PLACENTAL INSUFFICIENCY AND FETAL HEART: DOPPLER ULTRASONOGRAPHIC AND BIOCHEMICAL MARKERS OF FETAL CARDIAC DYSFUNCTION

KAARIN MÄKKIKALLIO

Department of Obstetrics and Gynaecology, University of Oulu
Department of Physiology, University of Oulu

OU LU 2002
KAARIN MÄIKIKALLIO

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OULUN YLIOPISTO, OULU 2002
Abstract

The first aim of this study was to investigate the relationship between Doppler ultrasonographic parameters and biochemical markers of human fetal cardiac dysfunction and myocardial cell damage in pregnancies complicated by placental insufficiency and/or fetal growth restriction. Our second aim was to examine fetal central and peripheral hemodynamic characteristics associated with retrograde aortic isthmus net blood flow.

 Fetuses with significant myocardial cell damage (cTnT > 0.10 ng/ml) had increased pulsatility in the blood velocity waveforms of ductus venosus, left hepatic vein and inferior vena cava, and had more often atrial pulsations in the umbilical vein. Their umbilical artery NT-proANP concentrations were higher than in fetuses without myocardial cell damage. The proportion of left ventricular cardiac output of the combined cardiac output was greater and the corresponding proportion of the right ventricle was less than in fetuses with only increased NT-proANP levels (> 1145 pmol/l). Tricuspid regurgitation was present more often and the right ventricular fractional shortening was less in fetuses with myocardial cell damage than in fetuses with normal umbilical artery cTnT levels. In fetuses with placental insufficiency and/or growth restriction (n = 48), umbilical artery NT-proANP concentrations showed a significant positive correlation with ductus venosus, left hepatic vein and inferior vena cava pulsatility index values for veins. Fetuses with placental insufficiency and antegrade aortic isthmus net blood flow demonstrated a shift in their right ventricular cardiac output from the pulmonary to the systemic circulation, and foramen ovale volume blood flow made up the majority of the left ventricular cardiac output. Fetuses with retrograde aortic isthmus net blood flow failed to demonstrate these changes, and they had signs of increased left atrial pressure. In addition, right ventricular fractional shortening was decreased and the pulsatility in the ductus venosus blood velocity waveforms was increased.

 In conclusion, human fetal myocardial cell damage was associated with a rise in systemic venous pressure, a change in the distribution of cardiac output towards the left ventricle and a rise in right ventricular afterload. Fetuses with retrograde aortic isthmus net blood flow failed to rearrange the distribution of the cardiac output and they had signs of increased left atrial pressure. In addition, right ventricular afterload and pulsatility in the ductus venosus blood velocity waveforms were increased.

Keywords: atrial natriuretic peptide, placenta, fetal echocardiography, aortic isthmus, cardiac troponin T, fetal growth restriction, physiology
L‘essential est invisible pour les yeux.

Antoine de Saint-Exupéry

To Tuomas, Johannes and Elias
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West Newton, May 16th, 2002, Kaarin Mäkikallio
## Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AEDV</td>
<td>Absent end diastolic velocity</td>
</tr>
<tr>
<td>ANP</td>
<td>Atrial natriuretic peptide</td>
</tr>
<tr>
<td>AoV</td>
<td>Aortic valve</td>
</tr>
<tr>
<td>A-wave</td>
<td>Atrial contraction wave</td>
</tr>
<tr>
<td>BNP</td>
<td>B-type natriuretic peptide</td>
</tr>
<tr>
<td>CC</td>
<td>Cardiac circumference</td>
</tr>
<tr>
<td>CCO</td>
<td>Combined cardiac output</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CK-MB</td>
<td>Creatine kinase myocardial band isoenzyme</td>
</tr>
<tr>
<td>CNP</td>
<td>C-type natriuretic peptide</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross-sectional area</td>
</tr>
<tr>
<td>cTnT</td>
<td>Cardiac troponin T</td>
</tr>
<tr>
<td>DA</td>
<td>Ductus arteriosus</td>
</tr>
<tr>
<td>DAO</td>
<td>Descending aorta</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DV</td>
<td>Ductus venosus</td>
</tr>
<tr>
<td>E-wave</td>
<td>Early passive filling wave</td>
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<tr>
<td>FGR</td>
<td>Fetal growth restriction</td>
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<tr>
<td>FHR</td>
<td>Fetal heart rate</td>
</tr>
<tr>
<td>FO</td>
<td>Foramen ovale</td>
</tr>
<tr>
<td>IMP</td>
<td>Index of myocardial performance</td>
</tr>
<tr>
<td>IRT</td>
<td>Isovolumetric relaxation time</td>
</tr>
<tr>
<td>IVC</td>
<td>Inferior vena cava</td>
</tr>
<tr>
<td>LHV</td>
<td>Left hepatic vein</td>
</tr>
<tr>
<td>LVCO</td>
<td>Left ventricular cardiac output</td>
</tr>
<tr>
<td>LVeFo</td>
<td>Left ventricular ejection force</td>
</tr>
<tr>
<td>LVFS</td>
<td>Left ventricular fractional shortening</td>
</tr>
<tr>
<td>MCA</td>
<td>Middle cerebral artery</td>
</tr>
<tr>
<td>MR</td>
<td>Mitral valve regurgitation</td>
</tr>
<tr>
<td>MV</td>
<td>Mitral valve</td>
</tr>
<tr>
<td>NT-proANP</td>
<td>N-terminal peptide of proatrial natriuretic peptide</td>
</tr>
</tbody>
</table>
PI  Pulsatility index
PIV  Pulsatility index for veins
PPA  Proximal pulmonary artery
PV  Pulmonary valve
Q  Volume blood flow (ml/min)
Q_{DA}  Ductus arteriosus volume blood flow
Q_{FO}  Foramen ovale volume blood flow
Q_{P}  Pulmonary volume blood flow
REDV  Retrograde diastolic velocity
RI  Resistance index
RNA  Ribonucleic acid
RVCO  Right ventricular cardiac output
RVeFo  Right ventricular ejection force
RVFS  Right ventricular fractional shortening
SD  Standard deviation
S/D- ratio  Systolic to diastolic velocity- ratio
TC  Thoracic circumference
TR  Tricuspid valve regurgitation
TV  Tricuspid valve
TVI  Time-velocity- integral
UA  Umbilical artery
UtA  Uterine artery
List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:


