PhD THESIS

Programa de Doctorat en Medicina

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"FETAL PROGRAMMING OF CARDIOVASCULAR DYSFUNCTION IN INTRAUTERINE GROWTH RESTRICTION"

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A thesis submitted by Fàtima Crispi Brillas for the degree of Doctor of Medicine (Faculty of Medicine, University of Barcelona) including the mention of "European doctor" under the direction of Eduard Gratacós, Professor of Obstetrics and Gynecology in the University of Barcelona.

Signed: Fàtima Crispi Brillas

Barcelona, 2nd June 2009

Eduard Gratacós Solsona, Professor of Obstetrics and Gynecology in the University of Barcelona,

DECLARES:

That Fàtima Crispi Brillas has realized the work entitled "**Fetal programming of cardiovascular dysfunction in intrauterine growth restriction**" under my direction for the degree of Doctor of Medicine fulfilling the requisites for "European doctor" mention, and that the mentioned work is ready to be presented from the present day.

Signed: Eduard Gratacós Solsona

Barcelona, 2nd June 2009.

PRESENTATION

The present thesis has been structured following the normative for PhD thesis as a compendium of publications with "European doctor" mention (approved by the *Comisión de Doctorado de la Facultad de Medicina*, 19th April 2006). The projects included in this thesis belong to the same research line leading to four articles already published or submitted for publication in international journals:

1.Crispi F, Hernandez-Andrade E, Pelsers MAL, Plasencia W, Benavides-Serralde JA, Eixarch E, Le Noble F, Ahmed A, Glatz JFC, Nicolaides KH, Gratacós E. Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses. Am J Obstet Gynecol 2008;199:254.e1-254.e4.

State: published

Impact factor: 2.917

Quartile: 1st

2.Crispi F, Comas M, Hernandez-Andrade E, Eixarch E, Gomez O, Figueras F, Gratacós E. Does preeclampsia influence fetal cardiovascular function? Ultrasound Obstet Gynecol 2009 (in press)

State: accepted for publication

Impact factor: 2.288

Quartile: 1st

3.Hernandez-Andrade E, Crispi F, Benavides-Serralde JA, Plasencia W, Figueroa Diesel H, Eixarch E, Acosta-Rojas R, Figueras F, Nicolaides K, Gratacós E. Contribution of the myocardial performance index and aortic isthmus blood flow index to refine prediction of mortality in preterm growth restricted fetuses. Ultrasound Obstet Gynecol 2009 (in press).

State: accepted for publication

Impact factor: 2.288

Quartile: 1st

4.Crispi F, Bijnens B, Figueras F, Bartrons J, Eixarch E, LeNoble F, Ahmed A, Gratacós E. Fetal growth restriction results in remodelled and less efficient hearts in children. Lancet 2009 (submitted).

State: submitted, under review

Impact factor: 28.600

Quartile: 1st

TABLE OF CONTENTS

I. SUMMARY

Background

Fetal growth restriction (FGR), with a prevalence of 5-10% in newborns, is associated with increased cardiovascular mortality in adulthood, but the pathophysiological links of this relationship are only partially understood. The main hypothesis of this thesis was that FGR induces primary cardiac dysfunction and remodelling in utero that persists postnatally and leads to increased cardiovascular risk in adulthood.

Methods

Cardiovascular function was assessed in a cohort of FGR fetuses and correlated to the severity stages of FGR, presence of preeclampsia and also perinatal data in order to evaluate its potential utility in the clinical management of these fetuses. Finally, cardiac and vascular function was also assessed in childhood.

Results

In utero, FGR fetuses showed signs of subclinical cardiac dysfunction measured by echocardiography (increased E/A ratios and isovolumic times with normal cardiac output) from early stages. Cardiac dysfunction deteriorated further with the progression of fetal compromise, together with the appearance of biochemical signs of cell damage (increased heart-fatty acid binding protein concentrations in cord blood). Preeclampsia per se was not associated to cardiac function in FGR fetuses. Cardiac function parameters, such as ductus venosus and myocardial performance

index, were independently associated with perinatal death in preterm FGR. Therefore, a combination cardiac parameters may be useful in the clinical management of preterm FGR by stratifying the estimated probability of death. Children with FGR showed changes in cardiac shape (more globular morphology), subclinical cardiac dysfunction (increased heart rate and reduced stroke volume and myocardial peak velocities) and vascular remodelling (increased blood pressure and carotid intima-media thickness).

Conclusions

FGR present cardiovascular dysfunction in utero that persists postnatlly. These findings suggest that fetal growth restriction induces primary cardiac changes which could explain the increased predisposition to cardiovascular disease in adult life. Given its high prevalence in the general population, this might have to be taken into account in assessing cardiovascular risk factors and treatment.

II. INTRODUCTION

Fetal programming of cardiovascular disease in adulthood

Cardiovascular disease is the main cause of mortality in developed countries, with an estimated 23% of all the disease burden and over 4 million deaths yearly in Europe.[1] It is widely accepted that cardiovascular disease undergoes a long subclinical phase decades before the onset of clinical symptoms, which in a substantial proportion of individuals started from childhood.[2-3] Aside from well-described lifestyle factors and genetic predisposition, increasing evidence points to the notion that in a proportion of cases predisposition to cardiovascular disease has its origins in prenatal life.[4] Historical cohort studies[4] and animal models[5] have demonstrated associations between small size at birth and increased adult cardiovascular mortality, together with unfavorable effects on blood pressure, glucose tolerance, blood lipids and coagulation factors in both children and adults. Recent studies further confirm that this association is entirely mediated through fetal growth restriction (FGR),[6] and thus it is independent of low birth weight if this was caused by premature delivery. However, the precise mechanisms underlying of the relationship between FGR and cardiovascular disease remains elusive.[7] The main hypothesis of this thesis was that FGR induces cardiac dysfunction and remodelling in utero that persists postnatally and leads to increased cardiovascular risk in adulthood. In order to test this hypothesis, four different projects with specific aims were designed. This introduction explains and justifies each specific objective.

Fetal growth restriction and cardiac dysfunction in utero

FGR, caused by placental insufficiency, affects up to 10% of all pregnancies and is a main cause of perinatal mortality and severe morbidity.[8] The heart is a central organ in the fetal adaptive mechanisms to placental insufficiency and hipoxia. Several studies have shown an association between FGR and cardiac dysfunction in utero.[9-13] Elevated fetal levels of atrial and B-type natriuretic peptides and significant differences in echocardiographic parameters have been reported in small-for-date babies.[9-13] However the onset and progression of fetal cardiac dysfunction across stages of severity in FGR have not been established. Aside from cardiac function, it is unknown whether myocardial cell damage occurs at any stage of fetal deterioration. Cord blood troponin levels are within normal values in most fetuses with severe FGR, which suggests that cell necrosis may be uncommon.[12,14-15] However, in adults with heart failure, biomarkers of myocardial cell damage such as heart-fatty acid binding protein have been demonstrated to be more sensitive than troponins.[16-18] Additionally, high sensitivity C-reactive protein, a marker of tissue injury and inflammation, is being associated increasingly with chronic cardiac disease and damage in adults.[19-20] These cardiovascular biomarkers have not been evaluated in relation with fetal cardiac function in FGR. The first specific aim of this thesis was to characterize in utero cardiac dysfunction and eventual myocardial cell damage in a cohort FGR stratified into severity stages (**PROJECT 1**).

A potential concern in the interpretation of studies on fetal cardiovascular function in FGR is the common association with preeclampsia (PE).[21-23] Most studies on cardiovascular assessment in FGR have included without distinction pregnancies

with and without this condition.[9-13] PE is characterized by dysfunction of the maternal vascular endothelium, which leads to increased systemic vascular resistance and maternal hemodynamic changes.[24-26] Several studies have shown that PE is associated with features of endothelial dysfunction also in the fetus.[27-30] It is unknown to which extent these changes have any influence in fetal cardiovascular function. Our second aim was to evaluate whether the association with PE has any impact on cardiovascular function in preterm FGR fetuses (**PROJECT 2**).

Fetal cardiac function and prediction of perinatal outcome in FGR

Prediction of perinatal outcome is critical for clinical management in preterm FGR fetuses. As the heart is a central organ in the fetal adaptive mechanisms to placental insufficiency and hypoxia, monitoring of cardiac function has been proposed as an adjunct to current methods to predict adverse outcome and death in FGR.[31] However, suitable parameters remain to be established. In a recent large multicenter study,[32] ductus venosus emerged as a strongest predictor for poor perinatal outcome. However, its sensitivity for fetal and neonatal death is still 40 to 70 %.[32-34] Over the last years, new cardiovascular parameters such as aortic isthmus flow[35-38] and myocardial performance index[10,13,39-40] have been proposed for fetal assessment. However, no attempt has been made to estimate the potential contribution of these parameters alone or in combination with other Doppler indices as predictors of perinatal mortality. The third aim of this thesis was to evaluate the predictive value of cardiac function parameters for perinatal outcome in FGR. Moreover, the best combination of several currently available cardiovascular indices for the prediction of perinatal mortality in preterm FGR fetuses and the performance

of a clinical decision algorithm including the previously selected parameters was explored (**PROJECT 3**).

FGR and cardiovascular disease postnatally

Epidemiological evidence has long suggested a link between low birth weight and increased cardiovascular mortality in adulthood.[4] It is widely accepted that the association between FGR and cardiovascular mortality occurs through fetal programming.[7] Adaptive changes to a chronic adverse environment in a critical moment of human development lead to epigenetic changes that eventually persist into childhood and adulthood. A link between prenatal cardiovascular dysfunction and permanent cardiac changes postnatally would seem obvious, but this hypothesis has not been demonstrated and the mechanistic pathways underlying the relationship between FGR and cardiovascular risk still remain unsolved.[7] A number of studies support that it might be partially explained by fetal metabolic programming leading to diseases associated with cardiovascular disease, such as obesity, diabetes and hypertension.[7] However, it remains unclear whether FGR induces primary changes in the heart which might predispose to cardiovascular dysfunction later in life.

It has recently been reported that newborns[41] with FGR have significant changes in cardiac function parameters and natriuretic peptides, in spite of normal cardiac output. In addition, newborns with FGR have an increase in aortic intima-media thickness,[42-43] supporting the existence of preclinical atherosclerosis. Animal studies suggest that subclinical cardiovascular abnormalities in fetuses exposed to growth restriction persist into adulthood,[5] but it is unknown whether this effect occurs in humans. The fourth aim of this thesis was to evaluate the hypothesis that

adaptation to growth restriction induces persistent cardiovascular changes in children (**PROJECT 4**).

Clinical relevance of fetal cardiac programming of cardiovascular disease

Most factors leading to chronic cardiovascular disease are already present in childhood,[2-3] and the importance of early identification and intervention in pediatric cardiovascular risk factors is now well recognized.[44] FGR is not listed among the conditions presumed to increase cardiovascular risk in current consensus guidelines.[44-45] However, primary cardiac programming might be one of the causes of increased cardiovascular mortality in adults born with FGR, and, if confirmed, this may open new opportunities for monitoring and intervention in newborns and children affected with this condition. Since FGR affects 5-10% of all newborns, strategies to establish detect cardiovascular programming and assess the risk in these children might benefit thousands of children yearly.

III. METHODS

III.0. GENERAL HYPOTHESIS AND OBJECTIVE:

***Main hypothesis*:** FGR induces cardiac dysfunction and remodelling in utero that persists postnatally and leads to increased cardiovascular risk in adulthood

***Main objective*:** To assess cardiac function and structure in FGR fetuses and children

To achieve this main objective, four different projects were planned and performed as explained bellow:

SPECIFIC PROJECTS:

III.1. Project 1: FGR and cardiac dysfunction and damage in utero

***Hypothesis*:** FGR induces cardiac dysfunction and myocardial cell damage in utero

***Objective*:** To characterize cardiac dysfunction and myocardial cell damage in growth restricted fetuses

***Study design*:** prospective cohort study (three paired cohorts)

Study populations: fetuses classified as:

a/ **FGR** defined as birth weight below 10th percentile together with abnormal umbilical artery pulsatility index (>2SD) delivering or dying between 24 and 34 weeks of gestation. Sub classified into severity stages according to umbilical artery end-diastolic flow: stage 1, present; stage 2, absent; and stage 3, reversed.

b/ **Appropriate for gestational age** (AGA) fetuses delivering **at term** defined as birth weight above 10th percentile delivering after 37 weeks of gestation (paired with FGR by gestational age at ultrasound)

c/ **AGA** fetuses delivering **preterm** defined as birth weight above 10th percentile delivering between 24 and 34 weeks of gestation (paired with FGR by gestational age at cord blood collection)

Interventions:

-Signature of consent form.

-Functional echocardiography.

-Maternal and cord blood samples collection at delivery and posterior biomarkers analysis.

-Collection of perinatal data from the hospital database or by parental questionnaire.

-Data analysis

Measures:

-Functional echocardiography: ductus venosus (DV), myocardial performance index (MPI), isovolumetric contraction time (ICT), ejection time (ET), isovolumetric relaxation time (IRT), left and right E/A ratios, cardiac output, cardiac index.

-Biomarkers of cardiac function: B-type natriuretic peptide (BNP).

-Biomarkers of myocardial cell damage: troponin I, heart-fatty acid binding protein (H-FABP), high sensitivity C-reactive protein (hsCPR).

-Perinatal data: prenatal (pregnancy complications, gestational age ultrasound, estimated fetal weight, prenatal feto-placental Doppler, intrauterine mortality), perinatal (gestational age at delivery mode and indication of delivery, gender, birth weight, birth weight percentile, Apgar score, cord arterial and venous pH) and neonatal (days in neonatal intensive care, mechanical ventilation, need for O_2, neonatal morbidity and mortality) data.

***Predictive variables*:** presence of FGR, stages of severity.

***Outcome variables*:** presence of cardiac dysfunction (as measured by DV, MPI, ICT, ET, IRT, left and right E/A ratios, cardiac output, cardiac index, cord blood levels of BNP) and myocardial cell damage (as measured by cord blood levels of troponin I, H-FABP, hsCPR).

III.2. Project 2: Potential impact of PE on fetal cardiac function in FGR

***Hypothesis*:** Maternal preeclamptic symptoms affect fetal cardiac function in FGR

***Objective*:** To assess cardiac function in FGR fetuses with and without PE

***Study design*:** prospective cohort study (four paired cohorts)

Study populations: fetuses classified as:

a/ **Normotensive FGR:** defined as birth weight below 10th percentile together with abnormal umbilical artery pulsatility index (>2SD) delivering or dying between 24 and 34 weeks of gestation; together with maternal normotension.

b/ **PE + FGR:** defined as in the previous group, together with PE defined as maternal hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg measured in two occasions 4 hours apart) and proteinuria (proteinuria ≥ 300 mg in 24 hours urine)

c/ **AGA** fetuses delivering **at term** defined as birth weight above 10th percentile delivering after 37 weeks of gestation (paired with FGR by gestational age at ultrasound)

d/ **AGA** fetuses delivering **preterm** defined as birth weight above 10th percentile delivering between 24 and 34 weeks of gestation (paired with FGR by gestational age at cord blood collection)

Interventions:

-Signature of consent form.

-Functional echocardiography.

-Maternal and cord blood samples collection at delivery and posterior biomarkers analysis.

-Collection of perinatal data from the hospital database or by parental questionnaire.

-Data analysis

Measures:

-Functional echocardiography: DV, aortic isthmus flow index (IFI), MPI, left and right E/A ratios, cardiac output.

-Biomarkers of cardiac function: BNP.

-Perinatal data: prenatal (pregnancy complications, gestational age ultrasound, estimated fetal weight, prenatal feto-placental Doppler, intrauterine mortality), perinatal (gestational age at delivery mode and indication of delivery, gender, birth weight, birth weight percentile, Apgar score, cord arterial and venous pH) and neonatal (days in neonatal intensive care, mechanical ventilation, need for O_2, neonatal morbidity and mortality) data.

***Predictive variables*:** presence of PE.

***Outcome variables*:** presence of cardiac dysfunction (as measured by DV, IFI, MPI, left and right E/A ratios, cardiac output, cardiac index, cord blood levels of BNP).

III.3. Project 3: Cardiovascular indices in the prediction of mortality in FGR

Hypothesis: Fetal cardiac function parameters are useful to predict perinatal outcome in FGR

Objective: To evaluate the predictive value of cardiac function parameters for perinatal outcome in FGR

Study design**:** prospective cohort study (one cohort).

Study populations: FGR defined as birth weight below 10th percentile together with abnormal umbilical artery pulsatility index (>2SD) delivering or dying between 24 and 34 weeks of gestation.

Interventions:

-Signature of consent form.

-Functional echocardiography.

-Collection of perinatal data from the hospital database or by parental questionnaire.

-Data analysis

Measures:

-Functional echocardiography: DV, MPI, IFI.

-Perinatal data: prenatal (pregnancy complications, gestational age ultrasound, estimated fetal weight, prenatal feto-placental Doppler, intrauterine mortality), perinatal (gestational age at delivery mode and indication of delivery, gender, birth weight, birth weight percentile, Apgar score, cord arterial and venous pH) and neonatal (days in neonatal intensive care, mechanical ventilation, need for O_2, neonatal morbidity and mortality) data.

Predictive variables**:** DV, MPI, IFI.

Outcome variables**:** perinatal mortality (defined as intrauterine + neonatal mortality).

III.4. Project 4: FGR and cardiovascular remodelling in childhood

Hypothesis: Cardiac dysfunction in FGR persists postnatally and induces cardiac remodelling

Objective: To evaluate cardiac shape and function in children with FGR

***Study design*:** prospective cohort study (three paired cohorts)

Study populations: one to five years-old children classified as:

a/ **mild FGR** children defined as birth weight below 10th percentile together with normal umbilical artery pulsatility index (<2SD)

b/ **severe FGR** children defined as birth weight below 10th percentile together with abnormal umbilical artery pulsatility index (>2SD)

c/ **AGA** children defined as birth weight above 10th percentile (paired with FGR by gender and gestational age at delivery)

Interventions:

-Signature of parental consent form.

-Collection of epidemiological and perinatal data from the hospital database or by parental questionnaire.

-Echocardiography.

-Vascular assessment (blood pressure and carotid wall thickness measurement).

-Blood samples collection and posterior biomarkers analysis.

-Data analysis

Measures:

-Epidemiological data: maternal (age, body mass index, smoking and socioeconomic status, parity, ethnicity, history), paternal (age, body mass index, smoking and socioeconomic status) and dietary patterns (questionnaire by parents).

-Perinatal data: prenatal (pregnancy complications, exposure to glucocorticoids, prenatal Doppler and echocardiography, gestational age at delivery, mode and indication of delivery) and neonatal (gender, birth weight, birth weight percentile, Apgar score, cord arterial and venous pH, base excess, pO_2, days in neonatal intensive care, mechanical ventilation, need for O_2, neonatal morbidity, breastfeeding) data.

-Echocardiography: left and right ventricle morphometry (base-to-apex length and basal diameter), septal and left posterior wall thickness, left ejection fraction, cardiac output, cardiac index, MPI, left and right E/A ratios, E deceleration time, mitral and tricuspid motion by M-mode, myocardial peak velocities by tissue Doppler imaging, E/E' ratios.

-Vascular assessment: systolic and diastolic blood pressure, carotid intima-media thickness (cIMT), circumferential wall stress.

-Biomarkers of cardiac function: BNP.

-Others cardiovascular biomarkers: C-reactive protein, glucose, triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL).

***Predictive variables*:** presence of FGR, stages of severity.

***Outcome variables*:** presence of cardiac remodelling (as measured by left and right ventricle morphometry), cardiac dysfunction (as measured by left ejection fraction, cardiac output, cardiac index, MPI, left and right E/A ratios, E deceleration time, mitral and tricuspid motion by M-mode, myocardial peak velocities by tissue Doppler imaging, E/E' ratios, BNP) and vascular impairment (as measured by blood pressure and cIMT).

IV. RESULTS

IV.1. Project 1: FGR and cardiac dysfunction and damage in utero

The results of this project have been published in an international journal[46] and have been presented at the 28th Annual Meeting of The Society for Maternal-Fetal Medicine the 2nd February 2008 in Dallas (USA) (oral communication: *Crispi F, Hernandez-Andrade E, Pelsers MM, Benavides-Serralde JA, Eixarch E, Acosta J, Gratacós E. Biomarkers for cardiac dysfunction and cell damage across different stages of hemodynamic deterioration in intrauterine growth restricted fetuses*) receiving the award of research excellence of The Society of Maternal-Fetal Medicine.

Results

Study populations

The study included 80 term AGA, 40 preterm AGA and 81 FGR cases (26 at stage 1 of severity, 28 at stage 2 and 27 at stage 3). FGR fetuses from severity stages 2 and 3 showed lower 5-minute Apgar scores and umbilical artery pH values and higher rates of adverse perinatal outcome, compared with AGA fetuses.

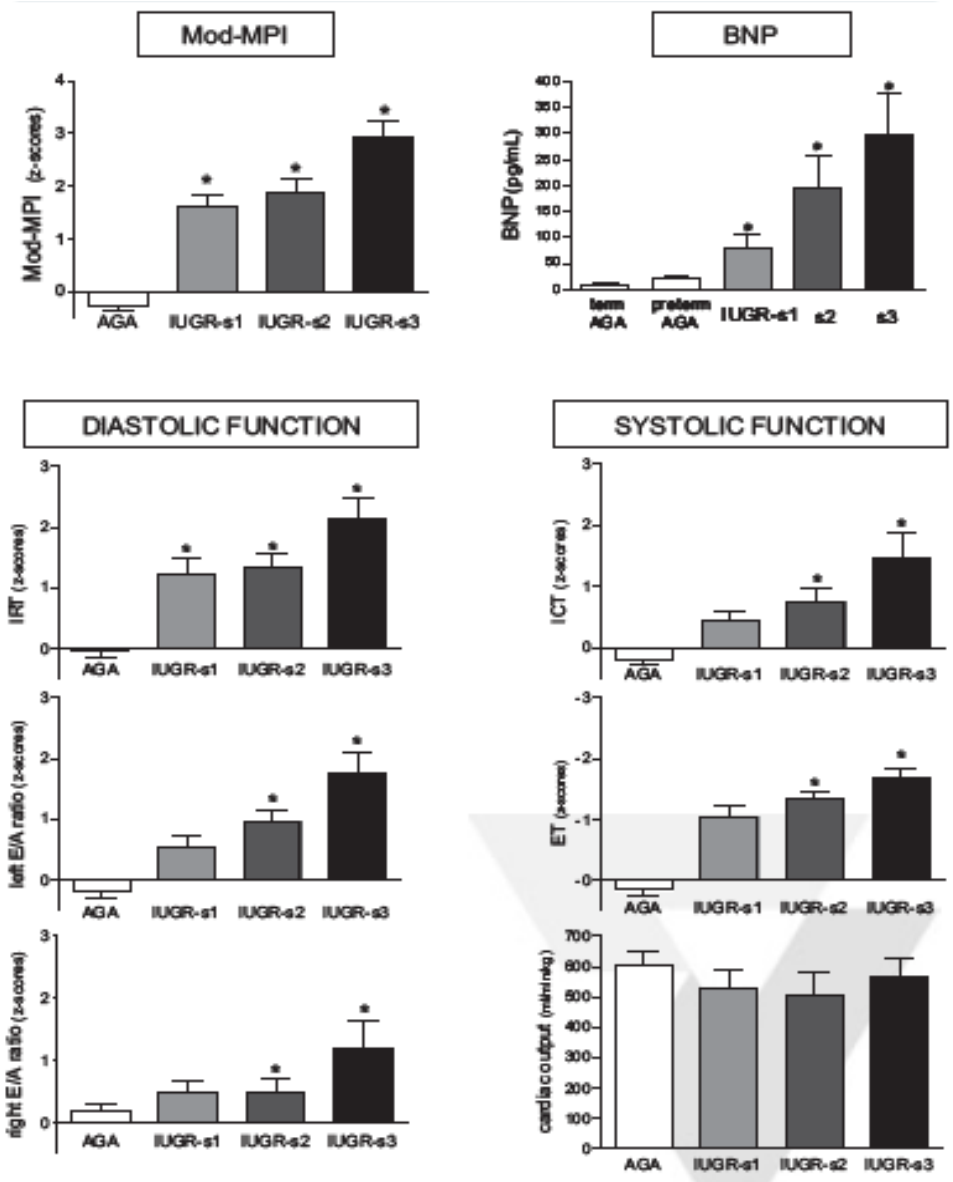
Cardiac function

Results are shown in Figure 1. MPI was significantly higher in stage 1 and showed a progressive increase through further stages of deterioration. All time periods that were used for calculation of the MPI (ICT, ET and IRT) were significantly different in FGR fetuses. E/A values in both atrioventricular valves were significantly higher from

stage 2 onwards. Cardiac output values were similar in control subjects and in FGR fetuses at all severity stages. BNP levels were significantly higher in fetuses at stage 1 and increased further across the stages of severity.

The statistical analysis for trend showed a significant tendency to different results with increasing stages of FGR for all parameters with the exception of cardiac output

Figure 1. Cardiac function parameters in AGA and FGR fetuses at different stages of severity



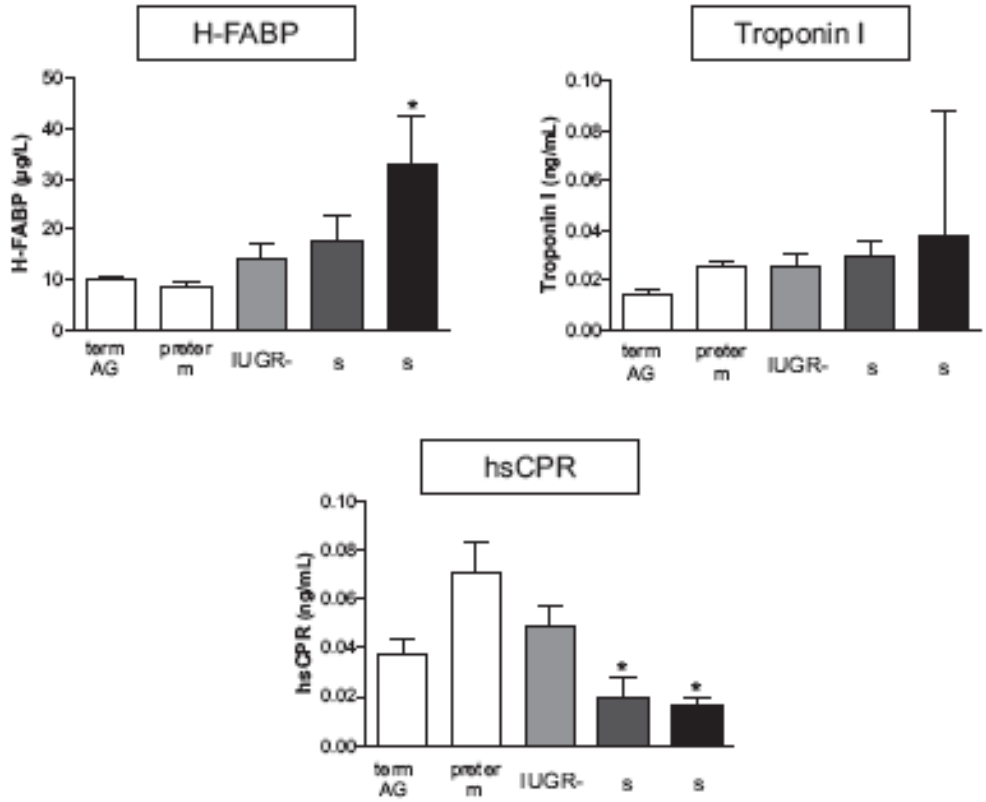
Data are given as mean ± SEM. The *asterisk* denotes a probability value of <.01, compared with AGA. *ET*, ejection time; *ICT*, isovolumetric contraction time; *IRT*, isovolumetric relaxation time; *s1*, stage 1; *s2*, stage 2; *s3*, stage 3.

