Cardiac dysfunction by tissue Doppler in early- and late-onset fetal growth restriction

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PhD THESIS

Departament d'Obstetrícia i Ginecologia, Pediatria, Radiologia i Medicina Física

Universitat de Barcelona

"CARDIAC DYSFUNCTION BY TISSUE DOPPLER IN EARLY- AND LATE-ONSET FETAL GROWTH RESTRICTION"

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A thesis submitted by Montserrat Comas Rovira for the degree of Doctor of Medicine (Faculty of Medicine, University of Barcelona) under the direction of Eduard Gratacós, Professor of Obstetrics and Gynecology in the University of Barcelona, and Fàtima Crispi.

Signed: Montserrat Comas Rovira

Barcelona, 29th March 2011.

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

DECLARE:

That Montserrat Comas Rovira has realized the work entitled "**Cardiac dysfunction by tissue Doppler in early- and late-onset fetal growth restriction**" under our direction for the degree of Doctor of Medicine and that the mentioned work is ready to be presented from the present day.

Signed: Eduard Gratacós Solsona and Fàtima Crispi Brillas.

Barcelona, 29th March 2011.

PRESENTATION

The present thesis has been structured following the normative for PhD thesis as a compendium of publications. The projects included in this thesis belong to the same research line leading to three articles already published or accepted for publication in international journals:

1. Comas M, Crispi F, Gómez O, Puerto B, Figueras F, Gratacós E. Gestational age and estimated fetal weight adjusted reference ranges for myocardial tissue Doppler indices at 24-41 weeks of gestation. Ultrasound Obstet Gynecol 2011; 37: 57-64.

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2. Comas M, Crispi F, Cruz-Martinez R, Martinez JM, Figueras F, Gratacós E. Usefulness of myocardial tissue Doppler vs conventional echocardiography in the evaluation of cardiac dysfunction in early-onset intrauterine growth restriction. Am J Obstet Gynecol 2010; 203: 45.e1-7

State: published

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Quartile: 1st

3. Comas M, Crispi F, Cruz-Martinez R, Figueras F, Gratacós E. Tissue-Doppler echocardiographic markers of cardiac dysfunction in small-for-gestational age fetuses. Am J Obstet Gynecol 2011.

State: accepted for publication

Impact factor: 3.278

Quartile: 1st

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ABREVIATIONS:

- FGR Fetal growth restriction
- TDI Tissue Doppler imaging
- UA Umbilical artery
- SGA Small for gestational age
- EFW Estimated fetal weight
- GA Gestational age
- MPI Myocardial performance index
- PW-TDI Pulsed-wave tissue Doppler imaging
- UA Umbilical artery
- PI Pulsatility index
- MCA PI Middle cerebral artery pulsatility index
- CPR Cerebro-placental ratio
- Ut PI Mean uterine artery pulsatility index
- DV PI Ductus venosus pulsatility index
- PVE Peak velocity in early diastole
- PVA Peak velocity during atrial contraction
- PVS Peak velocity during systole
- ICT Isovolumetric contraction time
- IRT Isovolumetric relaxation time
- ET Ejection time
- ICC Intraclass correlation coefficient

I. SUMMARY

Background

Fetal growth restriction (FGR) is present in 5-10% of the pregnancies and is associated to high perinatal and long-term cardiovascular morbidity. Subclinical cardiac dysfunction has previously been described in severe and early FGR cases, but not in milder forms of late-FGR. The main aim of this thesis was to assess cardiac function by new echocardiographic techniques on myocardial imaging as Tissue Doppler Imaging (TDI), in early- and late-onset FGR cases.

Methods

First, tissue Doppler was applied in a cohort of normally growth fetuses by TDI in order to describe its reproducibility and construct reference ranges for fetal annular peak velocities and myocardial performance index at 24-41 weeks of gestation. Secondly, cardiac function including conventional echocardiographic parameters and TDI was evaluated in a cohort of early-onset growth restricted fetuses with abnormal umbilical artery (UA) Doppler and, finally, in a cohort of late-onset small for gestational age (SGA) fetuses with normal UA Doppler.

Results

Fetal TDI measurements demonstrated a good reproducibility. GA and estimated fetal weight (EFW) adjusted reference ranges for tissue Doppler indices at 24-41 weeks of gestation were provided. TDI demonstrated the presence of both systolic and diastolic cardiac dysfunction in early-onset FGR fetuses. Late-onset FGR fetuses with normal UA were also associated with cardiac dysfunction detected by TDI.

Conclusions

Early- and late-onset growth restricted fetuses are associated with cardiac dysfunction. Subclinical cardiac dysfunction could be present from early stages of fetal deterioration and could be detected using TDI.

II. INTRODUCTION

The importance of cardiovascular diseases

Cardiovascular diseases constitute a main cause of mortality world-wide.[1] Growth and ageing of world population foreseen at the next decades support an increase of mortality due to cardiovascular diseases.[2,3] This tendency could be reduced by a combination of preventive interventions at population and individual level. For this purpose, early detection of high risk factors is critical.

Genetic predisposition and lifestyle have been tradicionally considered the main determinants of cardiovascular risk. Recently, several studies pointed out that a low birthweight could be considered a cardiovascular risk factor and suggested that cardiovascular disease has its origins in prenatal life, in a significative proportion of cases.[4-6] This concept has been named ***fetal programming of cardiovascular disease***.

Fetal programming

The concept of fetal programming was first introduced by Barker[7] and supports that insults during critical phases of development in utero (characterized by its plasticity) could produce permanent changes in organ development, including changes in cardiovascular structure and function. This changes appear as adaptative responses at intrauterine life but persist inappropiately at postnatal life and would predispose to cardiovascular disease at adulthood.[8] It has been hypothesized that different mechanisms based in epigenetic fenomens as DNA methilation and alternative splicing[9,10] are involved in this process.

The most commonly accepted theory is that fetal metabolic programming[11,12] leads to diseases associated with cardiovascular disease such as obesity, diabetes mellitus and hypertension and, secondarily, an increase cardiovascular risk is observed. However, a recent study of our group has demonstrated that FGR induces primary cardiac and vascular changes that persist into childhood.[13] These cardiovascular remodelling is present in both early- and late-onset FGR.

Early-onset FGR and cardiac dysfunction

Early-onset FGR, resulting from severe placental insufficiency affects less than 1% of deliveries and is recognized among the main causes of perinatal mortality and morbidity.[14] Prediction of mortality and morbidity is critical for clinical management of these fetuses.

The heart is a central organ in the fetal adaptive mechanisms to placental insufficiency and cardiac dysfunction is recognized among the central pathophysiologic features of FGR.[15-20] Several studies have demonstrated the presence of echocardiographic and biochemical signs of subclinical cardiac dysfunction, mainly related to diastolic function.[15,16,21]

While earlier studies suggested that cardiac parameters became abnormal only in severely affected fetuses,[21-23] more recent research strongly suggests that subclinical cardiac dysfunction would be present from early stages[15] and would progress further as the fetal condition deteriorates.

More sensitive cardiovascular parameters as TDI would be useful in the prediction of perinatal outcome and monitoring of growth restricted fetuses.

Late-onset FGR and cardiac dysfunction

Typically, small fetuses near term present normal UA Doppler and are defined as SGA. SGA fetuses have long been considered to be constitutionally small fetuses with a good prognosis.[24] However, recent evidence suggest that a proportion of these fetuses represent true forms of FGR where placental insufficiency is not reflected by UA Doppler. In fact, SGA have poorer perinatal results[25,26] and suboptimal neurodevelopment[27,28] and higher postnatal cardiovascular risk[5,6,13] compared with normal weight newborns of the same GA at delivery.

Although SGA has been associated with mild forms of adverse perinatal outcome, it is a rellevant condition because of its high prevalence, representing up to 10% of all pregnancies.

Most reports on cardiac function include altogether growth restricted fetuses at any GA with and without abnormal UA Doppler obtaining no significant results in conventional echocardiographic parameters. Although there are not many studies of cardiac function in SGA fetuses, preliminary data suggests that these fetuses might also present features of cardiac dysfunction.[16,20]

Since the identification of SGA fetuses with true growth restriction can not be based only on UA Doppler, cardiovascular assessment with sensitive techniques, such as TDI, could be used for these purposes.

Evaluation of cardiac function in FGR by conventional echocardiography

Several echocardiographic parameters have been explored in FGR including ejection fraction, ventricular ejection force, E/A ratios, cardiac output and, recently, myocardial performance index (MPI).[21,23,29-31]

Fetuses with early-onset FGR have been reported to have increased E/A ratios,[15,16,21] pointing out the presence of diastolic disfunction while parameters that evaluate systolic function as ejection fraction have a later deterioration.[21] Cardiac output is maintained within normal values, even in most severe stages when it is adjusted by fetal wheight.[15,17] Abnormalities in precordial veins, secondary to fetal heart failure, have also been described in placental insufficency. In this respect, ductus venosus has been considered as the best predictor for perinatal death in preterm FGR indicating the need for delivery.[32-34]. Most of these parameters became abnormal in severely affected fetuses. However, MPI, a marker of combined systolic and diastolic function, has been reported to be increased early in the clinical evolution of FGR and shows a progression across severity stages.[15,35]

Fetal cardiac function assessment has so far mainly been performed by conventional echocardiographic techniques such as M-mode, B-mode and pulsed Doppler ultrasound.[29] These paramaters are influenced by preload and afterload and are affected when cardiac dysfunction becomes obvious. New technologies that permit a more accurate evaluation of cardiac motion have been recently developed.

Evaluation of cardiac function in FGR by Tissue Doppler

New developments in echocardiography enable a much fuller assessment of cardiac function, including measurement of myocardial motion by TDI. TDI is a robust and reproducible echocardiographic tool which permits a quantitative assessment of motion and timing of myocardial events. This recent echocardiographic technique reflects better myocardial motion than conventional echocardiography, by evaluating cardiac function directly from the myocardium and being less influenced by loading conditions.[36] Two different techniques are available using TDI: color and pulsed-wave

tissue Doppler imaging (PW-TDI). Color-TDI requires an offline analysis that may be limited by fetal position movements and high heart rate. On the contrary, PW-TDI is obtained on line and most of the potential limitations of the fetal heart assessment could be avoided. Moreover, previous studies have reported lower reproducibility of color-TDI as compared to PW-TDI when applied to the fetal heart. Therefore, PW-TDI was selected for the projects of this thesis.

Myocardial velocities are a sensitive marker of mildly impaired systolic or diastolic function and therefore useful in the early identification of subtle cardiac dysfunction in preclinical stages.[37,38] In adults and children, TDI has demonstrated its utility as an early marker for preclinical cardiac dysfunction in the prediction of future cardiovascular diseases.[39,40]

Recently, TDI has been shown to be feasible in fetuses.[41-44] Harada [41] showed that TDI was technically possible in human fetuses in 1999. Age-related changes in fetal myocardial velocities were described[41,44,45] supporting the concept that myocardial velocities reflect maturational changes in fetal cardiac function. The results of preliminary studies suggest the use of TDI as a sensitive tool to demonstrate changes in cardiac function in fetuses with FGR,[46-48] hydrops[49] and from diabetic mothers.[50] Concerning FGR, a decrease of annular peak velocities have been described.[46-48]

The first specific aim of this thesis was to determine intra- and inter-observer reliability of this technique (**PROJECT 1**). Secondly, GA and fetal weight adjusted reference ranges for TDI parameters during gestation were constructed (**PROJECT 2**). Our final objective was to evaluate the presence of cardiac dysfunction in a cohort of fetuses with early- (**PROJECT 3**) and late-onset FGR (**PROJECT 4**).

III. HYPOTHESIS

***Main hypothesis*:** Early- and late-onset FGR are associated with in utero cardiac dysfunction that can be detected by TDI.

Secondary hypothesis:

1. TDI is a feasible and reproducible echocardiographic method to evaluate fetal cardiac function.
2. TDI can be used to demonstrate the presence of cardiac dysfunction in early-onset FGR.
3. TDI can be used to demonstrate the presence of cardiac dysfunction in late-onset FGR.
4. TDI may constitute a more sensitive tool than conventional echocardiography to evaluate cardiac dysfunction.

IV. OBJECTIVES

***Main objective*:** To assess cardiac function by conventional echocardiography and TDI in early and late growth restricted fetuses.

Specific objectives:

1. To determine intra- and inter-observer reliability of TDI measurements.
2. To construct GA- and fetal weight-adjusted reference ranges for TDI parameters during gestation.
3. To evaluate the presence of cardiac dysfunction by conventional echocardiography and TDI in a cohort of fetuses with early-onset FGR.
4. To evaluate the presence of cardiac dysfunction by conventional echocardiography and TDI in a cohort of fetuses with late-onset FGR.

To achieve these main and specific objectives, four different projects were planned and performed as explained below.

V. METHODS

V.1. GENERAL METHODOLOGY

Definitions early- and late-onset FGR and controls

a/ **Early-onset FGR** defined as an EFW below the 10th centile according to local reference curves[51] together with UA pulsatility index (PI) above 95th centile[52] delivering or dying between 24 and 34 weeks of gestation.

b/ **SGA** defined as an EFW below the 10th centile according to local reference curves[51] together with UA PI below 95th centile[52] delivering after 34 weeks of gestation.

c/ **control group** defined as normally grown fetuses defined as birth weight above 10th centile delivering at term.

Basic fetal ultrasound evaluation

Prenatal data

- GA at ultrasound: calculated based on the crown-rump length at first trimester ultrasound.[53]
- EFW: calculated according to the method described by Hadlock.[54]
- Sex: male/female

Feto-placental Doppler

- Umbilical artery pulsatility index (UA PI): obtained from a free–floating portion of the umbilical cord.

- Middle cerebral artery pulsatility index (MCA PI): 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.
- Cerebro-placental ratio (CPR): calculated as MCA-PI/UA-PI. [55]
- Mean uterine artery pulsatility index (Ut PI): identifying the vessel in an oblique scan with the sample volume distal to the crossing with the external iliac artery. Pulsatility indexes of the left and right arteries were measured and the mean PI was calculated.

Fetal echocardiography

Conventional echocardiography

- Ductus venosus pulsatility index (DV PI): 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. (Figure 1)
- Left and right E/A ratio: atrioventricular flows were obtained from a basal or apical four-chamber view placing the pulsed Doppler sample volume just below valve leaflets. The ratio between peak early (PVE) and late (PVA) transvalvular velocities was measured in each side.[29] (Figure 2)
- Aortic and pulmonary artery peak velocities: outflow tract velocities were obtained from a long- or short-axis view of the left and right ventricle respectively.

Figure 1. Normal flow velocity waveforms of the ductus venosus visualized in a sagittal section through the fetal abdomen. The first peak indicates sístole (S), the second early diastole (D) and the nadir of the waveform occurs during atrial contraction (a)

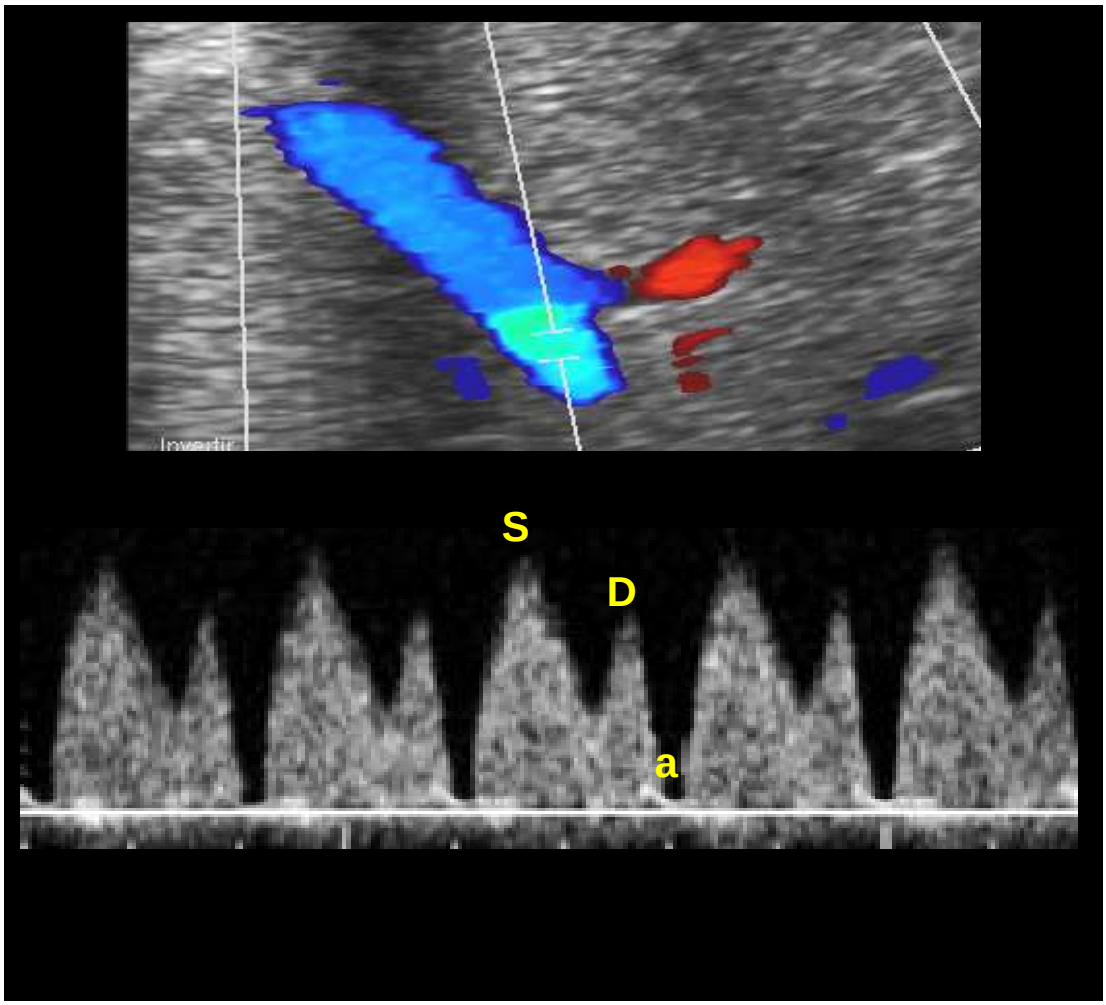
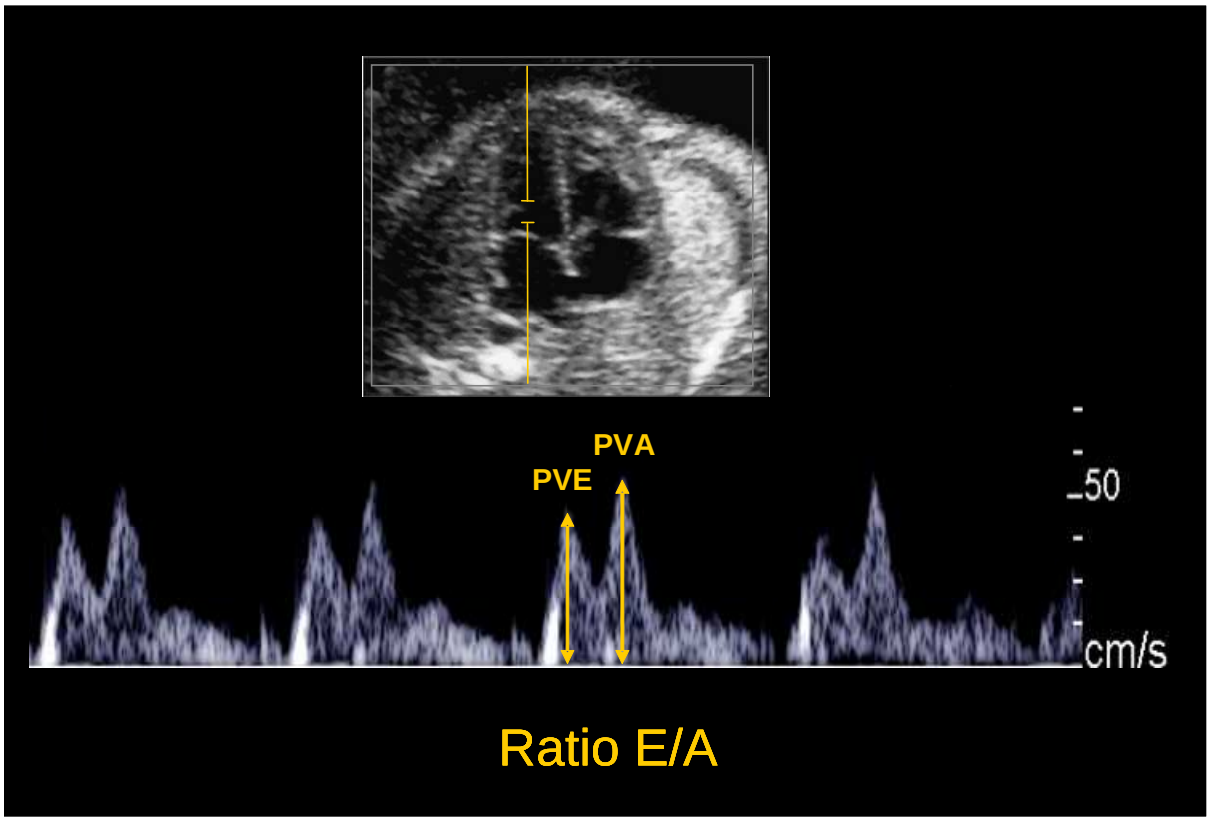
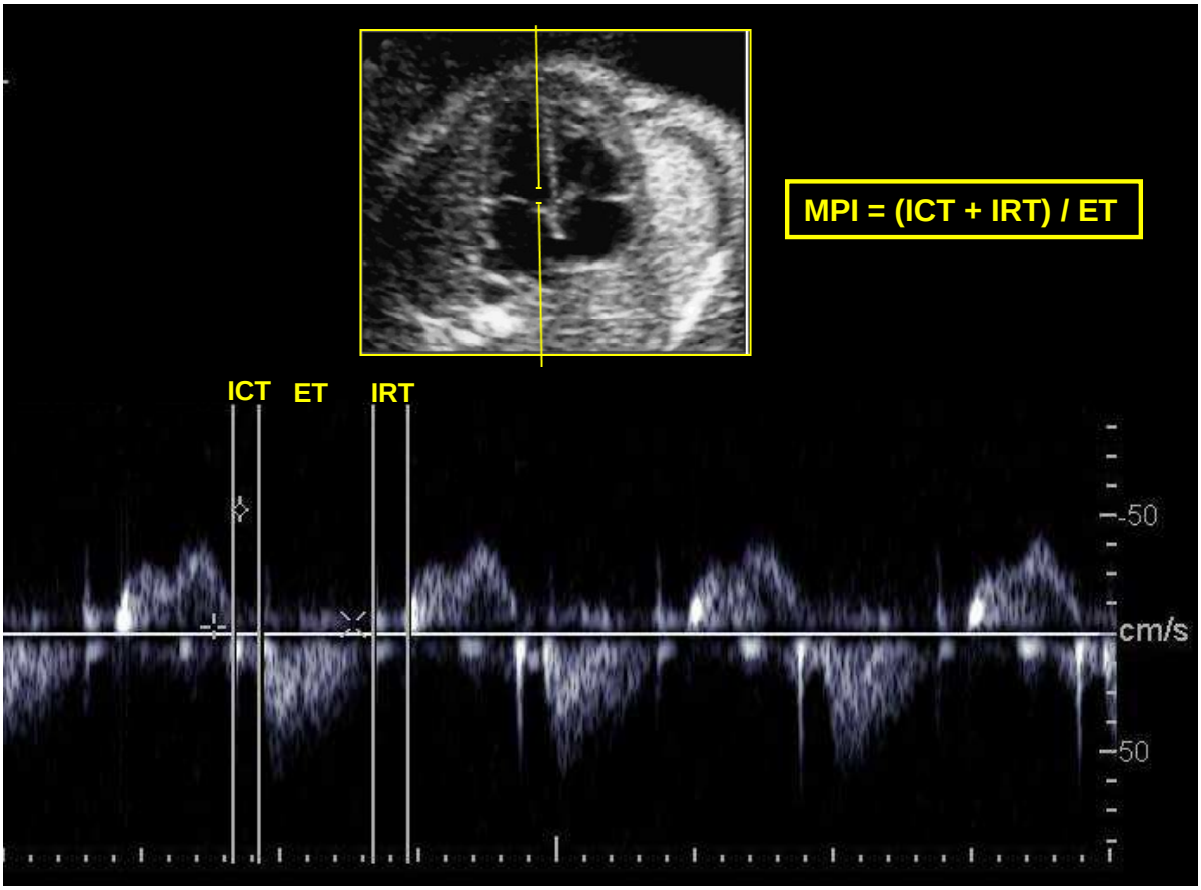


Figure 2. Example of measurement of left E/A ratio by conventional echocardiography. The sample volume is placed in the left ventricle just below the mitral valve and pulsed Doppler waveform is recorded obtaining peak transvalvular velocities.