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1 Introduction

Placental insufficiency and fetal growth restriction (FGR) are common obstetrical problems which may have long-term consequences. Intrauterine malnutrition may increase the risk of the development of diabetes, stroke, chronic hypertension, and death from coronary artery disease in adults (Barker et al. 1993, Eriksson et al. 2001). It can also permanently change lipid metabolism and the hemostatic factors leading to the increased risk of cardiovascular diseases (Barker et al. 1993).

Noninvasive Doppler ultrasonography enables the examination of placental, fetal central and peripheral hemodynamics (Jouppila & Kirkinen 1984, Giles et al. 1985a, Wladimiroff et al. 1986, Moise et al. 1988). Increased placental vascular impedance as determined by abnormal umbilical artery Doppler findings has been connected with decreased number of arterioles in the placental tertiary villae (Giles et al. 1985a). A rise in placental vascular impedance has been associated with increased perinatal morbidity and mortality (Rochelson et al. 1987a, Rochelson et al. 1987b). The presence of pathologic umbilical artery blood velocity waveforms indicates the need for closer fetal surveillance. In these cases, the timing of the delivery has often been based on pathologic fetal heart rate tracings (Arduini et al. 1993). In pregnancies complicated by chronic fetal hypoxia, an end-diastolic block in the blood velocity waveforms of thoracic aorta preceded pathologic cardiotocographic changes (Jouppila & Kirkinen 1984). Placental insufficiency may lead to cardiac dysfunction (Rasanen et al. 1989). Increased pulsatility in the blood velocity waveforms of fetal systemic veins has been associated with biochemical evidence of a rise in fetal systemic venous pressure (Capponi et al. 1997). A rise in the pulsatility in the human fetal inferior vena cava and ductus venosus blood velocity waveforms was closely related to abnormal biophysical assessment findings and other signs of fetal distress (Hecher et al. 1995a, Hecher et al. 1995b). In fetal circulation, the aortic isthmus has a dynamic role in connecting parallel circulations of the fetal ventricles. An increase in placental vascular resistance can change the fetal aortic isthmus blood flow profile before any change in umbilical artery Doppler velocimetry (Bonnin et al. 1993). In addition, oxygen delivery to the brain is diminished in fetal lambs with retrograde net blood flow in the aortic isthmus (Fouron et al. 1999).

Atrial natriuretic peptide (ANP) is predominantly expressed in the atria of the heart. Conditions with cardiac pressure and volume overload are associated with increased
plasma levels of ANP (Lang et al. 1985, Brenner et al. 1990). Cardiac troponin T (cTnT) is released rapidly after myocardial injury in direct proportion to the extent of injury (Gerhardt et al. 1991).

The purpose of this clinical study was to correlate Doppler ultrasonographic parameters of fetal central and peripheral hemodynamics with biochemical markers of fetal cardiac dysfunction and myocardial cell damage in human pregnancies complicated by placental insufficiency. In addition, the association between retrograde aortic isthmus net blood flow and fetal central and peripheral hemodynamics was investigated to understand the mechanisms leading to diminished oxygen delivery in cerebral circulation.
2 Review of the literature

2.1 Characteristics of fetal cardiovascular physiology

Some of the anatomic differences between the fetal and adult circulations were described by Harvey in 1628 (Harvey 1628). Fetal circulation is characterized by three shunts: ductus venosus (DV), foramen ovale (FO) and ductus arteriosus (DA) that permit the blood to bypass the liver and lungs, and shunt the most oxygenated blood from the right to the left side of the heart (Fig. 1).