Myocardial cell damage

Results are shown in Figure 2. H-FABP values were significantly increased in FGR fetuses at stage 3 together with a significant linear increment across severity stages. Troponin I values were similar in AGA and in FGR fetuses. Hs-CPR levels were significantly lower in FGR fetuses at stages 2-3, compared with preterm AGA.

Moreover, all biomarkers with the exception of troponin I showed a significant tendency to different results with increasing stages of FGR.

Figure 2. Myocardial cell damage markers in AGA and FGR fetuses at different stages of severity



Data are given as mean ± SEM. The *asterisk* denotes a probability value of <.01, compared with preterm AGA. *s1*, stage 1; *s2*, stage 2; *s3*, stage 3.

Association to perinatal death

MPI and E/A ratios and cord blood BNP, H-FABP and troponin I were significantly increased in FGR fetuses who died, in comparison with survivors (survivors vs. deaths: MPI z-scores median 1.7 (interquartile range 3) vs 2.5 (2), P=0.027; left E/A z-scores 0.8 (2.2) vs 2.4 (2.2), P=0.045; right E/A z-scores 1 (2.1) vs. 2.3 (5.2), P=0.002; BNP (pg/mL) 64 (127) vs 350 (456), P=0.029; H-FABP (µg/L) 11 (11) vs 23 (103) P<0.001; TnI (ng/mL) 0.02 (0.02) vs 0.07 (1.14) P=0.002).

Conclusions

FGR fetuses showed signs of cardiac dysfunction from early stages. Cardiac dysfunction deteriorates further with the progression of fetal compromise, together with the appearance of biochemical signs of cell damage.

IV.2. Project 2: Potential impact of PE on fetal cardiac function in FGR

The results of this project have been accepted for publication in an international journal[47] and have been presented at the 17th World Congress on Ultrasound on Obstetrics and Gynecology (8th October 2007, Firenze, Italy) (oral communication: *Rabanal A, Crispi F, Hernandez-Andrade E, Benavides-Serralde JA, Gomez O, Comas M, Figueras F, Gratacós E. Evaluation of the impact of the presence or absence of preeclampsia on fetal cardiac function in intrauterine growth restricted fetuses).* And it has also been presented in a national congress *(F Crispi, E Hernandez-Andrade, E Eixarch, E Gratacós. Progresión de marcadores de disfunción cardíaca y daño miocárdico en sangre de cordón de fetos CIR. XXV Congreso Nacional de la Sección de Ecografía de la Sociedad Española de Ginecología y Obstetricia, Murcia, 2 de octubre del 2008*).

Results

Study populations

The study included 31 normotensive FGR, 31 FGR+PE and 120 controls AGA fetuses (80 term AGA and 40 preterm AGA). All perinatal and Doppler parameters were similar among FGR and FGR+PE cases. A non-significant trend to higher perinatal mortality was observed in FGR as compared with FGR+PE.

Cardiac function

Results are shown in Figure 3 and 4. All measurements (DV, IFI, right and left E/A, and MPI) with the exception of cardiac output were significantly different in FGR

fetuses (with or without PE) as compared to term AGA. However, there were no differences in FGR as compared with FGR+PE.

BNP levels were significantly higher in FGR with or without PE as compared to AGA. However, BNP concentrations were similar among FGR cases with or without PE.

Figure 3. Echocardiographic results in AGA and FGR fetuses with and without PE

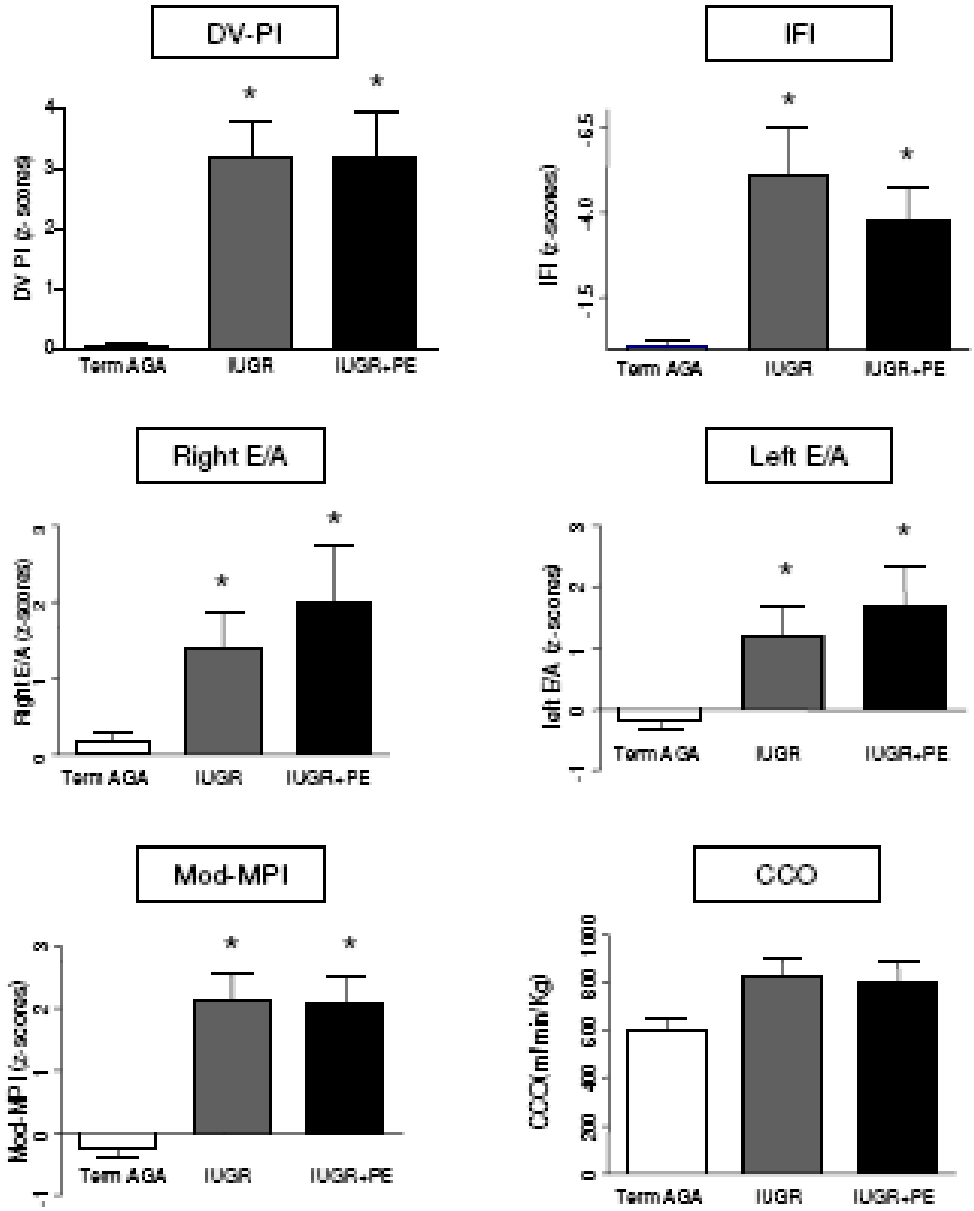
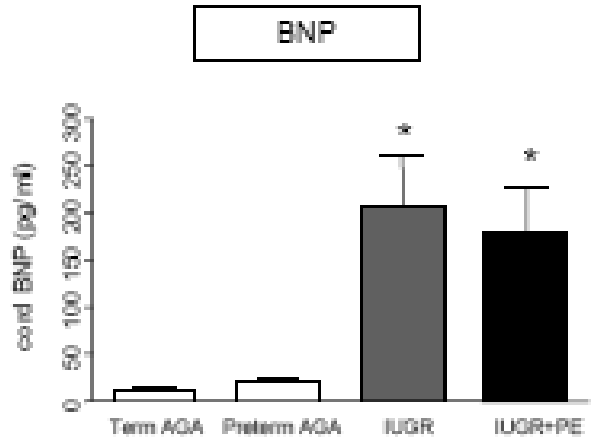


Figure 4. Cord blood BNP results in AGA and FGR fetuses with and without PE



Conclusions

FGR fetuses showed echocardiographic and biochemical signs of cardiac dysfunction. PE per se does not influence cardiac function in FGR fetuses.

IV.3. Project 3: Cardiovascular indices in the prediction of mortality in FGR

The results of this project have been accepted for publication in an international journal[48] and have been presented at the 18th World Congress on Ultrasound on Obstetrics and Gynecology (27th August 2008, Chicago, USA) (oral poster: *Crispi F, Hernandez-Andrade E, Benavides-Serralde JA, Plasencia W, Eixarch E, Acosta-Rojas R, Figueras F, Nicolaides KH, Gratacós E. Independent and combined contribution of cardiovascular Doppler parameters to short-term prediction of mortality in severe FGR. Ultrasound Obstet Gynecol 2008;32:358)* receiving the award at the best oral poster presentation. It has also been presented in a national congress (*F Crispi, E Hernandez-Andrade, E Eixarch, F Figueras, E Gratacós. Contribución de parámetros cardiovasculares en la predicción de mortalidad de fetos CIR severos (OP). XXV Congreso Nacional de la Sección de Ecografía de la Sociedad Española de Ginecología y Obstetricia. Murcia. 2 de octubre del 2008*) receiving the award Prof. Bonilla-Musoles for the best oral communication.

Results

Study population

A total of 97 preterm FGR cases were included. The median gestational age at delivery was 30 weeks and the median birth weight 956g. The overall perinatal mortality was 22% including 8 intrauterine and 14 neonatal deaths.

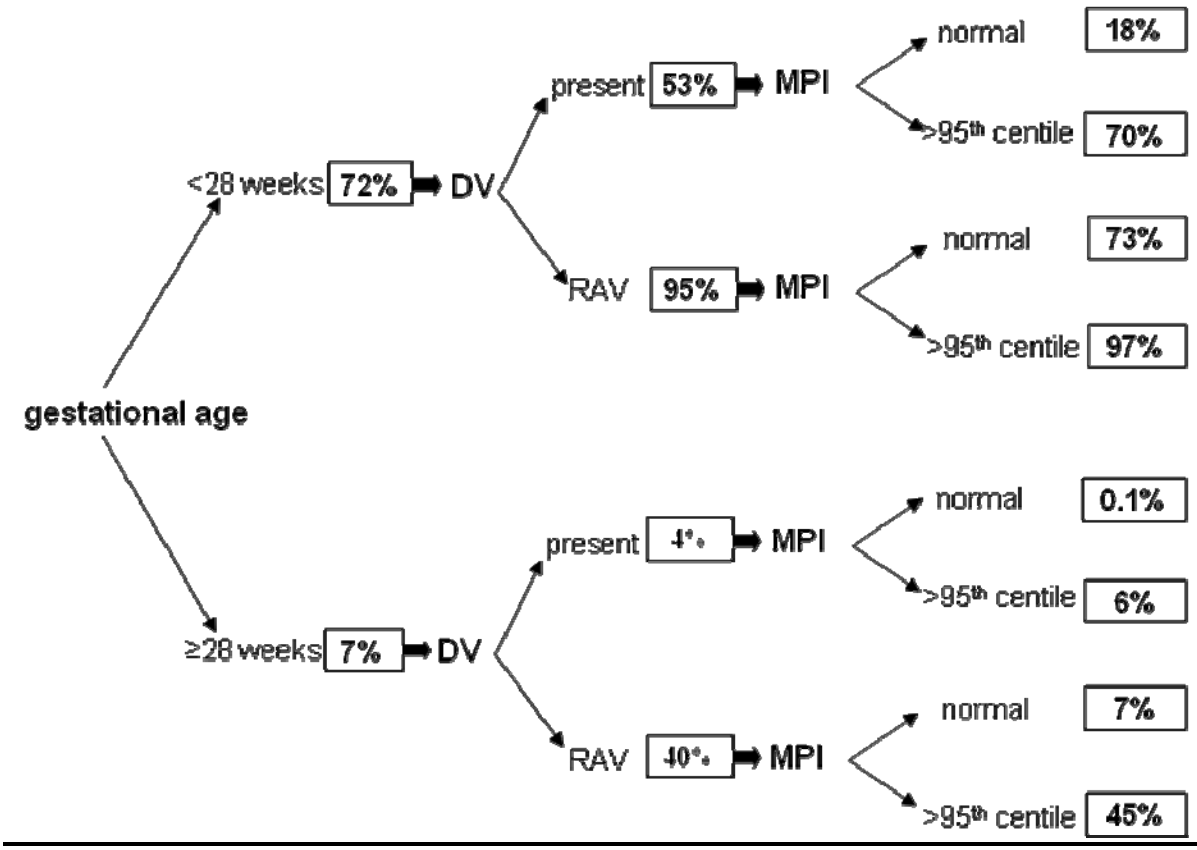
Prediction of perinatal mortality

Univariate analysis demonstrated that all Doppler indices (DV-PI, $P<0.0001$; UA-PI, $P<0.0001$; MPI, $P<0.0001$; IFI, $P<0.0001$) with the exception of MCA-PI ($P=0.068$) were significantly associated with perinatal mortality. Multivariate analysis identified

UA-PI (estimated odds ratio = 3.01, *P*=0.02), DV-PI (estimated odds ratio = 3.69, *P*=0.008) and MPI (estimated odds ratio = 1.39, *P*=0.02) as statistically significant independent predictors for perinatal mortality. IFI did not show any significant contribution to the explanation of perinatal mortality.

When gestational age was included in the model and variables were dichotomized to normal/abnormal, only gestational age (below 28 weeks), DV atrial flow (RAV) and MPI (abnormal) significantly and independently accounted for perinatal mortality. According to this model, risk was stratified for each combination of predictive variables (Figure 5) and a simplified score was modelled.

Figure 5. Estimated probability of death in FGR fetuses.



Conclusions

MPI is an independent predictor of perinatal death in preterm FGR with accuracy similar to DV. A combination of DV with MPI may better stratify the estimated probability of death. IFI does not add to the prediction of perinatal death when used in combination with DV.

IV.4. Project 4: FGR and cardiovascular remodelling in childhood

The results of this project have been submitted for publication in an international journal[49].

Results

Study populations

The study included 80 children with FGR (40 mild and 40 severe FGR) and 120 controls AGA (80 term and 40 preterm AGA). FGR children had higher occurrence of pregnancy complications, worse prenatal Doppler ultrasound findings, lower birth weight percentile, umbilical artery pH and longer admittance in neonatal intensive care unit. At the time of FGR children showed a linear tendency to lower height and weight values with similar results on body mass index as compared to AGA. Nutritional parameters and lipid/glucose profile showed similar values among all study groups.

Cardiac morphometry

Results are shown in Figure 6. Cardiac shape was significantly altered, with the left and right longitudinal-transverse ratios significantly decreased in mild and severe FGR children. Interventricular septum and left posterior wall thickness showed a significant linear trend to decreased values across FGR severity groups.

Cardiac function

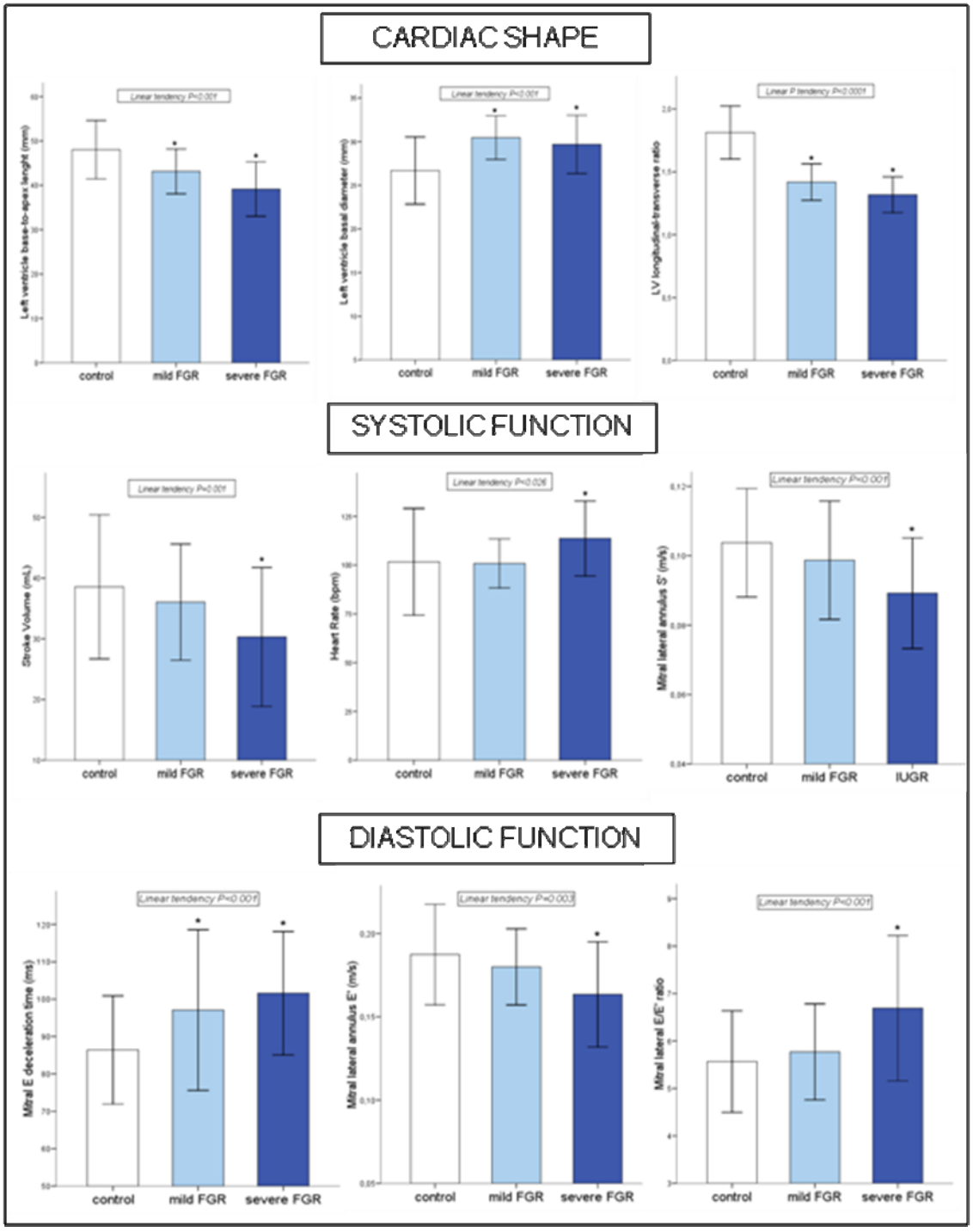
Results are shown in Figure 6.

While cardiac index, left ejection fraction and BNP, were similar among the study groups, stroke volume was significantly changed, compensated by a significantly increased heart rate to maintain output in severe FGR children, with a significant tendency across the FGR severity groups.

Systolic mitral and tricuspid ring displacements were significantly decreased in mild and severe FGR cases as compared with AGA. Severe FGR children showed significantly lower longitudinal S' in mitral lateral, mitral septal and tricuspid annulus as compared to AGA, with a significant linear tendency to lower values across FGR severity stages.

While mitral and tricuspid E/A ratios and IVRT were similar among study groups, mitral E deceleration time was significantly increased in mild and severe FGR with respect to controls. Tricuspid E deceleration time was significantly increased in severe FGR children, although there was a significant linear tendency across severity groups. Severe FGR children showed significantly lower longitudinal E' in mitral lateral, mitral septal and tricuspid annulus as compared to AGA with a significant linear tendency to lower values across FGR severity stages. Mitral lateral and septal E/E' ratios were significantly higher in severe FGR as compared to AGA, with a significant linear trend to higher values across severity stages.

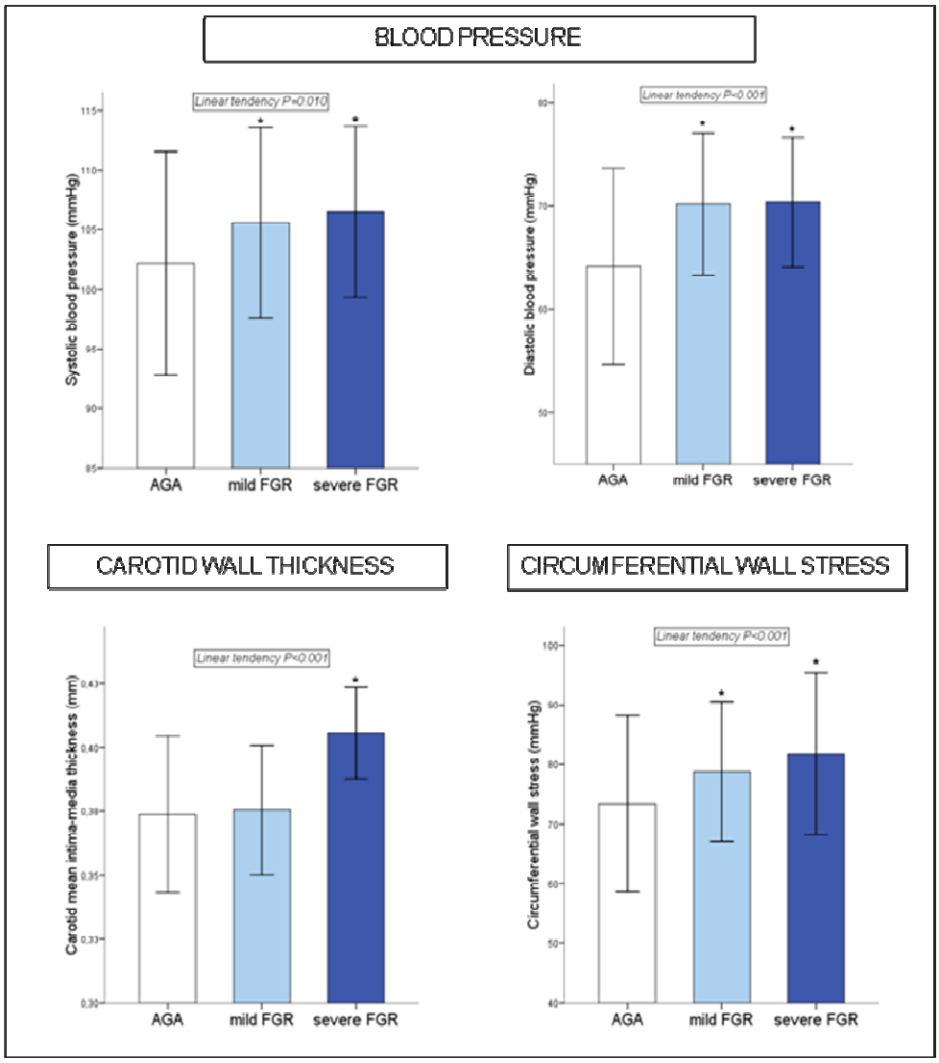
Figure 6. Cardiac results in controls and FGR children.



Vascular assessment

Systolic and diastolic blood pressure were significantly higher in mild and severe FGR groups, with a linear tendency to higher values in more severe forms of FGR. cIMT was significantly increased in severe FGR children, with a significant linear trend across severity stages. Circumferential wall stress values were significantly higher both in mild and severe FGR groups.

Figure 7. Vascular results in controls and FGR children



Conclusions

Children with FGR show changes in cardiac shape, subclinical cardiac dysfunction and vascular remodelling. For most parameters analyzed, the associations increased linearly with the severity of growth restriction.

V. DISCUSSION

This work provides evidence to support the concept that FGR induces cardiac dysfunction and remodelling in utero that persists postnatally. The results showed that cardiac dysfunction is present in utero from early stages and further deteriorates with the progression of fetal compromise, together with the appearance of myocardial cell damage in FGR. These changes seem not to be influenced by PE and may help to improve clinical management of FGR by estimating their probability of death. Finally, children with FGR showed changes in cardiac shape, subclinical cardiac dysfunction and vascular remodelling. These findings support the existence of direct cardiac programming in FGR and suggest a new mechanistic pathway for the association between fetal growth and cardiovascular disease.

V.1. Project 1: FGR and cardiac dysfunction and damage in utero

Cardiac dysfunction

The results provide evidence that subclinical cardiac dysfunction is an early and progressive event in severe FGR. Echocardiographic parameters and cord blood levels of BNP indicate that cardiac dysfunction increased progressively across the stages of fetal compromise.

MPI has been reported previously to be increased in FGR fetuses.[13,39,50-51] In this study we further described that MPI was elevated in fetuses at initial stages of hemodynamic compromise and progressed further with fetal deterioration. The progressive increment in MPI values was at the expense of all time periods that were involved in the calculation of this index, which suggests the existence of both systolic and diastolic subclinical dysfunction.

E/A ratio evaluates ventricular filling during the diastole. Fetuses with overt heart failure have been reported to have increased E/A ratios.[52] In FGR, earlier studies described unchanged or reduced E/A values,[9,11,53] but most recent studies found the ratios to be increased significantly with respect to control subjects.[10,12] We also observed a considerable increase in E/A values in fetuses with FGR in both atrioventricular valves, which was present from stage 2 and increased further with fetal Doppler deterioration. Increased E/A values were at the expense of reduced peak velocity in the A wave (data not shown). Fetal hypoxia is associated with a reduced diastolic compliance in the right ventricle, thus resulting in higher pressures during atrial contraction. The reduction in peak A wave velocity is consistent with the reduction/reversion of flow velocity occurring during atrial contration in venous vessels, such as ductus venosus or inferior cava vein, in hypoxic fetuses.