- Left MPI: obtained using the clicks of mitral and aorta valves as landmarks, as previously described.[31] The following time-periods were calculated: isovolumetric contraction time (ICT), ejection time (ET) and isovolumetric relaxation time (IRT). Finally, MPI was calculated as (ICT+IRT)/ET. (Figure 3)
- Right MPI: calculated by obtaining right ventricle inflow and outflow obtained in series from separate cardiac cycles.[35]

Figure 3. Example of measurement of left myocardial performance index by conventional echocardiography. In an apical four-chamber view, the sample volume is placed in the internal wall of the ascending aorta close to the internal leaflet of the mitral valve and below the aortic valve. The Doppler waveform shows the opening and closing clicks of both valves



Tissue Doppler imaging

TDI was obtained in real time using a 2-10 MHz phased-array transducer. First, a four-chamber-view was obtained in an apical or basal view. TDI was set to the pulsed-wave mode with a sample volume size between 2 and 4 mm. Sample volumes were placed in the basal part of the left ventricular wall (mitral annulus), interventricular septum and right ventricular wall (tricuspid annulus) (Figure 4). The

insonation ultrasound beam was kept at an angle of <30º to the orientation of the ventricular wall or the interventricular septum and no angle correction was applied.

- Left, right and septal annular peak velocity in early diastole (PVE')
- Left, right and septal annular peak velocity during atrial contraction (PVA')
- Left, right and septal annular peak velocity during systole (PVS')
- Left, right and septal E'/A' ratio (PVE'/PVA')
- Left and right E/E' ratio (PVE/PVE')
- Left, right and septal MPI': calculated as (ICT'+IRT')/ET'. Measurement of all MPI' components were made from the same cardiac cycle.[56] (Figure 5)

Figure 4. Locations of pulsed-wave tissue Doppler measurement. 1, left annulus; 2, septal annulus; 3, right annulus.

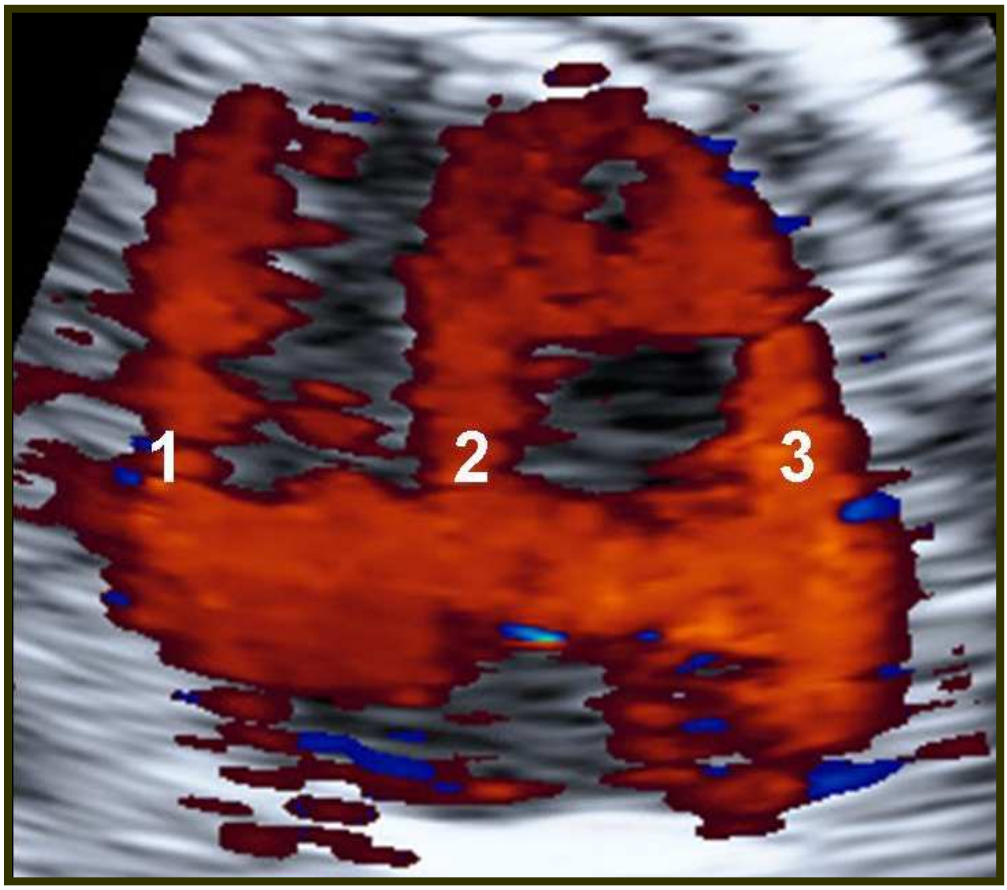
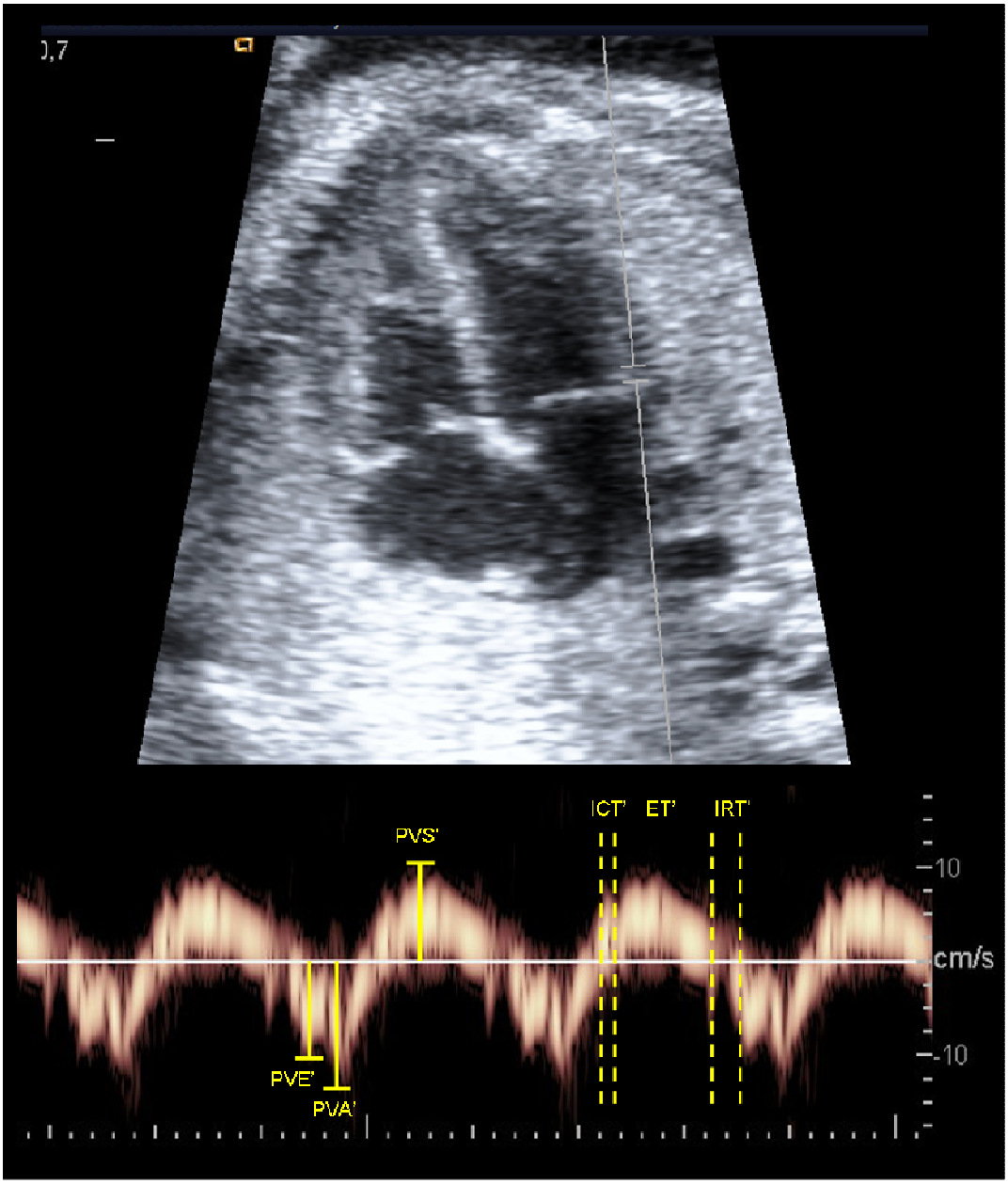


Figure 5. Example of measurement of annular peak velocities and performance index by pulsed-wave tissue Doppler in the left annulus. The sample volume is placed in the left annulus just below the mitral valve with an insonation angle of the ultrasound beam of <30º to the orientation of the ventricular wall. Tissue Doppler waveform is recorded and left peak annular velocities and times are obtained.



PVE', annular peak velocity in early diastole; PVA', annular peak velocity during atrial contraction; PVS', annular peak velocity in systole; ICT, isovolumetric contraction time; ET; ejection time; IRT, isovolumetric relaxation time; MPI'= (ICT'+IRT')/ET.

V.2. SPECIFIC METHODOLOGY FOR EACH PROJECT

V.2.1. Project 1: intra- and inter-observer reliability of tissue Doppler measurements

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

Study populations: 80 singleton pregnancies between 24-41 weeks of gestation (50 fetuses were evaluated were evaluated twice by the same operator and 30 fetuses by two independent operators) including controls and FGR.

Exclusion criteria: structural/chromosomal anomalies; evidence of fetal infection.

Interventions:

- Signature of consent form.
- Functional echocardiography.
- Collection of perinatal data from the hospital database or by parental questionnaire.
- Data analysis

Measures:

- Ultrasound prenatal data
- Conventional Doppler
- Functional echocardiography: conventional echocardiography and TDI
- Perinatal data: prenatal (maternal age at delivery, smoking status during gestation, body mass index at the beginning of gestation, ethnicity, parity), perinatal (GA at delivery, mode of delivery, birth weight, birth weight centile, 5-min Apgar, umbilical artery pH, preeclampsia, gestational diabetes and other complications of pregnancy)

***Outcome variables*:** intraclass and interclass correlation coefficient for agreement of TDI parameters (left, right and septal annular peak velocities, E'/A' ratios and MPI').

V.2.2. Project 2: Gestational age and fetal weight adjusted reference ranges for tissue Doppler parameters at 24-41 weeks of gestation

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

Study populations: singleton pregnancies between 24-41 weeks of gestation that attended the Maternal-Fetal Medicine Department at Hospital Clinic in Barcelona for routine pregnancy ultrasound scans.

Inclusion criteria: singleton pregnancy; normal fetal growth and uterine artery Doppler according to our local references[57] at 20 weeks of gestation; absence of risk factors for vascular disease including pregestational diabetes and immune or renal disease; no previous history of fetal growth restriction, preeclampsia or abruption.

Exclusion criteria: structural/chromosomal anomalies; evidence of fetal infection.

Interventions:

- Signature of consent form.
- Functional echocardiography.
- Collection of perinatal data from the hospital database or by parental questionnaire.
- Data analysis

Measures:

- Ultrasound prenatal data
- Conventional Doppler
- Functional echocardiography: conventional echocardiography and TDI

- Perinatal data: prenatal (maternal age at delivery, smoking status during gestation, body mass index at the beginning of gestation, ethnicity, parity), perinatal (GA at delivery, mode of delivery, birth weight, birth weight centile, 5-min Apgar, umbilical artery pH, preeclampsia, gestational diabetes)

***Outcome variables*:** Gestational age-adjusted normograns for left, right and septal PVE', PVA', PVS', E'/A' ratio, E/E' ratio, MPI'. As FGR are smaller for GA and annular peak velocities maybe be influenced by heart's size, EFW-adjusted normograms for PVE', PVA' and PVS' were also constructed.

V.2.3. Project 3: Association between early-onset FGR and cardiac dysfunction

***Study design*:** prospective cohort study (two cohorts).

Study populations: early-onset FGR and controls matched two to one with cases by gestational age at ultrasound (±1 week).

Exclusion criteria: structural/chromosomal anomalies; evidence of fetal infection.

Interventions:

- Signature of consent form.
- Functional echocardiography.
- Collection of perinatal data from the hospital database or by parental questionnaire.
- Data analysis

Measures:

- Ultrasound prenatal data
- Conventional Doppler
- Functional echocardiography: conventional echocardiography and TDI

- Perinatal data: prenatal (maternal age at delivery, smoking status during gestation, body mass index at the beginning of gestation, ethnicity, parity), perinatal (GA at delivery, mode of delivery, birth weight, birth weight centile, 5-min Apgar, umbilical artery pH, perinatal mortality), neonatal morbidity (bronchopulmonary dysplasia, hyaline membrane disease, neonatal intraventricular hemorrhage grade 3 or 4, necrotizing enterocolitis, sepsis, retinopathy grade 3 or 4).

Outcome variables: presence of cardiac dysfunction measured by conventional echocardiography (DV, left and right MPI, left and right E/A ratios, aortic and pulmonary artery peak velocities) and tissue Doppler (peak annular velocities, E'/A' ratios and MPI').

V.2.4. <u>Project 4</u>: Association between late-onset FGR and cardiac dysfunction

***Study design*:** prospective cohort study (two cohorts).

Study populations: SGA and controls matched one to one with cases by gestational age at ultrasound (±1 week).

Exclusion criteria: structural/chromosomal anomalies; evidence of fetal infection.

Interventions:

- Signature of consent form.
- Functional echocardiography.
- Collection of perinatal data from the hospital database or by parental questionnaire.
- Data analysis

Measures:

- Ultrasound prenatal data

- Conventional Doppler
- Functional echocardiography: conventional echocardiography and TDI
- Perinatal data: prenatal (maternal age at delivery, smoking status during gestation, body mass index at the beginning of gestation, ethnicity, parity), perinatal (GA at delivery, mode of delivery, birth weight, birth weight centile, 5-min Apgar, umbilical artery pH, preeclampsia).

Outcome variables: presence of cardiac dysfunction measured by conventional echocardiography (DV, left MPI, left and right E/A ratios) and tissue Doppler (peak annular velocities and MPI').

VI. RESULTS

VI.1. Project 1: intra- and inter-observer reliability of tissue Doppler measurements

The results of this project have been published in an international journal:[58]

Comas M, Crispi F, Cruz-Martinez R, Martinez JM, Figueras F, Gratacós E. Usefulness of myocardial tissue Doppler vs conventional echocardiography in the evaluation of cardiac dysfunction in early-onset intrauterine growth restriction. Am J Obstet Gynecol. 2010; 203: 45.e1-7.

The results have also been presented at the 19th World Congress on Ultrasound in Obstetrics and Gynecology, 15th september 2009, Hamburg (Germany) (oral poster: M. Comas, F. Crispi, R. Cruz, F. Figueras, E. Gratacós. *Intra- and interobserver reliability of tissue Doppler for measurement of fetal myocardial velocities*)

Results

Study populations

80 singleton pregnancies between 24-41 weeks of gestation (50 fetuses were evaluated were evaluated twice by the same operator and 30 fetuses by two independent operators).

Reliability of tissue Doppler parameters

Reliability analyses were performed by means of the intraclass correlation coefficient (ICC) for agreement. ICC was above 0.7 for most comparisons. (Table 1)

Table 1. Intra- and inter-observer reliability of annular peak velocities and myocardial performance index measured by tissue Doppler.

Parameters	Intraobserver reliability	Interobserver reliability
Diastolic parameters		
Left PVE'	0.79	0.86
Left PVA'	0.66	0.86
Left E'/A'	0.78	0.69
Right PVE'	0.87	0.82
Right PVA'	0.79	0.88
Right E'/A'	0.79	0.85
Septal PVE'	0.74	0.83
Septal PVA'	0.77	0.77
Septal E'/A'	0.57	0.81
Systolic parameters		
Left PVS'	0.79	0.81
Right PVS'	0.81	0.82
Septal PVS'	0.77	0.83
MPI		
Left MPI'	0.79	0.78
Right MPI'	0.77	0.70
Septal MPI'	0.71	0.70

Conclusions

Tissue Doppler calculation of annular peak velocities and performance indexes in fetuses is reliable enough for clinical or research purposes.

VI.2. Project 2: Gestational age and fetal weight adjusted reference ranges for tissue Doppler parameters at 24-41 weeks of gestation

The results of this project have been published in an international journal:[59]

Comas M, Crispi F, Gómez O, Puerto B, Figueras F, Gratacós E. Gestational age- and estimated fetal weight-adjusted reference ranges for myocardial tissue Doppler indices at 24-41 weeks' gestation. Ultrasound Obstet Gynecol. 2011; 37: 57-64.

The results have also been presented at the 20th World Congress on Ultrasound in Obstetrics and Gynecology, 10-14 October 2010, in Prague (Czech Republic) (Crispi F, Comas M, Cruz R, Gomez O, Figueras F, Gratacos E. *Reference ranges for fetal myocardial velocities and performance index using tissue Doppler at 24-41 weeks of gestation*).

Results

Study population

The study population included 213 singleton pregnancies with normal fetal growth and uterine artery Doppler according to our local references[51,57] and absence of previous risk factors for preeclampsia or FGR.

Mean GA at delivery was 39 weeks, mean birth weight was 3353 grams and mean birth weight centile was 53. The percentage of preeclampsia, preterm delivery and gestational diabetes was 2%,1% and 3%, respectively.

TDI assessment of the left ventricular wall, right ventricular wall and interventricular septum was successfully obtained in 94%, 97% and 95% of cases. (Table 2)

Table 2. Demographic characteristics and pregnancy outcome of the study population

Parameter	*Value*
Clinical characteristics	
Maternal age (years)	31 ± 5
Caucasian	72
Nulliparous	68
Maternal body mass index (kg/m^2)	23 ± 3
Cigarette smoker	9
Pregnancy outcome	
Gestational age at delivery (weeks)	39 ± 1
Cesarean section	20
Birth weight (g)	3353 ± 419
Birth-weight centile	52 ± 26
5-min Apgar score	10 ± 1
Umbilical artery pH	7.23 ± 0.07
Pre-eclampsia	2
Birth weight at delivery $< 10^{th}$ centile	4
Preterm delivery (< 34 weeks)	1
Gestational diabetes	3

Data given as mean ± SD or %.

Gestational age adjusted reference ranges for tissue Doppler parameters

The best model for most parameters was a first degree linear polynomial, with the exception of left PVE' (best modeled by a second degree linear polynomial) and septal E'/A' and right and septal MPI' that were constant across GA (Table 3).

Table 3. Regression equations for cardiovascular parameters obtained by tissue Doppler imaging

Parameter	*Mean*	*SD*
Myocardial peak velocities		
Log_e left PVE′ (cm/s)	$0.475 + (0.0106 \times GA) - (0.00002 \times GA^2)$	$0.071 + (0.00046 \times GA)$
Left PVA′ (cm/s)	$7.135 + (0.0046 \times GA)$	1.2733
Log_e left PVS′ (cm/s)	$1.619 + (0.0011 \times GA)$	0.1711
Right PVE′ (cm/s)	$3.64 + (0.0205 \times GA)$	$0.5846 + (0.0026 \times GA)$
Log_e right PVA′ (cm/s)	$2.123 + (0.0009 \times GA)$	$0.0399 + (0.0005 \times GA)$
Right PVS′ (cm/s)	$5.302 + (0.0094 \times GA)$	1.0249
Log_e septal PVE′ (cm/s)	$1.339 + (0.0018 \times GA)$	0.1828
Log_e septal PVA′ (cm/s)	$1.647 + (0.0012 \times GA)$	0.1791
Log_e septal PVS′ (cm/s)	$1.367 + (0.0015 \times GA)$	0.1575
Left E′/A′ ratio	$0.566 + (0.0013 \times GA)$	0.1114
Right E′/A′ ratio	$0.550 + (0.0012 \times GA)$	0.1106
Septal E′/A′ ratio	0.8377	0.0889
Left E/E′ ratio	$6.339 - (0.0048 \times GA)$	$0.1738 + (0.0046 \times GA)$
Right E/E′ ratio	$6.282 - (0.0043 \times GA)$	1.0877
Myocardial performance index (MPI′)		
Left MPI′	$0.435 + (0.0003 \times GA)$	0.0858
Right MPI′	0.4943	0.0793
Septal MPI′	0.5098	0.0683

All annular peak velocities showed a progressive increase with advancing gestation as well as left and right E'/A' and left MPI'. In contrast, left and right E/E' showed a progressive decline with advancing gestation. On the other hand, septal E'/A' and right and septal MPI' remained constant during the second half of pregnancy. (Figures 6,7,8,9)

Figure 6. Scatterplots of the left (a-c), right (d-f) and septal (g-i) annular peak velocities measured by tissue Doppler vs. gestational age in our population. Estimated 5th, 50th and 95th centile curves are shown.

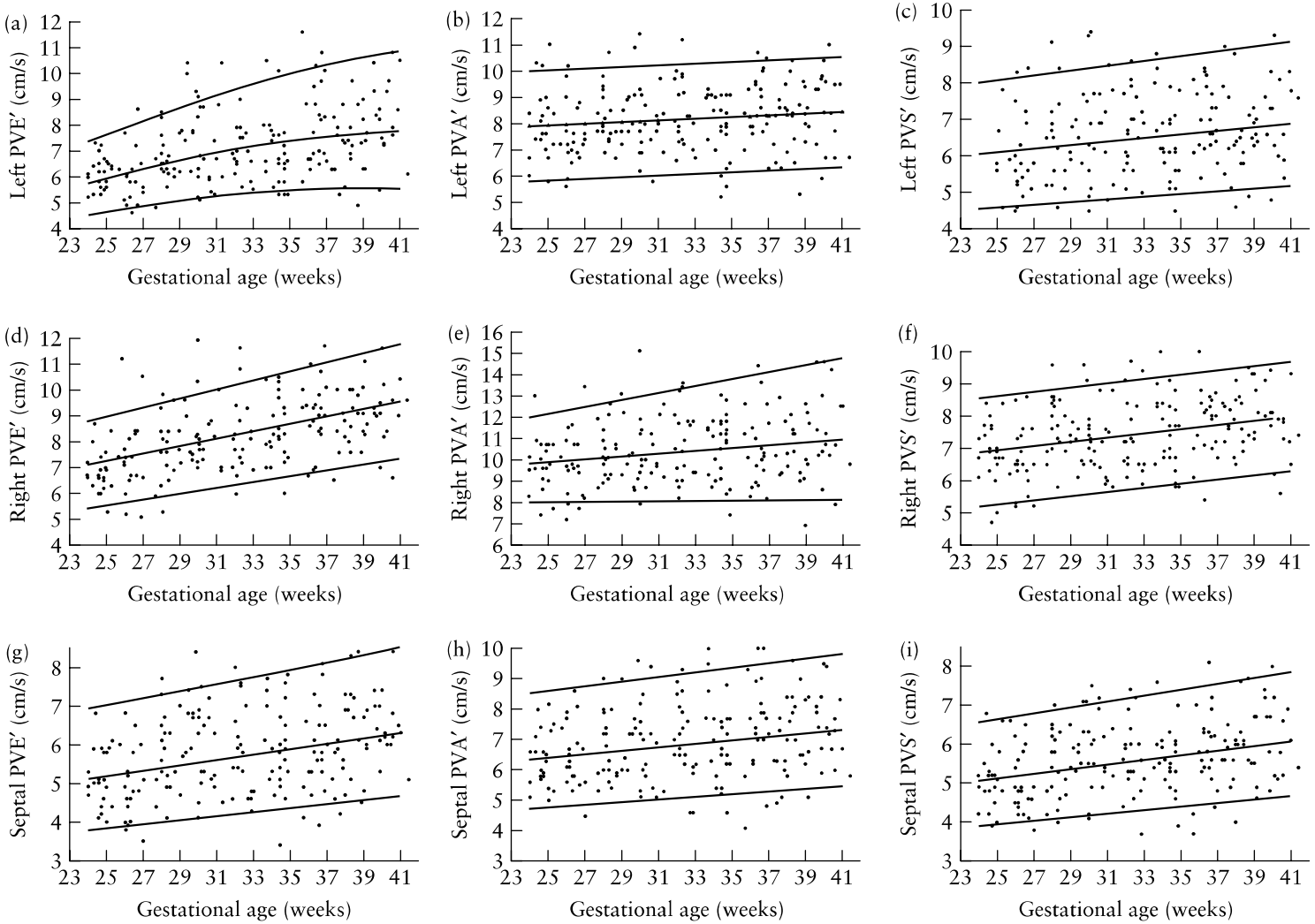


Figure 7. Scatterplots of the left (a), right (b) and septal (c) E/A ratios measured by tissue Doppler ultrasonography plotted against gestational age in the study population. Estimated 5th, 50th and 95th centile curves are shown.