2.1.1 Cardiac function

Contractility of the heart is markedly affected by contractile state, preload and afterload of the heart. The contractile or inotropic state of the muscle is defined in terms of its ability to shorten and generate tension. It also involves the rate at which shortening and tension develop and decay. The contractile state depends on the number of cross-bridges formed between actin and myosin, which in turn is related to the calcium concentration released from the sarcoplasmic reticulum by the action potential. Substances that alter the inotropic state may act by increasing the availability of calcium at contractile sites. They also appear to change the ability of troponin to bind calcium (Fig. 2). These mechanisms are responsible for the enhanced contractility. The catecholamines epinephrine and norepinephrine are potent inotropic agents. They also increase the heart rate (positive chronotropic effect). Both catecholamines shorten the refractory period of the myocardium and enhance conduction through the heart. Acetylcholine increases potassium and chloride permeability, and thus reduces heart rate and lengthens the conduction time and the refractory period (negative inotropic effect). (Pickoff 1998).
Fig. 2. Construction of actin-myosin complex. M=myosin fiber, A=actin fiber, I=troponin I, C=troponin C, T=troponin T and Tm=tropomyosin, Ca2+=calcium-ion.

2.1.1.1 Preload

Cardiac muscle responds to the stretching of its fibers (preload) by an increase in the strength of the subsequent contraction. This is called Frank-Starling's law. Premature atrial contraction is followed by a longer refractory period, allowing enhanced filling of the ventricle. This, in turn, leads to increased stroke volume in the subsequent ventricular contraction, demonstrating that Frank-Starling’s law is effective in human fetal heart (Kirkpatrick et al. 1976, Lingman & Marsal 1987). Furthermore, the inverse relationship between umbilical artery flow velocity and fetal heart rate suggests that the Frank-Starling mechanism regulates cardiovascular control as early as 10–15 weeks of human gestation (Ursem et al. 1998). The heart is protected from being overstretched by several factors: elasticity of the muscle fibers, noncompliant pericardium and functional regulation of the heart.

2.1.1.2 Afterload

Fetal ventricles must eject blood against their own inertia and that of blood, the impedance of central blood vessels, and the resistance of peripheral vessels (Teitel 1998). Parameters describing afterload are complicated because they should take into account all the forces against which the ventricle is contracting. More commonly, arterial mean pressure or systemic vascular resistance is used to approximate afterload. Fetal ventricles, especially the right ventricle, are more sensitive to afterload than adult ventricles (Gilbert 1982, Thornburg & Morton 1983) This may be a consequence of inefficient arterial elasticity rather than an actual sensitivity of the immature myocyte to afterload (Teitel 1998). On the other hand, aortic pressure has a strongly negative effect on fetal left
ventricular stroke volume when filling pressures remain constant. It seems that the fetal left ventricle has a limited ability to increase stroke volume above left atrial filling pressures of 5–7 mmHg with volume infusion. However, when aortic pressure is held relatively constant, left ventricular stroke volume continues to rise, even above left atrial pressures of 8–10 mmHg (Hawkins et al. 1989).

2.1.1.3 Myocardial blood flow

Myocardial blood flow is regulated by hydrostatic forces, anatomic factors, metabolic status and autoregulation. Intracardiac pressure is the most important determinant of the pressure gradient from the aorta across the coronary beds during diastole (Bellamy 1978, Dole & Bishop 1982, Pantely et al. 1984, Spaan 1985). Experiments performed in dogs and human adults show that in normal conditions, 70–85% of the left ventricular myocardial blood flow occurs during diastole (Klocke 1976, Hess & Bache 1976). An increase in the strength of heart contraction immediately increases coronary blood flow, while a decrease in the force will have an opposite effect. Autoregulation is due to the fact that the coronary arteries arise from the sinus in the base of the aorta. When the stroke volume increases, more blood is automatically forced into the coronary arteries. During diastole, part of the increased blood volume ejected to the aorta flows rapidly into the coronary vessels. However, there is experimental evidence that the autoregulatory reserve in subendocardial arteries is less than in subepicardial arteries (Sestier et al. 1978, Boatwright et al. 1980, Vatner 1984). Furthermore, several substances including oxygen, carbon dioxide, potassium and adenosine, as well as the autonomic nervous system have been proposed to have a role in vasoregulation of the myocardium (Reller et al. 1992a, Reller et al. 1992b, Reller et al. 1995).