Finally, this study confirmed previous observations that indicated that normalized **cardiac output** is maintained within normal values, even in most severe clinical stages of FGR.[10,54]

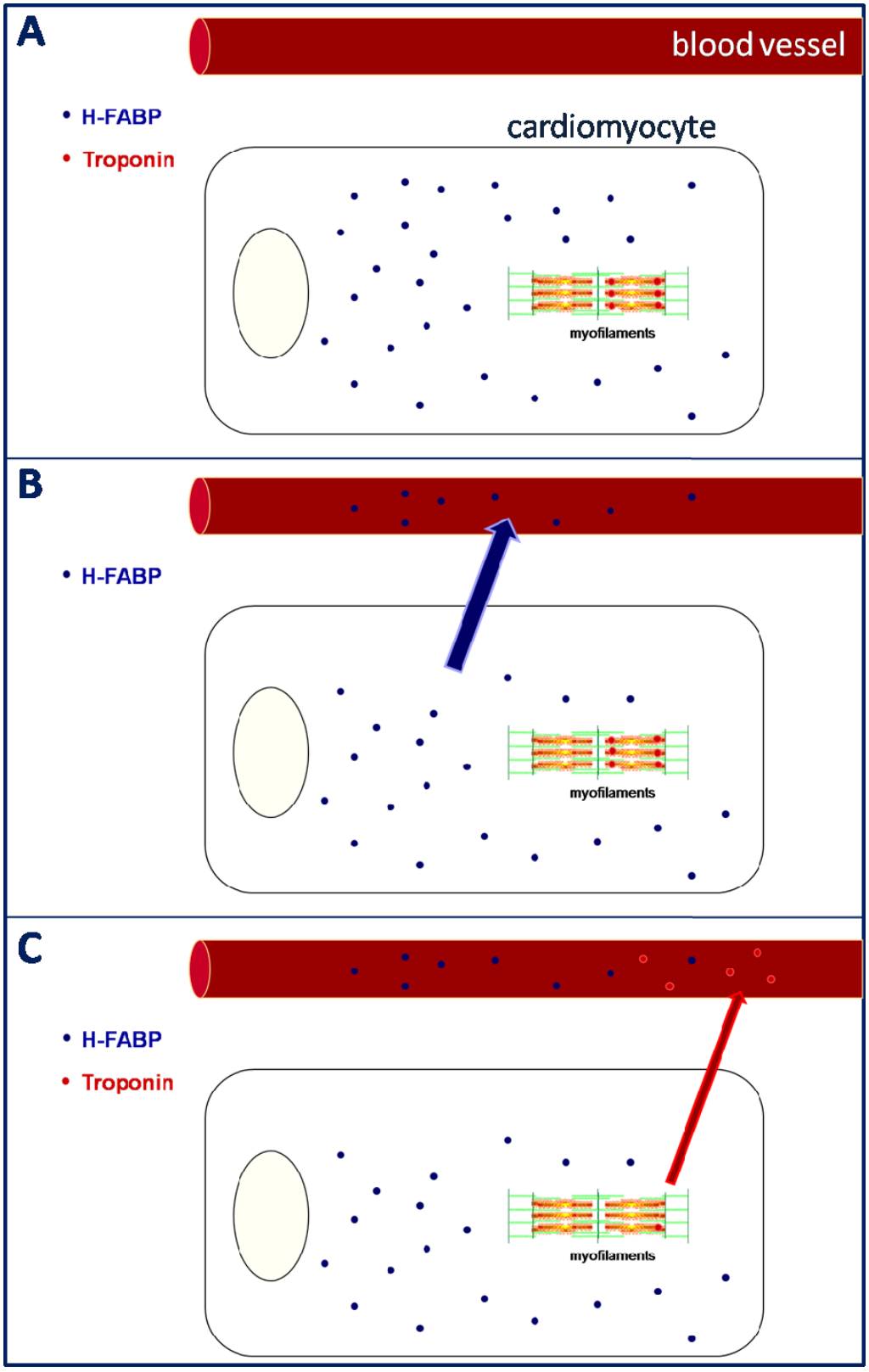
The progressive increase of **BNP** cord blood levels in FGR fetuses was consistent with that observed for echocardiographic parameters. BNP is a natriuretic peptide synthetized directly by myocardial cells as a response of wall tension or hipoxia. In adults, BNP is a gold standard biomarker for heart failure;[55] serum levels are elevated in early stages of subclinical diastolic dysfunction and increase in proportion to severity.[55] Our data in human fetuses with FGR are in agreement with those of Girsen et al.[10] who demonstrated that NT-pro-BNP was elevated in fetuses with growth restriction and increased across fetoplacental Doppler stages of fetal compromise. In another study, Leipala et al.[41] showed increased levels of BNP in

preterm FGR neonates during the first days of life, which points to a persistence of cardiac dysfunction postnatally.

Myocardial cell damage

The integrated evaluation of myocardial cell and tissue damage biomarkers offers new information for the understanding of fetal cardiac disease in FGR. Advanced fetal Doppler deterioration was associated with a significant increase in **H-FABP** levels, which supports the existence of myocardial cell damage, but to such a small extent that it could not be detected by cord blood levels of troponin I. H-FABP cord blood levels showed a significant linear increase across stages and were increased significantly in fetuses with reverse flow in the umbilical artery, which suggests the presence of myocardial cell damage in advanced stages of fetal deterioration. H-FABP is a cytosolic protein involved in the transport of fatty acids with a small size which results in early release into circulation in the presence of myocardial damage that confers a high sensitivity to detect myocardial cell damage.[18] It has proved not only to be an excellent marker for the early detection of cardiac injury in acute coronary syndromes but also showed to be sensitive enough for the detection of chronic minor myocardial injury in heart failure.[16,17] On the other hand, **troponins** are structural proteins attached to myofilaments that have been proved to be a well-established marker for myocardial infarction. However, 94% of cardiac troponin is bound to myofibrillar structures, and it has first to dissociate from its matrix before it is released (Figure 8). This explains a time delay for raised levels in plasma and a lower sensitivity to subtle cell damage, compared with H-FABP.[18] Consistent with this notion, the observed elevation of H-FABP in this study occurred in the absence of

Figure 8. A) H-FABP are cytosolic proteins and troponins structural proteins attached to myofilaments. B) H-FABP are rapidly released to circulation in the presence of cell damage. C) Troponins have first to dissociate from their matrix before they are released to circulation. This explains a time delay for raised levels in plasma and a lower sensitivity to subtle cell damage of troponins as compared to H-FABP.



significant changes in mean troponin levels. The latter finding is in line with previous studies that measured troponin in FGR.[14,15,51] It must be noted that, despite of the absence of overall differences, this and previous studies have observed individual cases of severe fetal growth restriction with high cord blood troponin values,[14,15,51] which suggests that extended cell damage may occur in very advanced stages of the disease. In this study, fetuses who died had significantly higher levels of troponin, compared with survivors.

Finally, we could not demonstrate any increase in **hsCPR** cord blood levels in severe FGR fetuses, compared with preterm AGA. On the contrary, there was a significant decrease of cord blood hsCPR in FGR stages 2 and 3, compared with preterm AGA, but not with term AGA fetuses. These differences could be explained by an abnormal increase in hsCPR levels in preterm AGA because of the accidental inclusion of cases with subclinical prenatal infection. Alternatively, it could reflect a true decrease in hsCPR levels in most severe forms of FGR that we cannot explain. HsCPR is a well-established acute marker for tissue injury, and the lack of elevation in severe FGR fetuses is consistent with the results that have been observed for troponin. On the other hand, hsCPR has also been described as a marker of chronic inflammation that leads to cardiovascular disease.[19,20] Thus, our data do not support the implication of this pathophysiologic mechanism in fetal cardiac disease that is related to growth restriction. The results in hcPCR are in disagreement with a recent study that described an increase in hsCPR levels in near-term small-for-gestational age fetuses.[56] Although the population is not comparable with that of the present study in terms of gestational age or in severity, we cannot find an explanation for the differences between both studies.

Association with perinatal death

The association between the risk of adverse outcome or perinatal death and the presence of abnormal echocardiographic parameters has been reported previously.[31,32] In this study, we confirm previous reports and further describe that those fetuses who died in utero or postnatally had significantly increased levels of H-FABP and troponin.[14] Ultrasonographic assessment of cardiac function could be integrated into clinical practice to improve the short-term prediction of fetal death. This information is essential to guide clinical decisions of fetuses with severe growth restriction, but no single Doppler ultrasound parameter has been demonstrated to have sufficient predictive value.[31,32,57] In the present study, MPI and E/A were higher in fetuses who died, but their predictive value for perinatal death in FGR remains to be evaluated in further studies (project 3).

Limitations

This study has several limitations. First, there was an elapsed period between the last ultrasound measurement and cord blood sampling. Although this did not exceed 48 hours, we acknowledge that, in a small number of cases, the severity stratification based on ultrasound parameters might have changed at the time of blood sampling. Second, sufficient cord blood for analysis could not be retrieved in approximately 20% of cases because of the inherent difficulty in obtaining samples from extremely preterm and small FGR fetuses. Furthermore, cord blood was not available in all intrauterine deaths. This may have biased the results by attenuating the differences on the levels of biomarkers between control subjects and FGR fetuses. Third, most biomarkers that were evaluated in this study are not fully cardio specific. This may raise concern for the interpretation of H-FABP data. This protein is expressed

abundantly in cardiomyocytes, but to a lesser extent also in skeletal muscle, distal tubular cells of the kidney, specific parts of the brain, lactating mammary glands, and placenta.[17,58] Although we postulate that cardiac damage is the most plausible reason for the observed increase in this study, we acknowledge that this concept cannot be demonstrated completely. Although the placenta can also produce HFABP, reduced maternal levels in FGR pregnancies support that placental production is unlikely to account for the observed increases in fetal blood. Future studies in animal models might help to clarify the origin of H-FABP in growth restricted fetuses. Finally, it is unknown whether the association with maternal preeclamptic symptoms has any effect on fetal cardiac function. This potential confounding effect is being investigated in further studies (project 2).

Conclusions

In summary, this study provides evidence that cardiac dysfunction is an early event in FGR and that its magnitude increases in proportion to the severity of the fetal condition. Thus, subclinical cardiac dysfunction is present in fetuses with FGR and mild degrees of Doppler deterioration, which currently is considered to have an overall good long-term outcome.[8,31,32] The data further suggest that advanced stages of fetal deterioration are associated with myocardial cell damage. It is tempting to speculate that the degree and duration of cardiac dysfunction and damage might be associated with distinct effects on fetal programming of cardiovascular function and disease in adulthood. The impact in long-term cardiovascular outcome of the changes described in this project will be established in long-term follow-up studies (project 4).

V.2. Project 2: Potential impact of PE on fetal cardiac function in FGR

This study provides evidence that FGR fetuses with and without PE had a similar degree of cardiovascular dysfunction. Since the study groups were comparable in terms of the degree of growth restriction and Doppler deterioration, the data support the concept that PE per se does not have an influence in the presence of cardiovascular dysfunction in FGR.

Cardiac function in FGR

The findings confirm previous studies reporting clear evidence of the existence of abnormal cardiovascular function in FGR. As expected and previously described,[33,46,59] DV was significantly increased in both study groups. DV reflects to a great extent myocardial impaired relaxation[59-61] and it is the strongest predictor of perinatal mortality and morbidity severe FGR.[32,33,59] Aside from DV, several ultrasound measurements, including IFI, E/A ratios and MPI, and cord blood BNP levels were abnormal in both study groups. Previous clinical series have reported that FGR fetuses with aortic isthmus reversed diastolic flow have a deterioration of cardiac function[51] and a poorer perinatal outcome.[35,37,51] Aortic isthmus has a dynamic role in connecting the right and left circulatory systems of the fetus and has been proposed as a potential monitoring tool for FGR fetuses.[35,37,51] Left and right E/A ratios have recently been described to be significantly increased in FGR with respect to controls,[10,12,46] supporting the existence of abnormal ventricular filling during diastole. MPI, a combined index of systolic and diastolic function, has been described to be elevated in FGR fetuses.[13,39,46] BNP is a gold standard marker for heart failure in adults[55] and children[62] that has been demonstrated to be increased in FGR from

early stages of fetal deterioration.[10,46] Of interest, the study confirmed previous observations indicating that cardiac output is maintained within normal values in FGR.[10,46,54]

Doppler in FGR with and without PE

Concerning umbilical artery and middle cerebral artery Doppler indices, similar differences with respect to controls were observed between study groups. Several studies have evaluated feto-placental Doppler parameters in FGR fetuses with and without PE. Harrington et al.[63,64] showed a similar pattern of changes in UA and MCA in preterm small for gestational age fetuses with or without PE. More recently, Mari et al.[65] showed that FGR fetuses without PE undergo a series of well-defined Doppler changes until fetal deterioration occurs or the fetus is delivered because non-reassuring testing. On the contrary, FGR fetuses with PE were often delivered for maternal indication before the full range of Doppler changes occur.[65]

Perinatal outcome in FGR with and without PE

Although perinatal outcome was similar in FGR with or without PE, a non significant trend to higher perinatal mortality in normotensive FGR fetuses was observed in this study. This finding is consistent with a previous report by Piper et al.[66] including 1012 preterm small for gestational age and showing that perinatal mortality was significantly higher in the normotensive than in the hypertensive group, even after controlling for potentially confounding factors. Recently, Mari et al.[65] also described an increased rate of fetal demise in FGR without PE. This may be explained by an

earlier diagnosis and elective delivery in those FGR cases with severe maternal symptoms.

Limitations

This study has several limitations. Firstly, although the fetuses were followed longitudinally, only the data of the last ultrasound before delivery were analyzed. This prevented to assess the potential existence of a different temporal sequence on cardiac changes in isolated FGR with respect to FGR with PE. We and others have previously reported that cardiac function parameters in FGR become abnormal from very early stages of fetal deterioration.[10,12,46] Therefore the existence of such longitudinal differences between FGR with and without PE may seem unlikely, although we acknowledge it can not be excluded it conclusively. Secondly, the study mostly included severe preterm FGR. Again, although we would suggest it is unlikely, the potential influence of PE in less severe forms of FGR later in pregnancy can not completely be excluded. Finally, it was impossible to construct a study group with a meaningful sample size of isolated preterm PE defined by PE with normally grown fetuses together with normal umbilical and middle cerebral artery Doppler evaluation. Identification of such cases at the gestational ages covered by this study proved to be a challenge, since in most instances PE is associated with some degree of FGR and/or fetal Doppler deterioration.

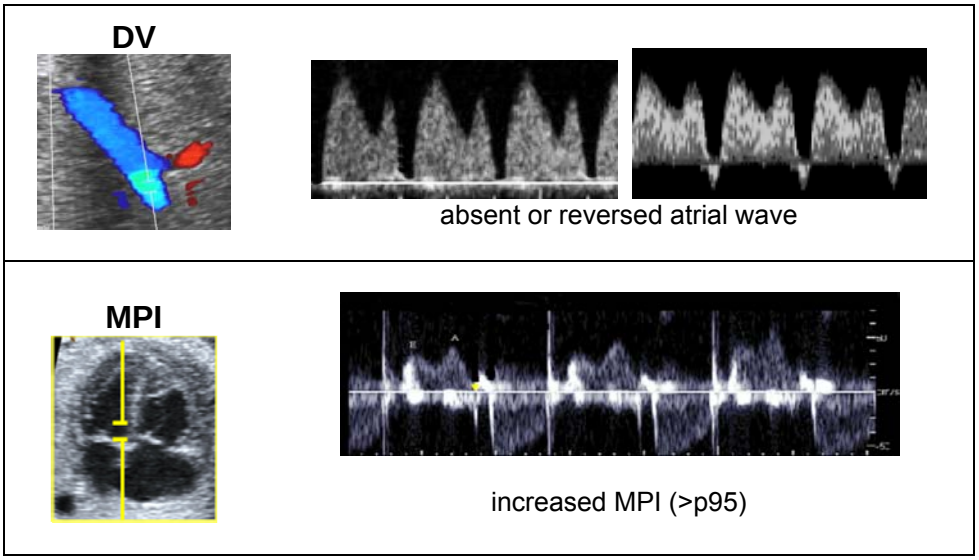
Conclusions

In conclusion, FGR fetuses with and without PE showed a similar degree of cardiovascular dysfunction. These results support the concept that PE per se is not causally related with the presence of cardiovascular dysfunction in FGR fetuses.

V.3. Project 3: Cardiovascular indices in the prediction of mortality in FGR

This study confirms that DV, together with gestational age at delivery, is a major determinant for perinatal mortality in FGR cases,[32,33] and further suggests that the combination of several cardiovascular parameters may improve prediction by stratifying FGR cases according to their estimated probability of death. Actually, DV and MPI were the only Doppler parameters with an independent association with perinatal mortality in our study population of preterm FGR cases.

Figure 9. Doppler parameters predictive of perinatal mortality in FGR (multivariate analysis).



Prediction of perinatal mortality in FGR

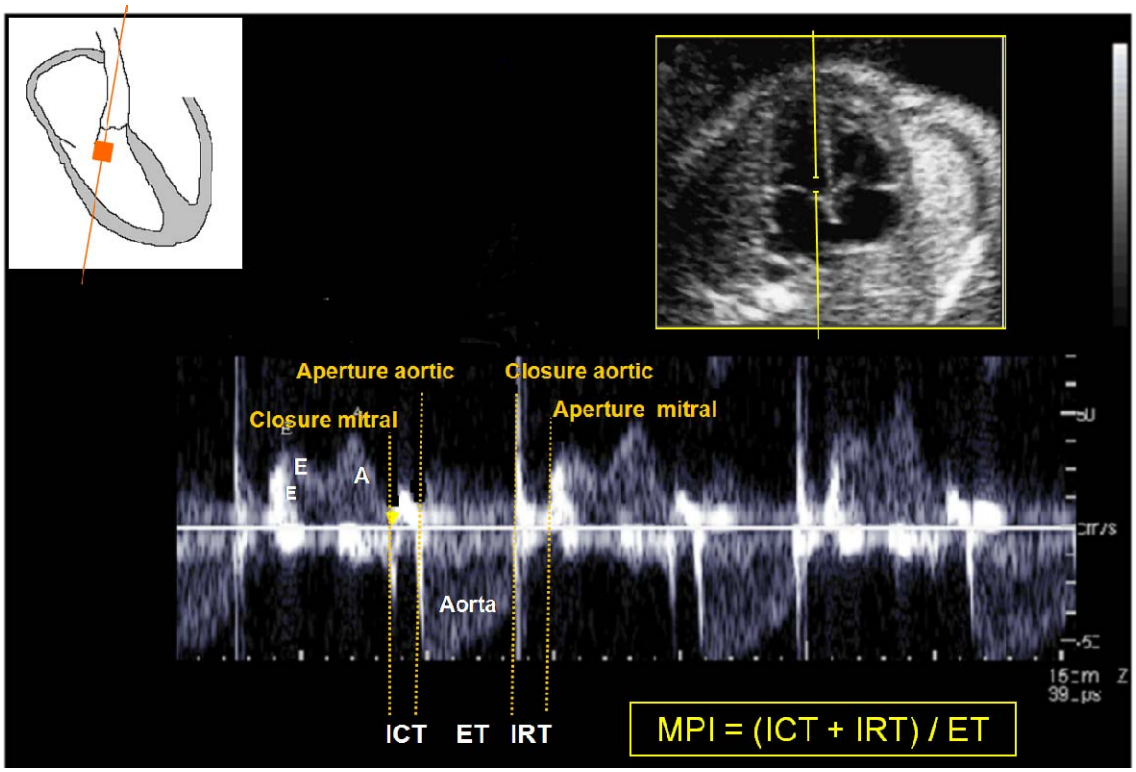
With the exception of MCA, all Doppler indices evaluated in this study showed a significant association with perinatal mortality as isolated parameters. However, only UA, DV and MPI remained as independent predictors of mortality in a multivariate analysis. When variables were dichotomized to explore their predictive value in clinical practice, only reversed atrial flow in DV and abnormal MPI showed an independent association with perinatal mortality, which persisted after adjusting for gestational age, so they could be used to construct a clinical-decision algorithm. Our

results are in line with previous studies integrating several Doppler indices and showing DV as the best predictor for perinatal death in preterm FGR.[32-34] The data are also in agreement with previous studies on UA and MCA, where a lack of improvement in prediction of mortality after adjustment for other Doppler parameters and gestational age was shown.[32,33,67,68] Finally, as previously reported,[32-34] gestational age at delivery was so strongly influencing perinatal outcome that the explored algorithm had to incorporate this variable to obtain meaningful results.

Myocardial performance index

This study first reports that MPI is independently associated with perinatal mortality and that its combination with DV could improve the predictive value of current fetal Doppler evaluation. MPI is cardiac parameter that evaluates both systolic and diastolic function by including isovolumetric and ejection times[69] (Figure 10).

Figure 10. Myocardial performance index

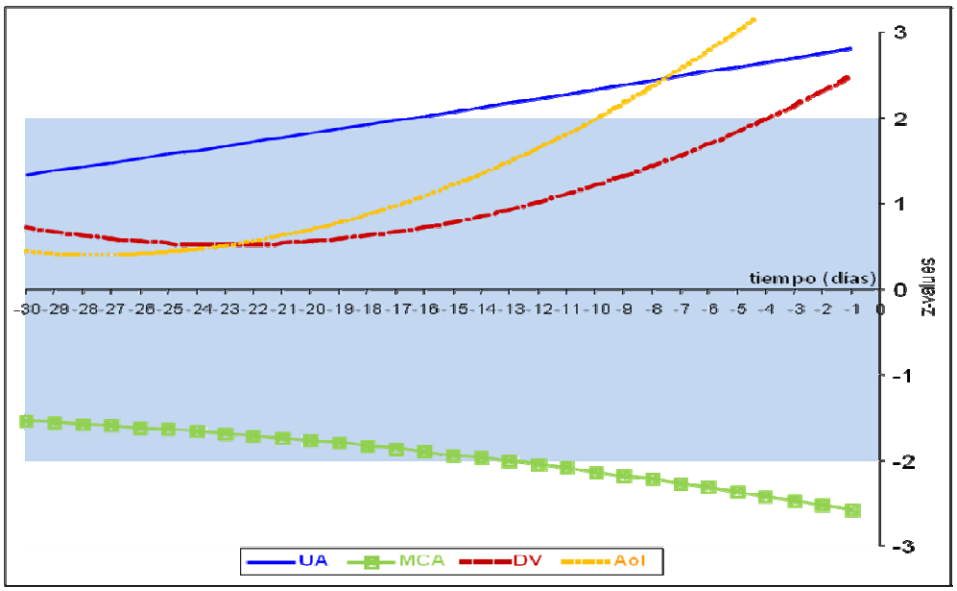


In addition, the modified method to calculate MPI in fetuses (Mod-MPI), which includes the Doppler recording of the clicks of the valves to estimate the time-periods, enhances the reproducibility of the measurement,[70] MPI correlates with the clinical hemodynamic deterioration of FGR fetuses and with progressively increased levels of cardiac dysfunction biomarkers such as BNP.[46] As with other fetal cardiovascular parameters, MPI measurement requires specific training by the examiner in order to be reproducible,[70] and this may be a limitation for clinical application. In addition, in the absence of purpose designed software, the ratio must be manually calculated. Studies are underway to evaluate the predictive value of the time periods used to calculate the index, as measured individually, in order to explore ways to simplify its clinical application.

Aortic isthmus

The evaluation of IFI as an isolated parameter in this study was consistent with previous series reporting that FGR cases with aortic isthmus reversed diastolic flow have a deterioration of cardiac function[51] and a poorer perinatal outcome.[35-37,51] However, the multivariate analysis demonstrated that IFI does not add to DV in the prediction of mortality. These findings seem to be explained by an intrinsic correlation of aortic isthmus with DV and other cardiovascular parameters.[51] Furthermore, in a longitudinal analysis of the evolution of the aortic isthmus and other Doppler indices in preterm FGR, we demonstrated that the IFI becomes abnormal earlier than DV[71] in the sequence of fetal hypoxic deterioration (Figure 11).

Figure 11. Deterioration pattern of Doppler indices in FGR (trend over time before delivery)



This may also partially explain a lower predictive capacity of IFI as an acute marker for a late event such as mortality. It must be noted that our findings do not preclude the strong predictive value of IFI when used as an isolated parameter.[35-37,51] On the other hand, this study focused on perinatal death, but not on neurological outcome. IFI has been reported to constitute a strong predictor of morbidity, mainly long-term neurodevelopmental adverse outcome,[37-38] an aspect not evaluated in this study.

Clinical algorithm to estimate probability of death in FGR

In the multivariate analysis using dichotomized variables to explore their predictive value in clinical practice, only gestational age below 28 weeks, reversed atrial flow in DV and abnormal MPI showed an independent association with perinatal mortality. The combination of these three parameters permitted to construct a clinical-decision algorithm to estimate the probability of death in FGR cases (Figure 5). As previously reported, gestational age at delivery is the major determinant for perinatal outcome in FGR cases. However, prematurity is not the only determinant factor in FGR fetuses. DV and MPI allowed a more accurate stratification of cases according to their

estimated probability of death. DV with present atrial flow together with normal Mod-MPI yielded to a low probability of death (0.1% in cases above 28 weeks, and 18% below 28 weeks), while both abnormal parameters yielded to much higher risk (45% and 97%, respectively). Therefore, an algorithm as proposed in this study may provide very useful information in clinical practice helping in the counseling and monitoring of these fetuses.

Limitations

This study has several limitations. The managing clinicians were not blinded to UA, MCA and DV results. Actually, reversed atrial flow in DV was a delivery criterion and therefore we acknowledge that this may have distorted the expected perinatal outcome. On the other hand, the study evaluated the predictive value of Doppler indices, but the data were not combined with other proposed means for fetal monitoring of preterm FGR, such as biophysical profile and computerized cardiotocography, and this may limit clinical applicability of these findings in certain clinical settings.

Conclusions

In conclusion, this study suggests that the combination of cardiovascular parameters such as DV and MPI may refine considerably the short-term prediction of perinatal mortality at different gestational ages at delivery in preterm FGR cases. Larger prospective studies are required to further confirm these results and to evaluate its potential clinical value. Additionally, its correlation and potential interaction with other clinical parameters such as biophysical profile and computerized cardiotocography remains to be ascertained in further studies.