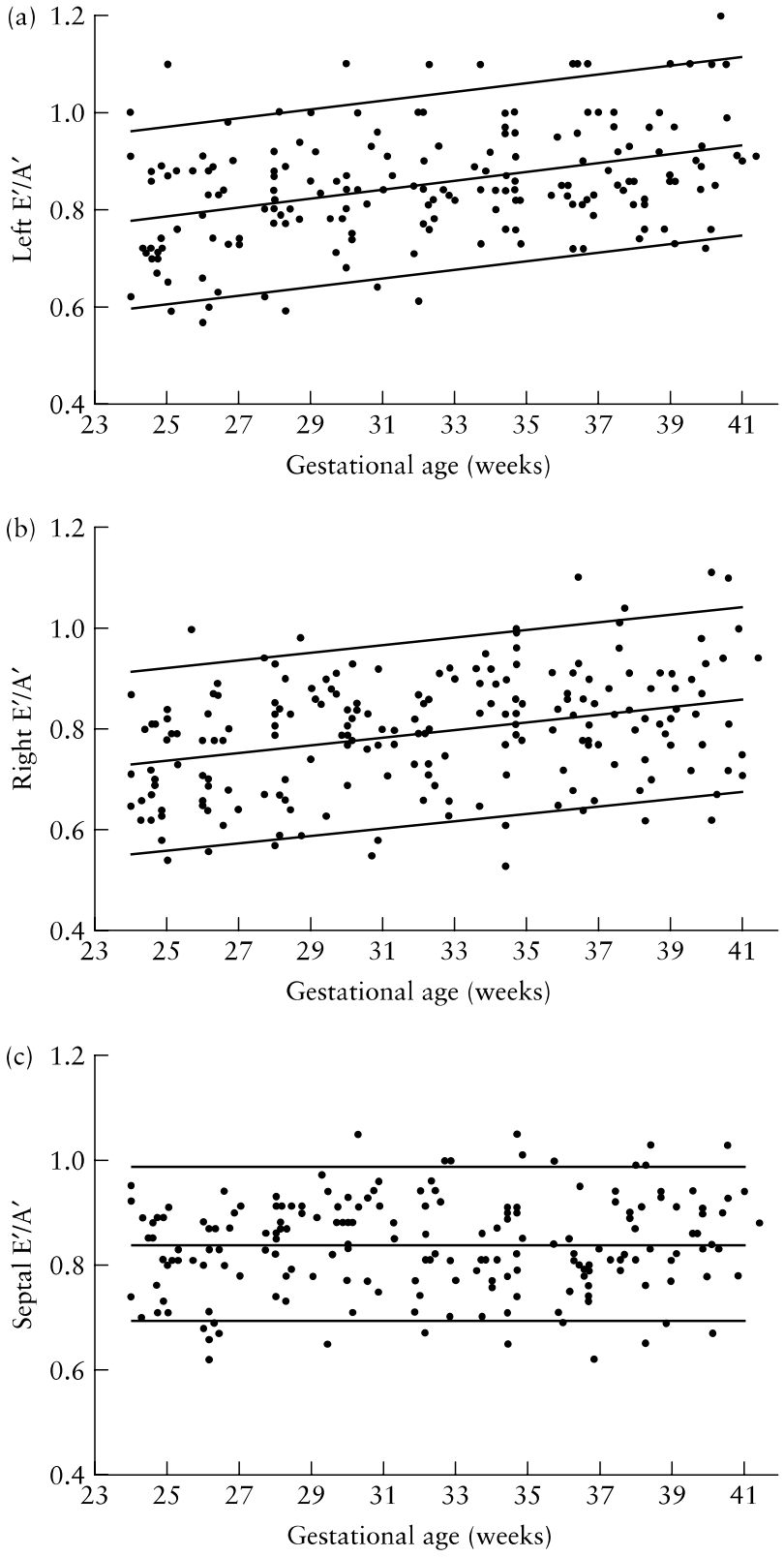


Figure 8. Scatterplots of the left (a) and right (b) E/E' ratios measured by convencional echocardiography and tissue Doppler ultrasonography vs. gestational age in the study population. Estimated 5th, 50th and 95th centile curves are shown.

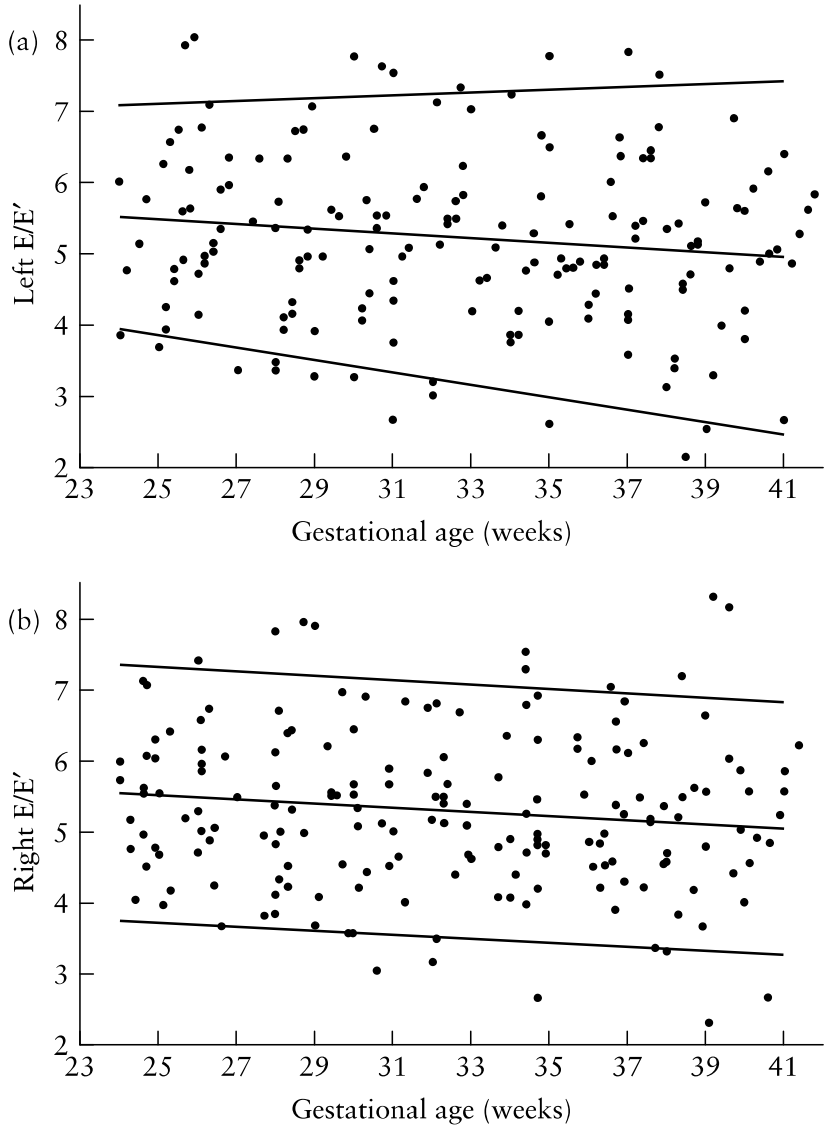
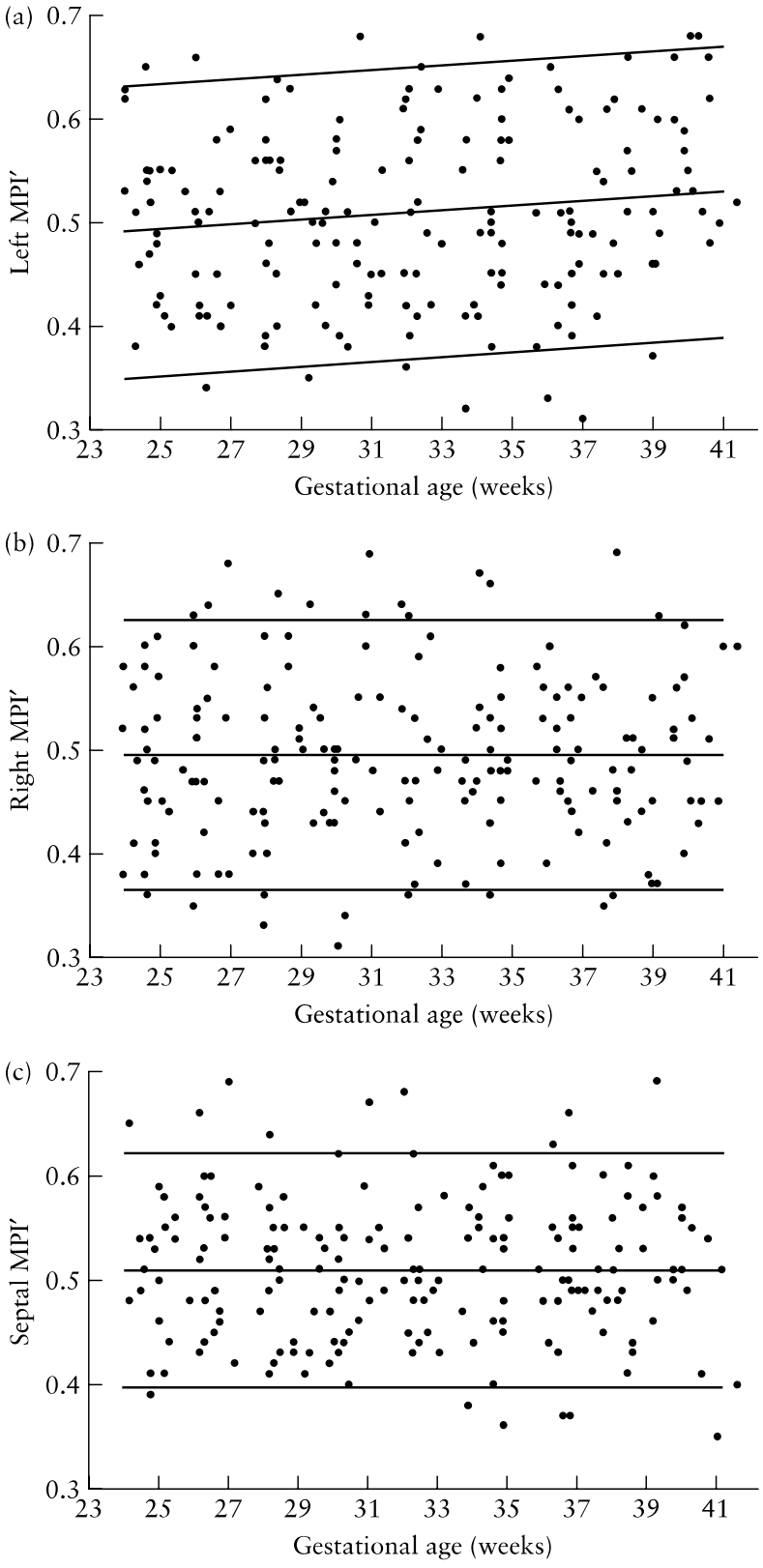


Figure 9. Scatterplts of the left, right and septal MPI' measured by tissue Doppler imaging vs. gestational age in our population. Estimated 5th, 50th and 95th centile curves are shown.



Fetal weight adjusted reference ranges for tissue Doppler parameters

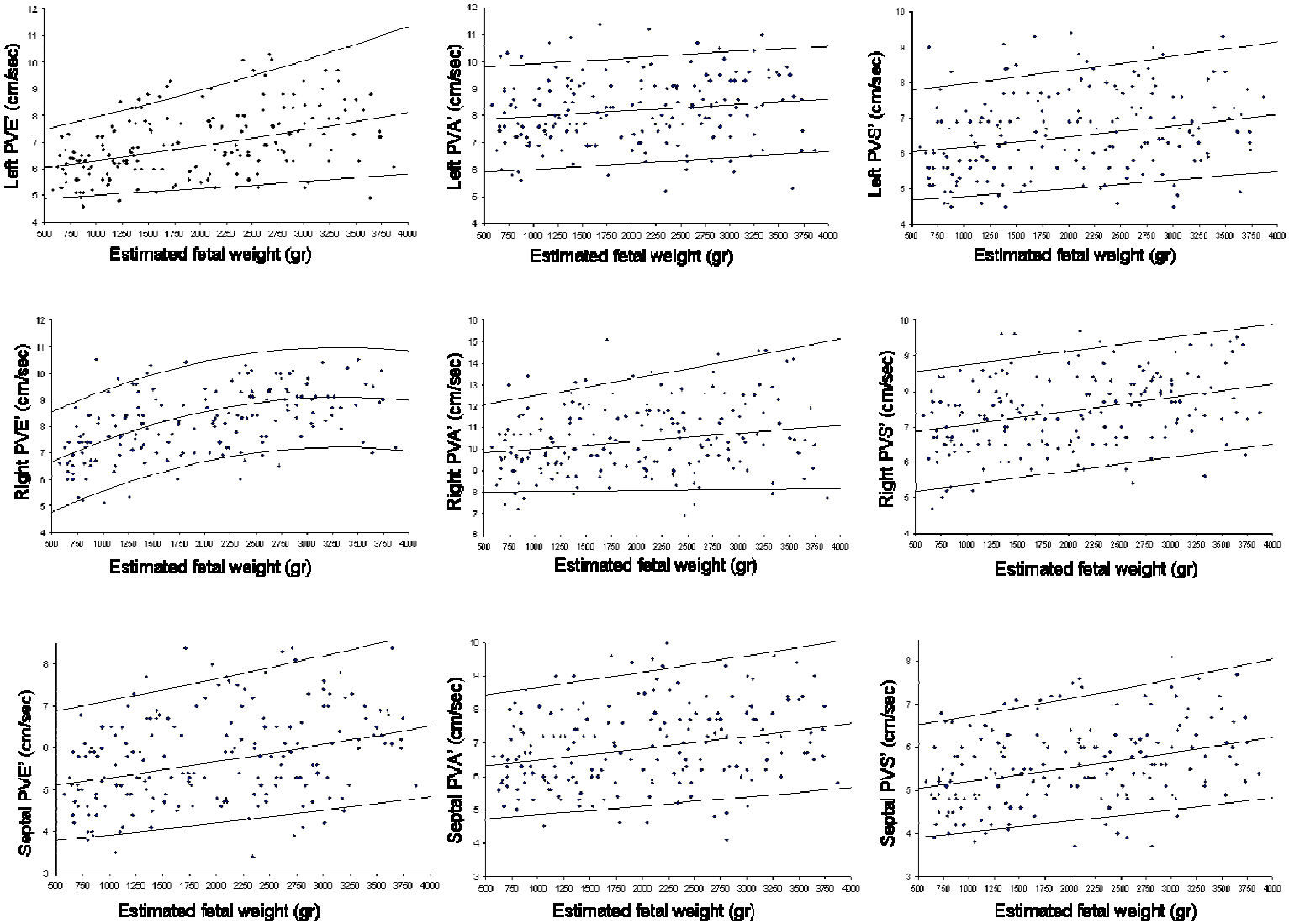
All annular peak velocities showed a progressive increase with increasing fetal weight (figure 10). Regression equations for PW-TDI parameters are given in Table 4.

Table 4. Regression equations for cardiovascular parameters obtained by tissue Doppler imaging

Parameters	**Mean**	**SD**
Myocardial peak velocities		
Log_e Left PVE' (cm/s)	1.758 + (0.0837 x EFW)	0.1202 + (0.0207 x EFW)
Left PVA' (cm/s)	7.749 + (0.2161 x EFW)	1.1896
Log_e Left PVS' (cm/s)	1.774 + (0.0462 x EFW)	0.1554
Right PVE' (cm/s)	5.721 + (2.022 x EFW – 0.303 x EFW)	1.1465
Log_e Right PVA' (cm/s)	2.267 + (0.0354 x EFW)	0.1168 + (0.0178 x EFW)
Right PVS' (cm/s)	6.674 + (0.382 x EFW)	1.0267
Log_e Septal PVE' (cm/s)	1.595 + (0.07 x EFW)	0.1821
Log_e Septal PVA' (cm/s)	1.817 + (0.052 x EFW)	0.1764
Log_e Septal PVS' (cm/s)	1.590 + (0.06 x EFW)	0.1554

EFW, estimated fetal weight (Kg); PVE', annular peak velocity in early diastole; PVA', annular peak velocity during atrial contraction; PVS', annular peak velocity in systole.

Figure 10. Scatterplot of the left, right and septal annularl peak velocities measured by tissue Doppler vs. estimated fetal weight in our population. Estimated 5th, 50th and 95th centile curves are shown.



Moreover, an excel file to calculate Z-scores for tissue Doppler parameters adjusted by gestational age or estimated fetal weight was constructed and can be consulted on the internet.[60]

Conclusions

Normal data of fetal annular peak velocities, their ratios and MPI' by tissue Doppler adjusted by GA and EFW were provided. The reported reference values may be useful in research or clinical studies and, specially, in fetuses with FGR.

VI.3. Project 3: Association between early-onset FGR and cardiac dysfunction

The results of this project have been published in an international journal:[58]

Comas M, Crispi F, Cruz-Martinez R, Martinez JM, Figueras F, Gratacós E. Usefulness of myocardial tissue Doppler vs conventional echocardiography in the evaluation of cardiac dysfunction in early-onset intrauterine growth restriction. Am J Obstet Gynecol. 2010; 203: 45.e1-7.

The results have also been presented at the 19th World Congress on Ultrasound in Obstetrics and Gynecology, 15th september 2009, Hamburg (Germany) (oral poster: M. Comas, F. Crispi, R. Cruz, F. Figueras, E. Gratacós. Cardiac function assessed by tissue Doppler myocardial velocities in fetuses with growth restriction).

Results

Study populations

The study population included 25 early-onset growth restricted fetuses and 50 controls with normal growth. Preeclampsia was present in 54% of the FGR pregnancies. Compared to controls, pregnancies with FGR presented lower GA at delivery, birth weight, Apgar score and umbilical artery pH, and higher rates of cesarean section, perinatal mortality and adverse outcome. (Table 5)

Conventional Doppler

UA PI, MCA PI and CPR were significantly different in growth restricted fetuses compared to controls. Among the growth restricted fetuses, 10 had UA absent-end diastolic flow and one had UA reverse diastolic flow.

Table 5. Baseline characteristics and perinatal outcome of the study populations

Characteristics	Controls	IUGR
n	50	25
Clinical characteristics		
Maternal age, y	31 (5)	32 (5)
White, %	70	65
Nulliparous, %	70	52
Maternal body mass index, g/m^2	23 (5)	24 (6)
Smoker, %	9	19
Preeclampsia, %	0	54[a]
Basic Doppler data		
Gestational age at ultrasound, wk	30 (3)	30 (3)
Umbilical artery PI	1.06 (0.24)	1.89 (0.34)[a]
Middle cerebral artery PI	2.08 (0.38)	1.37 (0.30)[a]
Cerebroplacental ratio	2.05 (0.55)	0.77 (0.24)[a]
Ductus venosus PI	0.56 (0.16)	0.79 (0.35)[a]
Perinatal outcome		
Gestational age at delivery, wk	39 (1)	31 (2)[a]
Cesarean section, %	18	91[a]
Birthweight, g	3347 (453)	993 (330)[a]
Birthweight percentile	53 (29)	5 (4)[a]
5-min Apgar	10 (1)	8 (2)[a]
Umbilical artery pH	7.25 (0.07)	7.21 (0.09)[a]
Perinatal death, %	0	15[a]
Adverse perinatal outcome, %	2	30[a]

Values are mean (standard deviation) or proportions

[a]*P*<0.05 as compared with controls

Conventional echocardiography

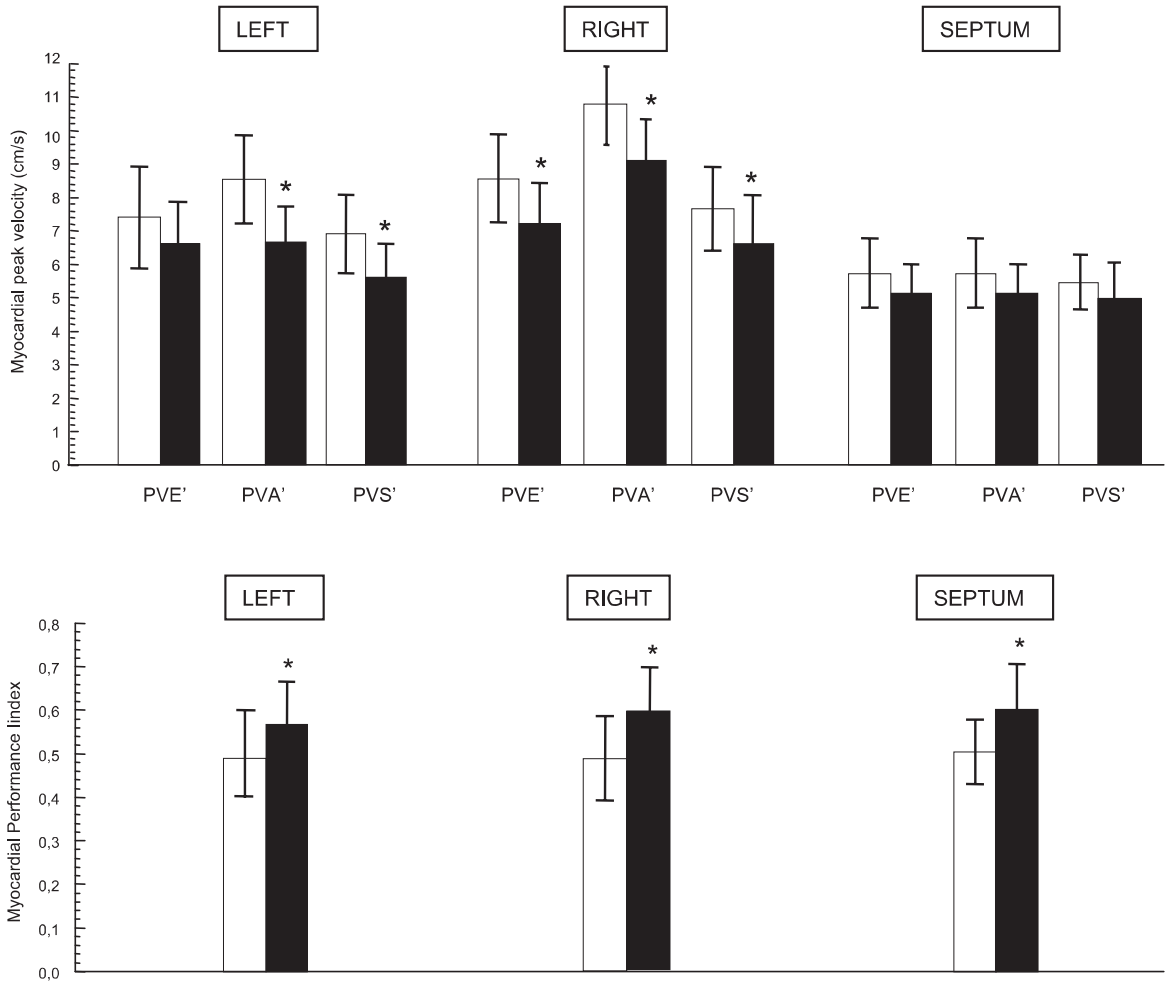
DV PI was significantly higher in growth restricted fetuses compared to controls. Among the growth restricted fetuses, two had absent or reverse flow in the ductus venosus. E and A velocities were significantly reduced in FGR. However, E/A ratios

were not significantly different in FGR as compared to controls. Aortic and pulmonary artery peak velocity were reduced with respect to controls, but the difference was not statistically different when adjusting by fetal weight. While growth restricted fetuses showed increased left MPI values, right MPI values were similar among cases and controls.

Tissue Doppler imaging

Results are shown in Figure 11. After adjusting for fetal weight, left PVA' and PVS', and right PVE', PVA' and PVS' were significantly reduced in the early-onset FGR. Left E'/A' ratio was higher in growth restricted fetuses compared to controls. Left, right and septal MPI' were significantly higher in FGR.

Figure 11. Tissue Doppler results in controls and early-onset FGR



***** *P*<0.05 as compared with controls

Conclusions

TDI demonstrated the presence of both systolic and diastolic cardiac dysfunction in early-onset FGR. TDI may constitute a more sensitive tool than conventional echocardiography to evaluate cardiac dysfunction in FGR.