Animal studies have demonstrated that increased wall stress, especially during the diastole, leads to increased ventricular myocardial oxygen consumption (Sarnoff 1958, Sonnenblick et al. 1965, Pool et al. 1968, Sonnenblick & Skelton 1971, Rooke & Feigl 1982). Due to the elliptical structure of the ventricles, regional wall stress and myocardial oxygen consumption are not homogeneous throughout the ventricle. The wall tension is greater in the subendocardial region than in the subepicardial region (Berman & Gamble 1975, Holtz et al. 1977). In a lamb model, an increase in right ventricular pressure was associated with a marked accumulation of products of reactive O$_2$ species generation in the right ventricular myocardium (Fouron et al. 2001a). The wall stress and myocardial oxygen consumption per gram of tissue will be restored to normal values when hypertrophy of the cardiac muscle occurs. However, hypertrophy compromises coronary vascular reserve.
2.1.2 Cardiac output and blood pressure

The cardiac output (stroke volume x fetal heart rate (FHR)) is affected by contractility, peripheral resistance, blood volume and viscosity of the blood. Venous blood return (preload) controls the cardiac output. The cardiac response depends on the sympathetic stimulation and on the contractile characteristics of the myocardium. Heart rate is influenced by the balance between sympathetic and parasympathetic impulses, ionic concentration, pH and secretion of several hormones. Stroke volume is affected by the same factors that affect myocardial contractility, preload and afterload of the heart, and heart rate. The dynamics of blood flow have a considerable effect on cardiac output and the work done by the heart. The law of Laplace states that the tension in the wall increases when the distending intraventricular pressure and/or the intraventricular volume increase. When intraventricular volume is increased, a greater tension must be developed in the myocardium to produce any given pressure. The amount of energy needed increases as pressure increases (Romero et al. 1972).

With advancing gestation fetal stroke volume, cardiac output and weight-indexed systemic vascular resistance increase (Rasanen et al. 1996a). Between 14 and 28 gestational weeks, left and right ventricular systolic and end diastolic pressures increased significantly in a linear fashion (Johnson et al. 2000). Ventricular systolic pressure increased from 13 mmHg to 37 mmHg and end diastolic pressure from 3 mmHg to 10 mmHg. No difference between left and right ventricular pressures was observed. In addition, atrial pressures were equal and remained constant between 14 and 28 gestational weeks (Johnson et al. 2000). Studies on fetal lambs have shown that the right ventricle ejects a larger stroke volume than the left ventricle, and represents a greater proportion of the combined cardiac output (CCO) (Rudolph 1985). According to studies on middle to late gestation lamb fetuses, right ventricular stroke volume (55%–67% of CCO) exceeds left ventricular stroke volume for any given physiologic filling pressure (Rudolph 1985, Reller et al. 1987). The weight-indexed fetal cardiac output is significantly higher than that of an adult person (Severi et al. 2000). In fetal lambs, CCO in term gestation was about 500 ml/min/kg and about 2.5% of CCO was directed to the heart (Itskovitz et al. 1987).

Mean (SD) umbilical venous oxygen saturation has been shown to be 63% (16%) and the corresponding umbilical artery value 24% (15%) (Richardson et al. 1998). Highly oxygenated blood from the placenta is directed via DV through FO to the left ventricle (Fig. 1) (Kiserud et al. 1991). In a study on near-term fetal lambs, about 44% of the umbilical venous blood was distributed to DV. With a 50% reduction in umbilical flow, this proportion increased to 72%, resulting in a marked decrease in umbilical blood flow distributed to the fetal liver (Itskovitz et al. 1987). In fetal lambs, the left ventricle delivers the majority of its output to the heart, brain and the upper parts of the body, with only less than 10% crossing through the aortic isthmus to the descending aorta (DAo) (Rudolph 1985). The fetal right ventricle receives mainly poorly saturated blood from the superior vena cava, coronary sinus and inferior vena cava (IVC) (Rudolph 1985). The majority of the right ventricular cardiac output (RVCO) is delivered across the DA to the DAo.
2.1.3 Peripheral circulation

Poiseuille described in 1864 the relationship between the factors that govern the nonpulsatile flow of homogeneous liquids through rigid tubes. The driving force that causes the fluid to flow from one end to the other is the pressure gradient (dP) between the two ends. Several factors provide the resistance (R) to volume flow (Q) including the viscosity of the fluid (η), the radius of the tube (r), and the length of the tube (L). Poiseuille’s law expresses this relationship in the equation: \[ Q = \frac{dP r^4}{4 \pi \eta L} \]. In clinical practice, the relationship among volume flow, pressure and resistance is expressed as \[ Q = \frac{P}{R} \]. Most of the vascular resistances in humans are in parallel. Even minor changes in the diameter of the blood vessel have significant effects on the conductance of blood through the vessel. A change in the blood vessel caliber has the most important effect on vascular resistance. In vessels connected in series, the resistances are additive. In parallel circulations, the pressure is maintained in each vessel and the rate of blood flow can be affected by selective opening or closing of the individual vessels.

Viscosity, which is the inner friction between the molecules in the adjacent layers or laminae, affects the resistance to flow. In laminar flow, the laminae move parallel to each other in longitudinally-oriented concentric sleeves, each moving at a different rate. The viscosity of the blood depends, for example, on the number of circulating blood cells and the bore of the vessels.