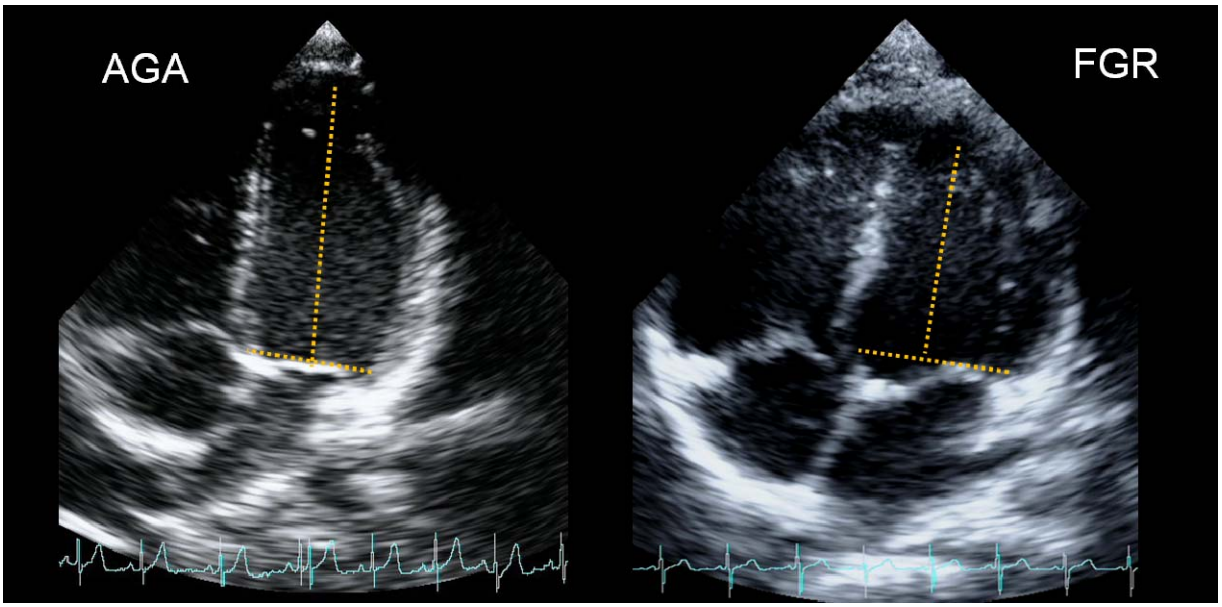
V.4. Project 4: FGR and cardiovascular remodelling in childhood

This study provides direct clinical evidence that FGR children show changes in cardiac morphology, subclinical cardiac longitudinal dysfunction and arterial remodelling, all of which increase linearly with the severity of growth restriction. The findings support the existence of direct cardiac programming in FGR and suggest a new mechanistic pathway for the association between fetal growth and cardiovascular disease.

Cardiac remodelling

The most striking finding was that children with FGR have a distinct cardiac geometry and shape, with less elongated and more globular ventricles. Morphometrical measurements confirmed quantitatively an overall increase in transverse cardiac diameters, leading to apparent dilatation of the ventricular cavities with thinner walls. The data are in line with post-mortem studies in human FGR describing hypoplasia in myocardial fibers.[78] These findings are also in agreement with our recent animal studies showing the persistence of dilated cardiomyopathy-like features in utero into adulthood in chick model of FGR under chronic hypoxia.[5]
The globular cardiac shape with thinner ventricular walls observed in children with FGR is most likely the result of changes in the cardiac development, induced by its fetal working conditions. The intrauterine chronic hypoxic and under nutrition state,[79] together with the increased placental vascular resistance,[80] result in a combined pressure and possibly volume overload of the fetal heart,[80-82] which induces abnormal cardiac function[10,46,80] and changes the wall stress on the developing myocardial fibres.

Figure 12. An example of cardiac shape changes in FGR as compared to AGA



Myocardial wall stress is determined by the ventricular pressure, the local radius of curvature and (inversely by) the wall thickness.[83] Increased wall stress will result in a tendency towards reducing this by myocardial remodelling. In acquired mild pressure overload such as hypertension, (local) hypertrophy in the region of highest stress (basal septum) results in a local stress normalisation.[84] However, in the developing heart under hypoxia and under nutrition, this locally increased wall stress could also be compensated for by changes in the local radius of curvature (with a smaller longitudinal curvature towards a more spherical the cavity).

The fetal alterations in shape are likely to induce a stabilised change in muscle fibre architecture[85] in the ventricular wall since the resulting myocardial shape and fiber orientation are importantly determined by the stress and strain conditions they are exposed to.[86,87] Therefore, as observed in our study, children that were exposed to important changes in fetal loading condition will have intrinsically differently shaped hearts. When these hearts work under normal loading conditions after birth, they are able to generate the required cardiac output without initial problems. However, the

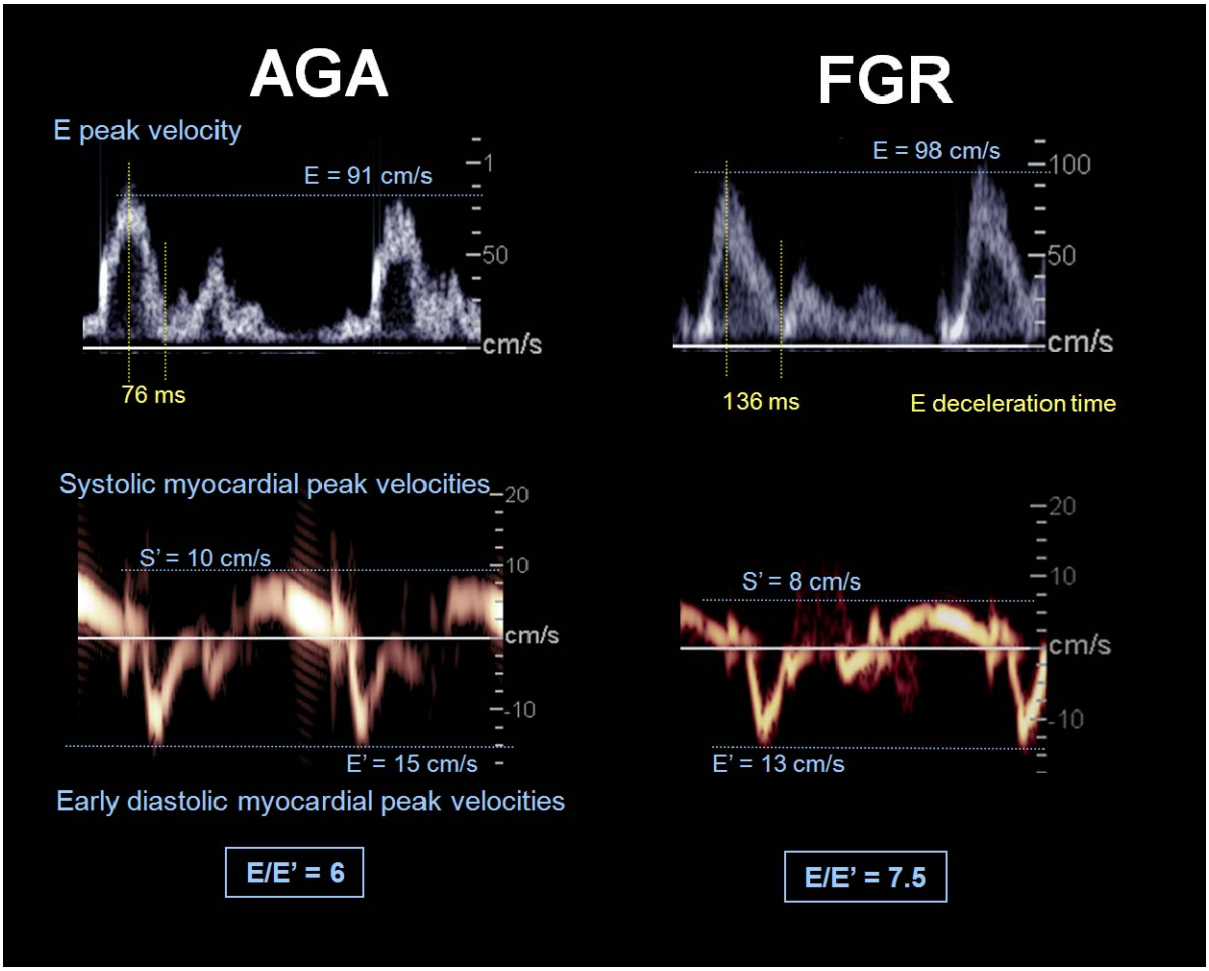
more globular shaped ventricle, with potentially a different architecture, is not as efficient in generating the normal longitudinal displacement and stroke volume,[88] resulting in the need for an increased heart rate to maintain cardiac output (as observed). Since diastolic function depends on ventricular shape and torsion, as generated by the normal fibre architecture,[89] it is not unexpected to find additional changes in diastolic parameters. Whereas the remodelled ventricles can compensate for their lower efficiency in childhood, any additional changes in its working conditions (hypertension, ischemia, arrhythmias) at later age will result in an abnormally high increase in local wall stress and dilatation since the potential for shape adaptation of the normal, ellipsoid, ventricle is not possible. Therefore, the initial cardiac remodelling, resulting from FGR, might explain the increased risk of cardiovascular disease described in epidemiological studies on FGR.[4]

Cardiac dysfunction

Regarding cardiac function, FGR children are asymptomatic and therefore as expected and previously reported[72] ejection fraction, cardiac index and BNP results were similar to those of controls. However, consistent with cardiac geometry findings, FGR children showed a subclinical decrease in longitudinal function as measured by TAPSE and tissue Doppler showing a significant reduction in myocardial velocities and increase in E/E' and E deceleration time. Decreased myocardial velocities and increased E/E' ratio are a markers of early subclinical dysfunction and it has been demonstrated to precede clinical cardiac dysfunction in a substantial number of conditions.[73-77] Furthermore, cardiac function results in FGR are consistent with those

of spherical heart geometry described in dilated cardiomyopathy, ischemic heart disease and mitral regurgitation.[75]

Figure 13. An example of mitral atrioventricular filling waves and septal annular myocardial velocities in AGA and FGR

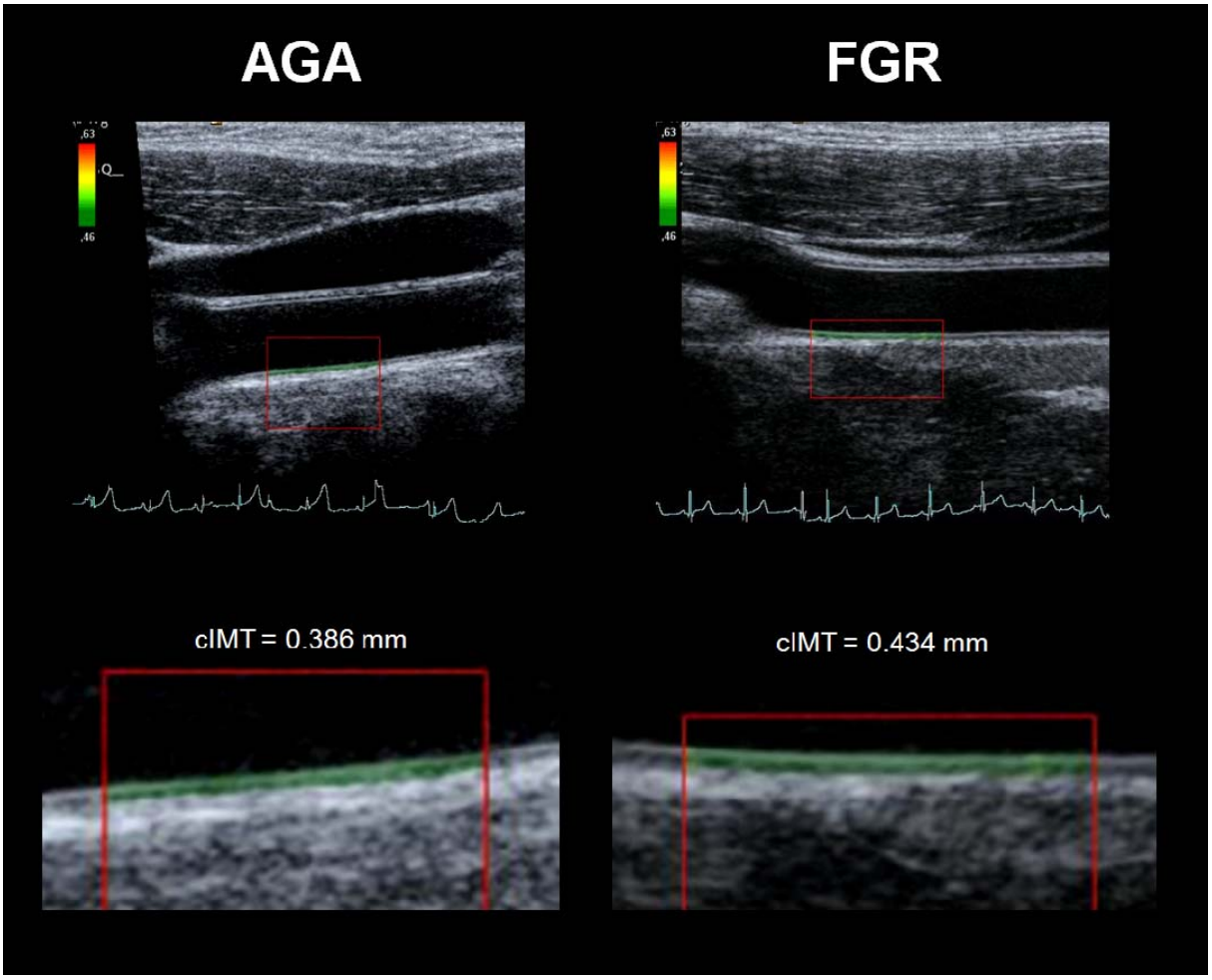


Vascular remodelling

This study confirms and extends previous studies reporting significant increased carotid wall thickness[42-43] in children suffering FGR. Increased cIMT had previously been reported in newborns with FGR[42-43] and our findings demonstrate that these changes persist into childhood. Since the hearts of FGR children did not present

hypertrophic changes, characteristic of hypertensive cardiomyopathy, the arterial changes in children with FGR are primary and not secondary. The increased arterial wall thickness is most likely the result of the overall pressure and possible volume overload in the FGR fetal circulation, where vascular wall stress (pressure overload) and increased shear stress (volume overload) induces hypertrophy of the intima-media layer. In childhood, the remodelled arteries, now working under normal loading conditions, will result in an increased peripheral resistance and an elevation in blood pressure.[90] Both blood pressure and cIMT are additional, accepted, risks factors for future cardiovascular disease.

Figure 13. An example of carotid intima-media thickness in AGA and FGR



Limitations

There are several limitations and considerations in the present study. The changes here reported are subclinical and the long-term association with adult cardiovascular function and disease remains to be proven. The study was not designed to assess the effect of other factors on cardiovascular function. In this study cardiac changes were independent of obesity or abnormal lipid profile, but the prevalence of these risk factors was very low in our setting. The existence of metabolic programming in FGR is well demonstrated,[7] and the potential interactions between metabolic and cardiac programming in the increased risk of cardiovascular disease in these patients remains to be elucidated. The impact of gender was also addressed, and cardiovascular differences were equally observed in males and females (data not shown), but we acknowledge that we may have lacked statistical power to detect subtle gender-associated differences. FGR children born preterm were compared with cases born at similar gestational age. Our findings are in line with recent studies that suggest that prematurity is not associated with fetal cardiovascular programming.[6] Exposure to prenatal glucocorticoids was also similar, although the influence of corticoids in cardiac function has recently been discarded in a large cohort study.[91] Finally, other potential confounders, such as socioeconomic status, race, familiar early cardiovascular history, breastfeeding, parity and parental smoking were also similar among study groups.

Clinical relevance of fetal cardiac programming of cardiovascular disease

Primary cardiac programming might be one of the causes of increased cardiovascular mortality in adults born with FGR, and this may open new

opportunities for monitoring and intervention in newborns and children affected with this condition. FGR affects 5-10% of all newborns and therefore the findings of this study involve thousands of children yearly. The importance of early identification and intervention in pediatric risk factors for cardiovascular disease is now well recognized.[44] However, FGR is not listed among the conditions presumed to increase cardiovascular risk in current consensus guidelines.[44,45] We believe that the results of this study merit further investigation in appropriately designed large cohort studies. If these findings are confirmed, the impact of lifestyle strategies[44,92-93] with beneficial effects on cardiac remodelling should be explored in FGR children.

Conclusions

In summary, this study provides evidence of an association between FGR and cardiac and vascular remodelling in childhood, which shows a linear increase with the severity of growth restriction and is independent of gestational age at delivery, lipid profile or body mass index. The findings support the existence of direct cardiac programming in FGR and suggest a new mechanistic pathway for the association between fetal growth and cardiovascular disease.

VI. CONCLUSIONS

1. In utero, FGR fetuses showed signs of cardiac dysfunction from early stages. Cardiac dysfunction deteriorates further with the progression of fetal compromise, together with the appearance of biochemical signs of cell damage.

2. PE does not have an influence *per se* on cardiac function in FGR fetuses.

3. MPI is an independent predictor of perinatal death in preterm FGR with an accuracy similar to DV. A combination of DV with MPI may better stratify the estimated probability of death. IFI does not add to the prediction of perinatal death when used in combination with DV.

4. Children with FGR show changes in cardiac shape, subclinical cardiac dysfunction and vascular remodelling. These findings support the existence of direct cardiac programming in FGR.

VII. REFERENCES

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IX. APPENDICES

Letter of acceptance (paper project 2):

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Date:07-May-2009

Ref.: UOG-2009-0016.R1

Dear Dr Gratacos

Thank you for submitting a revised version of your manuscript, "Does preeclampsia influence fetal cardiovascular function?". I am pleased to inform you that it has now been accepted for publication in Ultrasound in Obstetrics and Gynecology.

When the manuscript has been assigned an issue, it will be forwarded for typesetting and you will receive the proof via e-mail containing a link to a pdf file. Further instructions will be sent with the proof.

Thank you for choosing Ultrasound in Obstetrics and Gynecology for publication of your work.

Yours sincerely

Kurt Hecher
Editor, Ultrasound in Obstetrics and Gynecology

Letter of acceptance (paper project 3):

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De: onbehalfof@scholarone.com [mailto:onbehalfof@scholarone.com] En nombre de uog@isuog.org
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Dear Eduard

Thank you for submitting a further amended version of your manuscript, "Contribution of the myocardial performance index and aortic isthmus blood flow index to refine prediction of mortality in preterm growth restricted fetuses.". I am pleased to inform you that it has now been accepted for publication in Ultrasound in Obstetrics and Gynecology.

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Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses

Fatima Crispi, MD; Edgar Hernandez-Andrade, MD; Maurice M.A.L. Pelsers, PhD; Walter Plasencia, MD; Jesus Andres Benavides-Serralde, MD; Elisenda Eixarch, MD; Ferdinand Le Noble, PhD; Asif Ahmed, PhD; Jan F.C. Glatz, PhD; Kypros H. Nicolaides, MD; Eduard Gratacos, MD

OBJECTIVE: The purpose of this study was to assess cardiac function and cell damage in intrauterine growth-restricted (IUGR) fetuses across clinical Doppler stages of deterioration.

STUDY DESIGN: One hundred twenty appropriate-for-gestational-age and 81 IUGR fetuses were classified in stages 1/2/3 according umbilical artery present/absent/reversed end-diastolic blood flow, respectively. Cardiac function was assessed by modified-myocardial performance index, early-to-late diastolic filling ratios, cardiac output, and cord blood B-type natriuretic peptide; myocardial cell damage was assessed by heart fatty acid–binding protein, troponin-I, and high-sensitivity C-reactive protein.

RESULTS: Modified-myocardial performance index, blood B-type natriuretic peptide, and early-to-late diastolic filling ratios were increased in a stage-dependent manner in IUGR fetuses, compared with appropriate-for-gestational-age fetuses. Heart fatty acid–binding protein levels were higher in IUGR fetuses at stage 3, compared with control fetuses. Cardiac output, troponin-I, and high-sensitivity C-reactive protein did not increase in IUGR fetuses at any stage.

CONCLUSION: IUGR fetuses showed signs of cardiac dysfunction from early stages. Cardiac dysfunction deteriorates further with the progression of fetal compromise, together with the appearance of biochemical signs of cell damage.

Key words: cardiac function, Doppler ultrasound, heart fatty acid–binding protein, intrauterine growth restriction, myocardial damage

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Severe intrauterine growth restriction (IUGR) because of placental insufficiency affects 1% of pregnancies and contributes to 30% of total perinatal loss and severe morbidity.[1] The heart is a central organ in the fetal adaptive mechanisms to placental insufficiency and hypoxia. Elevated fetal levels of atrial and B-type natriuretic peptides and significant differences in echocardiographic parameters have been reported in small-for-date babies.[2-6] Monitoring of cardiac function is proposed as an adjunct to current methods to predict adverse outcome and death in IUGR[7]; however, suitable parameters remain to be established. Furthermore, fetal cardiac dysfunction might have important consequences in fetal programming of postnatal cardiac disease later in adulthood. Epidemiologic studies and animal models have established that low-birthweight babies have an increased risk of cardiovascular disease later in life.[8,9]

Clinically, IUGR fetuses are stratified in stages of severity, according to the sequential deterioration of fetoplacental Doppler patterns.[1,10] However, the onset and progression of fetal cardiac dysfunction across stages of severity in IUGR have not been established. Recently, Girsen et al[6] evaluated cord blood atrial and B-type natriuretic peptides in fetuses at different stages of Doppler deterioration and suggested that subclinical cardiac dysfunction might constitute an early event in the course of the disease.

Aside from cardiac function, it is unknown whether myocardial cell damage occurs at any stage of fetal deterioration.

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TABLE 1
Baseline characteristics of the study populations

	AGA		IUGR[a]		
Variable	**Term**	**Preterm**	**Stage 1**	**Stage 2**	**Stage 3**
Patients (n)	80	40	26	28	27
Clinical characteristics					
White (%)	96	71	88	81	84
Smoker (%)	12	27	27	44[b]	53[b]
Preeclampsia (%)	0	0	61[b,c]	76[b,c]	43[b,c]
Current Doppler data[d]					
Umbilical artery pulsatility index	−0.3 (1.3)	0 (2)	4.2 (1.7)[b,c]	8.4 (3.9)[b,c]	14.5 (19.2)[b,c]
Middle cerebral artery pulsatility index	0.1 (1.3)	−0.1 (1.2)	−2.1 (1.4)[b,c]	−2.1 (1)[b,c]	−2.4 (1.2)[b,c]
Cerebroplacental ratio	0 (1.2)	0 (1.2)	−2.6 (0.7)[b,c]	−3.2 (0.9)[b,c]	−3.3 (0.6)[b,c]
Ductus venosus pulsatility index	0 (0.9)	0.2 (0.5)	1 (1.9)[b,c]	1.6 (2.3)[b,c]	4.3 (4.4)[b,c]

[a] IUGR fetuses are classified according to the last ultrasound before delivery or death.

[b] $P < .01$, compared with term AGA.

[c] $P < .01$, compared with preterm AGA.

[d] Values are median (interquartile range).

Crispi. Cardiac dysfunction and cell damage. Am J Obstet Gynecol 2008.

Cord blood troponin levels are within normal values in most fetuses with severe IUGR, which suggests that cell necrosis may be uncommon.[3,11,12] However, in adults with heart failure, biomarkers of myocardial cell damage (such as the recently described heart-fatty acid binding protein [H-FABP]) have been demonstrated to be more sensitive than troponins.[13-15] Additionally, high sensitivity C-reactive protein (hsCPR), a marker of tissue injury and inflammation, is being associated increasingly with chronic cardiac disease and damage in adults.[16,17] These cardiovascular biomarkers have not been evaluated in relation with fetal cardiac function in IUGR.

Here we document the evolution of cardiac dysfunction and cell damage across clinical stages of severity in IUGR by conducting a longitudinal study that integrated echocardiographic evidence with biomarkers of cardiac dysfunction and myocardial damage in a cohort of 81 fetuses with well-documented severe fetal growth restriction.

Materials and Methods

Study populations

Eligible cases were singleton pregnancies that were selected from women who attended the Maternal-Fetal Medicine Department at Hospital Clinic and at Harris Birthright Research Centre for Fetal Medicine. The study protocol was approved by the Ethics Committee at each participating institution, and patients provided written informed consent.

IUGR was defined as an estimated fetal weight below the 10th percentile according to local reference curves[18] together with a Doppler pulsatility index in the umbilical artery of >2 standard deviations.[19] For the purposes of this study only, those IUGR infants who died or were delivered between 24 and 34 weeks of gestation were included. IUGR fetuses were longitudinally followed-up with serial ultrasound scans. At each examination fetuses were classified in stages of severity according to the end-diastolic flow (EDF) in the umbilical artery as: stage 1 was defined by presence of EDF; stage 2 was absence of EDF; and stage 3 reversed EDF. Ultrasound scans were performed on a weekly basis for fetuses in stage 1 and every 48-72 hours from stage 2 onwards. If the IUGR fetus remained in the same severity stage as defined earlier during several examinations, the median of each cardiac parameter that was obtained at that stage was considered to be the representative value. Maternal and cord blood samples were collected at delivery for biochemical marker analysis. Exclusion criteria were birthweight >10th percentile, evidence of fetal infection, or structural/chromosomal abnormalities. Echocardiographic or biochemical data were not available to the managing clinicians. *Adverse perinatal outcome* was defined by the presence of perinatal death,[10] bronchopulmonary dysplasia,[20] hyaline membrane disease, neonatal intraventricular hemorrhage grade 3 or 4, necrotizing enterocolitis, sepsis, or retinopathy grade 3 or 4.

Two different populations of appropriate-for-gestational age (AGA) fetuses were selected as control subjects. Ultrasonographic measurements were performed in 80 normal grown fetuses who were delivered at term (term AGA) matched for gestational age at enrolment (±2 weeks) with cases. In addition, maternal and cord blood biomarkers were measured at delivery not only in 30 of these control subjects who were delivered at term but also in 40 AGA fetuses who were delivered preterm without signs of infection (preterm AGA) and matched for gestational age at delivery (±2 weeks) with cases.

TABLE 2
Perinatal outcomes of the study populations

Populations	Term AGA	Preterm AGA	IUGR-s1	IUGR-s2	IUGR-s3
N (patients)	*80*	*40*	*26*	*28*	*27*
Cesarean section (%)	16	35[a]	96[a,b]	100[a,b]	100[a,b]
Gestational age at delivery (weeks)	39 (1)	30 (6)[a]	32 (3)[a]	30 (4)[a]	28 (3)[a,b]
Birth weight (g)	3110 (475)	1605 (1087)[a]	1160 (400)[a,b]	980 (360)[a,b]	600 (352)[a,b]
Birth weight percentile	45 (38)	50 (54)[a]	0 (0.5)[a,b]	0 (0.1)[a,b]	0 (0.1)[a,b]
5-min Apgar	10 (0)	10 (1)	10 (1)	9 (2)[a]	8 (2)[a,b]
Umbilical artery pH	7.30 (0.07)	7.27 (0.08)	7.27 (0.13)	7.21 (0.07)[a]	7.16 (0.1)[a,b]
Intrauterine death	0% (0/80)	0% (0/40)	0% (0/26)	7% (2/28)	33% (9/27)[a,b]
Neonatal death	0% (0/80)	3% (1/40)	4% (1/26)	0% (0/28)	26% (7/27)[a,b]
Perinatal death	0% (0/80)	3% (1/40)	4% (1/26)	7% (2/28)	59% (16/27)[a,b]
Adverse perinatal outcome	0% (0/80)	32% (13/40)[a]	36% (10/26)[a]	42% (12/28)[a]	78% (21/27)[a,b]

IUGR fetuses are classified according to the last ultrasound before delivery or death.
Values are median (interquartile range) or proportions. Adverse perinatal outcome defined by the presence of perinatal death, bronchopulmonary dysplasia, hyaline membrane disease, neonatal intraventricular haemorrhage grade 3 or 4, necrotizing enterocolitis, sepsis or retinopathy grade 3 or 4.
AGA, appropriate for gestational age; *IUGR*, intrauterine growth restriction; *s1*, stage 1; *s2*, stage 2; *s3*, stage 3; *PI*, pulsatility index.

