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Left ventricular isovolumic relaxation and renin-angiotensin system in the growth restricted fetus

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Abstract

Objective: To determine left ventricular isovolumic relaxation time (LV IRT) in normally developing and growth restricted fetuses (FGR) as an indicator of fetal cardiac afterload and neonatal systolic blood pressure.
Study design: A prospective longitudinal study in 124 normally developing and 47 growth restricted fetuses (FGR). LV IRT, fetal heart rate (FHR) and umbilical artery pulsatility index (PI) were determined at 2–3 week intervals starting at 22–26 weeks of gestation until delivery. Renin and angiotensin I levels were measured by radioimmunoassay in umbilical venous blood after delivery. Systolic blood pressure was measured at day 1 and day 5 of postnatal life. To evaluate the association between LV IRT, gestational age and FHR, bivariate regression analyses were performed.
Results: Mean LV IRT (62 ± 8 ms) was 29 percent longer in FGR as compared to the normal subset (47 ± 6 ms) at all gestational ages ($p < 0.001$). Mean postnatal active plasma renin level ($7.78 \pm$ S.D. 1.03 ng/ml) and postnatal angiotensin I level (4.21 ± 0.70 ng/ml) in the FGR subset were significantly higher ($p < 0.001$) than in the normal subset (4.81 ± 1.04 ng/ml, renin and 2.69 ± 0.44 ng/ml, angiotensin I). There was a significant difference ($p < 0.01$) in systolic blood pressure between the two subsets on postnatal day 1 (FGR 52 ± 6 mmHg vs. normal 46 ± 4 mmHg) and day 5 (FGR 76 ± 5 mmHg vs. normal 60 ± 6 mmHg).
Conclusion: Left ventricular isovolumic relaxation time may act as a sensitive index of increased arterial afterload in the growth retarded fetus and may herald raised systolic blood pressure in the early neonatal period.

Keywords: Isovolumic relaxation time; Growth restricted fetus; Renin; Angiotensin

1. Introduction

Fetal growth restriction (FGR) complicates 10%–15% of all pregnancies [1]. The cause of FGR is multifactorial. Pregnancy induced hypertension (PIH), preeclampsia and maternal nutritional deficiencies are among the most common causes of FGR [1,2]. Although perinatal complications of FGR are well documented, it is only recently that researchers are focused on the long-term morbidity which is associated with this phenomenon. Numerous epidemiological studies have described the relationship between low birth weight and high blood pressure in adult life. It was proposed that factors associated with FGR could program the development of the cardiovascular system and act as a risk for essential hypertension, hyperlipidemia and death from cardiovascular disease in later life [3–5]. However, there is no information about the mechanisms of this programming. Whether these mechanisms start during intrauterine life or factors of intrauterine life could serve

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as a background for the early development of hypertension in adults and adolescents is still unknown.

Current management of FGR fetuses consists of serial ultrasound examinations to assess growth as well as Doppler velocimetry in the umbilical artery and several other vessels [6–8]. FGR is usually characterized by an elevated umbilico-placental vascular resistance which may also affect the afterload of the fetal heart [7]. The specific sequence of Doppler changes and hemodynamic modifications in FGR fetuses has been shown, including a change in peripheral arterial impedance resulting in redistribution of blood flow to the vital organs (heart and brain) which is followed by progressive impairment of cardiac function and abnormal venous flow patterns [6,8].

The effects of experimental restriction of placental function and fetal growth on fetal arterial blood pressure regulation during late gestation have been investigated in fetal sheep [9]. A special role of endogenous renin-angiotensin system in the maintenance of blood pressure in growth restricted fetus was determined by captopril infusion [9].

Earlier we have shown the increase in left ventricular isovolumic relaxation time (LV IRT) in FGR fetuses [10]. It is well known that the main determinant of IRT is ventricular afterload [11]. Direct measurements of intraventricular pressure and time course of isovolumic relaxation demonstrated a strong correlation between LV IRT and end-systolic LV pressure (afterload) in sheep [12]. A higher end-systolic pressure value means longer period of isovolumic relaxation. During this time intraventricular pressure should decay from end-systolic pressure (systolic blood pressure) to the value about zero mmHg, which is essential for the initiation of ventricular filling.

We speculated that LV IRT could serve as a noninvasive index of human fetal cardiac afterload. To test this hypothesis we studied the changes of fetal left ventricular isovolumic relaxation time in normally growing and growth restricted fetuses and measured the activity of the renin-angiotensin system which could be involved in the maintenance of arterial blood pressure in the normal sized and growth restricted newborn.

