terminal differentiation, and accelerated proliferation.[5] In addition, disease states such as cardiac failure or hypertrophy can alter the relative and absolute myosin heavy chain isoform expression levels.[23] In humans, it has been shown that the relatively small V1 isoform of myosin heavy chain expression found in the normal heart is significantly downregulated in the failing heart.[24,25] These structural, ultrastructural, and mechanical changes related to remodeling of the LV could explain diminished IVCV acceleration and LV contractility. However, despite this diminished LV IVCV acceleration, the stroke volume and cardiac output were greater than in the control fetuses. In the present study LV IVRV deceleration was reduced in fetuses with chronic anemia. This suggests that LV remodeling can adversely affect active myocardial relaxation processes during the isovolumic phase of diastole.

In fetuses with chronic anemia, RV tissue Doppler indices did not differ significantly from those measured in the control fetuses. The RV at the midlevel is mainly composed of transverse fibers in the subepicardium and longitudinal fibers in the subendocardium.[26] It seems that chronic anemia-related RV remodeling does not affect RV myocardial longitudinal velocities. Our study demonstrates that fetal ventricular responses to chronic anemia are different with signs suggestive of abnormalities in LV contractility and relaxation during the isovolumic phases of the cardiac cycle. We propose that the RV can adapt to chronic anemia-related volume load better than the LV.

Angiotensin II infusion increased cardiac afterload and led to a significant improvement in the LV IVCV acceleration in the anemic fetuses. Angiotensin II increases systemic arterial pressure and vascular resistance by vasoconstriction. In addition, it has a direct inotropic effect on cardiomyocytes.[10] However, the inotropic response to AT II in cardiac muscle can vary; the responsiveness seems to be greater in the normal healthy myocardium than in the failing muscle.[11] Our results demonstrate that the cardiomyocytes in the remodeled LV free wall have the capacity to produce a positive inotropic response to AT II. This is in agreement with a previous study on sheep, demonstrating that AT II infusion similar to this present study significantly increased LV dP/dTmax and LV IVCV and its acceleration in fetuses with acidemia.[27] In addition, A′ wave velocity increased significantly in the LV during AT II infusion, demonstrating that LV expansion during atrial contraction improves. This also suggests that atrial contraction is augmented by AT II infusion. In the control fetuses, AT II infusion significantly decreased RVFS and VCFS, while these parameters remained unchanged in the anemic fetuses. Even though ventricular fractional shortening can be a measure of ventricular contractility, it is sensitive to changes in ventricular loading conditions. Our findings are in agreement with an experimental study in fetal sheep, demonstrating that acute ductal occlusion with an increase in pulmonary artery pressure immediately led to decreased RVFS mainly by increasing RV end-systolic dimension.[28] However, despite significantly decreased fractional shortening, RV pressure generation was preserved. Furthermore, in the present study the control fetuses were able to maintain the RVCO during AT II infusion. In humans, it has been shown that ventricular longitudinal and oblique fibers contract first, followed by the contraction of circumferential fibers, and ventricular long-axis shortening velocities and amplitude correlate with overall ventricular function as assessed by ejection fraction.[29] In adults, long-axis shortening tends to decline earlier than radial function in disease states.[30] On the other hand, the anemic fetuses were able to maintain RVFS during an increase in the ventricular afterload. It appears that RV remodeling in chronic anemia allows the RV not only to carry increased volume load but also to tolerate increased afterload, at least in short-term conditions.

We found that at baseline conditions anemic fetuses had significantly increased serum NT-proANP concentrations demonstrating the activation of the cardiac hormonal system by increased volume load. Secretion of proANP-derived peptides

can reduce cardiac work load by natriuresis and diuresis, vasodilation, and inhibition of the renin–angiotensin–aldosterone system. In the anemic fetuses, an increase in serum NT-proANP concentration was related to improved RV relaxation during early diastole. In adults, cardiac natriuretic peptide concentrations are used to evaluate the severity of heart failure and high levels of cardiac peptides identify those at greatest risk of future serious cardiovascular events.[31] Our results suggest that in the anemic fetus, an increase in ANP production could be a sign of successful myocardial adaptation. On the other hand, serum BNP concentrations were not affected by increased volume load in anemic fetuses demonstrating that increased preload is not the primary stimulus for BNP-derived peptides.

Our experimental study has limitations. The surgical procedures may constitute a significant stress to the examined fetuses. However, the recovery period after surgery should be long enough for the recovery of fetal myocardial function.[32] As regard the use of general anesthesia, blood pressure can be slightly lower than in unanesthetized fetal sheep. On the other hand, it has been shown that uterine and umbilical artery volume blood flows prior to and after the induction of general anesthesia are similar, suggesting conditions close to a physiologic circulatory state.[33] Although isoflurane may modify fetal cardiovascular regulation, newborn lamb under isoflurane anesthesia can increase cardiovascular performance during stress.[34] Fetal metabolic acidosis can impair myocardial contractility and relaxation during the isovolumic phases of the cardiac cycle.[9] It is unlikely that mild metabolic acidosis in the anemic fetuses could explain our findings, because RV tissue Doppler-derived parameters were unaffected. Validation studies in fetal sheep have shown that invasive and Doppler echocardiographic volume flow calculations correlate well.[35] In addition, the intraobserver variabilities of the Doppler ultrasonographic parameters of fetal sheep cardiovascular hemodynamics have been shown to be comparable with those in previous human fetal studies during the second half of gestation.[36,37] Furthermore, intraobserver variabilities of tissue Doppler-derived indices found in the present study were comparable to those reported in human fetuses.[38]

In conclusion, our experimental sheep model with chronic fetal anemia demonstrated that fetal myocardial adaptation was associated with impaired LV early relaxation and contraction. However, the anemic LV was capable of improving its contractility during inotropic stimulus, even in the presence of increased afterload.

Acknowledgments

The authors wish to acknowledge Loni Socha and Robert Webber for their excellent technical assistance and Pasi Ohtonen, MSc, for his assistance in statistical analyses. These studies were supported by NIH HL45043, NICHHD P01 HD 034430, and R01NHL106769.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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