[a] $P < 0.01$ compared to term AGA.

[b] $P < 0.01$ compared to preterm AGA.

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Fetal echocardiography

Echocardiographic measurements included left myocardial performance index (MPI), early and late diastolic filling (E/A) ratios and cardiac output. We used a modified MPI (Mod-MPI) that was adapted to fetal assessment, which results in a substantial improvement in interobserver variability.[21] Mitral and tricuspid peak velocities of E/A ratios were estimated in an apical 4-chamber view, with the Doppler sample volume just below the atrioventricular valves.[22] Left and right cardiac outputs were calculated as $\pi \times$ (aortic or pulmonary valve diameter)$^2 \times$ (aortic or pulmonary artery systolic time-velocity integral) $\times$ heart rate. The combined cardiac output was calculated as the sum of both.[3,23]

Because of their correlation with gestational age, all individual ultrasonographic data were normalized by conversion of the measurements into *Z*-scores (standard deviation from the gestational age mean), with the exception of cardiac output, which was normalized by estimated fetal weight.[19,22-25]

Biomarkers in fetal and maternal blood

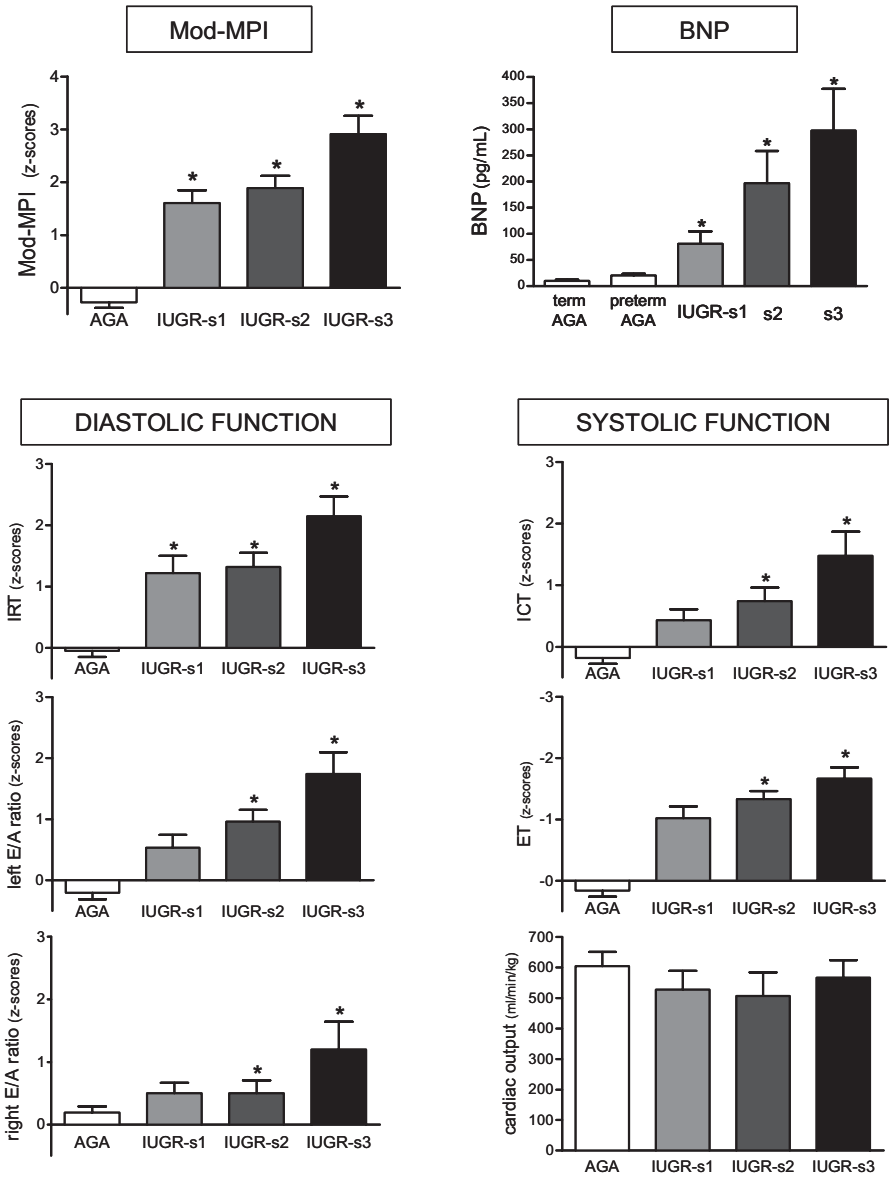
Fetal umbilical EDTA-treated blood was obtained from the umbilical vein after cord clamp at delivery. Maternal samples were drawn from the cubital vein at the time of fetal sampling. All samples were processed within 1 hour. Plasma was separated by centrifugation at 3000 rpm for 10 minutes at 4°C; samples were stored immediately at −80°C until assay.

Cord blood levels of B-type natriuretic peptide (BNP) were measured with an immunoassay system (ADVIA Centaur BNP; Siemens Healthcare Diagnostics, Deerfield, IL), as described previously.[26] Plasma concentrations of H-FABP were measured with a commercially available assay (Hycult Biotechnology, Uden, The Netherlands).[27] Troponin I and hsCPR levels were measured with commercially available assays with Centaur CP (Siemens Healthcare Diagnostics) troponin I assay and ADVIA 2400 Chemistry system (Siemens Healthcare Diagnostics), respectively.

Data analysis

Data were analyzed with the SPSS statistical software (version 13.0; SPSS Inc, Chicago, IL). Results were expressed as median ± interquartile range. Comparisons of perinatal outcome among groups were performed with analysis of variance based on log-transformed data that were adjusted with Bonferroni's post-hoc test. Comparisons of cardiac parameters among groups were performed with the use of regression (robust variance) to take into account repeated measurements per subject, with stage as a categoric variable. In addition, the Cuzick nonparametric test for trend across ordered groups was used to assess the existence to progressively different values within severity stages of IUGR.[28] Finally, the association of variables with perinatal death was assessed with the use of the last scan before delivery and cord blood biomarker levels at delivery by *T*-test. Differences were considered significant with probability values of $<.01$.

FIGURE 1
Cardiac function parameters in AGA and IUGR fetuses at different stages of severity



Data are given as mean ± SEM. The *asterisk* denotes a probability value of <.01, compared with AGA. *ET*, ejection time; *ICT*, isovolumetric contraction time; *IRT*, isovolumetric relaxation time; *s1*, stage 1; *s2*, stage 2; *s3*, stage 3.

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Results

Characteristics of the study populations

Clinical and perinatal data are shown in Tables 1 and 2. IUGR fetuses from severity stages 2 and 3 showed lower 5-minute Apgar scores and umbilical artery pH values and higher rates of adverse perinatal outcome, compared with AGA fetuses.

Fetal echocardiography

A total of 62, 65, and 47 ultrasound explorations were performed in IUGR-stages 1, 2, and 3, respectively. Values of echocardiographic parameters in term AGA and in IUGR fetuses are shown in Figure 1. Mod-MPI was significantly higher in stage 1 and showed a progressive increase through further stages of deterioration. All time periods that were used for calculation of the MPI were significantly different in IUGR fetuses. E/A values in both atrioventricular valves were significantly higher from stage 2 onwards. Cardiac output values were similar in control subjects and in IUGR fetuses at all severity stages. The statistical analysis for trend showed a significant tendency to different results with increasing stages of IUGR for all parameters with the exception of cardiac output (probability value of tendency: Mod-MPI, isovolumetric contraction, ejection time, relaxation time, left E/A, and right E/A, $P < .001$; cardiac output, $P = .375$).

Biomarkers in fetal blood

Maternal and cord blood samples were collected in 59 IUGR, 40 preterm AGA, and 30 term AGA fetuses. Data on cord blood biomarker levels are shown in Figures 1 and 2. BNP levels were significantly higher in fetuses at stage 1 and increased further across the stages of severity. H-FABP values were significantly increased in IUGR fetuses at stage 3 together with a significant linear increment across severity stages. Troponin I values were similar in AGA and in IUGR fetuses. Hs-CPR levels were significantly lower in IUGR fetuses at stages 2-3, compared with preterm AGA. Moreover, all biomarkers with the exception of troponin I showed a significant tendency to different results with increasing stages of IUGR (probability value tendency: BNP, H-FABP, and hsCPR, $P < .001$; troponin I, $P = .154$)

Biomarkers in maternal blood

All biomarkers showed similar maternal plasma levels among AGA and IUGR at any severity stage (BNP, $P = .717$; H-FABP, $P = .88$; troponin I, $P = .09$; hsCPR, $P = .673$). No significant correlation was measured between maternal and cord blood levels of any biomarker, with the exception of hsCPR (BNP, $R^2 = -0.008$, $P = .754$; H-FABP, $R^2 = 0.012$, $P = .129$; troponin I, $R^2 = -0.014$, $P = .910$; hsCPR, $R^2 = .261$, $P = .025$).

Association with perinatal death

Mod-MPI and E/A ratios and cord blood BNP, H-FABP and troponin I were significantly increased in IUGR fetuses who

died, in comparison with survivors (Tables 3 and 4).

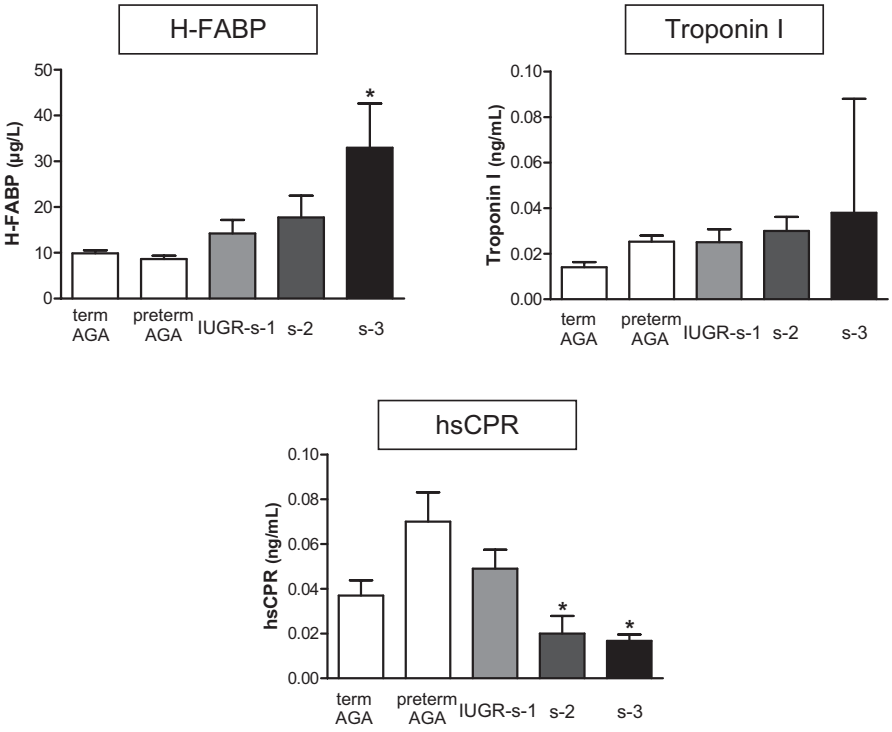
Comment

This study documents the evolution of cardiac dysfunction and cell damage in fetal growth restriction in relation with Doppler stages of severity that are used widely in clinical practice. It provides evidence that subclinical cardiac dysfunction is an early and progressive event in severe IUGR. Echocardiographic parameters and cord blood levels of BNP indicate that cardiac dysfunction increased progressively across the stages of fetal compromise. Advanced fetal Doppler deterioration was associated with a significant increase in H-FABP levels, which supports the existence of myocardial cell damage, but to such a small extent that it could not be detected by cord blood levels of troponin I.

Echocardiographic parameters that indicate subclinical cardiac dysfunction increased through the Doppler stages of fetal deterioration that were defined in this study. MPI has been reported previously to be increased in IUGR fetuses.[5,29] In this study we further described that Mod-MPI was elevated in fetuses at initial stages of hemodynamic compromise and progressed further with fetal deterioration. The progressive increment in Mod-MPI values was at the expense of all time periods that were involved in the calculation of this index, which suggests the existence of both systolic and diastolic subclinical dysfunction. E/A ratio evaluates ventricular filling during the diastole; in adults, decreased values are considered a sign of diastolic dysfunction. Contrary to adults, fetuses with overt heart failure have been reported to have increased E/A ratios.[30] In IUGR, earlier studies described unchanged or reduced E/A values,[2-4,31] but most recent studies found the ratios to be increased significantly with respect to control subjects.[3,6] We also observed a considerable increase in E/A values in fetuses with IUGR in both atrioventricular valves, which was present from stage 2 and increased further with fetal Doppler deterioration. Finally, this study confirmed previous observations that indicated that cardiac output is maintained within normal values, even in most severe clinical stages of IUGR.[6,23]

The progressive increase of BNP cord blood levels in IUGR fetuses was consistent with that observed for echocardiographic parameters. In adults, BNP is the gold standard biomarker for heart failure[32]; serum levels are elevated in early stages of subclinical diastolic dysfunction and increase in proportion to severity.[32] Our data in human fetuses with IUGR are in agreement with those of Girsen et al[6] who demonstrated that N-

FIGURE 2
Myocardial cell damage markers in AGA and IUGR fetuses at different stages of severity



Data are given as mean ± SEM. The *asterisk* denotes a probability value of <.01, compared with preterm AGA. *s1*, stage 1; *s2*, stage 2; *s3*, stage 3.

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TABLE 3
Echocardiographic parameters and perinatal deaths in IUGR fetuses

Variable	Survivors (n = 62)	Perinatal deaths (n = 19)	*P* value
Mod-MPI	1.7 (3)	2.5 (2)	.027
Isovolumetric contraction time	0 (2.2)	0.2 (2.7)	.063
Ejection time	−1.1 (1.4)	−2.1 (1.6)	.130
Isovolumetric relaxation time	1 (3.1)	2.1 (3.4)	.067
Left E/A	0.8 (2.2)	2.4 (2.2)	.045
Right E/A	1 (2.1)	2.3 (5.2)	.002
Cardiac output (mL/min/kg)	750 (274)	816 (248)	.484

Values are median (interquartile range). Doppler values are expressed in *Z*-scores, with the exception of cardiac outputs normalized by fetal weight.

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TABLE 4
Cord blood cardiac biomarkers and perinatal death in IUGR fetuses

Variable	Survivors (n = 51)	Perinatal deaths (n = 8)	*P* value
Cardiac function			
BNP (pg/mL)	64 (127)	350 (456)	.029
Myocardial cell damage			
H-FABP (μg/L)	11 (11)	23 (103)	<.001
troponin I (ng/mL)	0.02 (0.02)	0.07 (1.14)	.002
hsCPR (ng/mL)	0.02 (0.04)	0.01 (0.02)	.176

Values are median (interquartile range).

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terminal peptide of proBNP was elevated in fetuses with growth restriction and increased across fetoplacental Doppler stages of fetal compromise. In another study, Leipala et al[33] showed increased levels of BNP in preterm IUGR neonates during the first days of life, which points to a persistence of cardiac dysfunction postnatally.

The integrated evaluation of myocardial cell and tissue damage biomarkers offers new information for the understanding of fetal cardiac disease in IUGR. H-FABP cord blood levels showed a significant linear increase across stages and were increased significantly in fetuses with reverse flow in the umbilical artery, which suggests the presence of myocardial cell damage in advanced stages of fetal deterioration. H-FABP is a novel biochemical marker with a relatively small size that confers a high sensitivity to detect myocardial cell damage.[15] It has proved not only to be an excellent marker for the early detection of cardiac injury in acute coronary syndromes but also showed to be sensitive enough for the detection of chronic minor myocardial injury in heart failure.[13,14] On the other hand, troponin is a well-established marker for myocardial infarction. However, 94% of cardiac troponin is bound to myofibrillar structures, and it has first to dissociate from its matrix before it is released. This explains a time delay for raised levels in plasma and a lower sensitivity to subtle cell damage, compared with H-FABP.[15] Consistent with this notion, the observed elevation of H-FABP in this study occurred in the absence of significant changes in mean troponin levels. The latter finding is in line with previous studies that measured troponin in IUGR.[3,11,12] It must be noted that, despite of the absence of overall differences, this and previous studies have observed individual cases of severe fetal growth restriction with high cord blood troponin values,[3,11,12] which suggests that extended cell damage may occur in very advanced stages of the disease. In this study, fetuses who died had significantly higher levels of troponin, compared with survivors.

Finally, we could not demonstrate any increase in hsCPR cord blood levels in severe IUGR fetuses, compared with preterm AGA. On the contrary, there was a significant decrease of cord blood hsCPR in IUGR stages 2 and 3, compared with preterm AGA, but not with term AGA fetuses. These differences could be explained by an abnormal increase in hsCPR levels in preterm AGA because of the accidental inclusion of $\geq$1 cases with subclinical prenatal infection. Alternatively, it could reflect a true decrease in hsCPR levels in most severe forms of IUGR that we cannot explain. HsCPR is a well-established acute marker for tissue injury, and the lack of elevation in severe IUGR fetuses is consistent with the results that have been observed for troponin. On the other hand, hsCPR has also been described as a marker of chronic inflammation that leads to cardiovascular disease.[16,17] Thus, our data do not support the implication of this pathophysiologic mechanism in fetal cardiac disease that is related to growth restriction. The results in hcPCR are in disagreement with a recent study that described an increase in hsCPR levels in near-term small-for-gestational age fetuses.[34] Although the population is not comparable with that of the present study in terms of gestational age or in severity, we cannot find an explanation for the differences between both studies.

This study has several limitations. First, there was an elapsed period between the last ultrasound measurement and cord blood sampling. Although this did not exceed 48 hours, we acknowledge that, in a small number of cases, the severity stratification based on ultrasound parameters might have changed at the time of blood sampling. Second, sufficient cord blood for analysis could not be retrieved in approximately 20% of cases because of the inherent difficulty in obtaining samples from extremely preterm and small IUGR fetuses. Furthermore, cord blood was not available in all intrauterine deaths. This may have biased the results by attenuating the differences on the levels of biomarkers between control subjects and IUGR fetuses. Third, most biomarkers that were evaluated in this study are not fully cardiospecific. This may raise concern for the interpretation of H-FABP data. This protein is expressed abundantly in cardiomyocytes, but to a lesser extent also in skeletal muscle, distal tubular cells of the kidney, specific parts of the brain, lactating mammary glands, and placenta.[14,35] Although we postulate that cardiac damage is the most plausible reason for the observed increase in this study, we acknowledge that this concept cannot be demonstrated completely. Although the placenta can also produce H-FABP, reduced maternal levels in IUGR pregnancies support that placental production is unlikely to account for the observed increases in fetal blood. Future studies in animal models might help to clarify the origin of H-FABP in growth-restricted fetuses. Finally, it is unknown whether the association with maternal preeclamptic symptoms has any effect on fetal cardiac function. This potential confounding effect is being investigated in further studies.

The association between the risk of adverse outcome or perinatal death and the presence of abnormal echocardiographic parameters has been reported previously.[7,10] In this study, we confirm previous reports and further describe that those fetuses who died in utero or postnatally had significantly increased levels of H-FABP and troponin. Ultrasonographic assessment of cardiac function could be integrated into clinical practice to improve the short-term prediction of fetal death. This information is essential to guide clinical decisions of fetuses with severe growth restriction, but no single Doppler ultrasound parameter has been demonstrated to have sufficient predictive value.[7,10,36] In the present study, MPI and E/A were higher in fetuses who died, but their predictive value for perinatal death in IUGR remains to be evaluated. In a preliminary study, a composite cardiovascular score that integrated MPI with other Doppler indices improved the short-term prediction of fetal death, compared with ductus venosus Doppler alone.[37]

In summary, this study provides evidence that cardiac dysfunction is an early event in fetal growth restriction and that its magnitude increases in proportion to the severity of the fetal condition. Thus, subclinical cardiac dysfunction is present in fetuses with IUGR and mild degrees of Doppler deterioration, which currently is considered to have an overall good long-term outcome.[1,7,10] The data further suggest that advanced stages of fetal deterioration are associated with myocardial cell damage. It is tempting to speculate that the degree and duration of cardiac dysfunction and damage might be associated with distinct effects on fetal programming of cardiovascular function and disease in adulthood. The impact in long-term cardiovascular outcome of the changes here described remains to be established in long-term follow-up studies. ■

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Expanded Methods

Delivery indications and perinatal outcome definitions

The indications for delivery included decompensation of maternal disease (severe preeclampsia), decelerative fetal heart rate pattern, and deterioration of fetal venous indices (absent or reversal of atrial flow in the ductus venosus). When delivery was indicated for fetuses with gestational age of <26 weeks of gestation or with estimated weights of <500 g, patients where counseled regarding the high mortality rate and adverse sequelae, and the option of expectant management was contemplated. At delivery, gestational age, birthweight, Apgar scores, and umbilical pH were recorded. *Perinatal death* was defined as either intrauterine death or neonatal death within the first 28 days of life.[14] *Bronchopulmonary dysplasia* was defined by an oxygen dependency at 36 weeks of corrected gestational age plus compatible clinical and radiographic changes.[25] *Neonatal intraventricular hemorrhage* was defined according to Papile's criteria.[26,27] Necrotizing enterocolitis was classified according to modified Bell's stages.[28] *Adverse perinatal outcome* was defined by the presence of perinatal death, bronchopulmonary dysplasia, neonatal intraventricular hemorrhage grade 3 or 4, or necrotizing enterocolitis.

Ultrasonographic evaluation

The ultrasound studies were performed with a Siemens Sonoline Antares system (Siemens Medical Systems, Malvern, PA, USA) or a Voluson 730 Expert system (GE Medical Systems, Milwaukee, WI) with 6-4 MHz linear curved array probes. All estimations were done in the absence of fetal corporal and respiratory movements and with the mother in voluntary suspended respiration. The angle of insonation was kept <30 degrees in all measurements. The mechanical and thermal indices were maintained at <1, and the wall filter was set to 70 Hz. Routine ultrasound examination included fetal weight calculation, amniotic fluid index, and Doppler examination of the umbilical artery in a free loop of the umbilical cord, middle cerebral artery in a transverse view of the fetal skull at the level of the circle of Willis, and ductus venosus in a mid sagittal view of the fetal abdomen before its entrance to the inferior vena cava.

Echocardiographic measurements

Cardiac function was assessed by ultrasound with 2-dimensional and Doppler ultrasound modes in 80 term AGA and 81 IUGR fetuses. For term AGA fetuses, only 1 ultrasound scan was performed. For IUGR fetuses, there was a median of 3 examinations in the 2-week interval before delivery. If the IUGR fetus remained in the same severity stage as defined earlier during several examinations, the median of each cardiac parameter that was obtained at that stage was considered as the representative value.

Echocardiographic measurements included left MPI, E/A ratios, and cardiac output. Mod-MPI was obtained in a cross-sectional image of the fetal thorax, with the Doppler sample volume placed on the medial wall of the ascending aorta to include the leaflets of the aortic and mitral valves.[29] The clicks of the valves that registered in the Doppler trim were used as landmarks to calculate the isovolumetric contraction and relaxation times and the ejection time. Mod-MPI was calculated in the following manner: (isovolumetric contraction + isovolumetric relaxation time)/ejection time. Right and left E/A ratios were estimated in an apical 4-chamber view with the Doppler sample volume placed just below the atrioventricular valves.[30] Diameters of the aortic and pulmonary valves were measured 3 times in frozen real-time images during systole by the leading-edge method and with the mean as representative.[8] Aortic systolic time-velocity integral was obtained in a long axis of the left ventricle, and pulmonary artery systolic time-velocity integral was obtained in a short-axis view of the fetal heart. Then, left and right cardiac outputs were calculated in the following manner: $\pi \times$ (aortic or pulmonary valve diameter)$^2 \times$ (aortic or pulmonary artery systolic time-velocity integral) $\times$ heart rate. The combined cardiac output was calculated as the sum of both.[11,31]

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Does preeclampsia influence fetal cardiovascular function?

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Does preeclampsia influence fetal cardiovascular function?

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OBJECTIVE. Increasing evidence shows that intrauterine growth restriction (IUGR) is associated with fetal cardiac dysfunction. Most studies include altogether IUGR with and without preeclampsia (PE). Our objective was to evaluate whether the association with PE has any impact on cardiac function in IUGR fetuses

METHODS. 31 normotensive IUGR cases and 31 IUGR with preeclampsia (PE+IUGR) below 34 weeks of gestation were included. IUGR was defined as a birth weight below the 10 centile together with an umbilical artery pulsatility index above 2SD. Fetal cardiac function was assessed by measuring ductus venosus pulsatility index, modified-myocardial performance index, aortic isthmus blood flow, E/A ratios and cardiac output. Moreover, the presence of fetal cardiac dysfunction was also assessed by measuring cord blood brain-natriuretic peptide (BNP) levels collected at birth. Echocardiographic data were compared with those in 80 term appropriate for gestational age (AGA) fetuses from normotensive mothers matched by gestational age at ultrasound. Cord blood BNP levels were compared with those in 40 preterm AGA matched by gestational age at delivery with IUGR cases.

RESULTS. All IUGR cases (with or without PE) showed echocardiographic and biochemical signs of cardiac dysfunction compared to AGA. However, no differences were observed between IUGR and PE+IUGR cases neither in echocardiographic or in biochemical parameters. IUGR with or without PE had similar perinatal results.

CONCLUSIONS. IUGR fetuses showed echocardiographic and biochemical signs of cardiac dysfunction. Preeclampsia per se does not influence cardiac function in IUGR fetuses.

KEY WORDS: IUGR, preeclampsia cardiovascular function, echocardiography, B-type natriuretic peptide.

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INTRODUCTION

Intrauterine growth restriction (IUGR) affects 1-3% of all pregnancies and constitutes an important cause of perinatal mortality and morbidity.[1,2] Increasing evidence shows that IUGR is associated with fetal cardiovascular dysfunction. Several studies have reported significant differences in echocardiographic parameters, mainly related to diastolic function,[3-6] and elevated fetal levels of B-type natriuretic peptide (BNP).[6,7] The presence of abnormal cardiac function is associated with poorer perinatal outcome in IUGR fetuses[6,8-10] and this has prompted research on new cardiovascular parameters which might improve *in utero* monitoring.[11,12] In addition, epidemiological studies and animal models have established that low birth babies have an increased risk of developing cardiovascular disease later in life, including hypertension and coronary disease.[13,14] Thus, characterization of fetal cardiac dysfunction may be relevant for clinical management and for the understanding of fetal cardiac programming.