Blood pressure is almost directly proportional to volume distribution and vascular resistance. The arterioles have the ability to regulate the resistance of systemic circulation. Arteriolar resistance is affected by sympathetic stimulation. Blood vessel diameter increases as the internal pressure rises. The degree of distensibility varies considerably according to the thickness and composition of the vessel wall, and the degree of filling. The veins are referred to as the volume storers or capacitance vessels of the circulation, while the arteries, due to their ability to expand and recoil, are the pressure storers, storing the pressure during contraction of the heart and releasing it during cardiac relaxation.

2.1.4 Placental circulation

Maternal nutrient delivery depends upon placental mass, villous surface, and fetoplacental vasculature. Uteroplacental vascular lesions associated with FGR include incomplete uteroplacental vascular adaptation, fibrinoid necrosis or atherosis of the vessel wall, persistence of endovascular trophoblasts in the basal plate vasculature, uteroplacental thrombosis and chronic uteroplacental vasculitis (Salafia 1997). These lesions predispose to placental infarcts. It has been suggested that FGR may not be due to any particular placental lesions but to underperfusion of the placenta (Naeye 1989, Salafia et al. 1995). The presence of placental infarct more likely indicates overall poor perfusion with failed collateral flow. Poor uteroplacental perfusion may compromise terminal villous arborization due to effects of intervillous pressure on capillary net or by actual destruction of villous capillaries (Giles et al. 1985a).
The oxygen partial pressure ($pO_2$) of the fetal arterial blood, which normally ranges from 25 to 30 mmHg, is considerably lower than that of maternal arterial blood (Soothill et al. 1986). Even though oxygen tension in fetal blood is only one-fifth to one-fourth that of adult blood, fetal arterial blood oxygen content and oxyhemoglobin saturation are not much lower than those of an adult. Fetal hemoglobin ($\alpha_2, \delta_2$) is structurally different from adult hemoglobin ($\alpha_2, \beta_2$). It has a greater affinity for oxygen than has adult hemoglobin (Delivoria-Papadopoulos & McGowan 1998). Consequently, fetal hemoglobin combines more rapidly with oxygen at low tension than does adult hemoglobin. With advancing gestation, progressive decrease in umbilical artery $pO_2$ is associated with an increase in fetal hemoglobin concentration, thus maintaining fetal oxygen content constant (Soothill et al. 1986). Studies on moderately and severely anemic fetal lambs show that a high hemoglobin-oxygen affinity is critical for normal metabolism in fetuses subjected to a hypoxic stress (Edelstone et al. 1989). In fetal lambs, mean (SD) oxygen extraction increased from 33.6% (4.8%) to 67.7% (11.3%) during a 75% reduction of umbilical blood flow (Itskovitz et al. 1983). Similarly in fetal lambs, diminished oxygen delivery due to restriction of uterine artery blood flow increased mean (SD) oxygen extraction significantly from 0.33 (0.04) to 0.43 (0.04) and 0.54 (0.05) at 1 and 24 hours, respectively. Overall fetal oxygen consumption remained unchanged from control values (Bocking et al. 1992). Oxygen consumption in normal human fetuses between 28 and 40 weeks of gestation varied between 5.4–6.8 ml/kg/min (Bonds et al. 1986), while adult oxygen consumption is estimated to be about 3.0–4.0 ml/kg/min in resting state. Thus, oxygen consumption per kilogram in a normal fetus is almost double the consumption of the adult.

### 2.1.6 Hypoxemia and hyperoxemia

Inadequate fetal oxygen supply may result from inadequate maternal blood oxygen content, a fall in uterine blood flow or from disturbed placental circulation. A reduction in either uterine or umbilical blood flow will also interfere with carbon dioxide removal from the fetus resulting in fetal respiratory acidemia. In studies on fetal lambs, the proportion of cardiac output directed to the placenta rose from 41% to 48% in hypoxic fetuses and from 41% to 57% in acidemic fetuses, respectively (Cohn et al. 1974). In hypoxic fetuses, cerebral and coronary circulations and blood flow to the adrenals increased two- to three-fold, while pulmonary, renal, splenic, gut and carcass blood flows decreased. These changes were of a greater magnitude in fetuses with acidemia (Cohn et al. 1974). After initial fetal tachycardia during hypoxemia, the fetal arterial pressure increases and FHR decreases. Combined cardiac output is maintained (Cohn et al. 1980, Reuss et al. 1982, Court et al. 1984) unless the fetus becomes acidemic (Cohn et al. 1974). Thus, it seems that a moderate reduction in oxygen delivery to the fetus stimulates chemoreceptor activity that results in vasoconstriction in certain peripheral vascular beds, hypertension, and a transient or persistent bradycardia in the fetus (Peeters et al. 1979, Itskovitz & Rudolph 1982).
Maternal hyperoxygenation increases fetal oxygen partial pressure (Morin & Egan 1992). During maternal hyperoxygenation in the second half of the pregnancy, fetal middle cerebral artery (MCA) PI values increased and PI values of the aortic isthmus decreased with no change in the umbilical artery PI values (Almstrom & Sonesson 1996). In addition, increased absolute velocities in DV have been noted during maternal hyperoxygenation (Soregaroli et al. 1993). In experiments on fetal sheep in early pregnancy, maternal hyperoxygenation did not cause these changes (Kiserud et al. 2001). In studies on fetal lambs, increasing maternal oxygen tension increased pulmonary blood flow 10-fold in the third trimester fetuses but only 0.2-fold in late second trimester fetuses (Morin & Egan 1992). At the normal low oxygen tension of the fetus, pulmonary blood flow does not increase between these two points of gestation in the fetal lamb despite the increase in vessel density in the lungs.