2. Patients and methods

A total 171 consecutive singleton pregnancies was enrolled in the study which was approved by the local Ethics Review Board of the Mother and Child Care Institute. All women consented to participate. The gestational age at enrolment ranged between 22 and 26 weeks of gestation. Gestational age was determined from the patient's last menstrual period and was confirmed by a first-trimester fetal crown-rump length or early second-trimester fetal biparietal diameter measurement. Fetal biometry and Doppler velocimetry measurements were carried out at 2–3 week intervals until delivery.

Biometry and Doppler measurements were performed using a real-time pulsed Doppler ultrasound system (Aloka SSD 1400, Aloka Industry Corp., Tokyo). The Doppler carrier frequency was 3.5 MHz. The four-chamber view of the heart was visualized on a transverse cross-section. The Doppler sample was then placed immediately distal to the mitral valve leaflets. The high-pass filter was set at 100 Hz. Doppler tracings were accepted when the angle between the Doppler cursor and the assumed flow direction was 10^0 or less. On the Doppler waveform traces the left ventricular isovolumic relaxation time (LV IRT; ms) was determined from the artifact of aortic valve closure to the onset of transmitral flow [10] (Fig. 1). The latter was taken as the point where the signal rose above the filter. Fetal heart rate (bpm) was calculated from the time difference between the onsets of transmitral flow in two consecutive cardiac cycles.

Doppler flow velocity waveforms were recorded from the umbilical artery [6,7].

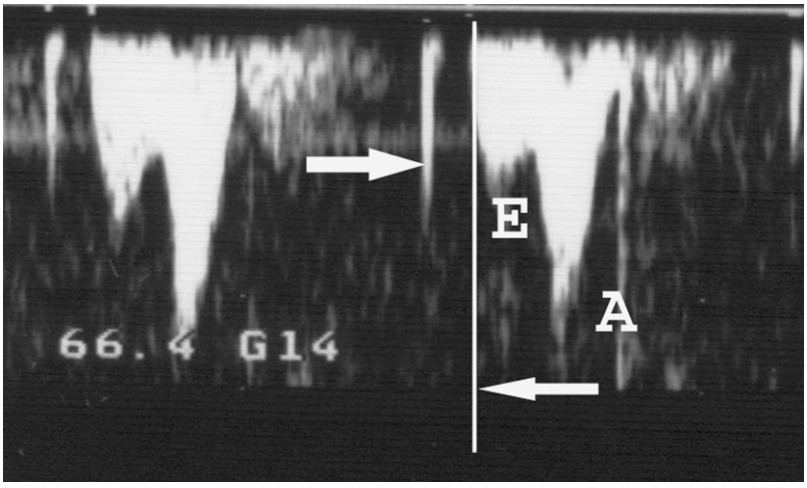

Fig. 1. Pulsed Doppler velocity waveforms from normally developing fetus at 34 weeks of gestation. Left isovolumic relaxation time is determined from the interval between aortic valve closure artifact and the onset of E component of transmitral flow.

For each subject the mean of three consecutive cardiac cycles of acceptable technical quality was taken for calculation of the LV IRT (ms) and umbilical artery Pulsatility Index (UA PI).

Aliquots of fetal blood were collected from the umbilical vein at delivery in prechilled tubes and placed immediately on ice. Plasma was then extracted by centrifugation at 1000 × *g* for 10 min at 4 °C, and store at −70 °C until assay. Active renin and angiotensin I concentrations were measured in plasma using a commercially available radioimmunoassay system (Amersham International, Buckinghamshire, UK). In our laboratory this assay has inter-assay and intra-assay coefficients of variation of 11% and 9%, respectively. Venous blood pH data were obtained with an automated blood gas analyzer (Radiometer Instruments BMS3 Mk2, Denmark).

Systolic blood pressure in newborn infants was measured by a Dinamap monitor on day 1 and day 5 after delivery according to the standard protocol for blood pressure measurements in the newborn [13].