VI.4. Project 4: Association between late-onset FGR and cardiac dysfunction

The results of this project have been published in an international journal:[61]

Comas M, Crispi F, Cruz-Martinez R, Figueras F, Gratacós E. Tissue-Doppler echocardiographic markers of cardiac dysfunction in small-for-gestational age fetuses. Am J Obstet Gynecol 2011 (in press).

The results have also been presented at the 20th World Congress on Ultrasound in Obstetrics and Gynecology, 10-14 October 2010, in Prague (Czech Republic) (Crispi F, Comas M, Cruz R, Martinez-Crespo JM, Eixarch E, Figueras F, Gratacos E. Cardiac dysfunction is present in small-for-gestational age fetuses with normal umbilical artery Doppler).

Results

Study populations

The study population included 58 SGA and 58 controls. Pregnancies with SGA showed significantly lower birth weight and birth weight percentile. GA at delivery, Apgar score and umbilical artery pH were similar between SGA pregnancies and controls. Growth restricted cases showed a non-significant trend to higher rates of of intervention for fetal distress, cesarean section and preeclampsia, which was present in 10% of the SGA pregnancies. (Table 6)

Conventional Doppler

UA PI and MCA PI were similar in growth restricted fetuses and controls. Only 10% of SGA fetuses presented brain vasodilation. Mean uterine artery PI was higher in growth restricted fetuses compared to controls.

Table 6. Baseline characteristics and perinatal outcome of the study populations

Characteristics	**controls**	**SGA**	**p**
N	58	58	
Clinical characteristics			
GA at ultrasound (weeks)	38 (2)	38 (1)	0.62
Estimated fetal weight (gr)	3024 (413)	2235 (330)*	**< 0.001**
Centile	45 (27)	4 (8)*	**<0.001**
Umbilical artery PI	0.92 (0.19)	1.01 (0.2)	0.1
Middle cerebral artery PI	1.62 (0.34)	1.60 (0.31)	0.89
Cerebro-placental ratio	1.79 (0.49)	1.65 (0.45)	0.13
Mean uterine artery PI	0.73 (0.2)	0.92 (0.41)*	**0.02**
Pregnancy outcome			
GA at delivery (weeks)	40 (1)	38 (1)	0.08
Birth weight (gr)	3353 (418)	2379 (304)*	**<0.001**
Birth weight centile	52 (26)	4 (3)*	**<0.001**
Cesarean section	17%	31%	0.1
Intervention for fetal distress	7%	16%	0.23
5 minutes Apgar	9.9 (0.1)	10 (0)	0.37
Umbilical artery pH	7.23 (0.07)	7.23 (0.07)	1
Preeclampsia	2%	10%	0.12
Days in neonatal care unit	0.1 (0.5)	0.8 (2.1)*	0.018

Data were expresed as mean (SD) or proportions.
*P-value <0.05 as compared with controls.

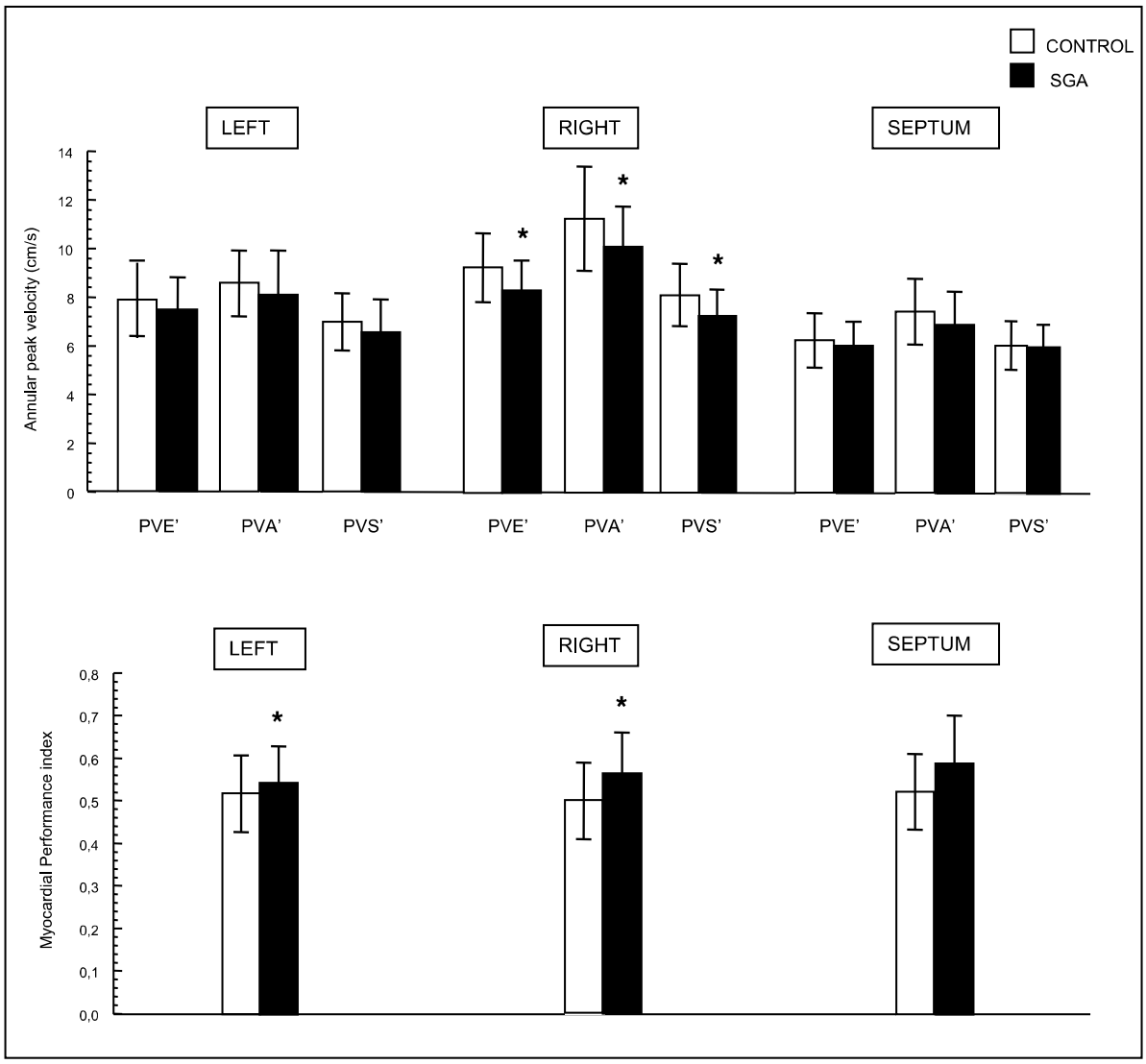
Conventional echocardiography

Ductus venosus PI, left and right E velocities and E/A ratios were similar among cases and controls. Both A velocities were significant lower in SGA as compared with controls even after adjusting by fetal weight. SGA cases showed a non-significant trend to increased left MPI values as compared with controls.

Tissue Doppler imaging

Results are shown in Figure 12. All peak velocities in tricuspid annulus were significantly lower in SGA as compared with controls, even after adjusting for fetal weight. Left and septal annular velocities showed a non-significant trend to lower values in SGA cases. Left and right MPI' were significantly higher in SGA.

Figure 12. Assessment of annular peak velocities and myocardial performance index measured by Tissue Doppler Imaging in controls and SGA.



*P-value <0.05 as compared with controls.

The proportion of cases with abnormal annular peak velocities and performance index according to GA-based reference ranges[59] was calculated in both groups. 15-20% and 30-40% of late SGA had annular peak velocities < 10th centile and MPI' > 90th centile respectively.

Conclusions

The findings of this project further support that a proportion of SGA have true late-onset FGR, which is associated with subclinical cardiac dysfunction, as previously described for early-onset intrauterine growth restriction.

VII. DISCUSSION

This work provides evidence to support that early- and late-onset FGR are associated with cardiac dysfunction. Fetal cardiac dysfunction that can be detected by a sensitive echocardiographic tool such as TDI. This new echocardiographic technique is reliable enough for clinical or research purposes. Normal data of fetal annular peak velocities, their ratios and MPI' by TDI and their changes related to GA and fetal weight were also provided.

Although **early-onset FGR** affects less than 1% of deliveries, it is recognized among the main causes of perinatal mortality and morbidity.[14,62] Prediction of mortality and morbidity is critical for clinical management of these fetuses. Other cardiovascular parameters such as DV and MPI have been previously proposed to predict their outcome and therefore as a clinical tool to decide when to deliver them.[32-34,63] However, these conventional echocardiographic parameters usually appear in late stages of deterioration being useful only to predict mortality or severe morbidity. The present work has demonstrated that tissue Doppler evaluation in early FGR is more sensitive to detect systolic and diastolic function as compared conventional echocardiography. Therefore, tissue Doppler parameters may be useful to detect and monitor earlier stages of cardiac dysfunction in FGR. Its correlation and potential interaction with perinatal and long-term outcome remains to be evaluated in further studies.

On the other hand, **SGA** are detected among 5-10% of all near term deliveries. Despite being considered as constitutionally small for some authors, the present study and others demonstrate that SGA babies have poorer perinatal outcome[25,26]

and also signs of cardiac dysfunction.[16,20] Unfortunately, UA is not useful to detect those late-onset small babies with worse results and future research should provide other parameters to improve the detection of **late-onset FGR**. The present work shows that a proportion of SGA have true growth restriction, which is associated with subclinical cardiac dysfunction. Therefore, one of the main clinical implication of this thesis is the potential use of cardiac assessment in the detection of those small-for-gestational fetuses with poorer outcome. Fetal cardiac evaluation using TDI showed that a considerable proportion of SGA (15 to 40%) had abnormal annular peak velocities or MPI' results, conversely to only 10% of vasodilatation or <10% of abnormal ductus venosus. These findings could suggest a higher sensitivity of TDI to detect late-onset FGR. Future research is warranted to explore whether it might have a value, alone or in combination within other markers, in improving the identification of fetuses with true late-onset FGR, which present poorer perinatal and postnatal outcome.

VII.1. Project 1: intra- and inter-observer reliability of tissue Doppler measurements

This study shows a good intra- and inter-observer reliability of TDI measurements supporting the concept that in experienced hands TDI may constitute a valid tool for research and clinical purposes. As with any other echocardiographic measurement, TDI requires an experienced examiner, but in this study TDI measurements could be successfully obtained in all cases and showed a good reproducibility.

Reliability of annular peak velocities

The present study shows a similar or better reliability of annular peak velocities as compared to previous studies using PW-TDI.[41,44,45] Reliability of PW-TDI parameters has been assessed in previous studies showing similar values. Harada et al.[41] was the first author who tested TDI in fetuses. To determine intra- and inter-observer variability, annular peak velocities were remeasured by the same and by an independent observer in 10 randomly selected fetuses. Intra- and interobserver variability of PVE', PVA' and PVS' was calculated as the difference in two measurements divided by the mean value and results were below 5% in all cases. Chan et al.[44] calculated intra- and interobserver variability of annular velocities in 10 fetuses using the ICC, showing a good reproducibility. However, this work provided variability of PVE', PVA' and PVS' without considering the annulus location. Gardiner et al.[45] provided two measurements for PVE', PVA' and PVS' made by the same operator in 10 fetuses and by the same operator in 17 different fetuses and reported the median absolute and median absolute relative deviation to assess reproducibility. Although data were generally below the acceptable level, intraoperator variability was high, explained by the presence of small number of high outliers.

In conclusion, our results are in line with most previous studies showing acceptable reproducibility of annular peak velocities.

On the other hand, Nii et al[64] reported intra- and inter-observer variability of 0.5% using color-TDI, ,reflecting the lower reliability of this offline technique compared to PW-TDI.

Reliability of myocardial performance index

Our results on reliability for left and right MPI' is consistent with a previous study by Achariya et al.[56] where reporting a ICC ranging between 0.76 and 0.94 in a group of 15 fetuses with normal cardiac structure. No previous reports have evaluated septal MPI' reproducibility.

VII.2. Project 2: Gestational age and fetal weight adjusted reference ranges for tissue Doppler parameters at 24-41 weeks of gestation

The study provides GA- and EFW- adjusted reference ranges of annular peak velocities, E'/A' ratios, E/E' ratios and MPI' measured by TDI in normal fetuses. This study first provides the mean, 5th and 95th centiles for TDI parameters together with the regression formulas.

Gestational age adjusted reference ranges for tissue Doppler parameters

The obtained reference charts for peak myocardial velocities show similar values to those previously reported, though certain differences remain that could be explained by the use of different echocardiographic systems.[65]

Our study confirms previous data on a positive correlation between diastolic and systolic **peak annular velocities** and GA, showing similar values to those previously reported. Chan et al.[44] described that PVE', PVA', PVS' increased from 19 to 37 weeks of gestation at the left and right ventricular wall and interventricular septum, and Gardiner et al.[45] showed a positive relationship with gestation for all annular peak velocities except for the left PVA'. Reference ranges for annular peak velocities using color-TDI were previously published by Nii et al,[64] showing that PVE', PVA', PVS' increased throughout gestation.

Our data suggest a positive correlation between left and right **E'/A' ratio** and GA, while septal E'/A' ratio was constant throughout second half of pregnancy. This increase is due to a faster increase in early when compared with late diastolic velocities. Previously published reference values for E'/A' ratios showed an increased throughout gestation in all three locations.[44] E/A ratios increased with GA at all sites using color TDI.[64]

The study describes a negative correlation between left and right **E/E' ratio** and GA. PVE' increased progressively with advancing fetal age at a faster rate than PVE. As a consequence, E/E' ratios decreased exponentially. This finding is consistent previously reported data using pulsed-wave and color-TDI.[44,64] The observed decrease in E/E' ratio, which reportedly correlate well with ventricular filling pressure,[66] could be interpreted as an indirect reflection of increased compliance due to myocardial maturation during pregnancy, and in these respects it is consistent with the progressive increase observed in E'/A'. Table 7 summarize previous data on TDI studies.

The study first reports reference ranges for **MPI'** measured by TDI. MPI' values by TDI are generally higher than those obtained by standard pulsed Doppler and this bias is consistently observed among adults[67] children[68] and fetuses.[31] These two indices are rather different because they are related to different mechanisms: MPI is based on blood flow events and MPI' is based on myocardial motion events. As has been observed with pulsed Doppler MPI,[69] MPI' values showed a mild tendency to increase with GA.

Table 7. Comparison of previous data on tissue Doppler values according to gestational age.

	Chan et al.[44] (2005) PW-TDI ATL HDI 5000		Gardiner et al.[45] (2006) PW-TDI ALOKA			Nii et al.[64] (2006) Color-TDI GE VIVID-7			Comas et al.[59] (2010) PW-TDI SIEMENS ANTARES		
GA (weeks)	19	37	24-29	30-34	35-38	25-29	30-34	35-42	24-29	30-34	35-41
Left PVE' (cm/s)	3.3±0.5	7.2±2.1	6.6[1.2]	7.7[1.2]	8.6[1.2]	2.8±0.8	3.8±0.7	4.2±1	6.2±1.2	7.1±1.2	7.6±1.2
Left PVA' (cm/s)	6.3±1.7	7.9±0.8	9.4[2.3]	9.4[2.3]	9.4[2.3]	4.8±0.9	5.3±1	4.9±1.5	8±1.3	8.2±1.3	8.4±1.3
Left PVS' (cm/s)	3.8±0.6	6±0.9	5.2[1.3]	5.8[1.3]	6.2[1.3]				6.2±1.3	6.4±1.3	6.7±1.2
Right PVE' (cm/s)	3.9±0.6	8.3±1.8	5.2[1.3]	5.8[1.3]	6.2[1.3]	3.7±0.8	4.9±1.4	5.5±1.5	7.4±1.1	8.2±1.2	9.1±1.3
Right PVA' (cm/s)	7.7±1.3	10.6±2.1	12.2[2.4]	13.3[2.4]	14.2[2.4]	6.6±1.2	6.9±1.5	7.5±1.1	9.9±1.1	10.3±1.2	10.7±1.2
Right PVS' (cm/s)	4.2±1.1	7.6±1.2	6.8[1.7]	7.4[1.7]	7.8[1.7]				7±1	7.4±1	7.8±1
Septal PVE' (cm/s)	3.2±0.5	5±0.8	7[0.9]	7.1[0.9]	8[0.9]	2.6±0.6	3±0.7	3.2±0.5	5.3±1.2	5.7±1.2	6.1±1.2
Septal PVA' (cm/s)	5.5±1.2	5.9±1.6	8.1[1.6]	8.7[1.6]	9.1[1.6]	4±1	4.5±0.8	4.5±0.8	6.5±1.2	6.8±1.2	7.1±1.2
Septal PVS' (cm/s)	3.3±0.6	5.6±1.1	5[1.2]	5.6[1.2]	6[1.2]				5.2±1.2	5.5±1.2	5.9±1.2
Left E'/A'	0.55±0.1	0.91±0.3				0.59±0.2	0.74±0.2	0.92±0.4	0.8±0.11	0.85±0.11	0.9±0.11
Right E'/A'	0.51±0.1	0.79±0.1				0.57±0.1	0.73±0.2	0.74±0.2	0.75±0.11	0.79±0.11	0.84±0.11
Septal E'/A'	0.61±0.1	0.76±0.2				0.67±0.1	0.68±0.1	0.72±0.1	0.84±0.09	0.84±0.09	0.84±0.09
Left E/E'	9.8±2.1	7.4±3.8							5.4±1	5.2±1.2	5±1.4
Right E/E'	9.7±2	6.4±2							5.5±1.1	5.3±1.1	5.1±1.1

Data are expressed as mean±SD or mean[SE]

Fetal weight adjusted reference ranges for tissue Doppler parameters

Reference charts for annular peak velocites adjusted for EFW were also provided, showing similar patterns to those based on GA. Since annular peak velocities have a positive correlation with subject's and heart's weight, we suggest that these normality curves will therefore be particularly useful in fetuses with FGR. There is good evidence that in a normally functional heart, myocardial velocities are essentially depending on heart/body size[70-73]. This relationship is also applicable to most cardiovascular parameters including cardiac output and blood flow velocitites (such as umbilical or aortic flow) that are usually adjusted by fetal weight. In normally grown fetuses, it would be irrelevant to use normal ranges calculated by GA or EFW as there is a strong correlation between them. However, measurement of these cardiovascular parameters in growth restricted fetuses could lead to the false assumption of abnormal results whether cardiac index (cardiac output adjusted by fetal weight) has been demonstrated to not being different in growth restricted and normally growth fetuses as demonstrated by several authors[15,17]. In support of this notion, annular peak velocities must also be adjusted by fetal weight. This concept would be true for peak velocitites, but not for ratios (E'/A' and E/E') and times (MPI') that are not affected by changes in body weight.

VII.3. Project 3: Association between early-onset FGR and cardiac dysfunction

In this study TDI demonstrated the presence of both systolic and diastolic cardiac dysfunction in early-onset growth restricted fetuses, suggesting that TDI is a more sensitive tool than conventional echocardiography to evaluate fetal cardiac function. The results are in line with echocardiographic studies in adults and children,[39,40] where TDI has demonstrated to be an earlier marker of cardiac disease.

Conventional echocardiography

Using conventional echocardiography, most of the parameters evaluated were similar among FGR and controls, which illustrates the relatively poor sensitivity of conventional echocardiography with respect to TDI in the detection of subclinical cardiac dysfunction.

Despite ventricular filling velocities were significantly lower in growth restricted fetuses, **E/A ratio** show similar values between cases and controls. E/A ratio evaluates ventricular filling during the diastole and represents the standard echocardiographic parameter to evaluate diastolic function.[29] Earlier studies in growth restricted fetuses have reported similar,[22] reduced[23,74] E/A ratios whereas recent studies have reported increased[15,16,21] values, which is considered a sign of diastolic dysfunction in fetal life.[75] A recent study demonstrated that E/A ratios are only significantly increased in cases with reverse flow in the UA.[15] Therefore, the lack of significant differences could be explained by the mix of the population and the fact that we included only one case with reversed diastolic flow in the UA.

Aortic and **pulmonary artery peak velocities** were not statistically different among groups after adjusting by fetal weight. Outflow velocities are normally recorded to calculate cardiac output, and our observations are consistent with previous reports

showing no significant changes in cardiac output adjusted by fetal weight in growth restricted fetuses.[15,17]

Left MPI was significantly elevated in early growth restricted fetuses. Left MPI is considered a marker of combined systolic and diastolic function. The data are in agreement with previous reports demonstrating that MPI is abnormal in FGR[18,76-78] and increases from early stages of fetal deterioration.[15] Additionally, a recent study reported that MPI is independently associated with perinatal mortality.[63] We have used the modified method (Mod-MPI) that includes the Doppler recording of the clicks of the valves to estimate the time-periods and improve reproducibility.[31]

ln contrast, **right MPI** was similar among groups. Previous studies have shown inconsistent results with right MPI in FGR,[35,56] which may be due to the difficulties in recording this parameter with conventional echocardiography, since it requires measurements from different cardiac cycles and it thus may be affected by fetal heart rate fluctuations.

Tissue Doppler imaging

In contrast with conventional echocardiography, TDI showed significant differences between FGR and control fetuses in almost all systolic and diastolic recorded parameters. TDI requires an experienced examiner, but the findings of this study support the notion that in experienced hands it may constitute a valid tool for research and clinical purposes. Moreover, not only diastolic but also systolic cardiac dysfunction has been demonstrated using TDI.

Annular peak velocities were significantly lower in most recorded locations, which was consistent with other previous studies. Myocardial tissue Doppler velocities reflect shortening and lenghening velocities of the long-axis fibers lying

predominantly in the ventricular subendocardium. In adults and children, they constitute a sensitive preclinical marker of impaired cardiac function[39,40] and a strong predictor of poor outcome in several major cardiac diseases.[79] There were no significant differences of annular peak velocities in the septal annulus. This could be explained by the reduced long-axis fibers in the interventricular septum compared to the ventricular free wall,[80] which contributes towards lower velocities.

The observed differences between early-onset growth restricted and normal fetuses are consistent with two previous studies:

Larsen et al[47] evaluated left and right PVS' using color-TDI in a group of 20 growth restricted fetuses of 26 to 34.6 weeks and compared their values with 42 normally grown fetuses of 18.6 to 39.1 weeks. Growth restricted fetuses had significantly lower left PVS' than normal group, especially those with reversed diastolic UA flow. In our study, a comparison was performed creating two subgroups with the 11 IUGR fetuses with umbilical artery absent (10) or reversed (1) diastolic flow and the 14 IUGR fetuses with normal flow, but no statistically significant differences were found. The lack of differences observed could be due to the fact that there was only one case with reversed UA flow.

The second study[48] included 14 growth restricted fetuses with abnormal UA, MCA and/or uterine arteries. Left and septal E'/A' ratio were higher in FGR compared to AGA fetuses with or without hypertension. In constrast with our study, the authors did not find lower PVE' and PVA' in the FGR group.

Early-onset growth restricted fetuses showed increased values of **MPI'** measured by TDI in mitral, tricuspid and septal annulus. Although no previous reports had evaluated MPI' by TDI in FGR, these results are consistent with the differences observed in MPI measured with conventional Doppler in growth restricted

fetuses.[15,18,76-78]. Increased right MPI' has also been described in fetuses with heart failure.[49]

VII.4. Project 4: Association between late-onset FGR and cardiac dysfunction

This study provides evidence that SGA fetuses with normal UA Doppler present signs of subclinical cardiac dysfunction by means of conventional echocardiography and by tissue Doppler imaging, which is consistent with previous studies suggesting the existence of true forms of gro

wth restriction among SGA fetuses. A proportion of SGA fetuses would be exposed to placental insufficiency and chronic restriction of nutrients and oxygen,[81,82] that would affect myocardial fibers, which is reflected by TDI.

Conventional echocardiography

Left and right **E/A ratios** were no significantly increased in late-onset growth restricted fetuses. These results are similar than those reported by Girsen[16] in a small group of SGA fetuses with normal UA Doppler. Furthermore, these results are in line with those of Crispi et al.[15] in early-onset FGR, that demonstrated that E/A ratios were significantly increased only in growth restricted fetuses with absent o reversed end-diastolic flow in the UA.

Similarly, **MPI** showed a non-significant trend to higher values among late-onset growth restricted fetuses. This results were also in line with those reported by Girsen.[16] This parameter of global cardiac function increases since early stages of fetal deterioration, for instance, in early-onset FGR with abnormal but present end-diastolic flow in UA.[15] Although this study contains the largest sample of SGA fetuses investigated to date, the absence of significant differences with conventional Doppler echocardiography would be due to sample size.

Tissue Doppler imaging

In contrast with conventional echocardiography, TDI could detect significant differences between SGA and controls, with regards to annular peak velocities and MPI'. The findings illustrate the higher sensitivity of TDI in relation with conventional echocardiography for detecting subclinical fetal cardiac dysfunction.