2.2 Ultrasonographic methodology

2.2.1 Doppler ultrasonography

Doppler phenomenon states that the pitch of the sound waves of a moving object is altered when the distance between the observer and the source of sound changes. The change in relative motion between the observer and the object is known as the Doppler shift. The Doppler shift (F_d) can be calculated with the following formula: \( F_d = \frac{v \cos \theta}{c} \), where \( v \) is the speed of the moving target, \( f \) is the frequency of the emitted pulse (i.e. the frequency of the transducer), \( \theta \) is the angle between the direction of emitted sound wave and the direction of the moving target, and \( c \) is the average speed of sound within the tissue (Kremkau 1990, Kremkau 1992). Doppler shift is dependent on the speed of blood flow, the angle between the transducer and the blood vessel, and the operating frequency of the Doppler transducer (Kremkau 1990, Kremkau 1992).

2.2.1.1 Continuous wave Doppler ultrasonography

In a continuous wave Doppler system, a sound wave is continuously transmitted and received with two different transducers. The transmitted and reflected beams begin to overlap a short distance from the surface of the probe, and the overlap extends until the beams attenuate. When several vessels are focused within the sensitive volume, the Doppler signals are superimposed and detected simultaneously (Gill 1987). This explains why the investigations of certain locations are not possible. On the other hand, continuous Doppler ultrasonography is not dependent on the depth of the location and speed of the blood flow.
2.2.1.2 Pulsed wave Doppler ultrasonography

A Doppler system with range resolution allows the selection of location where the Doppler signals are obtained. This is possible by sending the ultrasound waves in pulses, and thus only waves from certain areas return back before the next pulse is transmitted. In order to analyse reflected waves during a certain time period after pulse transmission, it is possible to set a sample volume located in predetermined range (Gill 1987). The axial length of the sample volume is determined by the time period the gate is open. Changing frequency of waves reflected from a moving target limits the use of pulsed Doppler. The maximum measurable Doppler shift frequency is related to half of the pulse repetition frequency (Nyqvist limit). The pulse repetition frequency must be increased with high velocities, and thus the depth reached with the ultrasound decreases. The velocity of the blood flow and the depth of the object are limitations of pulsed Doppler ultrasonography.

2.2.1.3 Color Doppler ultrasonography

Phase quadrature detection in the demodulators enable the observer to distinguish higher or lower received signals from the transmitted frequency, corresponding to Doppler shifts toward or away from the transducer. Flow toward the transducer is visually demonstrated as red and flow away from the transducer as blue, and non-moving targets remain grey. The saturation of the color is related to the velocity of the flow (Burns 1993). The limitations of color flow imaging are similar to pulsed Doppler ultrasonography.

2.2.1.4 Analysis of Doppler spectra

The presence or absence of blood flow, and the shape of the blood velocity waveform are analysed qualitatively. As a result of different physical, anatomic and morphologic factors, blood velocity waveform profiles differ from one vessel to another. The presence of a diastolic blood velocity waveform component is detected in arteries supplying low-resistance vascular systems. As the peripheral impedance increases, the diastolic component disappears. The presence of a diastolic notch in uterine arteries has been used to predict adverse pregnancy outcome (Harrington et al. 1996). In the umbilical vein, blood flow is normally nonpulsatile and the appearance of atrial pulsations has been thought to indicate the deterioration of fetal well-being (Reed 1997).

Semiquantitative analysis of Doppler spectra involves the evaluation of the relation between systolic and diastolic blood velocity waveform components. The most common indices are the pulsatility index (PI = (peak systolic velocity – end diastolic velocity) / time-averaged maximum velocity over the cardiac cycle) and the resistance index (RI = (peak systolic velocity – end diastolic velocity) / peak systolic velocity). The correlation between these indices is highly significant as long as the diastolic blood flow velocity is present (Thompson 1987). These angle independent indices are thought to describe
downstream impedance, which is resistance to pulsatile flow (O'Rourke 1982). However, they are only indirect estimations of true blood flow (Dickey 1997). No correction for heart rate, if it is within normal physiologic range, or blood pressure is required (Kofinas et al. 1989, Burns 1993, Dickey 1997).