A potential concern in the interpretation of studies on fetal cardiovascular function in IUGR is the common association with preeclampsia (PE).[15-19] Most studies on cardiovascular assessment in IUGR have included without distinction pregnancies with and without this condition.[4,5,20] PE is characterized by dysfunction of the maternal vascular endothelium, which leads to increased systemic vascular resistance and maternal hemodynamic changes.[22,23,24] Several studies have shown that PE is associated with features of endothelial dysfunction also in the fetus.[25-28] It is unknown to which extent these changes have any influence in fetal cardiovascular function.

The aim of this study was to evaluate whether the association with PE has any impact on cardiovascular function in preterm IUGR fetuses.

METHODS

Study populations

The study included 31 normotensive IUGR, 31 IUGR+PE and 120 controls appropriate-for-gestational age (AGA) fetuses (80 term AGA and 40 preterm AGA). The study protocol was approved by the local Ethics Committee and patients provided their written informed consent. Exclusion criteria were structural/chromosomal anomalies or evidence of fetal infection. All fetuses were delivered or died below 34 weeks of gestation. In all pregnancies gestational age was calculated based on the crown-rump length at first trimester ultrasound. IUGR was defined as an estimated fetal weight below the 10th centile according to local reference curves[29] together with umbilical artery (UA) pulsatility index (PI) above 2 standard deviations.[30] PE was diagnosed in the presence of hypertension associated with proteinuria.[31] Hypertension was defined as resting blood pressure ≥ 140 mm Hg (systolic) and/or ≥ 90 mm Hg (diastolic) on two occasions at least 4 hours apart after the 20th week of gestation in women known to be normotensive beforehand. Proteinuria was defined as excretion of 300 mg or more in a 24 hours specimen or a 2+ urine dipstick.[31] Forty-three cases reported here have been included as part of a previous study on IUGR.[6]

All cases underwent Doppler ultrasonographic examination of fetal cardiovascular hemodynamics every 48 to 72 hours until their delivery or death. Doppler and cardiovascular parameters obtained at the last examination before death or delivery were used for statistical analysis. The ultrasound studies were performed using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA) or a Voluson 730 Expert (GE Medical Systems, Milwaukee, WI, USA) with

6-4 MHz linear curved array probes. All estimations were done in the absence of fetal movements and, when required, with the mother in voluntary suspended respiration. An angle of insonation of <30º between the vessel and the Doppler beam was accepted for analysis. The mechanical and thermal indices were maintained below 1, and the wall filter was set to 70Hz. Doppler parameters were obtained from three or more successive waveforms in each vessel. Basic Doppler examination included UA and middle cerebral artery (MCA). UA-PI was measured from a free loop of the umbilical cord. MCA-PI was measured distally to the junction of the internal carotid artery in a transverse view of the fetal skull at the level of the circle of Willis. The cerebroplacental ratio was calculated as MCA-PI/UA-PI. Complete echocardiographic assessment was also performed at each ultrasound examination. At delivery, gestational age, birth weight, birth weigh centile, Apgar scores and umbilical pH were recorded. Perinatal mortality was defined as either intrauterine death or neonatal death within the first 28 days of life.[9] Additionally, cord blood from umbilical vein was also collected to measure the concentration of B-type natriuretic peptide (BNP).

Echocardiographic assessment

Cardiovascular function was assessed by ultrasound using 2-D and Doppler ultrasound modes. Cardiovascular measurements included ductus venosus (DV) PI, aortic isthmus blood flow index (IFI), E/A ratios, modified-myocardial performance index (Mod-MPI) and combined cardiac output (CCO).

DV-PI was measured either in a mid sagittal view of the fetal thorax or in a transversal plane through the upper abdomen prior to its entrance to the inferior vena cava, positioning the Doppler gate at the DV isthmic portion.[32] Aortic isthmus was

sampled downstream of the left subclavian artery and just upstream of the ductus arteriosus connection in a sagittal view simultaneously visualizing the aortic arch. IFI was calculated by dividing the sum of the systolic and diastolic Doppler flow integrals by the systolic flow integral.[33] Atrioventricular flows were obtained from a basal or apical four-chamber view placing the pulsed Doppler sample volume just below valve leaflets. Right and left E/A ratios were estimated calculating the ratio between early ventricular filling (E wave) to late ventricular filling (A-wave).[34] Mod-MPI was obtained in a cross-sectional image of the fetal thorax placing the Doppler sample volume on the medial wall of the ascending aorta and including the leaflets of the aortic and mitral valves.[35,36] The clicks of the valves registered in the Doppler trim were used as landmarks to calculate the following time-periods: isovolumetric contraction time (ICT) from the closure of the mitral valve to the opening of the aortic valve, ejection time (ET) from the opening to the closure of the aortic valve, and isovolumetric relaxation time (IRT) from the closure of the aortic valve to the opening of the mitral valve. Finally, the Mod-MPI was calculated as (ICT+IRT)/ET. Left and right cardiac outputs were calculated as π x (aortic or pulmonary valve diameter)^2 x (aortic or pulmonary artery systolic time-velocity integral) x heart rate. Then, CCO was calculated as the sum of both.[37,38] Diameters of the aortic and pulmonary valves were measured 3 times in frozen real-time images during systole by the leading-edge–to-leading-edge method and their mean value was used for further analysis.[39] Aortic systolic time-velocity integral was obtained in a long axis of the left ventricle, and pulmonary artery systolic time-velocity integral in a short-axis view of the fetal heart and were calculated by planimetering the area underneath the Doppler spectrum.

Cord blood sampling and BNP assessment

Cord blood samples were collected immediately after delivery. Plasma was separated by centrifugation at 3000 rpm for 10 minutes at 4ºC, and samples were immediately stored at -80ºC until assayed. Levels of BNP were measured using Siemens ADVIA Centaur® BNP assay.[40]

Statistical analysis

A sample size of 31 patients in each study group was calculated by expecting differences between cases and controls in MPI and BNP above 20%, for a given 5% alpha-error and 80% power. The case cohort was constructed by consecutive IUGR cases attended at our institution between January 2006 to December 2007. For each case of the IUGR group, the next patient in the cohort with the diagnosis of IUGR and PE delivering within the same study period at the same gestational age (+/- 2 weeks) was selected. Controls were randomly selected from our general population. Two different control populations of AGA fetuses were selected. To compare echocardiographic parameters, 80 AGA fetuses who delivered at term (term AGA) were matched by gestational age at ultrasound (± 2 weeks) with cases. Finally, 40 AGA delivering preterm (preterm AGA) were matched by gestational age at delivery (± 2 weeks) in order to compare cord blood levels of BNP.

Data were analyzed with the SPSS 13.0 statistical package (SPSS, Chicago, Illinois, USA). Ultrasound measurements were converted into Z-scores (standard deviation from the gestational age mean), with the exception of cardiac output which was normalized by estimated fetal weight.[30,32-34,36,37] Results were expressed as median and interquartile range (IQR). Comparisons among groups were performed with analysis of variance based on log-transformed data adjusted with Bonferroni's post-hoc test. Differences were considered significant when *P*-values < 0.05.

RESULTS

Characteristics of the study populations

The characteristics of the study populations are reported in Table 1. As expected, IUGR cases with or without PE had higher UA-PI, and lower MCA-PI, cerebroplacental ratio and birth weight percentile as compared to term and preterm AGA. All perinatals and Doppler parameters were similar among IUGR and IUGR+PE cases. A non-significant trend to higher perinatal mortality was observed in IUGR as compared to IUGR+PE.

Echocardiographic assessment

Values of cardiovascular parameters are shown in Table 2 and Figure 1. All measurements with the exception of cardiac output were significantly different in IUGR fetuses (with or without PE) as compared to term AGA. However, there were no differences in IUGR as compared with IUGR+PE.

Cord blood BNP

Data on cord blood BNP levels are shown in Figure 2. BNP levels were significantly higher in IUGR with or without PE as compared to AGA. However, BNP concentrations were similar among IUGR cases with or without PE.

CONCLUSIONS

This study provides evidence that IUGR fetuses with and without PE had a similar degree of cardiovascular dysfunction, as measured by means of echocardiographic and biochemical parameters. The study groups were comparable in terms of the degree of growth restriction and Doppler deterioration. The data support the concept

that PE per se does not have an influence in the presence of cardiovascular dysfunction in IUGR.

The findings confirm previous studies reporting clear evidence of the existence of abnormal cardiovascular function in IUGR. As expected and previously described,[6,41-43] DV-PI was significantly increased in both study groups. DV-PI reflects to a great extent myocardial impaired relaxation[41,44,45] and it is the strongest predictor of perinatal mortality and morbidity severe IUGR.[9,41,42] Aside from DV-PI, several ultrasound measurements, including IFI, E/A ratios and MPI, and cord blood BNP levels were abnormal in both study groups. Previous clinical series have reported that IUGR fetuses with AoI reversed diastolic flow have a deterioration of cardiac function[46] and a poorer perinatal outcome.[10,46,47] Aortic isthmus has a dynamic role in connecting the right and left circulatory systems of the fetus and has been proposed as a potential monitoring tool for IUGR fetuses.[10,46,47] Left and right E/A ratios have recently been described to be significantly increased in IUGR with respect to controls,[5-7] supporting the existence of abnormal ventricular filling during diastole. MPI, a Doppler index of combined systolic and diastolic function, has been recently described to be elevated in IUGR fetuses.[6,48,49] BNP is a gold standard marker for heart failure in adults[50] and children[51] that has been demonstrated to be increased in IUGR from early stages of fetal deterioration.[6,7] Of interest, the study confirmed previous observations indicating that cardiac output is maintained within normal values in IUGR.[6,7,37]

Concerning umbilical artery and middle cerebral artery Doppler indices, similar differences with respect to controls were observed between study groups. Several studies have evaluated feto-placental Doppler parameters in IUGR fetuses with and without PE. Harrington et al.[52,53] showed a similar pattern of changes in UA and MCA

in preterm small for gestational age fetuses with or without PE. More recently, Mari et al.[21] showed that IUGR fetuses without PE undergo a series of well-defined Doppler changes until fetal deterioration occurs or the fetus is delivered because non-reassuring testing. On the contrary, IUGR fetuses with PE were often delivered for maternal indication before the full range of Doppler changes occur.[21]

Although perinatal outcome was similar in IUGR with or without PE, a non significant trend to higher perinatal mortality in normotensive IUGR fetuses was observed in this study. This finding is consistent with a previous report by Piper et al.[54] including 1012 preterm small for gestational age and showing that perinatal mortality was significantly higher in the normotensive than in the hypertensive group, even after controlling for potentially confounding factors. Recently, Mari et al.[21] also described an increased rate of fetal demise in IUGR without PE. This may be explained by an earlier diagnosis and elective delivery in those IUGR cases with severe maternal symptoms.

This study has several limitations. Firstly, although the fetuses were followed longitudinally, only the data of the last ultrasound before delivery were analyzed. This prevented to assess the potential existence of a different temporal sequence on cardiac changes in isolated IUGR with respect to IUGR with PE. We and others have previously reported that cardiac function parameters in IUGR become abnormal from very early stages of fetal deterioration.[5-7] Therefore the existence of such longitudinal differences between IUGR with and without PE may seem unlikely, although we acknowledge it can not be excluded it conclusively. Secondly, the study mostly included severe preterm IUGR. Again, although we would suggest it is unlikely, the potential influence of PE in less severe forms of IUGR later in pregnancy can not completely be excluded. Finally, it was impossible to construct a study group with a

meaningful sample size of isolated preterm PE defined by PE with normally grown fetuses together with normal umbilical and middle cerebral artery Doppler evaluation. Identification of such cases at the gestational ages covered by this study proved to be a challenge, since in most instances PE is associated with some degree of IUGR and/or fetal Doppler deterioration.

In conclusion, IUGR fetuses with and without PE showed a similar degree of cardiovascular dysfunction. These results support the concept that PE per se is not causally related with the presence of cardiovascular dysfunction in IUGR fetuses.

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Figure 1. Echocardiographic assessment in the study populations.

Data given as mean±SEM. *P<0.05 compared to term AGA. AGA, appropriate for gestational age; IUGR, intrauterine growth restriction; PE, preeclampsia; DV-PI, ductus venosus pulsatility index; IFI, aortic isthmus blood flow index; Mod-MPI, modified myocardial performance index CCO, combined cardiac output.

Figure 2. Cord blood B-type natriuretic peptide (BNP) levels in the study populations.

Data given as mean±SEM. *P<0.05 compared to term AGA. AGA, appropriate for gestational age; IUGR, intrauterine growth restriction; PE, preeclampsia.

Table 1. Characteristics of the study populations.

	Term AGA	Preterm AGA	IUGR	IUGR+PE
N	*80*	*40*	*31*	*31*
Basic Doppler measurements				
Gestacional age at ultrasound (weeks)	29 (7)	30 (6)	29 (6)	30 (3)
Umbilical artery mean PI	0.01 (1.3)	0 (2.04)	7 (9)*†	8 (11.25)*†
Middle cerebral artery PI	0.1 (1.34)	-0.01 (1.23)	-2.53 (1)*†	-2.61 (1.32)*†
Cerebroplacental ratio	0.07 (1.26)	0.03 (1.16)	-3.54 (1.41)*†	-3.08 (0.61)*†
Perinatal outcome				
Gestational age at delivery (weeks)	39 (1)	30 (6)*	31 (4)*	30 (4)*
Birth weight (g)	3110 (475)	1605 (1087)*	1030 (470)*†	995 (274)*†
Birth weight percentile	45 (38)	50 (54)	1 (1)*†	1 (1)*†
5-min Apgar	10 (0)	10 (1)	9 (1)	9 (2)
Arterial cord pH	7.30 (0.07)	7.27 (0.08)*	7.21 (0.07)*	7.22 (0.1)*
Perinatal mortality	0%	3%	19%*†	13%*

Data presented as median (IQR) or %. Doppler values expressed in Z-scores.

**P*<0.05 as compared with term AGA. †*P*<0.05 as compared with preterm AGA.

AGA, appropriate for gestational age; IUGR, intrauterine growth restriction; PE, preeclampsia; PI, pulsatility index.

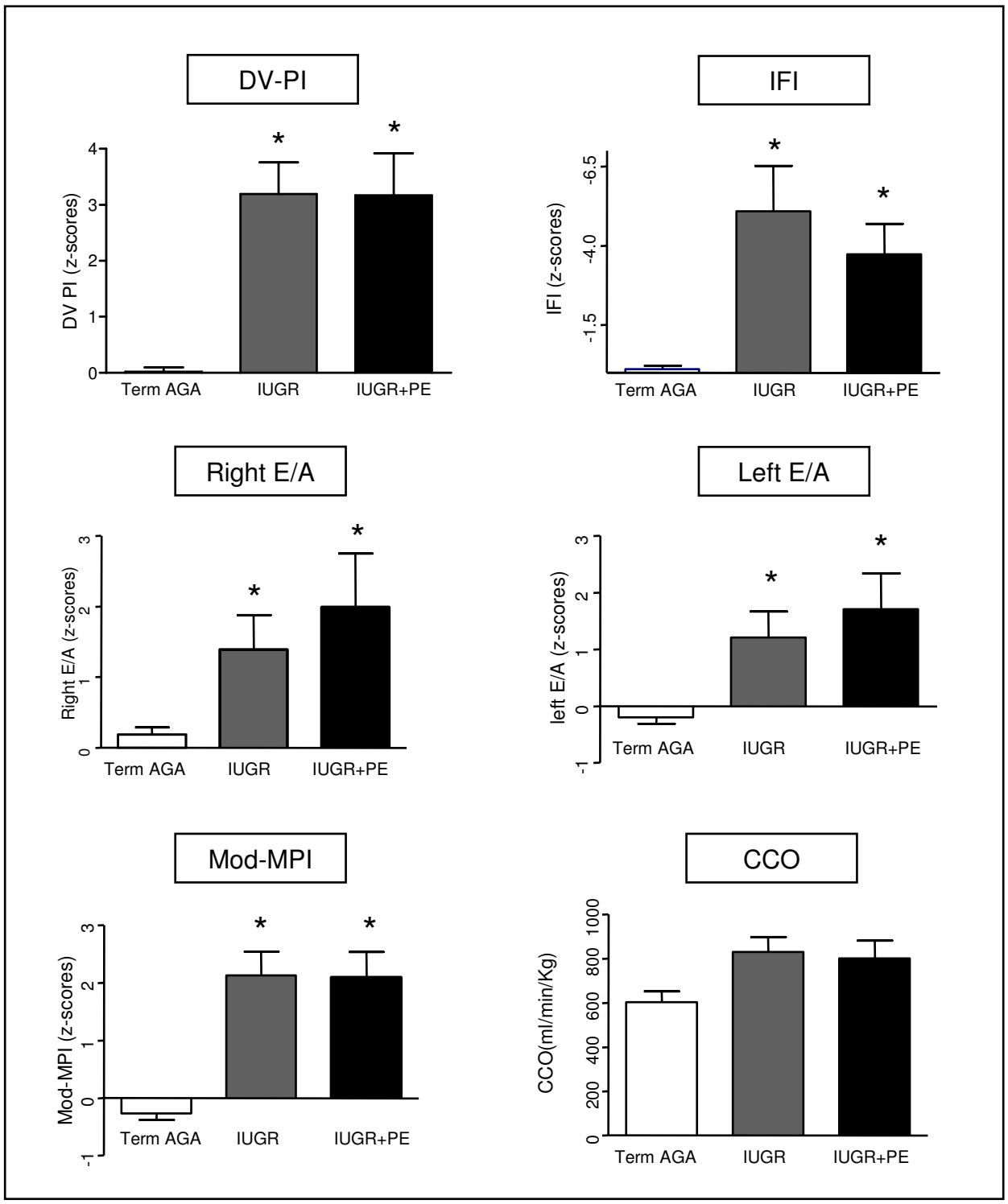
Table 2. Echocardiographic parameters in the study populations.

	Term AGA	**IUGR**	**IUGR+PE**
N	*80*	*31*	*31*
Ductus venosus PI	0.02 (1)	2.3 (4)*	2.1 (5)*
Aortic isthmus flow index	-0,1 (0.9)	-1,7 (8.3) *	-1.7 (7.1)*
Left E/A	-0.3 (1.4)	0.8 (3.4)*	1.2 (2.9)*
Right E/A	0.1 (0.9)	1.6 (3.1)*	1.2 (1.4)*
Mod-MPI	-0.4 (1.2)	1.8 (2.5)*	2.1 (3.4)*
CCO (ml/min/kg)	549 (56)	751 (279)	801 (178)

Doppler values are expressed in Z-scores with the exception of CCO normalized by fetal weight.

*$P<0.05$ compared to term AGA

AGA, appropriate for gestational age; IUGR, intrauterine growth restriction; PE, preeclampsia; PI, pulsatility index; Mod-MPI, modified myocardial performance index; CCO, combined cardiac output.


DV-PI
DV PI (z-scores)
0
1
2
3
4
*
*
Term AGA
IUGR
IUGR+PE
IFI
IFI (z-scores)
-6.5
-4.0
-1.5
*
*
Term AGA
IUGR
IUGR+PE
Right E/A
Right E/A (z-scores)
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Mod-MPI
Mod-MPI (z-scores)
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3
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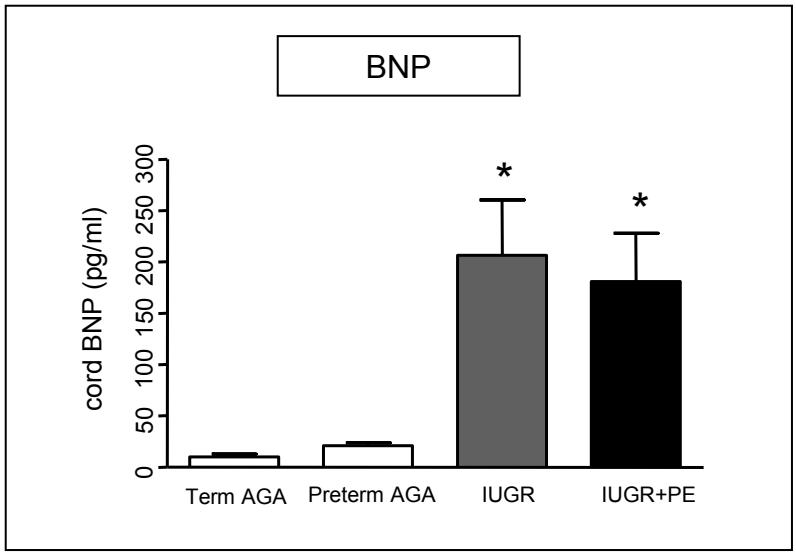
IUGR
IUGR+PE
CCO
CCO(ml/min/Kg)
0
200
400
600
800
1000
Term AGA
IUGR
IUGR+PE


BNP
cord BNP (pg/ml)
0
50
100
150
200
250
300
*
*
Term AGA
Preterm AGA
IUGR
IUGR+PE

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Contribution of the myocardial performance index and aortic isthmus blood flow index to refine prediction of mortality in preterm growth restricted fetuses.

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Contribution of the myocardial performance index and aortic isthmus blood flow index to refine prediction of mortality in preterm growth restricted fetuses.

Short title: **Doppler to predict death in IUGR**

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ABSTRACT

Objectives: To evaluate the predictive value for perinatal death of myocardial performance index (MPI) and aortic isthmus flow index (IFI), as isolated parameters and in a combined model including currently used Doppler indices in preterm growth restricted (IUGR) fetuses.

Methods: Umbilical artery, middle cerebral artery (MCA), ductus venosus (DV), IFI and MPI were measured in a cohort of 97 preterm IUGR fetuses. Logistic regression was performed to select those variables that were independently associated to perinatal mortality, and an algorithm to estimate probabilities of death was constructed including the best combination of parameters.

Results: With the exception of MCA, all Doppler indices were significantly associated with perinatal death as isolated parameters, but only DV and MPI remained independently associated after multivariate analysis. An algorithm combining DV atrial flow (positive or absent-reverse) and MPI (normal or above 95th percentile) had a better predictive accuracy than any single parameter. The risk for death in IUGR fetuses below 28 weeks with: normal DV and normal MPI was 18%, with either abnormal 70-73%, and with both abnormal, 97%. The risk for death in IUGR fetuses above 28 weeks was: with normal DV and normal MPI 0.1%; with either abnormal 6-7%; and with both abnormal 45%.

Conclusions: MPI is an independent predictor of perinatal death in preterm IUGR with accuracy similar to DV. A combination of DV with MPI may better stratify the estimated probability of death. IFI does not add to the prediction of perinatal death when used in combination with DV.

INTRODUCTION

Prediction of perinatal mortality is critical for decision-making in preterm intrauterine growth restricted (IUGR) fetuses. While gestational age is a major predictor for neonatal outcome[1-2], it needs to be combined with further parameters in order to weight the risks of prematurity versus stillbirth. Several approaches have been proposed to monitor these fetuses including arterial and venous Doppler[1-4], cardiotocography (CTG)[5-6] and biophysical profile score (BPP)[5-7] but to date no management standard for these cases has been established[8-10]. Longitudinal studies have documented that ductus venosus (DV) deteriorates earlier than BPP and could be a more sensitive parameter to predict perinatal mortality in IUGR cases[11-12]. In a recent large multicenter study[1], DV emerged as the strongest predictor for poor perinatal outcome. However, its sensitivity for fetal and neonatal death is still 40 to 70 %[1-3].

Over the last years, new cardiovascular parameters have been proposed for fetal assessment. Several experimental and clinical studies have demonstrated the association of aortic isthmus (AoI) flow pattern with adverse outcome[13-14] and later neonatal neurodevelopmental status[15-16] in IUGR fetuses. Additionally, the myocardial performance index (MPI), a Doppler index of combined systolic and diastolic function, has shown to be increased in IUGR fetuses[17-19] with a linear correlation with the hemodynamic severity stage[20-21]. However, no attempt has been made to estimate the potential contribution of these parameters either isolated or in combination with other Doppler indices as predictors of perinatal mortality.

This study evaluated the predictive value for perinatal death of the AoI and MPI, as isolated parameters and in a combined model including currently used Doppler indices. Secondarily, we explored the best combination of currently available cardiovascular indices for the prediction of perinatal mortality in preterm IUGR fetuses and the performance of a clinical decision algorithm including the previously selected parameters.

METHODS

Study population

Eligible cases were singleton pregnancies prospectively selected from women attending the Maternal-Fetal Medicine Department at Hospital Clínic (n=82), and at Harris Birthright Research Centre for Fetal Medicine (n=15) from January 2006 to May 2008. The study protocol was approved by the Ethics Committee at each participating institution, and patients provided their written informed consent. Thirty-seven cases reported here have previously been included as part of a previous study on cardiac function in IUGR[21].IUGR was defined as an estimated fetal weight below the 10th centile according to local reference curves[22-23] together with a Doppler pulsatility index (PI) in the umbilical artery (UA) above 95th percentile[24]. For the purposes of this study only those IUGR who died or were delivered between 24 to 34 weeks' were included. Exclusion criteria for the final analysis were birth weight >10th centile, evidence of fetal infection or structural/chromosomal abnormalities.

Ultrasound assessment

Ultrasound (US) assessment was performed using a Siemens Sonoline Antares (Siemens Medical Systems, Erlangen. Germany) or a Voluson 730 Expert (GE Medical Systems, Milwaukee, WI, USA) with 6-4 or 6-2 MHz curved array probes. Routine US examination included a complete morphological examination, fetal weight and amniotic fluid index calculations.

All Doppler estimations were done in the absence of fetal body movements and, if required, with maternal voluntary suspended respiration. The angle of insonation was kept <30º and the wall filter set to 70Hz to avoid sound artefacts. The mechanical and thermal indices were maintained below 1. Because of their correlation with gestational age, all individual Doppler data were normalized by converting the measurements into Z-scores (standard deviation from the gestational age mean)[24-28]. The following Doppler parameters were recorded: UA, middle cerebral artery (MCA), DV, AoI and MPI (MPI).