There is only a previous study of cardiac function using TDI in a small group of 12 SGA fetuses.[46] In this study growth restricted fetuses were defined by EFW below 10th centile and UA Doppler was not determined. Left and right PVE', PVA' and E/E' ratio were similar between cases and 38 normally grown fetuses.

From a pathophysiologic viewpoint, the results are consistent with previous evidence that a proportion of late-onset growth restricted fetuses with normal UA are exposed to placental insufficiency[81,82], which leads to the presence of cardiac dysfunction features. Chaiworapongsa et al.[20].demonstrated that 4 % of neonates born small for GA had detectable cardiac troponin I in umbilical cord blood, suggesting subclinical myocardial injury before birth. Girsen et al.[16] evaluated 13 SGA fetuses with normal UA Doppler and found significantly increased levels of ANP, although echocardiographic markers were not significantly different from controls. Therefore, TDI evaluation could constitute a non-invasive method to detect late-onset FGR. Its potential contribution to clinical practice requires future studies.

VIII. LIMITATIONS AND TECHNICAL CONSIDERATIONS

- **Availability of the technique**

TDI requires special software not available in all ultrasound machines and it requires formal training. Even in experienced hands, TDI measurements can be challenging. For instance, data were successfully obtained on average in 95% of cases and the main reason for unsuccessful TDI measurement was fetal position preventing an isonation angle less than 30º, which is critical to obtain waveforms of enought quality to allow meaningful measurements. As further research demonstrates its potential value to evaluate cardiac function in FGR and other conditions, TDI might become incorporated into obstetric ultrasound devices.

- **Resolution of ultrasound machines**

Annular peak velocities are remarkably lower in fetuses as compared with children and adults. Even the lowest available scale size of most ultrasound machines is often too large. In these circumstances waveforms are often displayed with suboptimal resolution which may hamper accuracy. This also could result into not enough resolution to detect significant differences between groups.

- **Evaluation of a limited subset of cardiac function parameters**

TDI was assessed in real time, which may confer a better feasibility as compared with offline analysis using color-TDI or more complex techniques as 2D speckle tracking, but does not permit to evaluate other deformation indices such as strain or strain-rate. However, fetal life conditions as higher heart rate, fetal and respiratory movements and varied body position may limit the off-line analysis and therefore,

future studies are already warranted to validate the use of off-line myocardial imaging techniques in fetuses.

- **Correlation between annular peak velocities and fetal weight**

It could be argued that the reduction of annular peak velocities in growth restricted fetuses might be explained by the smaller weight of these fetuses. This increase could be explained by the bigger size of the fetus and its heart across gestation.[41-43,59] and has been demonstrated as a part of this thesis. Therefore, these fetuses had absolute lower velocities than normal growth fetuses of the same GA just because they were smaller. For this reason all echocardiographic values were adjusted for fetal weight, and most differences remained significant.

- **Evaluation of cardiovascular outcome after birth**

Recently, it has been demonstrated that FGR children show subclinical cardiac dysfunction using TDI.[13] The findings support the existence of direct cardiac programming in FGR. However, the correlation between fetal echocardiographic results and cardiovascular outcome after birth could not be evaluated because of the short period of follow-up.

- **Differences in the tricuspid annulus**

Changes in TDI parameters were more prominent when measured in the tricuspid annulus, as compared with left and septal walls. While this might truly reflect higher peak velocities in the right ventricle, which is the predominant one in fetal life, we can not exclude a systematic technical bias since Doppler insonation of the right ventricle is normally more straightforward in the fetus.

IX. CONCLUSIONS

1. Fetal TDI measurements have demonstrated a good reproducibility in trained hands.

2. GA- and EFW- adjusted reference ranges of annular peak velocities, their ratios and MPI' measured by TDI in normal fetuses, between 24 and 41 weeks of gestation, were successfully provided.

3. Early and late-onset FGR fetuses were associated with the presence of both systolic and diastolic cardiac dysfunction detected by TDI.

4. TDI would be a more sensitive tool than conventional echocardiography to evaluate fetal cardiac function in growth restricted fetuses.

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XII. ANNEXES

XII.1. Annex 1. Acceptance letter article 3

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XII.2. Annex 2. Informed consent – Patient information

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ESTUDI DE LA DISFUNCIÓ CARDÍACA EN FETUS AMB RESTRICCIÓ DE CREIXEMENT PER INSUFICIÈNCIA PLACENTÀRIA

La convidem a participar en un estudi que té com a principal objectiu investigar l'associació entre la restricció de creixement intrauterina de causa placentària i l'alteració de la funció cardíaca fetal. La seva participació a l'estudi li suposarà un estudi del cor fetal mitjançant ecocardiografia convencional i noves tècniques que permeten detectar alteracions subtils de la funció cardíaca fetal com ara el Doppler tissular. L'examen ecogràfic es realitzarà entre les 24 i les 40 setmanes.

Se li proposa participar en aquest estudi perquè vostè és una gestant normal sense problemes de creixement fetal ni d'hipertensió en l'embaràs i per tant s'ofereix com a control.

Les seves dades seran utilitzades sempre de forma anònima i absolutament confidencial, disposant d'accés a la informació obtinguda exclusivament els membres autoritzats. Si decideix NO participar en aquest seguiment, se li oferirà el seguiment estàndard del control de l'embaràs .

Jo, _____

He llegit el full d'informació que m'ha sigut entregat. He pogut fer preguntes sobre els possibles beneficis i inconvenients de participar en l'estudi, i he rebut suficient informació sobre el mateix.

He parlat amb:_____ ,comprenc que la meva participació és voluntària i que puc retirar-me de l'estudi en qualsevol moment, sense haver de donar explicacions, i sense que repercuteixi en les atencions mèdiques.

Data: _____Signatura:_____ Pacient

Signatura: _____ Metge

CLÍNIC Corporació Sanitària

FULL DE CONSENTIMENT INFORMAT

CLÍNIC BARCELONA Hospital Universitari

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Se li proposa participar en aquest estudi perquè el seu embaràs presenta criteris de restricció del creixement intrauterina (pes fetal estimat inferior al percentil 10 ± un índex de pulsatilitat de l'artèria umbilical superior al percentil 95).

Les seves dades seran utilitzades sempre de forma anònima i absolutament confidencial, disposant d'accés a la informació obtinguda exclusivament els membres autoritzats. Si decideix NO participar en aquest seguiment, se li oferirà un seguiment correcte i a discreció de l'equip que atén el seu embaràs.

Jo, _____

He llegit el full d'informació que m'ha sigut entregat. He pogut fer preguntes sobre els possibles beneficis i inconvenients de participar en l'estudi, i he rebut suficient informació sobre el mateix.

He parlat amb:_____ , comprenc que la meva participació és voluntària i que puc retirar-me de l'estudi en qualsevol moment, sense haver de donar explicacions, i sense que repercuteixi en les atencions mèdiques.

Data: _____Signatura:_____ Pacient

Signatura: _____ Metge

XII.3. Annex 3. Data form

A. IDENTIFICATION	
NHC |__|__|__|__|__|__|__|	LMP (US) |__|__| |__|__| |__|__|__|__| LMP (mother) |__|__| |__|__| |__|__|__|__| Parity: nulliparous / multiparous
Surname 1: _____	Smoking (cig/day) No (0) yes |__||__|
Name: _____	Drugs: no/cannabis/cocain/opiacios/bdz/others_____
Date of birth |__|__| |__|__| |__|__|__|__| Adress: _____ Phone: |__|__|__|__|__|__|__|__|__| |__|__|__|__|__|__|__|__|__|	Ethnicity: Caucasian / latin-american / black / asian /others Socioeconomic status: low /high Educational status:elementary/secondary/higher education
	Height |__|__| Weight |__|__|

(1) MATERNAL AND PREGNANCY DATA
Cronic hypertension / DM / renal disease / coagulation disorder/ Autoinmune / previous PE / previous IUGR / previous abruptio / previous fetal death / _____ Multiple pregnancy: No Yes: DC-DA / MC-DA / MC-MA / triplet

(2) MATERNAL OUTCOME			
Preeclampsia:	no	yes:	gestational age at diagnosis |__|__|__|
Gestational hipertension:	no	yes:	gestational age at diagnosis |__|__|__|
IUGR:	no	yes:	gestational age at diagnosis |__|__|__|
SGA:	no	yes:	gestational age at diagnosis |__|__|__|
Placental abruptio:	no	yes:	gestational age at diagnosis |__|__|__|
Spontaneous preterm delivery:	no	yes:	gestational age at diagnosis |__|__|__|
Chorioamnionitis:	no	yes:	gestational age at diagnosis |__|__|__|
Gestational diabetes:	no	yes:	gestational age at diagnosis |__|__|__|
Abortion:	no	yes:	gestational age at diagnosis |__|__|__|
ILE:	no	yes:	gestational age at diagnosis |__|__|__|

(3) FETAL OUTCOME
Date of delivery: |__|__| |__|__| |__|__|__|__| **Gestational age at delivery :** |__|__|__| Place of delivery: ICGON / _____ Indication: spontaneous / elective. Mode: vaginal non-distress / vaginal for distress / cesarean section non-distress / cesaren section for distress Newborn gender: male /female Neonatal birth weight: |__|__|__|__| Apgar |__|__| |__|__| UA/UV pH |__|__|__| |__|__|__| Perinatal death: intrauterine / intrapartum / neonatal Admission in Neonatal Intensive Care Unit: no /yes days |__|__| Neonatal morbidity: no / yes IVH grade III-IV / hypoxic-ischemic encephalopathy/ convulsions / necrotizing enterocolitis / respiratory distress requiring intubation / sepsis/ periventricular leukomalacia

FETAL CARDIOVASCULAR FUNCTION

B. GENERAL DATA

Date of evaluation |__|__| |__|__| |__|__|

Nº evaluation |__|

Hospital ID |__|__|__|__|__|__|__|__|

Study ID |__|__|__|

Name _____

Surname 1_____**Surname**

LMP (US) |__|__| |__|__| |__|__|__|__|

Gestational age: |__|__|.|__|

C. BIOMETRIC DATA

BPD |__|__| mm

Cranial perimeter |__|__|__| mm

Abdominal perimeter: |__|__|__| mm

Femur: |__|__| mm

Estimated fetal weight: |__|__|__|__| g

Gender male /female

Centile: |__|__|

DOPPLER ULTRASOUND

UA: PI |__|.|__|__| End diastolic flow: |__| (1.Present 2.Absent 3.Reversed)

MCA: PI |__|.|__|__|

DV: PI |__|.|__|__| Atrial flow |__| (1.Present 2.Absent 3.Reversed)

Median Ut PI |__|.|__|__| Right Ut PI |__|.|__|__| Left Ut PI |__|.|__|__|

CARDIOVASCULAR EVALUATION

Left E/A ratio: |__|__|.|__| PV E |__|__|.|__| cm/s PV A |__|__|.|__| cm/s

MPI: |__|.|__|__| ICT (ms) |__|__| IRT (ms) |__|__| ET (ms) |__|__|__|

Right E/A ratio: |__|__|.|__| PV E |__|__|.|__| cm/s PV A |__|__|.|__| cm/s

MPI: |__|.|__|__| a (ms) |__|__|__| b (ms) |__|__|

TDI EVALUATION

Mitral:	E'/A' ratio |__|__|.|__|	PVE' |__|__|.|__| cm/s	PVA' |__|__|.|__| cm/s	PVS' |__|__|.|__| cm/s	
	MPI |__|.|__|__|	ICT (ms) |__|__|	IRT (ms) |__|__|	ET (ms) |__|__|__|	
Tricuspid:	E'/A' ratio |__|__|.|__|	PVE'|__|__|.|__| cm/s	PVA' |__|__|.|__| cm/s	PVS' |__|__|.|__| cm/s	
	MPI |__|.|__|__|	ICT (ms) |__|__|	IRT (ms) |__|__|	ET (ms) |__|__|__|	
Septum:	E'/A' ratio |__|__|.|__|	PVE'|__|__|.|__| cm/s	PVA' |__|__|.|__| cm/s	PVS' |__|__|.|__| cm/s	
	MPI |__|.|__|__|	ICT (ms) |__|__|	IRT (ms) |__|__|	ET (ms) |__|__|__|	

XIII. PAPERS

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Gestational age- and estimated fetal weight-adjusted reference ranges for myocardial tissue Doppler indices at 24–41 weeks' gestation

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KEYWORDS: cardiac function; echocardiography; myocardial peak velocities; myocardial performance index; tissue Doppler imaging

ABSTRACT

Objectives *To construct gestational age (GA)- and estimated fetal weight (EFW)-adjusted reference ranges for tissue Doppler cardiac function parameters from 24 to 41 weeks' gestation.*

Methods *This was a prospective cross-sectional observational study involving 213 singleton pregnancies between 24 and 41 weeks' gestation. Myocardial peak velocities and myocardial performance index (MPI′) were measured by tissue Doppler ultrasonography (values indicated by 'prime') in the left and right annulus and interventricular septum. Left and right atrioventricular parameters were also measured by conventional Doppler and ratios between the values found by the two methods calculated. Regression analysis was used to determine GA- and EFW-adjusted reference ranges and to construct nomograms for tissue Doppler parameters.*

Results *All myocardial peak velocities, left and right E′/A′ and left MPI′ showed a progressive increase with GA. In contrast, left and right E/E′ showed a progressive decline. Septal E′/A′, and right and septal MPI′ remained constant. Myocardial peak velocities showed a progressive increase with increasing fetal weight.*

Conclusions *Normal data of fetal myocardial peak velocities, their ratios and MPI′ by tissue Doppler adjusted by GA and EFW are provided. The reported reference values may be useful in research or clinical studies and can be used in fetuses with intrauterine growth restriction.*

INTRODUCTION

Evaluation of cardiac function is being increasingly used in fetal medicine as a clinical and research tool in a wide range of fetal diseases, such as intrauterine growth restriction (IUGR), hydrops, diabetes and structural heart defects.

Assessment of fetal cardiac function has so far mainly been performed by conventional echocardiographic techniques such as M-mode, B-mode and pulsed Doppler ultrasound[1]. However, new technologies that permit a more accurate evaluation of cardiac motion have recently been developed. Tissue Doppler imaging (TDI) is a robust and reproducible echocardiographic tool that uses Doppler principles to measure the velocity and timing of myocardial motion. In adults and children, TDI has demonstrated its utility as an early marker of preclinical cardiac dysfunction in the prediction of future cardiovascular disease[2,3], and it has also been shown to be feasible and reproducible in fetuses[4–8]. Furthermore, recent studies support the use of TDI as a sensitive tool for demonstrating changes in cardiac function in fetuses with IUGR[8–11] and hydrops[12] and those of diabetic mothers[13].

The purpose of this study was to construct gestational age (GA)-based reference ranges for myocardial peak velocities and myocardial performance index (MPI′) assessed by TDI (indicated by the use of a 'prime') at 24 to 41 weeks' gestation in an appropriately selected population. Since myocardial velocities may be substantially affected by body size[14], we also constructed estimated fetal weight (EFW)-based normality curves, which could be of particular use in fetuses with IUGR.

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Accepted: 17 September 2010

METHODS

Study population

The study population included 213 singleton pregnancies attending the Maternal–Fetal Medicine Department at Hospital Clinic in Barcelona for routine pregnancy ultrasound scans from July 2008 to September 2009. Inclusion criteria were: singleton pregnancy; normal fetal growth and uterine artery Doppler according to our local reference values at 20 weeks' gestation[15,16]; absence of risk factors for vascular disease including pregestational diabetes and immune or renal disease; and no previous history of fetal growth restriction, pre-eclampsia or abruption. Pregnancies with structural/chromosomal anomalies or evidence of fetal infection were excluded from the study. The study protocol was approved by the local ethics committee and pregnant women provided their written informed consent. In all pregnancies GA was calculated based on the crown–rump length at first-trimester ultrasound[17]. EFW was calculated at the time of echocardiography according to the method of Hadlock *et al.*[18]. The number of cases included per gestational week was roughly 15 (Figure S1).

All women underwent ultrasonographic examination using a Siemens Sonoline Antares machine (Siemens Medical Systems, Malvern, PA, USA). Basic Doppler examination included umbilical artery, middle cerebral artery and ductus venosus. At delivery, GA, mode of delivery, birth weight, birth-weight centile, Apgar score, umbilical artery pH and occurrence of pre-eclampsia, gestational diabetes or prematurity were recorded.

Echocardiography

Cardiac function was assessed in all fetuses by conventional echocardiography and spectral TDI. Conventional echocardiography included the measurement of peak early (E) and late (A) transvalvular filling velocities. Atrioventricular flows were obtained from a basal or apical four-chamber view, placing the pulsed Doppler sample volume just below the valve leaflets, and left and right E/A ratios were calculated[1].

TDI was obtained in real time using a 2–10-MHz phased-array transducer. Frame rate was above 100 frames per s in all cases. In a four-chamber-view, sample volumes were placed in the basal part of the left ventricular wall (mitral annulus), interventricular septum and right ventricular wall (tricuspid annulus). The insonation ultrasound beam was kept at an angle of $< 30°$ to the orientation of the ventricular wall or the interventricular septum. No angle correction was applied. Myocardial peak velocities were measured in early diastole (PVE′), atrial contraction (PVA′) and systole (PVS′). The ratio of E′ to A′ was calculated at each location. The ratio of E (by conventional echocardiography), and E′ (by TDI), was calculated in the left and right sides. To calculate MPI′ by TDI, the following time-periods were calculated: isovolumetric contraction time (ICT′), ejection time (ET′) and isovolumetric relaxation time (IRT′).

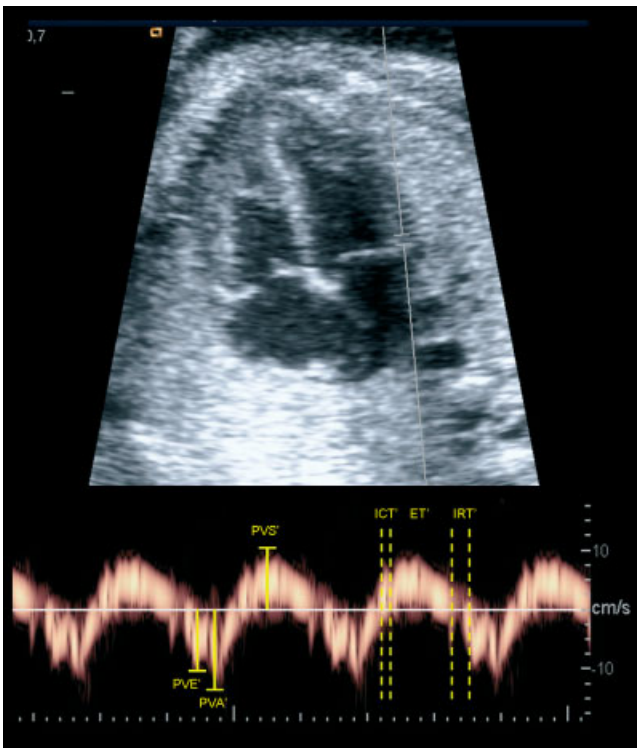

Figure 1 Pulsed tissue Doppler image of the mitral annulus showing measurement of myocardial peak velocities and performance indices. ET′, ejection time; ICT′, isovolumetric contraction time; IRT′, isovolumetric relaxation time; PVA′, myocardial peak velocity during atrial contraction; PVE′, myocardial peak velocity in early diastole; PVS′, myocardial peak velocity in systole.

Finally, left, right and septal MPI′ were calculated as (ICT′ + IRT′)/ET′. Measurement of all MPI′ components were made from the same cardiac cycle (Figure 1)[19]. The maximum allowed duration of the cardiac examination for the acquisition of all relevant measurements was 30 min.

Statistical analysis

The statistical model described by Royston and Wright was used to estimate reference intervals[20]. Separate linear, cubic and quadratic regression models were fitted to estimate the relationship between the TDI variables studied and GA and EFW. The best fitting model for each variable was selected. *Z*-scores ((measurement − mean)/SD) were calculated for assessing model fit. Normal distribution of *Z*-scores was checked with the Shapiro–Francia *W*-test, and natural logarithmic transformation of the data was used if appropriate. SD curves as functions of GA and EFW were calculated by means of quadratic polynomial regression procedure of absolute residuals of the measurement of interest. Equations of the polynomial regression curves were used to calculate mean and 5^{th} and 95^{th} centiles for each GA or EFW (centile = estimated mean ± 1.645 SD). Statistical procedures were performed using the SPSS 15.0 statistical package (SPSS, Chicago, IL, USA).

RESULTS

Clinical characteristics and pregnancy outcomes of the study population are shown in Table 1. TDI assessment of the left ventricular wall, right ventricular wall and interventricular septum was successfully performed in 94, 97 and 95% of cases, respectively.

Regression equations representing the relationships between the studied parameters and GA are shown in Table 2. The best model for most parameters was a first-degree linear polynomial, with the exception of left PVE′, which was best modeled by a second-degree linear polynomial, and septal E′/A′ and right and septal MPI′, which were constant across GA. The values for the median, 5th and 95th centiles at each GA for TDI parameters are included in the supporting information (Tables S1–S4).

All myocardial peak velocities showed a progressive increase with advancing gestation as did left and right E′/A′ and left MPI′. In contrast, left and right E/E′ showed a progressive decline with advancing gestation. Septal E′/A′ and right and septal MPI′ remained constant during the second half of pregnancy.

Figure 2 shows scatterplots of myocardial peak velocities – with the mean, 5th and 95th centile lines – plotted against GA, while Figures 3–5 show scatterplots of E′/A′ ratios, E/E′ ratios and MPI′, respectively – with the mean, 5th and 95th centile lines – plotted against GA.

The normality curves of myocardial peak velocities corrected for EFW are included in the supporting information (Figure S2, Tables S5 and S6). All myocardial peak velocities showed a progressive increase with increasing fetal weight.

Table 1 Demographic characteristics and pregnancy outcome of the study population

Parameter	*Value*
Clinical characteristics	
Maternal age (years)	31 ± 5
Caucasian	72
Nulliparous	68
Maternal body mass index (kg/m^2)	23 ± 3
Cigarette smoker	9
Pregnancy outcome	
Gestational age at delivery (weeks)	39 ± 1
Cesarean section	20
Birth weight (g)	3353 ± 419
Birth-weight centile	52 ± 26
5-min Apgar score	10 ± 1
Umbilical artery pH	7.23 ± 0.07
Pre-eclampsia	2
Birth weight at delivery < 10th centile	4
Preterm delivery (< 34 weeks)	1
Gestational diabetes	3

Data given as mean ± SD or %.

DISCUSSION

The study provides GA- and EFW-adjusted reference ranges for myocardial peak velocities, E′/A′ ratios, E/E′ ratios and MPI′ measured by TDI in normal fetuses and, for the first time, it gives the mean, 5th and 95th centiles for TDI parameters together with the regression formulae.

The reference charts for peak myocardial velocities show similar values to those previously reported[7,21]. Our results confirm previous data showing a positive correlation between diastolic and systolic myocardial velocities and GA. Chan *et al.*[7] showed that PVE′, PVA′ and PVS′ increased from 19 to 37 weeks' gestation at the

Table 2 Regression equations for cardiovascular parameters obtained by tissue Doppler imaging

Parameter	*Mean*	*SD*
Myocardial peak velocities		
Log_e left PVE′ (cm/s)	0.475 + (0.0106 × GA) − (0.00002 × GA^2)	0.071 + (0.00046 × GA)
Left PVA′ (cm/s)	7.135 + (0.0046 × GA)	1.2733
Log_e left PVS′ (cm/s)	1.619 + (0.0011 × GA)	0.1711
Right PVE′ (cm/s)	3.64 + (0.0205 × GA)	0.5846 + (0.0026 × GA)
Log_e right PVA′ (cm/s)	2.123 + (0.0009 × GA)	0.0399 + (0.0005 × GA)
Right PVS′ (cm/s)	5.302 + (0.0094 × GA)	1.0249
Log_e septal PVE′ (cm/s)	1.339 + (0.0018 × GA)	0.1828
Log_e septal PVA′ (cm/s)	1.647 + (0.0012 × GA)	0.1791
Log_e septal PVS′ (cm/s)	1.367 + (0.0015 × GA)	0.1575
Left E′/A′ ratio	0.566 + (0.0013 × GA)	0.1114
Right E′/A′ ratio	0.550 + (0.0012 × GA)	0.1106
Septal E′/A′ ratio	0.8377	0.0889
Left E/E′ ratio	6.339 − (0.0048 × GA)	0.1738 + (0.0046 × GA)
Right E/E′ ratio	6.282 − (0.0043 × GA)	1.0877
Myocardial performance index (MPI′)		
Left MPI′	0.435 + (0.0003 × GA)	0.0858
Right MPI′	0.4943	0.0793
Septal MPI′	0.5098	0.0683

E/E′, ratio between peak velocity in early diastole by conventional echocardiography and tissue Doppler; E′/A′, ratio between myocardial peak velocity during early diastole and atrial contraction; GA, gestational age (days); PVA′, myocardial peak velocity during atrial contraction; PVE′, myocardial peak velocity in early diastole; PVS′, myocardial peak velocity in systole.