Volume flow analysis is the most difficult to perform. It is dependent on the angle of insonation, accurate measurement of the vessel diameter, tortuousity of the vessel and analytical power of the ultrasonographic equipment. Due to these limitations, it is recommended for larger vessels (Dickey 1997). However, Kiserud et al. (Kiserud et al. 1999) have demonstrated by in vitro and in vivo measurements that ultrasonographic diameter assessment has a high reproducibility even for vessels of small dimensions when measurements are taken with a high-frequency ultrasound. The flow volume (Q (ml/min)) of the vessel can be calculated if the mean velocity (V mean = TVI x HR) and the cross-sectional area (CSA) of the vessel are known according to following formula: Q = TVI x HR x CSA, where CSA is derived from the diameter (r) of the vessel with the formula: CSA = \( \pi (r/2)^2 \), time-velocity- integral (TVI) is calculated by planimetering the area underneath the Doppler spectrum and HR is heart rate (Rizzo et al. 1995a).

### 2.2.1.5 Methodological problems

When measuring absolute velocity and volume, the angle of insonation is of great concern. For angles less than 20 degrees, the maximum error in calculating velocity and volume caused by 5% error in estimating the angle of insonation is equal or less than 1.5% (Dickey 1997). If the angle of insonation is kept at less than 30 degrees, the effect of the angle on the absolute velocities and volume is minimal (Tessler et al. 1990). In PI and RI calculations, an angle of less than 60 degrees is considered nonsignificant (Gudmundsson et al. 1990, Davies et al. 1990).

In volume flow calculations, the main source of error is the vessel CSA. Vessel diameter changes during the cardiac pulsations and this error is magnified when squared. Error in vessel diameter measurements becomes less as vessel diameter increases. However, at least three separate measurements of the diameter from sequential cardiac cycles are recommended (Dickey 1997).

### 2.2.2 M-mode echocardiography

M-mode imaging shows the instantaneous position of one interface with respect to other interfaces along the selected transmission line. Monitored interfaces will change each time the selected transmission penetrates through the area. As each transmission collects and displays its interface data with its own time reference, variations in the position of echoes received are recognized as motion displacements. This method is possible without any physical changes of the transducer when the transducer is set to collect data from one direction and from only one line of transmission. In fetal cardiology, it is used to estimate...
the diameters of ventricular cavities and walls and the fractional shortenings of the ventricles. It is also useful in the diagnosis of fetal arrhythmia (DeVore et al. 1984, Reed 1997).

2.2.3 Safety aspects

Mechanical index indicates cavitation potential in the tissues, and thus the amplitude of ultrasonographic pulses at any time. Increased pulse amplitudes result in proportionally higher mechanical index values. It is estimated that cavitation potential can be of major concern when intensities exceed 3300 W/cm³. Thermal index is an indicator for tissue heating. Thermal index is an estimate of the tissue temperature rise in °C, which might be possible in "reasonable worst case conditions". It is divided into soft tissue, bone and cranial thermal indices. Ultrasonographic equipment has to display the emitted energy in settings in which thermal and/or mechanical indices might be over or equal to 0.4 (European Committee for Medical Ultrasound Safety 1999a, European Committee for Medical Ultrasound Safety 1999b). Diagnostic exposure that produces a maximum in-situ temperature rise of no more than 1.5°C above normal physiologic level (37°C) may be used clinically without reservations on thermal grounds. Fetal temperature elevation above 41°C for 5 minutes or more is considered hazardous (Barnett et al. 1997).

In animal ultrasonographic studies during organogenesis and later pregnancy, no long-term ramifications, abortions, gross malformations, or stillbirths have been observed in the exposed animals (Tarantal & Hendrickx 1989a, Tarantal & Hendrickx 1989b, Tarantal et al. 1993, Tarantal et al. 1995). A follow-up study after human fetal ultrasonographic exposure revealed no association between ultrasound exposure in early fetal life and growth or impaired vision or hearing during childhood, at least until the age of seven years (Kieler et al. 1997). However, the results concerning the relationship between impaired fetal growth and repeated fetal ultrasound exposures are controversial (Newnham et al. 1993, Kieler et al. 1997). No association between diagnostic ultrasound exposure during pregnancy and childhood malignancies or neurological maldevelopment has been found (Salvesen & Eik-Nes 1999). Kieler et al. (Kieler et al. 1998) found no difference in non-right handedness between exposed and non-exposed children at the age of 8–9 years, but, separately, the boys exposed to ultrasound had higher prevalence of non-right handedness. There exists a consensus that frequently repeated ultrasonographic exposures should be of a clinical benefit (Kossoff 1997).
2.3 Doppler ultrasonographic assessment of fetal circulation