UA PI and end-diastolic flow (EDF) were obtained from a free loop of the umbilical cord. UA was dichotomized into three categories according to the EDF characteristics as: present, absent (ADEF) and reversed (REDF). MCA PI was measured in a transverse view of the fetal skull at the level of its origin from the circle of Willis. DV PI and atrial flow were obtained from a mid-sagittal or alternatively transverse section of the fetal abdomen[25]. DV was dichotomized into three categories according to the PI and atrial flow as: normal (PI below 95th percentile), increased pulsatility (PI above 95th percentile) and absent/reversed atrial flow (RAV). AoI blood flow velocity index (IFI) was obtained in a sagittal view of the fetal thorax with a clear visualization of the aortic arch placing the Doppler sample volume between the origin of the left subclavian artery and the confluence of the ductus arteriosus[26]. Alternatively IFI was also obtained in a cross sectional view of the fetal thorax at the level of the 3-vessel and trachea view placing the Doppler gate in the aorta just before the convergence of the arterial duct[27]. IFI was calculated as: (systolic+diastolic)/systolic velocity integrals[26]. Left MPI was obtained in a cross sectional image of the fetal thorax and an apical 4-chamber view, placing the Doppler sample volume on the medial wall of the ascending aorta including the aortic and mitral valve as previously described[29]. The movements (clicks) of the valves in the Doppler trim were used as landmarks to calculate the isovolumetric contraction (ICT) and relaxation times (IRT), and the ejection time (ET). MPI was calculated as (ICT+IRT)/ET. MPI was dichotomized into two categories as: normal (MPI below 95th percentile) and abnormal (MPI above 95th percentile)[28].

For analysis only Doppler estimations recorded within the last 72 hours before delivery or fetal demise were included.

Delivery criteria

The indications for delivery included deterioration of fetal venous indices (absent or reversed atrial flow in the DV), decelerative CTG, persistent abnormal BPP and maternal complications secondary to preeclampsia. When, according to these

criteria, elective delivery was indicated for fetuses at gestational age earlier than 26 weeks, the option of expectant management was discussed with parents and accepted if requested. The managing clinicians were blinded to the results of IFI and MPI. At delivery, gestational age, birth weight, Apgar scores and umbilical pH were recorded.

Data analysis

Variables were log- or square-transformed to achieve normal distribution. The diagnostic performance for perinatal mortality was evaluated by means of 2x2 tables where standard cut-offs for predictive variables were used.

Logistic regression was used to explore the association of UA, MCA, DV, IFI, MPI (as continuous variables) with perinatal mortality, defined as either intrauterine death or neonatal death within the first 28 days of life. Assumptions for logistic regression were checked by assessing the log-distribution of the residuals.

Additionally, a stepwise variable selection procedure was performed, where all predictive variables were introduced categorized (normal/abnormal). Standard rules were used for variable selection in the stepwise procedure[30]. Gestational age was also included in the final model as it has been demonstrated to be the strongest predictor for perinatal mortality in IUGR fetuses[1]. For each predictor, the cut-off best associated with perinatal mortality on the univariate analysis was used. The model coefficients were used to predict the mortality risk for each combination of the significant predictive variables resulting in a score risk. From the final model, risks were stratified for each combination of predictive variables for fetuses who delivered before and after 28 weeks.

Data were analyzed with the SPSS 13.0 statistical package (SPSS, Chicago, Illinois, USA). Differences were considered significant when *P*-values < 0.05.

RESULTS

Maternal characteristics, Doppler recordings and perinatal outcome of the study population are shown in Tables 1 and 2. A total of 97 preterm IUGR cases were included. The median gestational age at delivery was 30 weeks and the median birth weight 956g. The indications for delivery were deterioration of fetal venous indices (49 cases), decelerative CTG (12 cases), persistently abnormal BPP (5 cases), maternal complications secondary to preeclampsia (23 cases) and fetal death (8 cases). The overall perinatal mortality was 22% including 8 intrauterine and 14 neonatal deaths. All cases but 8 intrauterine deaths were delivered by cesarean section. Those IUGR fetuses that died were delivered earlier, and had lower birth weight as compared with survivors. All Doppler indices with the exception of MCA were significantly different in those IUGR fetuses that died as compared to survivors.

Univariate analysis demonstrated that all Doppler indices (DV-PI, $P<0.0001$; UA-PI, $P<0.0001$; MPI, $P<0.0001$; IFI, $P<0.0001$) with the exception of MCA-PI ($P=0.068$) were significantly associated with perinatal mortality (Table 3a). Multivariate analysis identified UA-PI (estimated odds ratio = 3.01, $P=0.02$), DV-PI (estimated odds ratio = 3.69, $P=0.008$) and MPI (estimated odds ratio = 1.39, $P=0.02$) as statistically significant independent predictors for perinatal mortality (Table 3b). IFI did not show any significant contribution to the explanation of perinatal mortality. The Hosmer & Lemeshow statistic was performed as a measure of goodness-of-fit (Chi-square = 9.292; *P=0.318*).

When gestational age was included in the model and variables were dichotomized to normal/abnormal, only gestational age (below 28 weeks), DV atrial flow (RAV) and MPI (abnormal) significantly and independently accounted for perinatal mortality (R^2 Nagelkerke 50%) (Table 4). The Hosmer & Lemeshow statistic was performed as a measure of goodness-of-fit (Chi-square = 2.135; *P=0.545*). Table 5 shows the diagnostic performance of different standard cut-offs of the predictive

variables for perinatal mortality. According to this model, risk was stratified for each combination of predictive variables (Figure 1) and a simplified score was modelled (Table 6).

DISCUSSION

This study evaluated the independent and combined contribution to the prediction of mortality of cardiovascular parameters currently not integrated in clinical practice. The findings confirmed that DV is the strongest Doppler predictor for perinatal mortality in preterm IUGR[1-2]. However, MPI showed an independent association with perinatal death, with a predictive accuracy similar to DV. The data suggest that a score combining DV and MPI might improve the predictive accuracy obtained with each parameter alone. These findings support the notion that combination of several cardiovascular indices may improve clinical management by better stratifying the estimated probability of death.

With the exception of MCA, all Doppler indices evaluated in this study showed a significant association with perinatal mortality as isolated parameters. However, only UA, DV and MPI remained as independent predictors of mortality in a multivariate analysis. When variables were dichotomized to explore their predictive value in clinical practice, only DV-RAV and abnormal MPI showed an independent association with perinatal mortality, which persisted after adjusting for gestational age, so they could be used to construct a clinical-decision algorithm. Our results are in line with previous studies integrating several Doppler indices and showing DV as the best predictor for perinatal death in preterm IUGR[1-3]. The data are also in agreement with previous studies on UA and MCA, where a lack of improvement in prediction of mortality after adjustment for other Doppler parameters and gestational age was shown[1-2,31-32]. Finally, as previously reported[1-3], gestational age at delivery was so strongly influencing perinatal outcome that the explored algorithm had to incorporate this variable to obtain meaningful results.

This study first reports that MPI is independently associated with perinatal mortality, and that its combination with DV could improve the predictive value of current fetal Doppler evaluation. MPI is a global cardiac parameter that evaluates both systolic

and diastolic function by including the measurement of isovolumetric and ejection times[33]. Inclusion of the Doppler clicks of aperture and closure of the mitral and aortic valves, to estimate the time-periods for MPI calculation, enhances the reproducibility of the measurement[29]. In a recent study we showed that MPI correlates with the clinical hemodynamic deterioration of IUGR fetuses and with progressively increased levels of cardiac dysfunction biomarkers such as B-type natriuretic peptide[21].

The evaluation of IFI as an isolated parameter in this study was consistent with previous series reporting that IUGR cases with AoI reversed diastolic flow have a deterioration of cardiac function[34] and a poorer perinatal outcome[13-15,34]. However, the multivariate analysis demonstrated that IFI does not add to DV in the prediction of mortality. These findings seem to be explained by an intrinsic correlation of AoI with DV and other cardiovascular parameters[34-36]. Furthermore, in a longitudinal analysis of the evolution of the AoI and other Doppler indices in preterm IUGR, we demonstrated that the IFI becomes abnormal earlier than DV[37] in the sequence of fetal hypoxic deterioration. This may also partially explain a lower predictive capacity of IFI as an acute marker for a late event such as mortality. It must be noted that our findings do not preclude the strong predictive value of IFI when used as an isolated parameter[13-15,34]. On the other hand, this study focused on perinatal death, but not on neurological outcome. IFI has been reported to constitute a strong predictor of morbidity, mainly long-term neurodevelopmental adverse outcome[15-16], an aspect not evaluated in this study.

This study has several limitations. The managing clinicians were not blinded to UA, MCA and DV results. Actually, DV-RAV was a delivery criterion and therefore we acknowledge that this may have distorted the expected perinatal outcome. However, according to available evidence[11,12] it would be unethical not to consider DV-RAV a delivery criterion, and this limitation is present in recent clinical studies evaluating fetal/neonatal mortality[1-3]. On the other hand, the study evaluated the predictive value

of Doppler indices, but the data were not combined with other proposed means for fetal monitoring of preterm IUGR, such as BPP and computerized CTG[4-7,38], and this may limit clinical applicability of these findings in certain clinical settings.

In conclusion, this study suggests that a combination of MPI with DV improves the predictive value of these parameters alone. The results support the notion that combined cardiovascular scores might refine considerably the prediction of perinatal mortality at different gestational ages at delivery in preterm IUGR cases. Gestational age remains the strongest predictor of mortality and any score should contemplate it. Larger prospective studies are required to further confirm these results and to evaluate its potential clinical value. Additionally, its correlation and potential interaction with other clinical parameters such as BPP and computerized CTG remains to be assessed.

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Table 1. Maternal characteristics and perinatal outcome of the study population ().

Maternal characteristics	
Maternal age (years)	31 (28 to 36; 92)
Caucasians (%)	70 (65/92)
Smokers (%)	17 (15/88)
Primiparity (%)	58 (56/96)
Preeclampsia (%)	59 (57/97)
Doppler recordings	
UA-PI	12 (5 to 15; 97)
MCA-PI	-2 (-3 to -2; 97)
DV-PI	3 (1 to 5; 97)
IFI	-3 (0 to -5; 97)
MPI	3 (0 to 4, 97)
Delivery data	
Gestational age at delivery (weeks)	30 (28 to 32; 97)
Birth weight (g)	956 (694 to 1170; 95)
Birth weight's percentile	1 (0 to 1; 95)
5 min Apgar<7 (%)	15 (13/86)
Umbilical artery pH<7.20 (%)	35(33/86)
Perinatal outcome	
Days in neonatal intensive care unit	32 (14 to 50; 86)
Perinatal death (%)	22 (22/97)
Bronchopulmonary displasia (%)	8 (7/87)
Intraventricular hemorrhage III-IV (%)	6 (5/80)
Necrotizing enterocolititis (%)	3 (3/87)
Adverse outcome (%)	30 (29/97)

Data are median (interquartile range;N) or proportions (N). Doppler values are expressed in z-scores
PI, pulsatility index; UA, umbilical artery; MCA, middle cerebral artery; DV, ductus venosus; IFI, aortic isthmus flow index; MPI, myocardial performance index. Adverse outcome was defined by the presence of perinatal death, bronchopulmonary dysplasia, neonatal intraventricular haemorrhage grade 3 or 4, or necrotizing enterocolitis.

Table 2. Doppler results and antenatal surveillance in intrauterine growth restriction fetuses.

Doppler recordings	**Survivors (n=75)**	**Perinatal deaths (n=22)**
UA-PI	6.3 (4 to 10; 75)	17.1 (13 to 88; 22)*
MCA-PI	-2.5 (-3 to -2; 75)	-1.9 (-2 to -2; 22)
DV-PI	1.4 (0 to 3; 75)	6.7 (4 to 10; 22)*
IFI	-1 (0 to 4; 75)	-6 (-2 to 9; 22)*
MPI	1.9 (0 to 4; 75)	3.3 (1 to 4; 22)*
Delivery data		
Gestational age at delivery (weeks)	31 (29 to 32; 75)	27 (26 to 28; 22)*
Birth weight (g)	1045 (794 to 1252; 75)	539 (400 to 590; 20)*
Birth weight's percentile	0 (0 to 1; 75)	0 (0 to 1; 20)
5 min Apgar<7 (%)	8 (6/73)	6 (8/13)*
Umbilical artery pH<7.20 (%)	31 (23/74)	80 (10/12)
Neonatal outcome		
Bronchopulmonary displasia (%)	4 (3/75)	33 (4/12)*
Intraventricular hemorrhage III-IV (%)	6 (4/68)	8 (1/12)*
Necrotizing enterocolititis (%)	4 (3/75)	0 (0/12)

Data are median (interquartile range; N) or proportions (N). Doppler values in z-scores.
*P<0.05 compared to survivors.
PI, pulsatility index; UA, umbilical artery; MCA, middle cerebral artery; DV, ductus venosus; IFI, aortic isthmus flow index; MPI, myocardial performance index.

Table 3. Estimated odds ratio for perinatal mortality by logistic regression.

3a. Univariate analysis

	Mean difference between survivors and deaths	***P-value***
UA-PI	17.91	*<0.0001*
MCA-PI	0.54	*0.068*
DV-PI	5.23	*<0.0001*
IFI	3.94	*<0.0001*
MPI	2.32	*<0.0001*

3b. Multivariate analysis

	Estimated odds ratio (95% confidence interval)	***P-value***
UA-PI	3.01 (1.19-7.64)	*0.02*
MCA-PI	-	*0.072*
DV-PI	3.69 (1.41-9.64)	*0.008*
IFI	-	*0.178*
MPI	1.39 (1.05-1.82)	*0.02*

UA, umbilical artery; PI, pulsatility index; MCA, middle cerebral artery; DV, ductus venosus; IFI, aortic isthmus flow index; MPI, myocardial performance index. Estimated odds ratios represent the increase in likelihood for each increased unit of standard deviation.

Table 4. Estimated odds ratio for perinatal mortality by logistic regression after dichotomizations of variables and inclusion of gestational age into the model.

	Estimated odds ratio (95% confidence interval)	***P-value***
Gestational age < 28 weeks	34.61 (6.58-182.04)	*<0.001*
DV-RAV	12.24 (1.94-77.31)	*0.008*
MPI >95th centile	10.42 (1.00-109.33)	*0.049*

DV, ductus venosus; RAV, reversed / absent atrial flow; MPI, myocardial performance index. Estimated odds ratios represent the increase in likelihood for each increased unit of standard deviation.

Table 5. Predictive values for perinatal mortality.

Variable	Detection rate (%)	False positive rate (%)	Positive likelihood ratio	Negative likelihood ratio
Gestational age < 28 weeks	87 (55-92)	11 (2-15)	7.91 (5-15)	0.14 (0.08-0.8)
DV-RAV	57 (32-76)	5 (2-13)	11.40 (7-15)	0.45 (0.2-1.4)
MPI >95th centile	95 (77-99)	52 (43-66)	1.82 (1.3-2.3)	0.10 (0.01-0.7)

Data are value (95% confidence interval). DV, ductus venosus; RAV, reversed / absent atrial flow; MPI, myocardial performance index.

Table 6. Performance of a cardiovascular score risk including DV and MPI for the prediction of perinatal mortality.

	Cardiovascular score risk	Estimated probability for perinatal death
Gestational age < 28 weeks	0	18%
	1	70-73%
	2	97%
Gestational age ≥ 28 weeks	0	0.1%
	1	6-7%
	2	45%

DV, ductus venosus; MPI, myocardial performance index.
Cardiovascular score risk was defined as: 0, present atrial flow in the DV together with normal values of MPI (below 95th percentile); 1, DV-reversed /absent atrial flow (RAV) or MPI > 95th centile; 2, DV-RAV and abnormal MPI.

Figure 1. Flowchart to estimate the probability of perinatal death according to gestational age, ductus venosus (DV) and myocardial performance index (MPI).

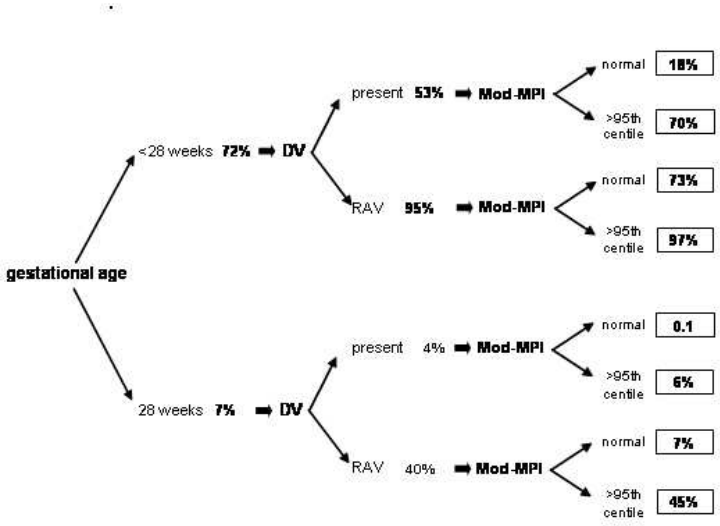

Figure 1

254x190mm (72 x 72 DPI)

Cardiac remodelling in children with fetal growth restriction

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Abstract

Background

Fetal growth restriction, with a prevalence of 5-10% in newborns, is associated with increased cardiovascular mortality in adulthood, but the pathophysiological links of this relationship are only partially understood. We evaluated the hypothesis that fetal growth restriction induced persistent cardiac structural and functional changes in children at 1 to 6 years of age.

Methods

In a cohort study of 25,530 pregnancies, delivering between 2002 and 2007, we randomly identified 80 subjects with fetal growth restriction and compared them with 120 normally grown fetuses, matched for gender, birth date and gestational age at birth. Cardiac morphometry and function by echocardiography, blood pressure and carotid wall thickness were assessed.

Findings

As compared with controls, children with fetal growth restriction had a different cardiac shape, with increased transversal diameters and more globular cardiac ventricles. This was associated with subclinical longitudinal systolic dysfunction (decreased myocardial peak velocities) and diastolic changes (increased E/E' ratio and E deceleration time). Fetal growth restriction was also associated with significantly higher blood pressure and signs of arterial remodelling (increased intima-media thickness). For all parameters evaluated, there was a linear increase with the severity of growth restriction, but the associations were independent of gestational age at delivery.

Interpretation

These findings suggest that fetal growth restriction induces primary cardiac changes, resulting in a different morphology of the heart, which could explain the increased predisposition to cardiovascular disease in adult life. Given its high prevalence in the general population, this might have to be taken into account in assessing cardiovascular risk factors and treatment.

Introduction

Cardiovascular disease is the main cause of death in adults. Most factors leading to chronic cardiovascular disease are already present in childhood.[1-2] Epidemiological evidence has long suggested a link between low birth weight and increased cardiovascular mortality in adulthood.[3] This association is essentially mediated through fetal growth restriction (FGR),[4] a condition affecting 5 to 10 % of all newborns.[5] The mechanistic pathways underlying the relationship between FGR and cardiovascular risk are poorly understood.[6] A number of studies support that it might be partially explained by fetal metabolic programming leading to diseases associated with cardiovascular disease, such as obesity, diabetes and hypertension.[6] However, it remains unclear whether FGR induces primary changes in the heart which might predispose to cardiovascular dysfunction later in life.

It has recently been reported that fetuses[7-8] and newborns[9] with severe forms of growth restriction have significant changes in fetal cardiac function parameters and natriuretic peptides, in spite of normal cardiac output. In addition, newborns with FGR have an increase in aortic intima-media thickness,[10-11] supporting the existence of vascular remodelling. Animal studies suggest that subclinical cardiovascular abnormalities in fetuses exposed to growth restriction persist into adulthood,[12] but it is unknown whether this effect occurs in humans.

In this study we evaluated the hypothesis that adaptation to growth restriction induces persistent cardiovascular changes in children. From a prospective perinatal registry, we selected a cohort of FGR children classified into mild or severe growth restriction, and a cohort of normally grown children matched for gestational age at delivery. We evaluated the association between FGR and echocardiographic structural and functional measurements at one to six years of age.

Methods

Study populations

The study design was a prospective cohort study including 80 children with FGR and 120 controls with birth weight appropriate for gestational age (AGA). The source population comprised all pregnancies cared for between January 2002 to October 2007 at a tertiary referral university hospital in Barcelona (Spain), which covers a inner city area of about 0.6 million inhabitants, and registered in a database prospectively constructed at the time of delivery (n=25,350). Cases were considered non-eligible in the presence of any of the following, congenital malformations and/or chromosomal defects, evidence of fetal infection, and multiple monochorionic pregnancy. Eligible cases were infants with a birth weight below the 10th centile according to local standards[13] of which full prenatal information was available including umbilical artery Doppler. For the purposes of this study, FGR was classified as mild when umbilical artery Doppler was normal (pulsatility index below 2 standard deviations) and severe FGR when it was abnormal (pulsatility index equal or above 2 standard deviations).[5] From the eligible population, cases were randomly sampled and offered to participate until a final study population of 40 cases with mild FGR and 40 with severe FGR was completed, according to the sample size requirements. A reference cohort of children born with a normal birth weight (> 10th percentile) randomly sampled from pregnancies delivering at our institution was selected as control group. Non-eligibility criteria for controls were the same as for cases. Controls were matched two-to-one with mild FGR cases and one-to-one with severe FGR according to gender, birth date (± 6 months) and gestational age at delivery (± 1 week) calculated by first-trimester crown–rump length measurement. The study protocol was approved by Hospital Clinic Ethics Committee, and written parental consent was obtained for all study participants. Figure 1 shows a diagram of flow of the study population.

Study protocol and follow-up

Parents accepting to participate in the study were given an appointment for a single visit in which all examinations contemplated in the study were performed. The study protocol consisted in a medical examination and a dietary questionnaire. A blood sample extraction was also proposed but, if parents rejected it, the case was not excluded from the study. The study was completed by a detailed echocardiography and ultrasound carotid assessment.

The follow-up team consisted of a research nurse trained to perform the medical evaluation (including height, weight and blood pressure), blood sample extraction and dietary questionnaire, and two experienced physicians (F.C., J.B.) who performed echocardiography and carotid assessment. Perinatal, including fetal ultrasound and Doppler exams, demographic and neonatal data were already recorded in the database, but were confirmed by review of medical records and clinical databases and by parental interview at the time of study evaluation.

Anthropometric data including child's height, weight and body mass index were measured at the time of the study examination, and their percentiles were calculated according to local reference values.[14] Nutritional status was assessed by questionnaires in which parents reported the child's weighted food and beverages diaries consumed for 3 days. Nutrient analysis for such diet was performed with DietSource Junior 1.1.23 software provided by Nestle Healthcare Nutrition S.A. (Esplugues de Llobregat, Spain). Total cholesterol, high-density lipoprotein, triglyceride, glucose and C-reactive protein concentrations were measured by standard methods on an automatic analyzer (Olympus AU-400, Germany) in fasting blood samples. Low-density lipoprotein concentrations were calculated according to the Friedewald formula.

Children with hypertension (blood pressure above 95th percentile for age, gender and height), abnormal lipid profile or suboptimal nutritional status were referred to their pediatrician for follow-up and treatment.

Cardiac morphometry and function

All echocardiographic exams were performed following a standardized protocol[15] using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA) with 2-10 MHz phased-array transducer. Electrocardiogram was registered continuously during echocardiography. A complete two-dimensional, M-mode and Doppler echocardiographic examination was performed initially to assess structural heart integrity and morphometry. Linear measurements of base-to-apex length and basal diameter of left and right ventricles were measured on 2D images from an apical four-chamber view at end-diastole (Figure 2), and the longitudinal-transverse ratio was calculated for each ventricle. Left ventricular end-diastolic septum and posterior wall thickness were measured by M-mode from a parasternal long-axis view.

Cardiac function was assessed by echocardiography. Besides the conventional echocardiographic examination, tissue Doppler (TDI) velocities were obtained in mitral lateral and septal annulus, and tricuspid lateral annulus from an apical four-chamber view and measured in real time during echocardiographic study. For systolic function, cardiac output, left ejection fraction, mitral and tricuspid ring displacement by M-mode and systolic myocardial velocities (S') were measured. Diastolic function was assessed by measuring peak early (E) and late (A) transvalvular filling velocities, E/A ratio, isovolumic left ventricle relaxation time (IVRT), deceleration time of E velocity, early diastolic peak myocardial velocities (E'), and E/E' ratios. When blood was available, B-type natriuretic peptide (BNP) concentrations were measured using Siemens ADVIA Centaur® BNP assay.

Vascular assessment

Systolic and diastolic blood pressure was obtained at the beginning of the medical evaluation by a trained nurse while the child was sitting after resting 5 to 10 minutes. Right and left carotid arteries were scanned following a standardized protocol[16] using 13MHz linear array transducer. Catotid intima-media thickness (cIMT) measurements were performed off-line with the assistance of a computerized program (Siemens Syngo® Arterial Health Package) (Figure 3). Circumferential wall stress was calculated as: (mean blood pressure x mean diastolic carotid diameter)) / 2 x cIMT.

Statistics

The primary outcomes were cardiac dimensions, function and vascular wall dimensions. The sample size was calculated to allow to observe a difference of 20 % in E/E' between the group of severe FGR and controls, with a 85% of power and a 5% of type I risk. Basal mean and within group standard deviation were estimated according elsewhere published normative data in children,[17] resulting in a required sample of 40 individuals in each group. Data are presented as mean±SD, median (interquartile range) or as percentage, as appropriate. Paired comparisons between the study and controls groups were adjusted for age, gender and gestational age at delivery by linear (GLM) or logistic regression. In addition, a polynomial contrast was also constructed for each model to test the hypothesis of a linear association across FGR severity groups. All reported P-values are two-sided. The software statistical package SPSS 15.0 (SPSS, Chicago, Illinois, USA) was used for the statistical analysis.

Results

Anthropometric, echocardiographic and vascular data were obtained from all patients included. Satisfactory nutritional assessment could be obtained in 95%, 95% and 92.5% of children in controls, mild and severe FGR groups respectively. Parents accepted blood sampling in 42.5 %, 55% and 82.5% respectively. Two children were excluded because of structural heart defect diagnosed at the time of the study and referred to the Department of Pediatric Cardiovascular Surgery of Hospital Sant Joan de Déu.

Baseline and follow-up characteristics

Baseline characteristics are shown in Table 1. The study groups were similar in terms of maternal, paternal and familiar characteristics, with the exception of lower parental height in mild FGR children as compared to AGA. As expected, FGR children had a higher occurrence of pregnancy complications, worse prenatal Doppler ultrasound findings, umbilical artery pH and longer admittance in neonatal intensive care unit.

Follow-up characteristics at the time of children assessment are shown in Table 2. At the time of FGR children showed a linear tendency to lower height and weight values with similar results on body mass index as compared to AGA. Nutritional parameters and lipid/glucose profile showed similar values among all study groups.

Cardiac morphometry and function

Results are shown in Table 3 and Figure 2. Cardiac shape was significantly altered, with the left and right longitudinal-transverse ratios significantly decreased in mild and severe FGR children. Interventricular septum and left posterior wall thickness showed a significant linear trend to decreased values across FGR severity groups.