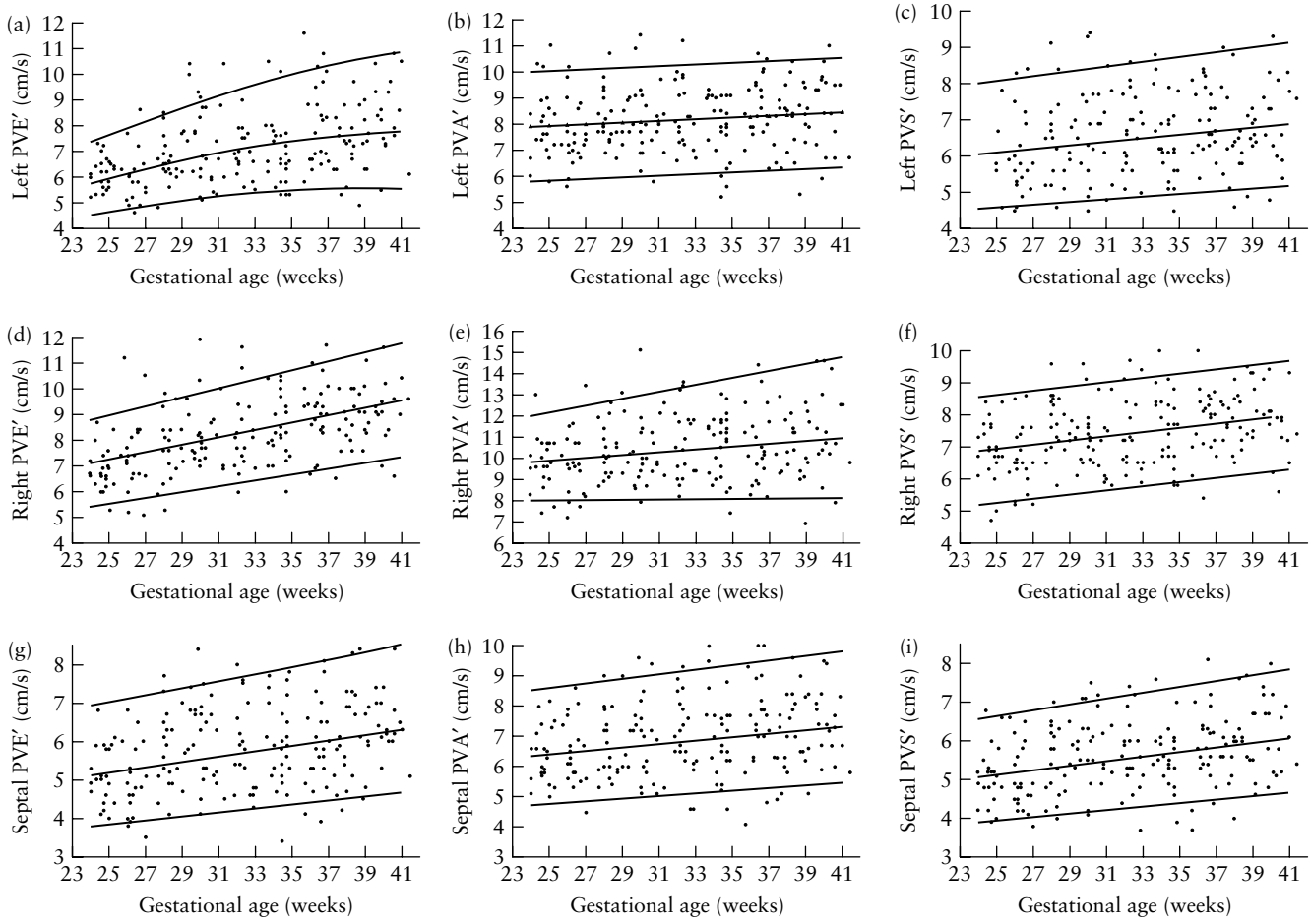

Figure 2 Scatterplots of the left (a–c), right (d–f) and septal (g–i) myocardial peak velocities measured by tissue Doppler ultrasonography plotted against gestational age in the study population. Estimated 5th, 50th and 95th centile curves are shown. PVA′, myocardial peak velocity during atrial contraction; PVE′, myocardial peak velocity in early diastole; PVS′, myocardial peak velocity in systole.

left and right ventricular wall and interventricular septum, and Gardiner *et al.*[21] showed a positive relationship with GA for all myocardial velocities except for the left PVA′. Additionally, our data suggest a positive correlation between left and right E′/A′ ratio and GA, while the septal E′/A′ ratio was constant throughout the second half of pregnancy, though previously published reference values for E′/A′ ratios showed an increase throughout gestation in all three locations, with a steeper slope[7]. Finally, we found a negative correlation between left and right E/E′ ratio and GA, which is consistent with previously reported data[7]. The observed decrease in E/E′ ratio throughout gestation could be interpreted as an indirect reflection of increased compliance due to myocardial maturation during pregnancy, and in this respect it is consistent with the progressive increase observed in E′/A′. Reference ranges for myocardial velocities and ratios using color TDI have been published previously by Nii *et al.*[22] in a group of 114 fetuses. Although values of myocardial velocities are lower using color TDI, the authors showed that PVE′, PVA′, PVS′ and E′/A′ ratios increased throughout gestation while the E/E′ ratio decreased. Table S7 (supporting information) summarizes previous studies on TDI in order to better compare them with our data. While tissue Doppler values are generally similar between studies, certain differences remain that could be explained by the use of different echocardiographic systems[23].

The present study is the first to report reference ranges for MPI′ measured by TDI. MPI′ values by TDI are generally higher than those obtained by standard pulsed Doppler, a bias that is consistently observed among adults[24,25], children[26] and fetuses[19]. MPI and MPI′ are based on the measurement of two different but related phenomena, which explains these differences: MPI measures blood flow events while MPI′ measures myocardial motion events[19]. MPI′ values showed a mild tendency to increase with GA in a similar fashion to that observed with pulsed Doppler MPI[27].

In this study, normal ranges for peak myocardial velocities adjusted for EFW are also provided, with similar patterns to those based on GA. The rationale for providing EFW-adjusted curves is that myocardial velocities have a positive correlation with the subject's size as well as that of their heart[28–31]. In this respect, while ratios and time periods used in the evaluation of cardiac function probably depend on maturation of the cardiac fiber, and therefore on GA, velocities seem to be mostly dependent on body size. This notion has been demonstrated in studies on IUGR fetuses with indices based on velocity, such as

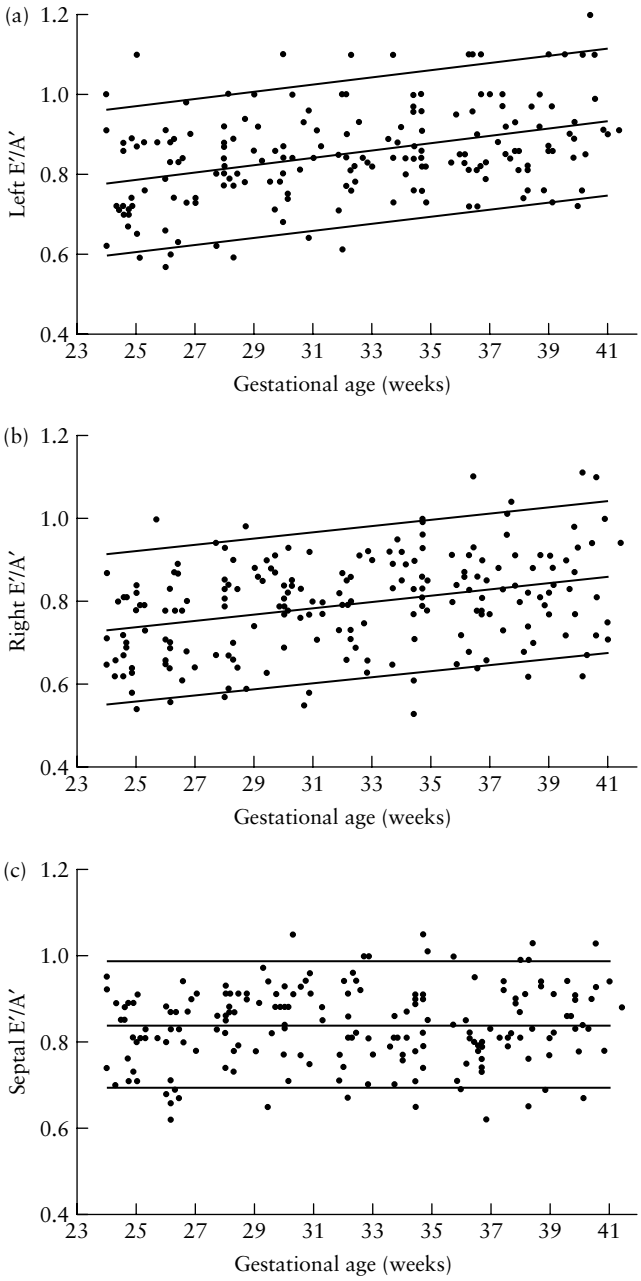

Figure 3 Scatterplots of the left (a), right (b) and septal (c) ratios between myocardial peak velocity during early diastole and atrial contraction (E′/A′ ratios) measured by tissue Doppler ultrasonography plotted against gestational age in the study population. Estimated 5th, 50th and 95th centile curves are shown.

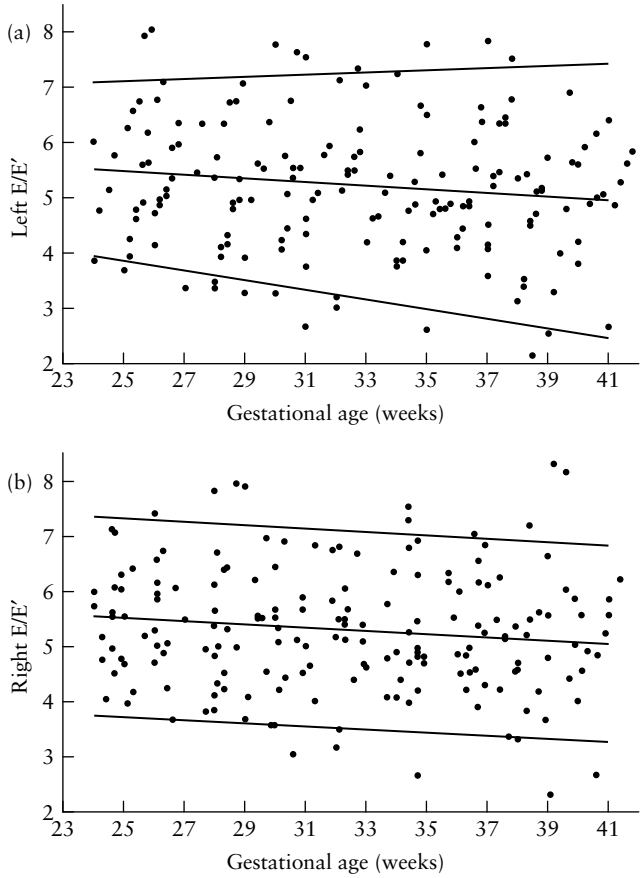

Figure 4 Scatterplots of the left (a) and right (b) ratios between peak velocity in early diastole measured by conventional echocardiography and tissue Doppler ultrasonography (E/E′ ratios) plotted against gestational age in the study population. Estimated 5th, 50th and 95th centile curves are shown.

cardiac output, where the use of GA-based curves might lead to biased estimates and the false assumption that IUGR reduces cardiac output when this is not the case, as shown when values are adjusted for fetal weight[32–34]. This limitation may be overcome by adjusting values by EFW, and therefore we believe that the use of EFW-based curves might be more appropriate for fetuses with IUGR.

The study has several limitations and technical considerations. TDI requires special software not available in all ultrasound machines and it requires formal training. Even in experienced hands, TDI measurements can be challenging. Data were successfully obtained on average in 95% of cases in this study. The main reason for unsuccessful TDI measurement was fetal position preventing an insonation angle less than 30°, which is critical for obtaining waveforms of sufficient quality to allow meaningful measurements. In addition, myocardial peak velocities are markedly lower in fetuses than in children and adults. Even the lowest available scale of most ultrasound machines is often too large. In these circumstances waveforms are often displayed with suboptimal resolution, which may hamper accuracy. In spite of these technical difficulties, in trained hands fetal TDI measurements have demonstrated good reproducibility[8], which supports their use for research and clinical purposes. In this study TDI was assessed in real time, which may be more feasible than by using off-line analysis, but it does not permit the evaluation of other deformation indices such as strain or strain rate. Although off-line techniques such as color TDI and two-dimensional (2D) speckle tracking allow the calculation of other functional parameters, they have some potential limitations for use in fetuses. These techniques were designed to estimate strain and strain rate in adults using an ECG co-registration. As compared with adults, fetuses have higher heart rates, fetal and respiratory movements and varied body position, and ECG cannot be performed. All these conditions

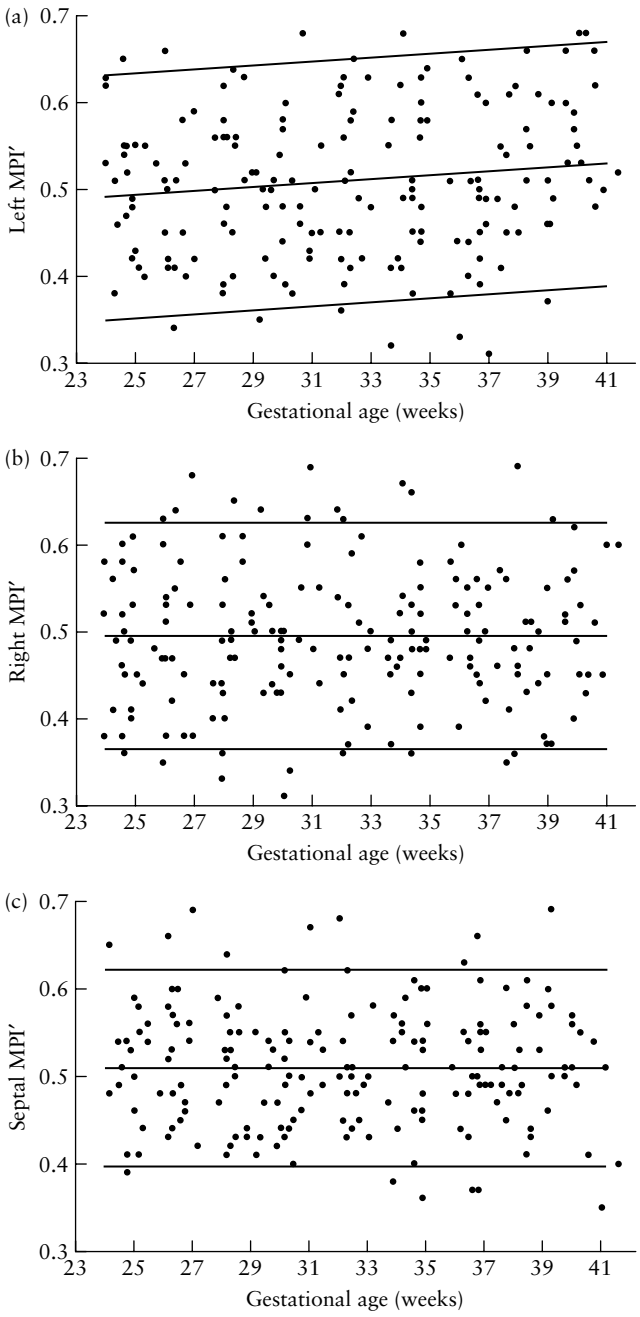

Figure 5 Scatterplots of the left (a), right (b) and septal (c) myocardial performance index (MPI′) measured by tissue Doppler ultrasonography plotted against gestational age in the study population. Estimated 5th, 50th and 95th centile curves are shown.

may limit off-line analysis, therefore fetal evaluation with color TDI requires further investigation.

Another recent echocardiographic method is 2D speckle tracking[35], which provides an off-line assessment of myocardial velocities, strain or strain rate without angle dependency with good feasibility and reproducibility in fetuses[36]. Future studies are warranted to validate the use of off-line myocardial imaging techniques in fetal life.

Finally, this study provides information limited to 24–40 weeks' gestation. Although this range covers most potential applications of TDI, we acknowledge that it would also be useful to ascertain reference values at lower GAs. From a clinical and research perspective, in spite of its technical challenges, TDI may constitute a more sensitive tool than does conventional echocardiography for the detection of fetal cardiac dysfunction, as suggested in recent studies in IUGR fetuses[8]. TDI is being used for research in fetal cardiac function in fetuses with IUGR[8,10,11] and heart failure[12] and in those of diabetic mothers[13]. GA- and EFW-adjusted reference values for the TDI parameters reported in this study could be useful for future research or clinical studies on fetal cardiac function. An Excel file to calculate *Z*-scores for GA- and EFW-adjusted tissue Doppler parameters is provided in the online supporting information of this article (Table S8).

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 Distribution of the study population across gestational age.

Figure S2 Scatterplot of the left, right and septal myocardial peak velocities measured by tissue Doppler vs. estimated fetal weight in our population. Estimated 5th, 50th and 95th centile curves are shown. PVA′, myocardial peak velocity during atrial contraction; PVE′, myocardial peak velocity in early diastole; PVS′, myocardial peak velocity in systole.

Table S1 Mean, 5th (*p5*) and 95th (*p95*) centiles at each gestational age for myocardial peak velocities by pulsed tissue Doppler.

Table S2 Mean, 5th (*p5*) and 95th (*p95*) centiles at each gestational age for left, right and septal E′/A′ ratios by pulsed tissue Doppler.

Table S3 Mean, 5th (*p5*) and 95th (*p95*) centiles at each gestational age for E/E′ ratios.

Table S4 Mean, 5th (*p5*) and 95th (*p95*) centiles at each gestational age for left, right and septal myocardial performance index by pulsed tissue Doppler.

Table S5 Regression equations for cardiovascular parameters obtained by tissue Doppler imaging and normalized by estimated fetal weight.

Table S6 Mean, 5th (*p5*) and 95th (*p95*) centiles regarding estimated fetal weight for myocardial peak velocities by pulsed tissue Doppler.

Table S7 Comparison of previous data on tissue Doppler parameters.

Table S8 Excel file to calculate *Z*-scores for tissue Doppler parameters adjusted by gestational age or estimated fetal weight.

OBSTETRICS

Usefulness of myocardial tissue Doppler vs conventional echocardiography in the evaluation of cardiac dysfunction in early-onset intrauterine growth restriction

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OBJECTIVE: To evaluate cardiac function by tissue Doppler imaging vs conventional echocardiography in intrauterine growth restriction.

STUDY DESIGN: A prospective study in 25 intrauterine growth restriction, and in 50 normally grown fetuses between 24 and 34 weeks. Conventional echocardiography (E/A ratios, outflow tract velocities and myocardial performance index), and tissue Doppler (myocardial peak velocities, E'/A' ratios and myocardial performance index') measurements were performed.

RESULTS: With conventional echocardiography, intrauterine growth restriction fetuses showed an increase in left myocardial performance index but similar values of E/A ratios, outflow tract velocities and right myocardial performance index as compared with controls. Tissue Doppler imaging demonstrated that intrauterine growth restriction fetuses had significantly lower systolic and diastolic myocardial velocities in mitral and tricuspid annulus, higher mitral E'/A' ratio and higher mitral, tricuspid and septal myocardial performance index' values.

CONCLUSION: Tissue Doppler imaging demonstrated the presence of both systolic and diastolic cardiac dysfunction in intrauterine growth restriction. Tissue Doppler imaging may constitute a more sensitive tool than conventional echocardiography to evaluate cardiac dysfunction in intrauterine growth restriction.

Key words: cardiac function, echocardiography, IUGR, myocardial performance index, tissue Doppler imaging

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Intrauterine growth restriction (IUGR) caused by placental insufficiency affects 1-3% of pregnancies and is associated with an increased risk of perinatal mortality and morbidity.[1] Cardiac dysfunction with maintained cardiac output has consistently been reported to be present in IUGR. [2-4] Although earlier studies suggested that cardiac parameters became abnormal only in severely affected fetuses, [5-7] more recent research strongly suggests that subclinical cardiac dysfunction could be present from early stages of fetal deterioration.[2] The identification and monitoring of cardiac dysfunction may be relevant for clinical purposes and to advance in the understanding of the relation between IUGR and long-term cardiovascular outcome.[8,9]

New developments in echocardiography enable a much fuller assessment of cardiac function, including measurement of myocardial motion by tissue Doppler imaging (TDI). TDI is a robust and reproducible echocardiographic tool that permits a quantitative assessment of motion and timing of myocardial events. Myocardial velocities are a sensitive marker of mildly impaired systolic or diastolic function and therefore useful in the early identification of subtle cardiac dysfunction in preclinical stages.[10,11] In adults and children, TDI has demonstrated its use in the prediction of future cardiovascular diseases.[12,13] Recently, TDI has been shown to be feasible in fetuses.[14-17] The results of preliminary studies in IUGR fetuses suggest that there is a reduction in myocardial velocities.[18-20] We postulated that TDI could constitute a more sensitive tool than conventional echocardiography to detect the presence of cardiac dysfunction in fetuses with IUGR.

We performed a prospective study to evaluate cardiac function parameters with TDI and with conventional echocardiography in a group of fetuses with early onset IUGR.

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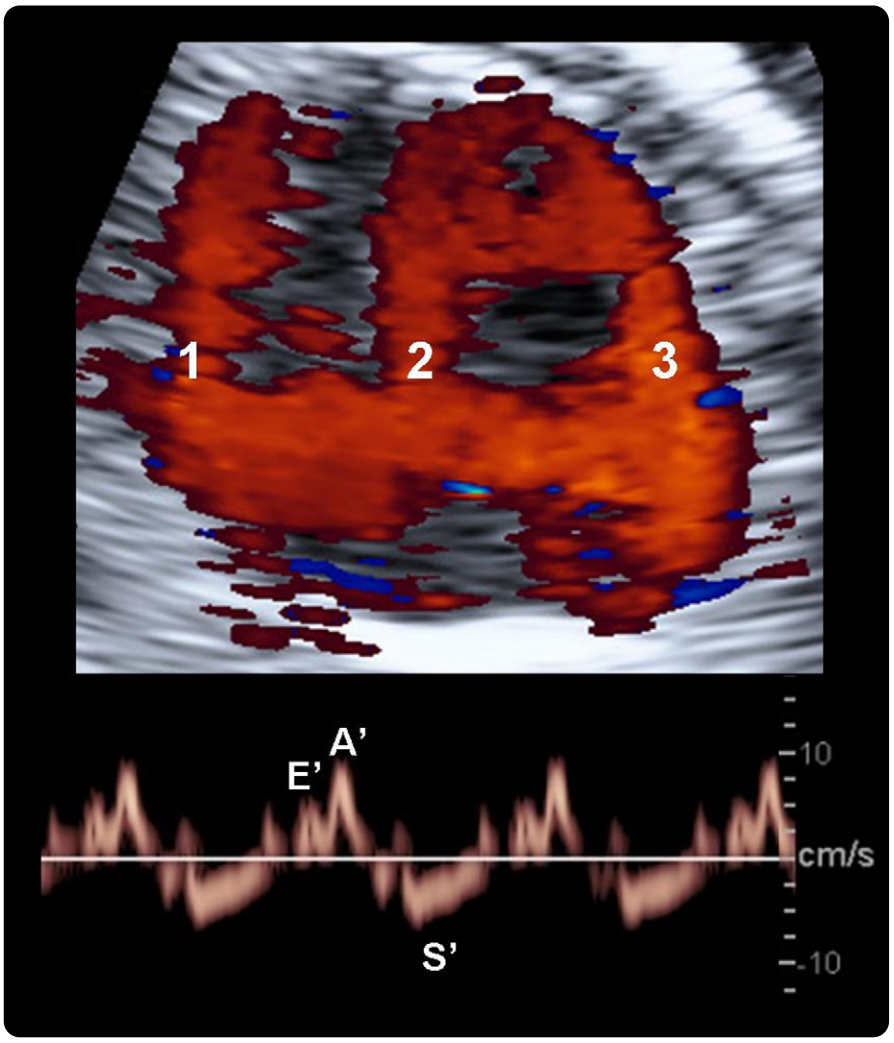
FIGURE 1
Myocardial velocities



Myocardial velocities in early diastole (E'), during atrial contraction (A'), and systole (S') by pulsed tissue Doppler in left (1), septal (2) and right (3) annulus.