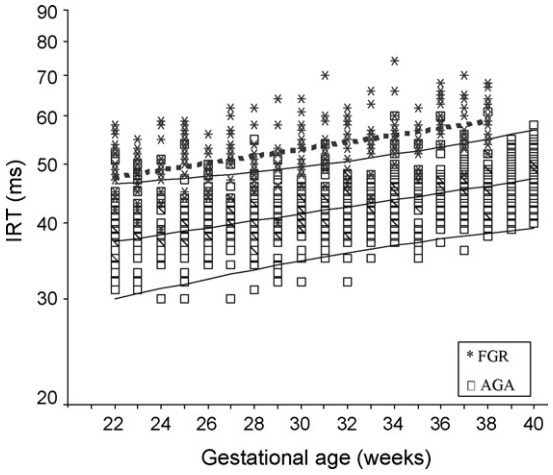

Fig. 2. Relationship between Isovolumic Relaxation Time (ms) and gestational age in normally developing appropriate for gestational age (AGA) and FGR fetuses. Solid and dotted straight lines represent regression lines for AGA and FGR, respectively. Bended curves represent upper and lower limits of 95% reference range for AGA. Note the logarithmically transformed vertical axis.

2.1. Statistical analysis

To evaluate the association between LV IRT and gestational age regression analysis was performed using mixed model Anova (random coefficients model). In this analysis IRT-values were logarithmically transformed to obtain approximately normal distributions. Differences in other investigated parameters between normally developing and growth restricted fetuses were determined using the *t*-test. All data are presented as mean ± S.D. The level of statistical significance was chosen as $p < 0.05$.

3. Results

Table 1 presents the clinical data concerning the normal and growth restricted fetuses. Pregnancy was uneventful in 124 of 171 women. Fetal abdominal circumference was always situated between 10th and 90th percentile [14]. There were no structural abnormalities in this subset.

Fetal growth restriction, as expressed by an abdominal circumference below the 10th percentile during all serial measurements [14], was diagnosed in the remaining 47 pregnancies. Pregnancy-induced hypertension (PIH), defined as a diastolic blood pressure of 90 mmHg or more on two occasions, was present in 5 out of 47 women. There were no structural anomalies. Maternal age in both subsets ranged between 20 and 36 years (median 28 years).

The mean gestational age at delivery was 36.4 weeks in the FGR subset and 38.8 weeks in the normally growing subset. There was no significant difference between subsets with respect to maternal age, parity or gestational age at the time of the study. Also, there were no significant differences in umbilical or vein pH and Apgar score (at 1 min) at birth.

Fig. 2 depicts the relationship between LV IRT data and gestational age in the normal and FGR subsets. LV IRT increases with advancing gestation in both subsets. The regression equations were as follows.

For the normally growing subset:

$$\log_{10}(\mathrm{IRT}_{[\mathrm{ms}]}) = 1.44 + 0.00575\mathrm{GA}_{[\mathrm{week}]},$$

and for the FGR subset:

$$\log_{10}(\mathrm{IRT}_{[\mathrm{ms}]}) = 1.55 + 0.00576\mathrm{GA}_{[\mathrm{week}]}$$

Table 1
Characteristics of the study population

Characteristic	AGA ($n = 124$)	SGA ($n = 47$)
Maternal Age (y)	26.1 ± 2.4	27.1 ± 3.1
Parity	0.6 ± 0.7	0.8 ± 0.7
Gestation at project inclusion (week)	23.5 ± 1.6	23.7 ± 1.4
Gestation at delivery (week)	38.8 ± 1.8	36.4 ± 1.6
Birth weight (g)	3345 ± 323	2284 ± 236 ($p < 0.01$)
Umbilical artery pH	7.310 ± 0.003	7.304 ± 0.004
Umbilical vein pH	7.398 ± 0.012	7.374 ± 0.011
Apgar score at 5 min < 7	0/124	1/47

Data are represented as mean ± standard deviation or *n*.

where GA denotes gestational age (week).

The slope was the same for both regression relationships. These results were not significantly affected by fetal heart rate. At each gestational age mean LV IRT value was 29 percent longer (95% CI: 25%–31%, $p < 0.001$) in FGR compared with the normal subset.

Prior to delivery, the mean LV IRT at term was 47 ± 6 ms in the normally growing subset as opposed to 62 ± 8 ms ($p < 0.001$) in the FGR subset.

LV IRT and Doppler PI in the umbilical artery were monitored serially during 12–14 weeks prior the delivery in all fetuses. In the FGR subset there was a significant increase in Doppler umbilical artery PI about 2 weeks prior to delivery. In the same subset a significantly raised LV IRT could already be demonstrated at the start of study which was about 8–10 weeks before the increase in umbilical artery PI.