While cardiac index, left ejection fraction and B-type natriuretic peptide, were similar among the study groups, stroke volume was significantly changed, compensated by a significantly increased heart rate to maintain output in severe FGR children, with a significant tendency across the FGR severity groups. Systolic mitral and tricuspid ring displacements were significantly decreased in mild and severe FGR cases as compared with AGA. Severe FGR children showed significantly lower longitudinal S' in mitral lateral, mitral septal and tricuspid annulus as compared to AGA, with a significant linear tendency to lower values across FGR severity stages.

While mitral and tricuspid E/A ratios and IVRT were similar among study groups, mitral E deceleration time was significantly increased in mild and severe FGR with respect to controls. Tricuspid E deceleration time was significantly increased in severe FGR children, although there was a significant linear tendency across severity groups. Severe FGR children showed significantly lower longitudinal E' in mitral lateral, mitral septal and tricuspid annulus as compared to AGA with a significant linear tendency to lower values across FGR severity stages. Mitral lateral and septal E/E' ratios were significantly higher in severe FGR as compared to AGA, with a significant linear trend to higher values across severity stages.

Vascular assessment

Results are displayed in Table 4 and Figure 3. Systolic and diastolic blood pressure was significantly higher in mild and severe FGR groups. cIMT was significantly increased in severe FGR children. Circumferential wall stress values were significantly higher both in mild and severe FGR groups. There was a significant linear trend for higher values in relation to severity of FGR for all parameters evaluated.

Discussion

This study provides direct clinical evidence that FGR children show changes in cardiac morphology, subclinical cardiac longitudinal dysfunction and arterial remodelling, all of which increase linearly with the severity of growth restriction. The findings support the existence of direct cardiac programming in FGR and suggest a new mechanistic pathway for the association between fetal growth and cardiovascular disease. The most striking finding was that children with FGR have a distinct cardiac geometry and shape, with less elongated and more globular ventricles. Morphometrical measurements confirmed quantitatively an overall increase in transverse cardiac diameters, leading to apparent dilatation of the ventricular cavities with thinner walls. The data are in line with post-mortem studies in human FGR describing hypoplasia in myocardial fibers.[18] These findings are also in agreement with our recent animal studies showing the persistence of dilated cardiomyopathy-like features in utero into adulthood in chick model of FGR under chronic hypoxia.[12]

The globular cardiac shape with thinner ventricular walls observed in children with FGR is most likely the result of changes in the cardiac development, induced by its fetal working conditions. The intrauterine chronic hypoxic and undernutrition state,[44] together with the increased placental vascular resistance,[43] result in a combined pressure and possibly volume overload of the fetal heart,[43,45,46] which induces abnormal cardiac function[7-8,43] and changes the wall stress on the developing myocardial fibres. Myocardial wall stress is determined by the ventricular pressure, the local radius of curvature and the wall thickness.[36] Increased wall stress will result in a tendency towards reducing this by myocardial remodelling. In acquired mild pressure overload hypertrophy in the region of highest stress results in a local stress normalisation.[37] However, in the developing heart under hypoxia and undernutrition, this locally increased wall stress could also be compensated for by changes in the local radius of curvature towards a more spherical the cavity.
The fetal alterations in shape are likely to induce a stabilised change in muscle fibre architecture[38] in the ventricular wall since the resulting myocardial shape and fiber orientation are importantly determined by the stress and strain conditions they are exposed to.[39,40] Therefore, as observed in our study, children that were exposed to important changes in fetal loading condition will have intrinsically differently shaped hearts. When these hearts work under normal loading conditions after birth, they are able to generate the required cardiac output without initial problems. However, the more globular shaped ventricle, with potentially a different architecture, is not as efficient in generating the normal longitudinal displacement and stroke volume,[41] resulting in the need for an increased heart rate to maintain cardiac output (as observed). Since diastolic function depends on ventricular shape and torsion, as generated by the normal fibre architecture,[42] it is not unexpected to find additional changes in diastolic parameters. Whereas the remodelled ventricles can compensate for their lower efficiency in childhood, any additional changes in its working conditions (hypertension, ischemia, arrhythmias) at later age will result in an abnormally high increase in local wall stress and dilatation since the potential for shape adaptation of the normal, ellipsoid, ventricle is not possible. Therefore, the initial cardiac remodelling, resulting from FGR, might explain the increased risk of cardiovascular disease described in epidemiological studies on FGR.[3]

This study confirms and extends previous studies reporting significant increased carotid wall thickness[10-11] in children suffering FGR. Increased cIMT had previously been reported in newborns with FGR[10-11] and our findings demonstrate that these changes persist into childhood. Since the hearts of FGR children did not present hypertrophic changes, characteristic of hypertensive cardiomyopathy, the arterial changes in children with FGR are primary and not secondary. The increased arterial wall thickness is most likely the result of the overall pressure and possible volume overload in the FGR fetal circulation, where vascular wall stress induces hypertrophy of the intima-media layer. In childhood, the remodelled arteries, now working under normal loading conditions, will result in an increased

peripheral resistance and an elevation in blood pressure[29]. Both blood pressure and cIMT are additional, accepted, risks factors for future cardiovascular disease.

There are several limitations and considerations in the present study. The changes here reported are subclinical and the long-term association with adult cardiovascular function and disease remains to be further proven. The study was not designed to assess the effect of other factors on cardiovascular function. In this study cardiac changes were independent of obesity or abnormal lipid profile, but the prevalence of these risk factors was very low in our setting. The existence of metabolic programming in FGR is well demonstrated,[6] and the potential interactions between metabolic and cardiac programming in the increased risk of cardiovascular disease in these patients remains to be elucidated. The impact of gender was also addressed, and cardiovascular differences were equally observed in males and females (Supplementary Data), but we acknowledge that we may have lacked statistical power to detect subtle gender-associated differences. FGR children born preterm were compared with cases born at similar gestational age. Our findings are in line with recent studies that suggest that prematurity is not associated with fetal cardiovascular programming.[4] Exposure to prenatal glucocorticoids was also similar, although the influence of corticoids in cardiac function has recently been discarded in a large cohort study.[31] Finally, other potential confounders, such as socioeconomic status, race, familiar early cardiovascular history, breastfeeding, parity and parental smoking were also similar among study groups.

In summary, this study provides evidence of an association between FGR and cardiac remodelling and longitudinal dysfunction in childhood, which shows a linear increase with the severity of growth restriction and is independent of gestational age at delivery, lipid profile or body mass index. Primary cardiac programming might be one of the causes of increased cardiovascular mortality in adults born with FGR, and this may open new opportunities for monitoring and intervention in newborns and children affected with this condition. FGR affects 5-10% of all newborns and therefore the findings of this study involve thousands of children yearly. The importance of early identification and intervention in pediatric risk factors for cardiovascular disease is now well recognized.[32] However, FGR is not listed among the conditions presumed to increase cardiovascular risk in current consensus guidelines.[32,33] We believe that the results of this study merit further investigation in appropriately designed large cohort studies. If these findings are confirmed, the impact of lifestyle strategies[32,34-35] with beneficial effects on cardiac remodelling should be explored in FGR children.

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Table 1: Baseline characteristics of the study groups

Characteristic	AGA (N=120)	mild FGR (N=40)	severe FGR (N=40)	Linear tendency P Value
Male sex (%)	46	40	38	0.560
White race (%)	95	98	90	0.342
Low socioeconomic level (%)	3	0	8	0.406
Familiar early cardiovascular history (%)†	17	10	27	0.121
Maternal characteristics				
Age (yr)	33±4	33±4	32±5	0.417
Height (cm)	163±55	160±68*	162±74	0.209
BMI (kg/m^2)‡	22 [21,25]	22 [20,24]	23 [21,27]	0.077
Smoking (cigarettes per day)	0 [0,3]	0 [0,10]	0 [0,7]	0.904
Primiparity (%)	64	73	70	0.539
Paternal characteristics				
Age (yr)	35±5	35±4	35±6	0.882
Height (cm)	177±67	174±55*	174±80	0.051
BMI (kg/m^2)‡	25 [24,27]	25 [24,27]	26 [24,28]	0.376
Smoking (cigarettes per day)	0 [0,10]	0 [0,5]	4 [0,15]	0.011
Pregnancy complications				
In vitro fecundation	4	3	5	0.845
Preeclampsia	1	3	38*	<0.001
Gestational diabetes	3	15*	3	0.016
Prenatal glucocorticoid exposure				
Born preterm (%)	85% (34/40)	-	88% (35/40)	0.378
Born at term (%)	0% (0/80)	0% (0/40)	-	-
Prenatal ultrasound				
Umbilical artery pulsatility index (Z-scores)	0 [-1,1]	0 [-1,1]	6 [4-8]*	<0.001
Middle cerebral artery pulsatility index (Z-scores)	0 [-1,1]	-1 [(-1,1)]*	-3 [-3,-2]*	<0.001
Ductus venosus pulsatility index (Z-scores)	0 [-1,1]	-1 [(-1,0])	1 [0,2]	0.241
Gestational age at delivery (weeks)	38 [34,40]	40 [39,40]*	32 [30,34]*	<0.001
Birthweight (g)	3150 [2300,3550]	2630 [2505,2738]*	1065 [875,1402]*	<0.001
Birthweight percentile	55 [31,81]	3 [1-3]*	0 [0,0] *	<0.001
Umbilical artery pH	7.30 [7.25,7.35]	7.24 [7.17,7.28]*	7.24 [7.17,7.27]*	0.002
Days in neonatal intensive care unit	0 [0,5]	0 [0,3]	30 [27,60]*	<0.001
Major neonatal morbidity (%)§	7	0	34*	<0.001
Breastfeeding (months)	4 [2,8]	5 [2-11]	4 [4-6]	0.927
Postnatal corticoid exposure (%)	4	8	8	0.585
Postnatal growth hormone treatment (%)	0	0	3	0.997

Data are mean±SD or median [interquartile range].
AGA = children with birth weight adequate for gestational age. FGR = fetal growth restriction. BMI = body mass index.
* P-value<0.05 as compared to AGA calculated by linear or logistic regression.
† Defined as early cardiovascular disease (including congenital heart disease, coronary disease, hypertension, diabetes or hypercholesterolemia) in expanded 1st degree pedigree (male<55 years; female <65 years).
‡ BMI calculated as weight in kilograms divided by the square of the height in meters.
§ Major neonatal morbidity defined by the presence of bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukomalacia, retinopathy, persistent ductus arteriosus or sepsis.

Table 2: Follow-up characteristics of the study groups

Characteristic	AGA (N=120)	Mild FGR (N=40)	severe FGR (N=40)	Linear tendency P Value
Age (yr)†	5 [3,6]	5 [3,5]	4 [2,5]	0.654
Anthropometric data	**(N=120)**	**(N=40)**	**(N=40)**	
Height (cm)	109 [88,117]	105 [96,110]	94 [80,107]*	0.002
Height percentiles	52 [48,56]	50 [47,52]	48 [43,51]*	0.001
Weight (kg)	18 [13,22]	16 [14,19]*	14 [11,18]*	0.369
Weight percentiles	49 [30,73]	37 [14,62]*	24 [9,47]*	0.212
Body mass index (kg/m^2)‡	16 [15,17]	15 [15,17]*	16 [15,17]	0.480
Obesity (%)§	5	0	5	0.352
Nutritional assessment	**(N=114)**	**(N=38)**	**(N=37)**	
Total energy (kcal)	1606±340	1522±374	1525±388	0.720
Proteins (%)	18±6	18±3	18±3	0.717
Carbohydrates (%)	48±9	49±7	49±7	0.448
Fats (%)	34±6	34±5	33±6	0.171
Saturated fatty acids (%)	38±7	38±7	40±7	0.058
Monounsaturated fatty acids (%)	34±6	36±5	34±5	0.644
Polyunsaturated fatty acids (%)	8±5	7±3	7±2	0.759
Lipid and glucose profile	**(N=51)**	**(N=22)**	**(N=33)**	
Total cholesterol (mg/dL)	160 [142,185]	174 [147,194]	168 [137,189]	0.739
High-density lipoproteins (mg/dL)	54 [41,61]	48 [40,60]	55 [41,60]	0.526
Low-density lipoproteins (mg/dL)	96 [75,114]	110 [79,128]	96 [71,109]	0.423
Triglyceride (mg/dL)	73 [59,98]	53 [43,110]	78 [63,110]	0.159
Glucose (mg/dL)	89 [82,95]	88 [84,92]	86 [84,92]	0.247
C-reactive protein (mg/dL)	0.75 [0,1]	1 [0,5]	0.30 [0,2]	0.330

Data are mean±SD or median [interquartile range].
AGA = birth weight adequate for gestational age. FGR = fetal growth restriction. BMI = body mass index.
* P-value<0.05 as compared to AGA calculated by linear or logistic regression adjusted by gender, age and gestational age at delivery.
† Children age is corrected by gestational age at delivery.
‡ BMI calculated as the weight in kilograms divided by the square of the height in meters.
§ Obesity defined as body mass index above 95th percentile for age and gender.

Table 3: Cardiac outcome of the study groups

Characteristic	AGA (N=120)	mild FGR (N=40)	severe FGR (N=40)	Linear tendency P Value
Cardiac morphometry†				
Left ventricle				
Base-to-apex length (mm)	49 [34,63]	43 [33,53]*	40 [27,50]*	<0.001
Basal diameter (mm)	26 [19,40]	30 [26,38]*	30 [22,37]*	<0.001
Longitudinal-transverse ratio	1.8 [1.3,2.3]	1.4 [1.1,1.7]*	1.3 [1.1,1.7]*	<0.001
Interventricular septum (mm)	7 [5,11]	7 [5,11]	6 [5,9]	0.004
Left posterior wall (mm)	7 [4,11]	7 [5,9]	6 [5,9]	0.010
Right ventricle				
Base-to-apex length (mm)	39 [25,53]	35 [28,49]	34 [22,45]	0.342
Basal diameter (mm)	25 (16,33]	26 [20,33]*	25 [17,33]*	<0.001
Longitudinal-transverse ratio	1.6 [1.2,2.4]	1.4 [1.1,1.9]*	1.4 [0.9,1.8]*	<0.001
Cardiac function				
Systolic function				
Left stroke volume (mL)	28 [13,52]	30 [18,47])	22 [11,43]*	0.001
Right stroke volume (mL)	37 [15,57]	34 [21,58]	30 [13,58]*	0.013
Heart rate (bpm)	94 [67,178]	101 [79,127]	112 [81,180]*	0.026
Global cardiac output (L/min)	6 [4,10]	7 [4,11]	5 [4,11]*	0.028
Cardiac index (L/(minxm^2))	9 [6,16]	10 [7,15]	10 [6,14]	0.070
Left ejection fraction (%)	69 [50,88]	70 [56,87]	72 [59,91]	0.749
Mitral ring displacement (mm)	13 [8,20]	10 [5,11]*	9 [7,13]*	<0.001
Tricuspid ring displacement (mm)	17 [10,26]	16 [11,19]*	12 [8,18]*	<0.001
Mitral annular S' (cm/s)	10 [8,17]	9 [8,15]	9 [6,13]*	<0.001
Mitral septal S' (cm/s)	10 [7,15]	9 [8,12]*	9 [7,11]*	<0.001
Tricuspid S' (cm/s)	15 [11,21]	15 [12,24]	14 [10,19]*	0.024
Diastolic function				
Mitral E/A ratio	1.7 [1.1,2.9]	1.7 [1.2,2.4]	1.5 [1.1,2.7]	0.053
Tricuspid E/A ratio	1.4 [1-3.5]	1.5 [0.9-2.2]	1.3 [1-2.2]	0.503
Left isovolumic relaxation time (ms)	56 [40,80]	60 [40,88]	56 [40,76]	0.416
Mitral E deceleration time (ms)	88 [52,128]	96 [56,148]*	100 [56,160]*	<0.001
Tricuspid E deceleration time (ms)	107 [56,160]	105 [64,156]	115 [52,160]*	0.003
Mitral annular E' (cm/s)	19 [12,28]	18 [13,23]	16 [10,24]*	0.003
Mitral septal E' (cm/s)	15 [11,19]	15 [12,18]	14 [11,24]*	0.004
Tricuspid E' (cm/s)	20 [11,29]	19 [11,23]	18 [14,26]*	0.002
E/E' (annular)	5.4 [3.9,8.3]	5.6 [3.3,8.5]	6.3 [4.1,10.8]*	<0.001
E/E' (septal)	6.7 [4.7,9.4]	6.8 [3.7,9.4]	7.4 [4.5,10.2]*	<0.001
B-type natriuretic peptide (pg/mL)	12 [0,63]	12 [0,39]	13 [0,41]	0.821

Data are median [interquartile range].
AGA = children with birth weight adequate for gestational age. FGR = fetal growth restriction.
* P-value<0.05 as compared to AGA calculated by linear or logistic regression adjusted by gender, age and gestational age at delivery.
† Cardiac morphometry results measured in end-diastole.

Table 4: Vascular outcome of the study groups

Characteristic	AGA (N=120)	mild FGR (N=40)	severe FGR (N=40)	Linear tendency P Value
Systolic blood pressure (mmHg)	100 [80,130]	105 [90,115]*	110 [90,120]*	0.009
Diastolic blood pressure (mmHg)	65 [45,90]	70 [57,85]*	70 [60,85]*	<0.001
Carotid mean intima-media thickness (mm)	0.37 [0.29,0.47]	0.38 [0.32,0.43]	0.41 [0.37,0.44]*	<0.001
Circumferential wall stress (mmHg)	72 [29,120]	78 [61,109]*	84 [58,102]*	0.010

Data are median [interquartile range].
AGA = children with birth weight adequate for gestational age. FGR = fetal growth restriction.
* P-value<0.05 as compared to AGA calculated by linear or logistic regression adjusted by gender, age and gestational age at delivery.

Legends to figures

Figure 1.
Flow diagram of children in the study groups.

Figure 2.
Values of linear cardiac size, mass and function in appropriate for gestational age (AGA) and severe fetal growth restricted (FGR) cases.

Figure 3.
Values of carotid intima-media thickness (cIMT) in AGA appropriate for gestational age (AGA) and severe fetal growth restricted (FGR) cases.

Figure 1.

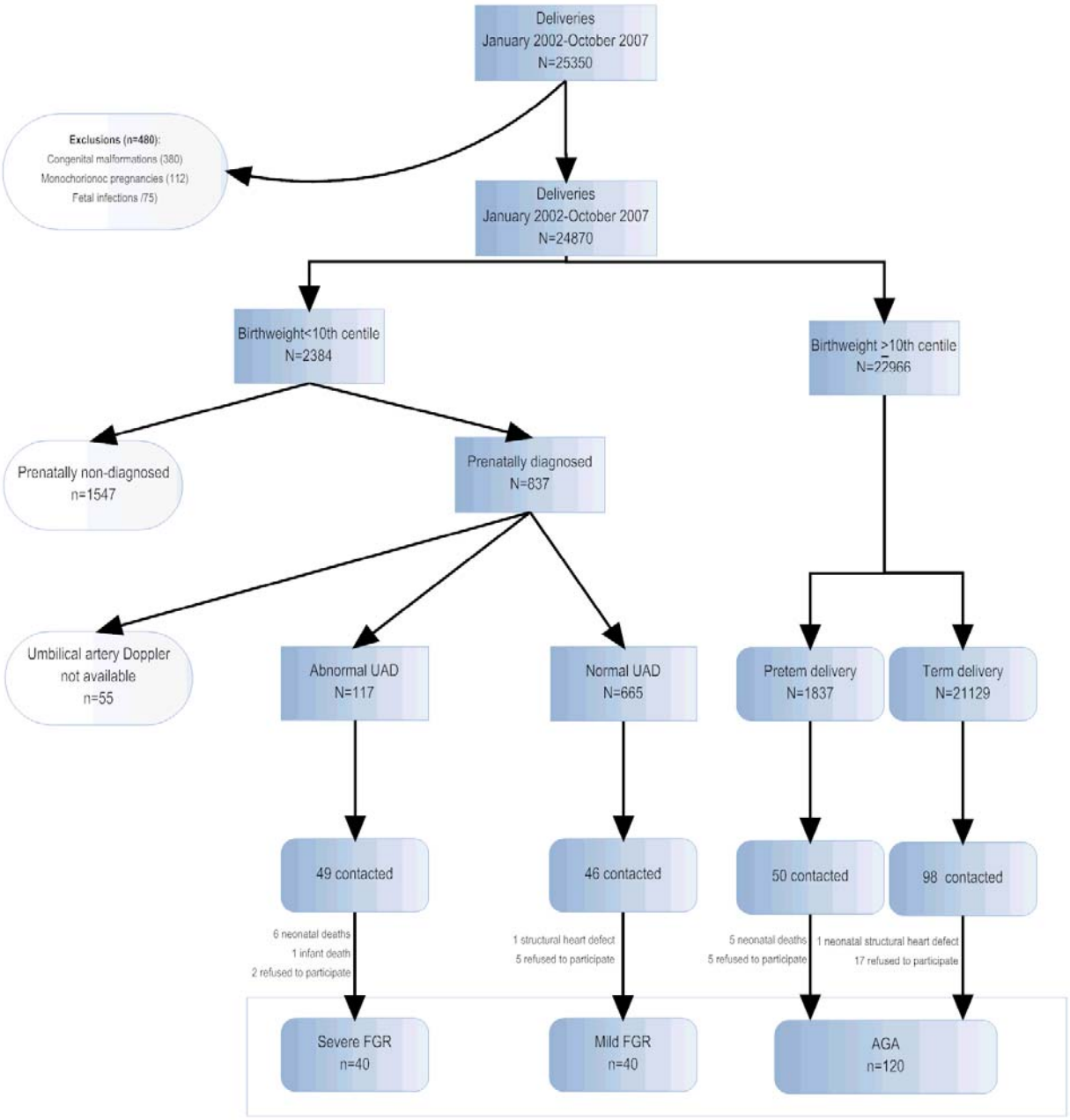
Deliveries
January 2002-October 2007
N=25350
Exclusions (n=480):
Congenital malformations (380)
Monochorionoc pregnancies (112)
Fetal infections /75)
Deliveries
January 2002-October 2007
N=24870
Birthweight<10th centile
N=2384
Birthweight >10th centile
N=22966
Prenatally non-diagnosed
n=1547
Prenatally diagnosed
N=837
Umbilical artery Doppler
not available
n=55
Abnormal UAD
N=117
Normal UAD
N=665
Pretem delivery
N=1837
Term delivery
N=21129
49 contacted
46 contacted
50 contacted
98 contacted
6 neonatal deaths
1 infant death
2 refused to participate
1 structural heart defect
5 refused to participate
5 neonatal deaths
5 refused to participate
1 neonatal structural heart defect
17 refused to participate
Severe FGR
n=40
Mild FGR
n=40
AGA
n=120

Figure 2.

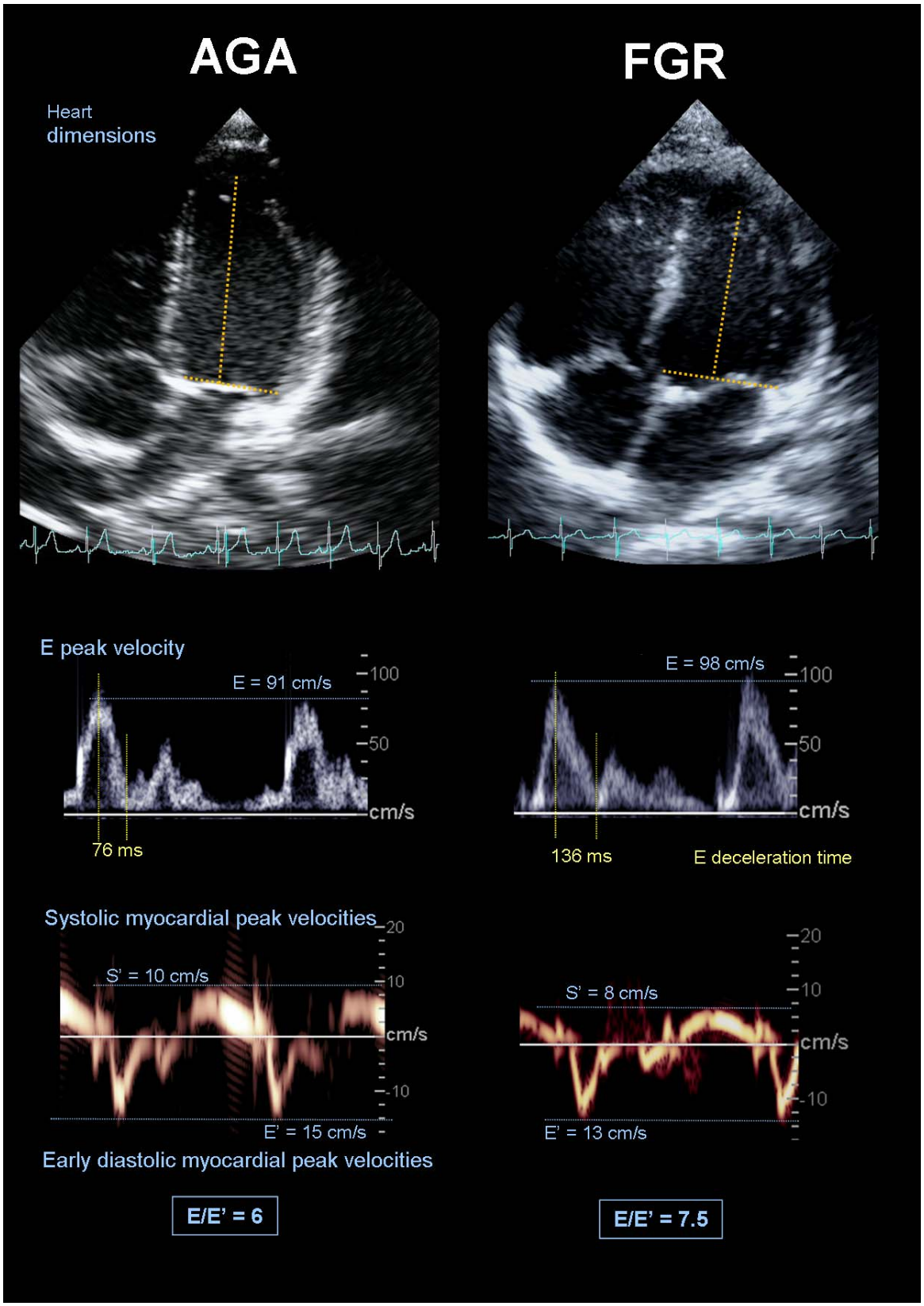
AGA
FGR
Heart
dimensions
E peak velocity
E = 91 cm/s
100
50
cm/s
76 ms
E = 98 cm/s
100
50
cm/s
136 ms
E deceleration time
Systolic myocardial peak velocities
20
10
S' = 10 cm/s
cm/s
-10
E' = 15 cm/s
Early diastolic myocardial peak velocities
E/E' = 6
20
10
S' = 8 cm/s
cm/s
-10
E' = 13 cm/s
E/E' = 7.5

Figure 3.