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Materials and Methods

Study populations

The study population included 25 IUGR fetuses and 50 controls. Patients were selected from women who attended the Maternal-Fetal Medicine Department at Hospital Clinic in Barcelona, Spain. The study protocol was approved by the local Ethics Committee and patients provided their written informed consent. In all pregnancies, gestational age was calculated based on the crown-rump length at first-trimester ultrasound.[21] IUGR was defined as an estimated fetal weight below the 10th percentile according to local reference curves,[22] together with umbilical artery (UA) pulsatility index (PI) above the 95th percentile.[23] For the purpose of this study, only patients who were delivered between 26 and 34 weeks of gestation were included. The control group consisted of 50 normally grown fetuses matched 2 to 1 with cases by gestational age at ultrasound (± 1 week). Exclusion criteria were structural/chromosomal anomalies or evidence of fetal infection.

All patients underwent ultrasonographic examination using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA). Basic Doppler examination included UA, middle cerebral artery, and ductus venosus. At delivery, gestational age, mode of delivery, birthweight, birthweight percentile, Apgar score, umbilical pH, and perinatal mortality and morbidity were recorded. Perinatal mortality was defined as either intrauterine death or neonatal death within the first 28 days of life.[24] Adverse perinatal outcome was defined by the presence of perinatal death, bronchopulmonary dysplasia, hyaline membrane disease, neonatal intraventricular hemorrhage grade 3 or 4, necrotizing enterocolitis, sepsis, or retinopathy grade 3 or 4.

Cardiac function was assessed in all cases and controls by conventional echocardiography and TDI.

Conventional echocardiography

Conventional echocardiography included peak early (E) and late (A) transvalvular filling and outflow tracts velocities and myocardial performance index (MPI). Atrioventricular flows were obtained from a basal or apical 4-chamber view, placing the pulsed Doppler sample volume just below valve leaflets, and left and right E/A ratios were calculated.[25] Aortic and pulmonary artery peak velocities were obtained from a long- or short-axis view of the left and right ventricle respectively. Left MPI was obtained using the clicks of mitral and aorta valves as landmarks, as previously described.[26] The following periods were calculated: isovolumetric contraction time (ICT), ejection time (ET), and isovolumetric relaxation time (IRT). Finally, the MPI was calculated as (ICT + IRT)/ET. Right MPI was calculated by obtaining right ventricle inflow and outflow obtained in series from separate cardiac cycles.[27,28]

TDI

TDI was obtained in real time using a 2-10 MHz phased-array transducer. First, a clear 4-chamber view was obtained in an apical or basal view. The TDI program was set to the pulsed-wave mode with a sample volume size between 2 and 4 mm. Sample volumes were placed in the basal part of the left ventricular wall (mitral annulus), interventricular septum and right ventricular wall (tricuspid annulus) (Figure 1). The in-

sonation ultrasound beam was kept at an angle of <30° to the orientation of the ventricular wall or the interventricular septum and no angle correction was applied. Peak annular velocities were measured in early diastole (PVE'), atrial contraction (PVA'), and systole (PVS'). The ratio of E' to A' was calculated in each location. Left, right, and septal MPI' were also measured by TDI. To calculate MPI by TDI (MPI'), the following periods were calculated: ICT', ET', and IRT'. Finally, left, right, and septal MPI' were calculated as (ICT' + IRT')/ET'. Measurement of all MPI' components were made from the same cardiac cycle.[29]

To determine TDI reliability, 50 fetuses were evaluated by the same operator and 30 fetuses by 2 independent operators.

Statistical analysis

Data were analyzed with the SPSS 15.0 statistical package (SPSS Inc, Chicago, IL). Results are expressed as mean ± standard deviation or proportions. Comparisons between groups were performed by *t* test, and echocardiographic parameters were also compared by logistic regression adjusted by estimated fetal weight. Reliability analyses were performed by means of the intraclass correlation coefficient for agreement.

RESULTS

Characteristics of the study populations

The characteristics of the study populations are reported in Table 1. Preeclampsia was present in 54% of the IUGR pregnancies. As expected, UA, middle cerebral artery, cerebroplacental ratio, and ductus venosus PI were significantly different in IUGR fetuses compared with controls. Among the IUGR fetuses, 10 had UA absent-end diastolic flow, 1 had UA reverse diastolic flow, and 2 had absent or reverse flow in the ductus venosus. Compared with controls, pregnancies with IUGR presented lower gestational age at delivery, birthweight, Apgar score, and umbilical artery pH, and higher rates of cesarean section, perinatal mortality, and adverse outcome.

TABLE 1
Baseline characteristics and perinatal outcome of the study populations

Characteristics	Controls	IUGR
n	50	25
Clinical characteristics		
Maternal age, y	31 (5)	32 (5)
White, %	70	65
Nulliparous, %	70	52
Maternal body mass index, g/m^2	23 (5)	24 (6)
Smoker, %	9	19
Preeclampsia, %	0	54[a]
Basic Doppler data		
Gestational age at ultrasound, wk	30 (3)	30 (3)
Umbilical artery PI	1.06 (0.24)	1.89 (0.34)[a]
Middle cerebral artery PI	2.08 (0.38)	1.37 (0.30)[a]
Cerebroplacental ratio	2.05 (0.55)	0.77 (0.24)[a]
Ductus venosus PI	0.56 (0.16)	0.79 (0.35)[a]
Perinatal outcome		
Gestational age at delivery, wk	39 (1)	31 (2)[a]
Cesarean section, %	18	91[a]
Birthweight, g	3347 (453)	993 (330)[a]
Birthweight percentile	53 (29)	5 (4)[a]
5-min Apgar	10 (1)	8 (2)[a]
Umbilical artery pH	7.25 (0.07)	7.21 (0.09)[a]
Perinatal death, %	0	15[a]
Adverse perinatal outcome, %	2	30[a]

IUGR, intrauterine growth restriction; *PI*, pulsatility index.
Values are mean (standard deviation) or proportions.
Body mass index calculated as weight in kilograms divided by the square of the height in meters. Adverse perinatal outcome defined by the presence of perinatal death, bronchopulmonary dysplasia, hyaline membrane disease, neonatal intraventricular hemorrhage grade 3 or 4, necrotizing enterocolitis, sepsis or retinopathy grade 3 or 4.

[a] $P < .05$ as compared with controls.

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Conventional echocardiography

Values of conventional echocardiographic parameters are shown in Table 2. E and A velocities were significantly reduced in IUGR. However, E/A ratios were not significantly different in IUGR as compared with controls. Aortic and pulmonary artery peak velocity were reduced with respect to controls, but the difference was not statistically different when adjusting by fetal weight. Although IUGR fetuses showed increased left MPI values, right MPI values were similar among cases and controls.

TDI

Satisfactory TDI measurements were successfully obtained from all fetuses. Values of myocardial velocities and MPI' measured by TDI are shown in Table 3 and Figure 2. After adjusting for fetal weight, left PVA' and PVS', and right PVE', PVA', and PVS' were significantly reduced in IUGR. Left E'/A' ratio was higher in IUGR fetuses compared with controls. Left, right, and septal MPI' were significantly higher in IUGR fetuses.

Table 4 illustrates the intra- and interobserver reliability of peak myocardial

TABLE 2
Cardiac function results by conventional echocardiography in controls and IUGR fetuses

Parameters	Controls	IUGR	*P* value[a]	Adjusted *P* value[b]
Diastolic parameters				
Left E velocity, cm/s	37 (5.4)	31 (7.4)	< .001	.002
Left A velocity, cm/s	50 (8.5)	41 (10.1)	< .001	< .001
Left E/A	0.74 (0.1)	0.78 (0.2)	.34	.07
Right E velocity, cm/s	43 (7.9)	32 (7.5)	< .001	< .001
Right A velocity, cm/s	57 (9.2)	39 (7.2)	< .001	< .001
Right E/A	0.76 (0.1)	0.81 (0.1)	.13	.2
Systolic parameters				
Aortic peak velocity, cm/s	94 (20.2)	81 (16.5)	.02	.245
Pulmonary artery peak velocity, cm/s	91 (20.9)	84 (24.5)	.4	.122
MPI				
Left MPI	0.45 (0.06)	0.52 (0.09)	.02	.006
Right MPI	0.47 (0.19)	0.45 (0.13)	.34	.38

A, atrial contraction; *E*, early diastole; *IUGR*, intrauterine growth restriction; *MPI*, myocardial performance index.
Values are mean (standard deviation).

[a] Calculated by *t* test; [b] Calculated by logistic regression adjusted by estimated fetal weight.

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TABLE 3
Cardiac function results by tissue Doppler in controls and IUGR fetuses

Parameters	Controls	IUGR	*P* value[a]	Adjusted *P* value[b]
Diastolic parameters				
Left PVE', cm/s	7.3 (1.3)	6.7 (0.8)	.03	.59
Left PVA', cm/s	8.5 (1.4)	6.2 (0.7)	< .001	< .001
Left E'/A'	0.85 (0.13)	1.09 (0.17)	.003	.001
Right PVE', cm/s	8.5 (1.3)	7.2 (1.3)	< .001	.007
Right PVA', cm/s	10.8 (1.5)	9.1 (1.2)	< .001	.01
Right E'/A'	0.79 (0.1)	0.82 (0.1)	.96	.44
Septal PVE', cm/s	6.3 (1.1)	5.4 (1)	.02	.3
Septal PVA', cm/s	7.4 (1.3)	6.2 (0.6)	< .001	.049
Septal E'/A'	0.86 (0.1)	0.88 (0.1)	.08	.26
Systolic parameters				
Left PVS', cm/s	6.9 (1.2)	5.6 (0.6)	< .001	.002
Right PVS', cm/s	7.6 (1.2)	6.6 (1.4)	.004	.049
Septal PVS', cm/s	5.8 (0.88)	5.3 (1.1)	.29	.28
MPI				
Left MPI'	0.49 (0.08)	0.56 (0.09)	.01	.007
Right MPI'	0.47 (0.09)	0.61 (0.11)	.001	.006
Septal MPI'	0.49 (0.06)	0.58 (0.05)	.001	< .001

IUGR, intrauterine growth restriction; *MPI*, myocardial performance index; *MPI'*, MPI by tissue Doppler; *PVA'*, myocardial peak velocity during atrial contraction; *PVE'*, myocardial peak velocity in early diastole; *PVS'*, myocardial peak velocity in systole.
Values are mean (standard deviation).

[a] Calculated by *t* test; [b] Calculated by logistic regression adjusted by estimated fetal weight.

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velocities and MPI'. Intraclass correlation coefficients were above 0.7 for most comparisons.

COMMENT

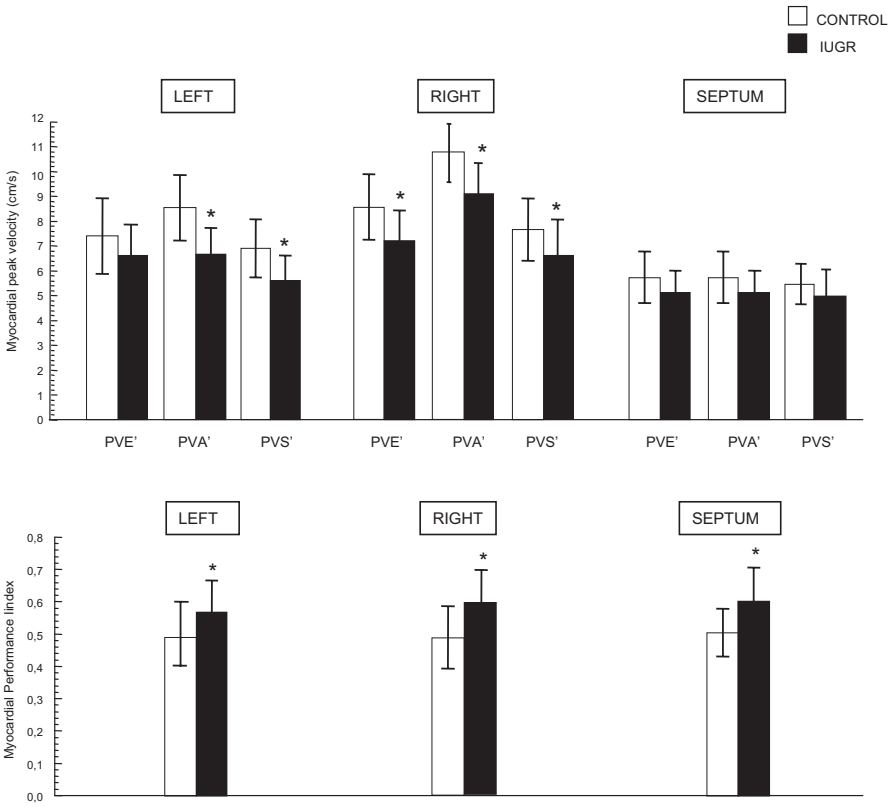
In this study, TDI demonstrated the presence of both systolic and diastolic cardiac dysfunction in IUGR fetuses, suggesting that TDI is a more sensitive tool than conventional echocardiography to evaluate fetal cardiac function. The results are in line with adult echocardiographic studies, in which TDI has demonstrated to be an earlier marker of cardiac disease, and support the use of this Doppler modality in pathophysiologic and clinical studies in fetuses.

Concerning conventional echocardiography, most of the parameters evaluated in this study were similar among IUGR and controls. These findings are concordant with previous data on IUGR fetuses.[2,6] Despite ventricular filling velocities were significantly lower in IUGR fetuses, E/A ratio showed similar values between cases and controls. E/A ratio is a standard echocardiographic parameter to evaluate diastolic function.[25] Previous studies have reported similar,[5] reduced,[7,30] or increased[2,3,6] E/A ratios in IUGR fetuses. A recent study demonstrated that E/A ratios are only significantly increased in cases with reverse flow in the UA,[2] and therefore the severity case mix of the population studied may influence the results. The lack of significant differences in our study is not surprising because we included only 1 case with reversed diastolic flow in the UA. Aortic and pulmonary artery peak velocities were not statistically different among groups after adjusting by fetal weight. Outflow velocities are normally recorded to calculate cardiac output, and our observations are consistent with previous reports showing no significant changes in cardiac output adjusted by fetal weight in IUGR fetuses.[2,4] Left MPI, an early marker of combined systolic and diastolic function, was significantly elevated. The data are in agreement with previous reports demonstrating that MPI is abnormal from early stages in IUGR fetuses.[2,31,32] In contrast, right MPI was similar among groups. Previous studies have shown inconsistent results with right MPI in IUGR,[28,31] which may be due to the difficulties in recording this parameter with conventional echocardiography, because it requires measurements from different cardiac cycles and it thus may be affected by fetal heart rate fluctuations.

In contrast with conventional echocardiography, TDI showed significant differences between IUGR and control fetuses in almost all systolic and diastolic recorded parameters. Decreased myocardial velocities are 1 of the earliest signs of systolic and diastolic dysfunction. They constitute a sensitive preclinical marker of impaired cardiac function[13,33] and a strong predictor of poor outcome in several major cardiac diseases.[12] In this study, myocardial peak velocities measured by real time TDI were significantly lower in most recorded locations. Likewise, left E'/A' ratio was significantly higher in IUGR fetuses. The observed differences between IUGR and normal fetuses are consistent with 2 previous studies, including 20 and 14 IUGR fetuses at gestational ages ranging from 25-36 weeks.[19,20] In contrast, Watanabe et al[18] failed to demonstrate significant differences in myocardial velocities using TDI in a group of 12 fetuses with IUGR defined only on the basis of fetal weight. A relatively small sample size and the potential inclusion of some constitutionally small fetuses may have influenced the lack of differences observed.

In the current study, we could not detect significant differences of most myocardial velocities in the septal annulus. This might be explained by the narrowness of myocardial tissue at septal annu-

FIGURE 2
Myocardial peak velocities and myocardial performance index



Myocardial peak velocities and myocardial performance index at left, right, and septal annulus measured by tissue Doppler in the study populations. Data given as mean ± standard deviation. *$P < .05$ compared with controls adjusted by fetal weight.

IUGR, intrauterine growth restriction; *PVA'*, myocardial peak velocity during atrial contraction; *PVE'*, myocardial peak velocity in early diastole; *PVS'*, myocardial peak velocity in systole.

Comas. Myocardial tissue Doppler vs conventional echocardiography. Am J Obstet Gynecol 2010.

TABLE 4
Intra- and interobserver reliability of myocardial peak velocities and MPI measured by tissue Doppler

Parameters	Intraobserver reliability	Interobserver reliability
Diastolic parameters		
Left PVE'	0.79	0.86
Left PVA'	0.66	0.86
Left E'/A'	0.78	0.69
Right PVE'	0.87	0.82
Right PVA'	0.79	0.88
Right E'/A'	0.79	0.85
Septal PVE'	0.74	0.83
Septal PVA'	0.77	0.77
Septal E'/A'	0.57	0.81
Systolic parameters		
Left PVS'	0.79	0.81
Right PVS'	0.81	0.82
Septal PVS'	0.77	0.83
MPI		
Left MPI'	0.79	0.78
Right MPI'	0.77	0.70
Septal MPI'	0.71	0.70

MPI, myocardial performance index; *MPI'*, MPI by tissue Doppler; *PVA'*, myocardial velocity during atrial contraction; *PVE'*, myocardial peak velocity in early diastole; *PVS'*, myocardial peak velocity in systole.
Values are intraclass correlation coefficient.

Comas. Myocardial tissue Doppler vs conventional echocardiography. Am J Obstet Gynecol 2010.

lus that normally results in lower velocity recordings. Concerning MPI' values, IUGR fetuses had consistently increased values in mitral, tricuspid, and septal annulus. Although no previous reports had evaluated MPI' by TDI in IUGR, our results are consistent with TDI studies in fetuses with heart failure[34] and with the differences observed in MPI measured with conventional Doppler in IUGR fetuses.

The clinical usefulness of TDI in IUGR fetuses remains to be assessed in future research. The findings of this study support the notion that in experienced hands it may constitute a valid tool for research and clinical purposes. As with any other echocardiographic measurement, TDI requires an experienced examiner, but in this study, TDI measurements could be successfully obtained in all cases and showed a good reproducibility. TDI requires special echocardiographic software and it is not readily available in obstetric ultrasound devices. However, if further studies demonstrated its use, incorporation of TDI to obstetric ultrasound would become more widespread.

This study has several limitations. TDI was assessed in real time that most probably confers a better feasibility as compared with offline analysis, but does not permit to evaluate other deformation indices such as strain or strain rate. In addition, the short period of follow-up limited the evaluation of a potential correlation between fetal echocardiographic results and postnatal cardiovascular outcome. Finally, it could be argued that the reduction of myocardial velocities in IUGR fetuses might be explained by the smaller weight of IUGR fetuses. It has been described that fetal myocardial velocities increase across gestational age,[14,17] and this is likely related to the increase in heart size. Therefore, it could be argued that IUGR fetuses had absolute lower velocities than normal growth fetuses of the same gestational age just because they were smaller. To counter this potential bias, the data were adjusted for fetal weight, and most differences remained significant.

In summary, our study confirms and extends previous evidence supporting the existence of early cardiac dysfunction in IUGR, which affects both diastolic and systolic function. The study further suggests that TDI could be a more sensitive technique to demonstrate fetal cardiac dysfunction in IUGR fetuses at early stages of severity. The potential clinical use of TDI remains to be established in long-term follow-up studies. ■

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Tissue-Doppler echocardiographic markers of cardiac dysfunction in small-for-gestational age fetuses

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Small-for-gestational age fetuses with normal umbilical artery are associated with cardiac dysfunction detectable by tissue Doppler imaging.

Tissue-Doppler echocardiographic markers of cardiac dysfunction in small-for-gestational age fetuses

OBJECTIVE: To evaluate echocardiographic markers of cardiac dysfunction in small-for-gestational age (SGA) fetuses with normal umbilical artery Doppler.

STUDY DESIGN: Cardiac function was evaluated in 58 SGA (mean gestational age 38 weeks) and 58 gestational-age matched normally grown fetuses by conventional echocardiography (E/A ratios and myocardial performance index (MPI)), and tissue Doppler imaging (TDI) (annular peak velocities and MPI').

RESULTS: With conventional echocardiography, SGA fetuses had a non significant trend to increased E/A ratios and left MPI as compared to controls. TDI demonstrated that SGA fetuses had significantly lower right E' and A' peak velocities and higher MPI' values.

CONCLUSIONS: These findings further support that a proportion of SGA fetuses have true late-onset intrauterine growth restriction, which is associated with subclinical cardiac dysfunction, as previously described for early-onset intrauterine growth restriction.

KEY WORDS: SGA, late-onset intrauterine growth restriction, tissue Doppler imaging, fetal cardiac function, fetal echocardiography, myocardial performance index.

INTRODUCTION

Intrauterine growth restriction (IUGR) due to placental insufficiency is recognized among the main causes of perinatal morbidity and mortality.[1] Umbilical artery (UA) Doppler has been the mainstay for diagnosing placental insufficiency for two decades. Consequently, fetuses with normal UA Doppler, normally defined as small for gestational age (SGA), have long been considered to be constitutionally small fetuses with a good prognosis. However, recent evidence strongly suggests that a remarkable proportion of SGA fetuses share clinical features with early-onset IUGR fetuses, which supports the existence of mild forms of placental insufficiency that are not reflected in the UA Doppler. Thus, SGA fetuses as a group have poorer perinatal results[2-3], suboptimal neurodevelopment[4-5] and higher postnatal cardiovascular risk[6-8] as compared with normal weight newborns of the same gestational age (GA) at delivery. This evidence stresses the need to characterize the pathophysiology and develop biomarkers to identify the subgroup of "late-onset" IUGR forms among the category of SGA.

Cardiac dysfunction is now recognized among the central pathophysiologic features of human growth restriction.[9-14] In addition, recent evidence supports that cardiac dysfunction might be one of the key mechanisms explaining cardiac programming and the long described increased cardiovascular mortality in adults who suffered growth restriction in utero.[8] Concerning early-onset IUGR, several studies have demonstrated the presence of echocardiographic and biochemical signs of subclinical cardiac dysfunction, which progress further as the fetal condition deteriorates.[9-13] Preliminary evidence suggests that SGA fetuses with normal UA Doppler might also present features of cardiac dysfunction. Chaiworapongsa et al.[14] demonstrated that 4 % of neonates born small for gestational age had detectable

cardiac troponin I in umbilical cord blood, suggesting subclinical myocardial injury before birth. Girsen et al.[9] evaluated 13 SGA fetuses with normal UA Doppler and found significantly increased levels of ANP, a biomarker of cardiac dysfunction, although echocardiographic markers were not significantly different from controls.

In this prospective study we aimed at confirming and extending previous evidence of the existence of cardiac dysfunction in SGA fetuses with normal UA Doppler. We evaluated cardiac function parameters by means of conventional echocardiography and by tissue Doppler imaging, which has been shown to have a higher sensitivity to detect subclinical fetal cardiac dysfunction than conventional Doppler.[15-18] We compared a group of 58 late-onset SGA fetuses with 58 normal fetuses matched for gestational age.

MATERIAL AND METHODS

Study populations

The study population included 58 SGA fetuses and 58 controls. Patients were selected from women who attended the Department of Maternal-Fetal Medicine at Hospital Clinic in Barcelona. The study protocol was approved by the local Ethics Committee and patients provided their written informed consent. In all pregnancies gestational age was calculated based on the crown-rump length at first trimester ultrasound.[19] SGA was defined as an estimated fetal weight below the 10th centile according to local reference curves[20] together with UA pulsatility index (PI) below 95th centile.[21] Last examination before delivery was used for statistical analysis. The control group consisted of 58 normally grown fetuses matched with cases by gestational age at ultrasound (±1 week). Exclusion criteria of were structural/chromosomal anomalies or evidence of fetal infection.

All patients underwent ultrasonographic examination using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA). Basic Doppler examination included UA, middle cerebral artery and uterine arteries. Cerebral vasodilation was defined as middle cerebral artery PI below 5th centile. Cerebro-placental ratio was calculated as described previously.[22] At delivery, gestational age, mode of delivery, birth weight, birth weight centile, Apgar score and umbilical pH were recorded.

Cardiac function was assessed in all cases and controls by conventional echocardiography and TDI.