Active plasma renin concentration (7.78 ± 1.03 ng/ml) and angiotensin I concentration (4.21 ± 0.70 ng/ml) were significantly higher ($p < 0.001$) in growth-restricted newborns than in normal sized newborns (4.81 ± 1.04 ng/ml and 2.69 ± 0.44 ng/ml). The ratio growth-restricted/normal subset of mean values for renin level was 1.62 and for angiotensin level 1.56.

A significant difference ($p < 0.01$) in systolic blood pressure existed between the FGR subset (52 ± 6 mmHg) and normal subset (46 ± 4 mmHg) on the first day of life.

The mean systolic blood pressure on the fifth day of life was 76 ± 5 mmHg in the FGR subset, which was approximately 25% higher ($p < 0.01$) than in the normal subset (60 ± 6 mmHg).

4. Discussion

The present study shows that in the growth restricted fetus an increase in LV IRT occurs well before changes in the umbilical artery PI. We suggest that Doppler measurement of LV IRT could serve as an early indicator of increase in fetal cardiac afterload. It is known that, in the mammalian myocardium the main determinant of isovolumic relaxation is afterload (systolic blood pressure) [12]. Therefore, we speculate that elevated afterload could be considered the main cause of LV IRT increase in the growth restricted fetus. Earlier, fetal hypertension was demonstrated in the ovine model of intrauterine growth restriction associated with chronic maternal hyperthermia [9].

We demonstrated that active renin and angiotensin I blood levels increase in FGR fetuses. This is in agreement with Konje et al. [15] who found a significant increase in renin levels in growth restricted fetuses as well as an inverse correlation between renin level and birthweight. They also demonstrated a reduction in kidney volume and significant morphological changes at renal level in FGR fetuses. Of interest is that the growth-restricted/normal renin concentration ratio in the study of Konje et al. (1.66) was approximately the same (1.62) as in our study.

The renin-angiotensin system (RAS) is an important regulator of arterial pressure and body fluid balance during life span including intrauterine development [16]. The importance of RAS in the evolution of hypertension in animal models of fetal programming of hypertension has been demonstrated. Early administration of angiotensin converting enzyme (ACE) inhibitors prevented development of hypertension in offspring from protein restricted dams [17].

We were unable to find any significant changes in blood pH in the presence of fetal growth restriction. This may suggest that early in growth restriction, chronic hypoxia is not the leading mechanism responsible for the renin-angiotensin system activation and fetal arterial hypertension. We can speculate that the initial cause of growth restriction could be changes in transplacental amino acid transport. Significant changes in placental amino acid transport were shown in growth restricted fetuses in monochorionic twins long before the onset of the twin-to-twin perfusion syndrome [18].

Regarding the underlying mechanism responsible for the early fetal circulatory adaptation, the increase in renin-angiotensin blood levels may have a beneficial hemodynamic effect on the fetus. A direct positive inotropic effect on the myocardium of angiotensin II has been demonstrated [19]. An early rise in arterial blood pressure and subsequent improvement of placental blood perfusion could protect umbilical arterial blood flow for a certain period of time. As a result one could not detect any significant changes in umbilical artery PI in the early stages of fetal growth restriction. However, early renin-angiotensin system (RAS) activation could be essential for the programming of the arterial hypertension in later life.

Fetal growth restriction as a consequence of compromised uteroplacental oxygen and nutrients transport is an important contributor to neonatal morbidity and long term health problems [20]. Several epidemiologic studies have implicated intrauterine growth factors as risk factors for essential hypertension in later life [21]. Hypertension is at all ages associated with low birth weight. From this follows that low birth weight is associated with a higher mortality rate from ischemic heart disease [4].

Human pathological studies that have been performed in growth restricted neonates have demonstrated the absence of a compensatory increase in nephron number as well as renal size [15,22]. Animal studies have shown that the induction of growth restriction in the rat fetus leads to a decrease in renal size, nephron number, and hypertension after birth [23], which could be an important contribution to hypertension programming in later life.

It is proposed that the hypertension, reflected in LV IRT increase, as a result of RAS activation could be considered a compensatory mechanism to protect placental perfusion and provide maximal sequestration of nutrients and oxygen from

placental vessels at the initial stage of fetal growth restriction. However, once activated during intrauterine life, the renin-angiotensin system could exist in a latent-activated state and be essential for the early development of hypertension in later life. It can be concluded that in the growth restricted fetus, LV IRT may act as a sensitive indicator of elevated arterial afterload, as it increases well before a rise in umbilical Pulsatility Index. It may herald raised systolic blood pressure in the early neonatal period.

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