Conventional echocardiography

Conventional echocardiography included ductus venosus PI (DV-PI), peak early (E) and late (A) transvalvular filling velocities and MPI. 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.[23] Atrioventricular flows were obtained from a basal or apical four-chamber view placing the pulsed Doppler sample volume just below valve leaflets, and left and right E/A ratios were calculated.[24] Left MPI was obtained using the clicks of mitral and aorta valves as landmarks, as previously described.[25] The following time-periods were calculated: isovolumetric contraction time (ICT), ejection time (ET) and isovolumetric relaxation time (IRT). Finally, the MPI was calculated as (ICT+IRT)/ET.

Tissue Doppler imaging

TDI was obtained in real time using a 2-10 MHz phased-array transducer. In a four-chamber-view, sample volumes were placed in the basal part of the left ventricular wall (mitral annulus), interventricular septum and right ventricular wall (tricuspid annulus). The insonation ultrasound beam was kept at an angle of <30º to the orientation of the ventricular wall or the interventricular septum. No angle correction was applied. Annular peak velocities were measured in early diastole (PVE'), atrial contraction (PVA') and systole (PVS'). To calculate MPI by TDI (MPI'), the following time-periods were calculated: ICT', ET' and IRT'. Finally left, right and septal MPI' was calculated as (ICT'+IRT')/ET' (Figure 1). Measurement of all MPI' components were made from the same cardiac cycle.[26]

Statistical analysis

Data were analyzed with the SPSS 15.0 statistical package (SPSS, Chicago, Illinois, USA). Results are expressed as mean ± standard deviation or proportions. Comparisons between groups were performed by t-test, and echocardiographic parameters were also compared by logistic regression adjusted by estimated fetal weight. Comparison of cases with abnormal tissue Doppler parameters according to reference values between groups were performed by Chi-square.

RESULTS

Characteristics of the study populations

The characteristics of the study populations are reported in Table 1. UA and middle cerebral artery PI were similar in SGA fetuses and controls. Only 10% of SGA fetuses presented brain vasodilation. Mean uterine artery PI was higher in SGA

fetuses compared to controls. As expected, pregnancies with SGA showed significantly lower birth weight and birth weight percentile. GA at delivery, Apgar score and umbilical artery pH were similar between SGA pregnancies and controls. SGA cases showed a non-significant trend to higher rates of intervention for fetal distress, cesarean section and preeclampsia, which was present in 10% of the SGA pregnancies. SGA babies lasted more days in neonatal unit than controls.

Conventional echocardiography

Values of conventional echocardiographic parameters are shown in Table 2. Ductus venosus PI, left and right E velocities were similar among cases and controls. Both A velocities were significant lower in SGA as compared with controls even after adjusting by fetal weight. Left E/A showed a non-significant trend to lower values in SGA fetuses. SGA fetuses showed a non-significant trend to increased left MPI values as compared with controls.

Tissue Doppler imaging

Values of annular peak velocities and MPI' measured by TDI are shown in Table 3 and Figure 2. All peak velocities in tricuspid annulus were significantly lower in SGA fetuses as compared with controls, even after adjusting for fetal weight. Left and septal annular velocities showed a non-significant trend to lower values in SGA cases. Left and right MPI' were significantly higher in SGA fetuses. Figure 3 show the proportion of cases with abnormal annular peak velocities (< 10^{th} centile) and MPI' (> 90^{th} centile) in both groups, according to gestational age-based reference ranges.[27] 15-20% and 30-40% of SGA fetuses showed abnormal annular peak velocities and MPI' results, respectively.

COMMENT

This study provides evidence that SGA fetuses with normal UA Doppler present signs of subclinical cardiac dysfunction, which is consistent with previous studies suggesting the existence of true forms of growth restriction among SGA fetuses.[2-8]

Using conventional Doppler, E/A ratios and MPI showed a non-significant trend to higher values among SGA fetuses. These results are in line with those reported by Girsen in a small group of SGA fetuses with normal UA Doppler.[9] In contrast, tissue Doppler imaging could detect significant differences between cases and controls with regards to annular peak velocities and MPI'. The findings provide further evidence to support the notion that fetuses with SGA present subclinical cardiac dysfunction, as previously suggested by studies using biochemical markers of cardiac dysfunction and injury.[9,14] In addition, the data illustrate the higher sensitivity of TDI in relation with conventional echocardiography for detecting subclinical fetal cardiac dysfunction. Similar differences between TDI and conventional echocardiography have previously been reported in early-onset IUGR[15] and in various cardiac conditions in children and adults.[28-29]

From a pathophysiologic viewpoint, the results are consistent with previous evidence that a proportion of SGA fetuses are exposed to placental insufficiency and chronic restriction of nutrients and oxygen.[30-31] TDI annular peak velocities mainly reflect the motion of longitudinal myocardial fibers which are mostly located in the subendocardial layer.[32] Experimental studies have shown that subendocardial fibers are the earliest to be altered in the presence of an oxygen decrease.[33] Thus, the data suggest that TDI could be particularly sensitive to detect subtle forms of fetal

hypoxia. From a clinical perspective this opens opportunities to explore the use of cardiac function parameters in SGA. The identification and monitoring of true forms of growth restriction among fetuses diagnosed as SGA will be a clinical need in future years, and studies to explore the potential contribution of TDI are now underway. Fetal cardiac evaluation using TDI showed that a considerable proportion of SGA fetuses (15 to 40%) had abnormal annular peak velocities or MPI' results, conversely to only 10% of vasodilatation or <10% of abnormal ductus venosus. These findings could suggest a higher sensitivity of TDI to detect late-onset FGR as a marker of fetal hypoxia/undernutrition. Future research would have to explore whether TDI might have a value, alone or in combination within other markers, in improving the identification of fetuses with true late-onset FGR.

The study has several limitations and technical considerations. Firstly, although this study contains the largest sample of SGA fetuses investigated to date, we acknowledge that the absence of significant differences with conventional Doppler echocardiography is most likely due to sample size. Indeed, the average differences in measurements between cases and controls were similar using conventional echocardiography or TDI (7 to 10%), however the dispersion of data was substantially lower with the latter technique. Secondly, TDI is not readily available in current obstetric ultrasound machines and this represents a clear limitation for its wider use in clinical practice or research. However, the technique has demonstrated a good reproducibility in trained hands[15] and does not entail a more complex training than conventional fetal cardiac Doppler. As further research demonstrates the potential value of evaluating fetal cardiac function in this and other clinical conditions, TDI might become incorporated into obstetric ultrasound devices. Thirdly, since the main hypothesis of this study was focused on the mere

demonstration of subclinical cardiac dysfunction, we investigated a limited subset of the parameters now available for TDI. More complex techniques to assess cardiac dysfunction now used in adults, such as strain and strain-rate measured by TDI or 2D speckle tracking techniques might provide further insights in the characterization of cardiac dysfunction in SGA fetuses.[34-35] Fourthly, it was not possible to perform placental pathology nor cord blood biomarkers in this study, although we acknowledge that it could help to better understand the placental component and degree of cardiac dysfunction of these cases. Finally, changes in TDI parameters were more prominent when measured in the tricuspid annulus, as compared with left and septal walls. While this might truly reflect higher peak velocities in the right ventricle, which is the predominant one in fetal life, we can not exclude a systematic technical bias since Doppler insonation of the right ventricle is normally more straightforward in the fetus.

In summary, our study suggests the existence of subtle cardiac dysfunction in SGA fetuses with normal UA. These results open new lines for further investigation on the characterization of cardiac dysfunction in late-onset growth restriction and the evaluation of its potential contribution to clinical practice.

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Legends to figures

Figure 1. Measurement of peak velocities and times by pulsed tissue Doppler imaging in the right annulus.

Figure 2. Assessment of annular peak velocities and myocardial performance index measured by Tissue Doppler Imaging in the study populations.

Data given as mean ± standard deviation. *p<0.05 compared to controls adjusted by fetal weight. SGA; Small-for-gestational-age. PVE', peak velocity in early diastole; PVA', peak velocity during atrial contraction; PVS', peak velocity in systole. MPI'; myocardial performance index by tissue Doppler.

Figure 3. Proportion of cases with abnormal annular peak velocities and myocardial performance index in both groups

Abnormal annular peak velocities and myocardial performance index were defined as < 10^{th} centile and >90^{th} centile, respectively

*p<0.05 compared to controls. SGA; Small-for-gestational-age. PVE', peak velocity in early diastole; PVA', peak velocity during atrial contraction; PVS', peak velocity in systole. MPI'; myocardial performance index by tissue Doppler.

Table 1. Baseline characteristics and perinatal outcome of the study populations

Characteristics	**controls**	**SGA**	**p**
N	58	58	
Clinical characteristics			
GA at ultrasound (weeks)	38 (2)	38 (1)	0.62
Estimated fetal weight (gr)	3024 (413)	2235 (330)*	**< 0.001**
Centile	45 (27)	4 (8)*	**<0.001**
Umbilical artery PI	0.92 (0.19)	1.01 (0.2)	0.1
Middle cerebral artery PI	1.62 (0.34)	1.60 (0.31)	0.89
Cerebro-placental ratio	1.79 (0.49)	1.65 (0.45)	0.13
Mean uterine artery PI	0.73 (0.2)	0.92 (0.41)*	**0.02**
Pregnancy outcome			
GA at delivery (weeks)	40 (1)	38 (1)	0.08
Birth weight (gr)	3353 (418)	2379 (304)*	**<0.001**
Birth weight centile	52 (26)	4 (3)*	**<0.001**
Cesarean section	17%	31%	0.1
Intervention for fetal distress	7%	16%	0.23
5 minutes Apgar	9.9 (0.1)	10 (0)	0.37
Umbilical artery pH	7.23 (0.07)	7.23 (0.07)	1
Preeclampsia	2%	10%	0.12
Days in neonatal care unit	0.1 (0.5)	0.8 (2.1)*	0.018

Data were expresed as mean (SD) or proportions. *P-value <0.05 as compared with controls. SGA, small-for-gestational age; GA, gestational age; PI, pulsatility index.

Table 2. Cardiac function results by conventional echocardiography in controls and SGA fetuses.

	controls	**SGA**	***p-value****	***adjusted p-value**†*
Ductus venosus PI	0.49 (0.19)	0.46 (0.15)	0.38	0.58
Left E velocity (cm/s)	38 (8)	36 (6)	*0.17*	*0.16*
Left A velocity (cm/s)	49 (8)	44 (6)	***<0.001***	***<0.001***
Left E/A	0.78 (0.12)	0.83 (0.13)	***0.01***	*0.07*
Right E velocity (cm/s)	47 (9)	43 (7)	***0.02***	*0.08*
Right A velocity (cm/s)	59 (10)	53 (9)	***0.001***	***0.007***
Right E/A	0.80 (0.08)	0.82 (0.11)	*0.20*	*0.12*
Left MPI	0.49 (0.08)	0.53 (0.11)	*0.22*	*0.07*

Values are mean (standard deviation).

*calculated by t-test

†calculated by logistic regression adjusted by estimated fetal weight

SGA, small-for-gestational age; PI, pulsatility index; E, early diastole; A, atrial contraction; MPI, myocardial performance index

Table 3. Cardiac function results by tissue Doppler in controls and SGA fetuses.

	controls	**SGA**	***p-value****	***adjusted p-value**†*
Annular peak velocities				
Left PVE' (cm/s)	7.89 (1.56)	7.60 (1.39)	*0.28*	*0.49*
Left PVA' (cm/s)	8.56 (1.37)	8.17 (1.86)	*0.18*	*0.14*
Left PVS' (cm/s)	6.94 (1.19)	6.64 (1.35)	*0.19*	*0.38*
Right PVE' (cm/s)	9.25 (1.42)	8.48 (1.47)	***0.003***	***0.039***
Right PVA' (cm/s)	11.23 (2.15)	10.13 (1.64)	***0.002***	***0.033***
Right PVS' (cm/s)	8.09 (1.29)	7.39 (1.29)	***0.003***	***0.049***
Septal PVE' (cm/s)	6.24 (1.13)	6.11 (1.07)	*0.52*	*0.57*
Septal PVA' (cm/s)	7.41 (1.37)	6.97 (1.34)	0.07	*0.60*
Septal PVS' (cm/s)	6.03 (1.02)	5.99 (1.14)	*0.83*	*0.22*
Myocardial performance index				
Left MPI'	0.52 (0.09)	0.55 (0.09)	*0.036*	***0.001***
Right MPI'	0.49 (0.09)	0.56 (0.10)	*<0.001*	***<0.001***
Septal MPI'	0.52 (0.09)	0.59 (0.11)	*<0.001*	*0.076*

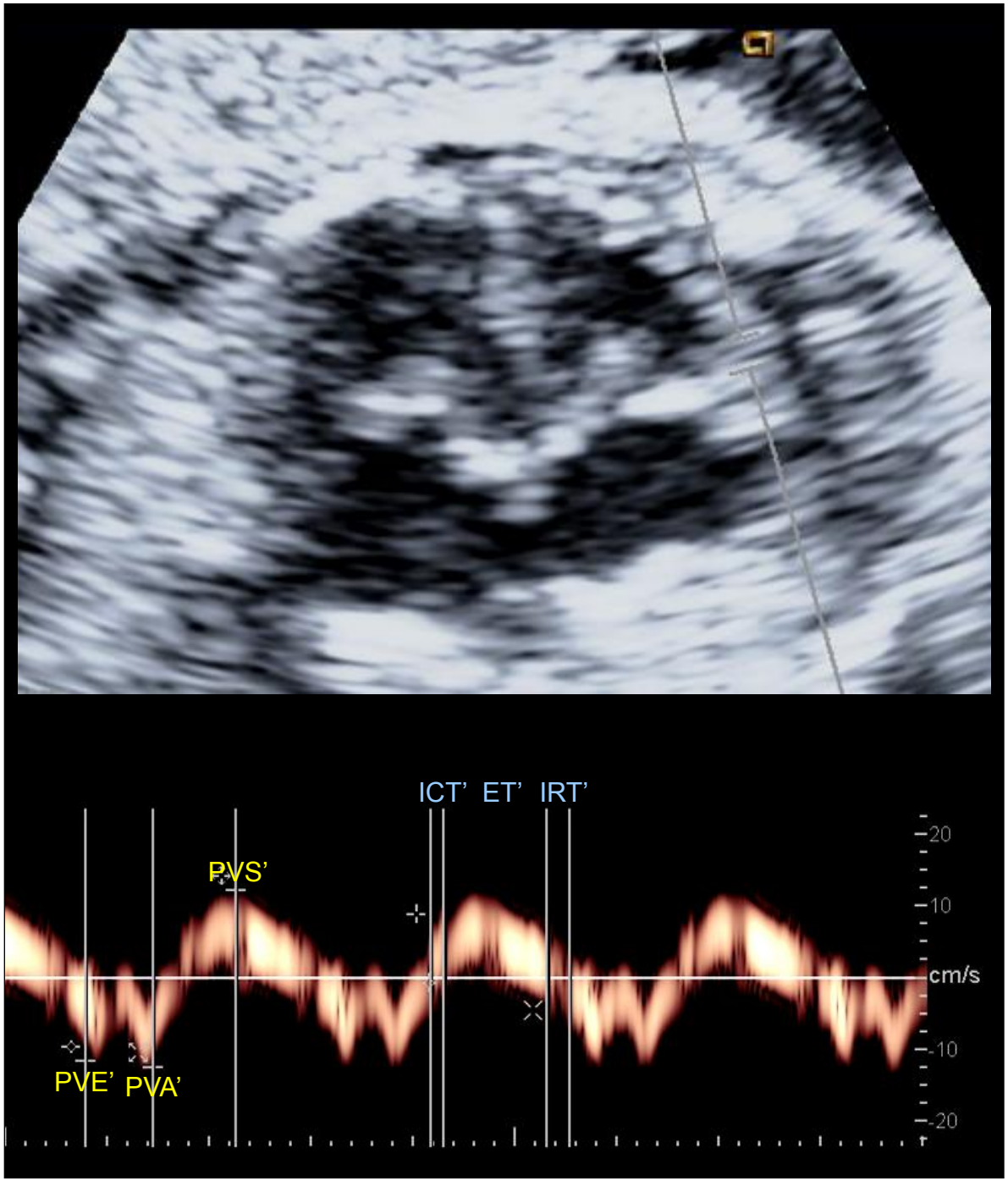
Values are mean (standard deviation).

*calculated by t-test

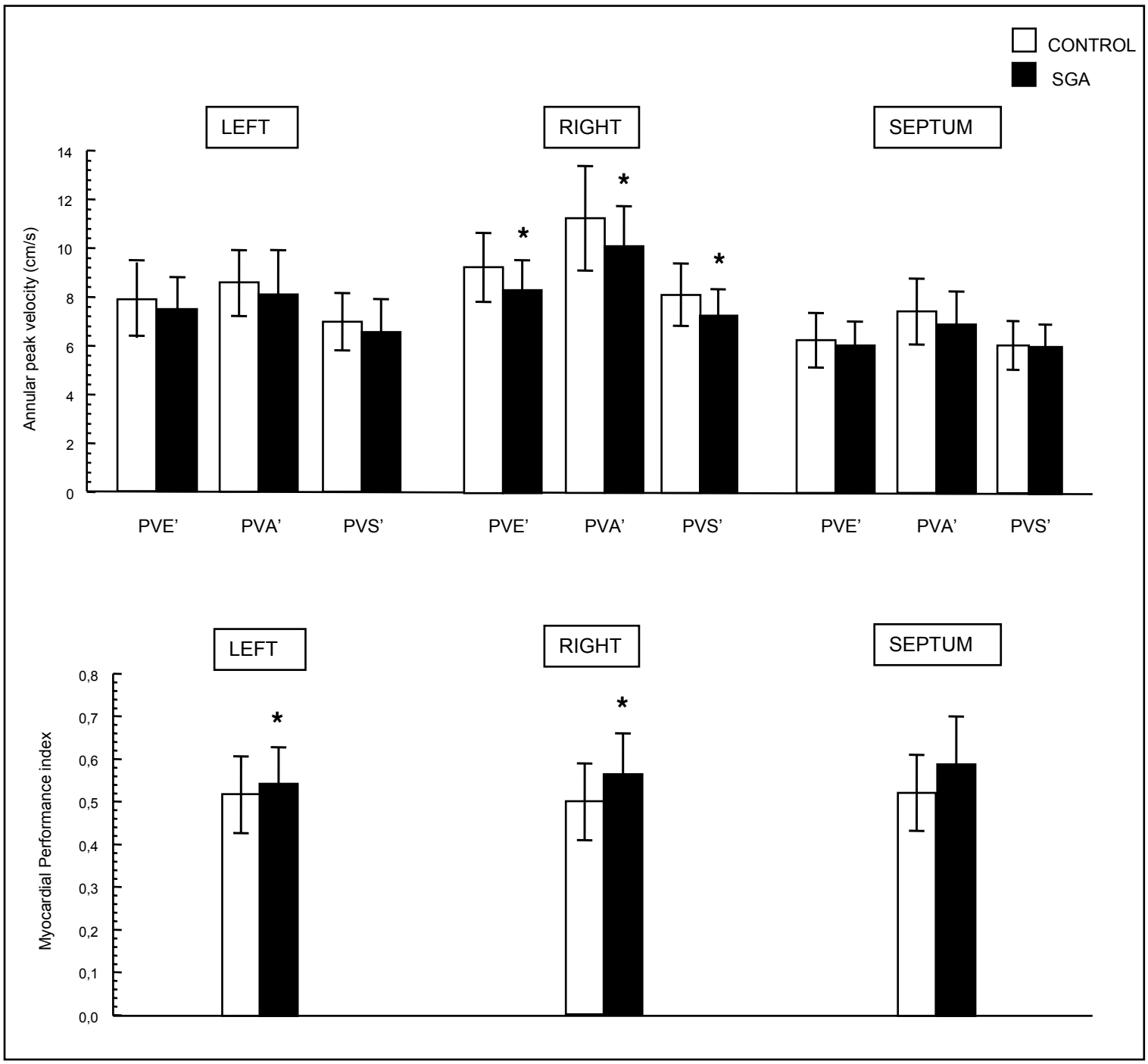
†calculated by logistic regression adjusted by estimated fetal weight.

SGA, small-for-gestational age; PVE', annular peak velocity in early diastole; PVA', annularl peak velocity during atrial contraction; PVS', annular peak velocity in systole; MPI', myocardial performance index by tissue Doppler.

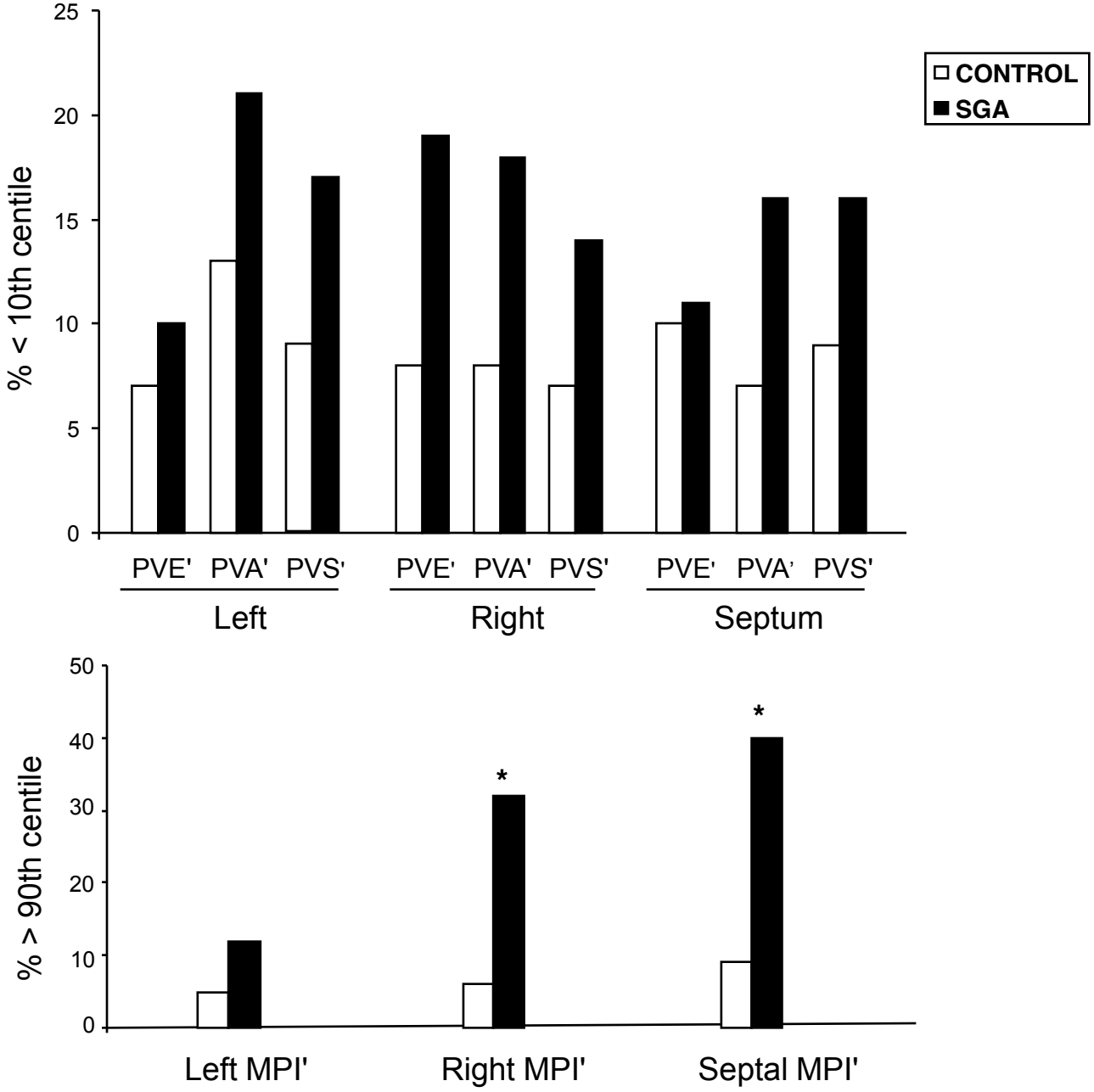
Figure



Figure


CONTROL
SGA
LEFT
RIGHT
SEPTUM
Annular peak velocity (cm/s)
14
12
10
8
6
4
2
0
PVE'
PVA'
PVS'
PVE'
PVA'
PVS'
PVE'
PVA'
PVS'
*
*
*
LEFT
RIGHT
SEPTUM
Myocardial Performance index
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
*
*

Figure


25
20
15
10
5
0
% < 10th centile
CONTROL
SGA
PVE' PVA' PVS'
PVE' PVA' PVS'
PVE' PVA' PVS'
Left
Right
Septum
50
40
30
20
10
0
% > 90th centile
*
*
Left MPI'
Right MPI'
Septal MPI'