2.3.1 Uterine hemodynamics

The major arterial blood supply to the uterus is derived from the uterine arteries. Blood velocity waveform indices in the uterine artery remain high and quite stable during the first trimester of pregnancy (Dickey & Hower 1995). In the beginning of the second trimester, Doppler indices decrease sharply and early diastolic notching of uterine artery disappears. However, a diastolic notch can be a normal finding until 26 weeks of gestation (Schulman et al. 1986). Little or no change is observed in either the uterine or arcuate artery indices after 26 weeks of gestation. The Doppler indices of the uterine artery on the placental side are significantly lower than on the nonplacental side. A centrally located placenta demonstrates similar Doppler indices on both sides (Bewley et al. 1989). Abnormal uterine artery blood velocity waveforms are identified by a persistent abnormal index, a persistent diastolic notch or an abnormal difference between the indices in the left and right uterine arteries (Fleischer et al. 1986, Thaler et al. 1992).

2.3.2 Umbilicoplacental hemodynamics

The umbilical circulation has been studied by Doppler ultrasonography from 7 weeks of gestation onwards (den Ouden et al. 1990, Wladimiroff et al. 1991). After 12 gestational weeks, the diastolic blood flow component becomes progressively present and should be present in all pregnancies after 16 weeks. From 20 gestational weeks onwards, the growth of the placental unit with an increase in the number of functioning vascular villae decreases its vascular impedance (Trudinger et al. 1985). In humans, umbilical blood flow increases in direct proportion to the increase in human fetal body weight, so that weight-indexed flow remains approximately constant at 110 ml/min/kg to 125 ml/min/kg over the last third of the pregnancy (Gill et al. 1981, Sutton et al. 1990). In near-term fetal sheep, it has been demonstrated that about 45% of fetal CCO is directed to the placenta (Itskovitz et al. 1987). In human studies, about 30% of CCO is estimated to be directed to the placenta (Sutton et al. 1991a).

Abnormal umbilical artery blood velocity waveforms in humans are associated with a decrease in the number of small muscular arteries in the placental tertiary stem villi (Giles et al. 1985a, Giles et al. 1985b). These morphologic changes may deteriorate the placental oxygen and other nutritional transport, and lead to FGR. Abnormal umbilical artery findings are demonstrated to correlate better with fetal acidosis than a non-stress test (Arduini et al. 1991). In addition, abnormal umbilical artery blood velocity waveforms are shown to precede pathologic non-stress tests (Donner et al. 1995). On the other hand, even profound fetal acidemia does not change the umbilical artery waveforms, unless the fetus becomes hypotensive (Muijsers et al. 1990, Morrow et al. 1990). Occlusion of placental arteriolar bed by plastic microspheres leads to increased placental vascular resistance and to a diminished umbilical artery diastolic blood flow.
component (Trudinger et al. 1987). It has been estimated that over 60% of the
intraplacental fetal vasculature must be occluded before significant changes in umbilical
artery blood velocity waveform pattern occur (Thompson & Trudinger 1990).

The diastolic blood flow component in umbilical artery increases with advancing
gestation. In late second and third trimesters, the systolic to diastolic velocity-ratio (S/D-ratio) is considered to be increased if it is ≥ 3.5 (Forouzan et al. 1991). Absent diastolic velocity (AEDV) or retrograde diastolic velocity (REDV) in the umbilical artery indicate extremely increased placental vascular impedance. Usually, AEDV persists and occasionally deteriorates into REDV. The median interval between the appearance of AEDV and abnormal fetal heart rate tracings is about one week with very wide individual variability (Arduini et al. 1993). Fetal condition cannot be inferred from AEDV alone because AEDV itself is not diagnostic for hypoxemia and acidemia at the delivery (Nicolini et al. 1990). By the time REDV is detected, severe fetal compromise with acidemia and high perinatal mortality is evident (Brar & Platt 1988, Kurkinen-Raty et al. 1997). In animal studies, REDV immediately preceded fetal death (Morrow et al. 1989). In humans, structurally normal fetuses with umbilical artery AEDV or REDV comprised 19% of all deaths occurring before 30 weeks of gestation. The perinatal mortality rate was significantly higher for fetuses with REDV (35.7%) than for fetuses with AEDV (8.9%) (Kurkinen-Raty et al. 1997). The incidence of FGR with AEDV has been reported to be 83% and over 50% of the pregnancies with AEDV are complicated by maternal hypertensive disorders. There is also an increased incidence of fetal abnormalities in pregnancies complicated by AEDV (Farine et al. 1994). In a meta-analysis, umbilical artery blood flow assessment in high-risk pregnancies reduced the odds of perinatal death by 38% without an increase in the rate of unnecessary obstetric interventions (Alfirevic & Neilson 1995, Divon 1996).

2.3.3 Fetal heart

2.3.3.1 Cardiac output and its distribution

In appropriately grown human fetuses, stroke volume increased exponentially from 0.7 ml at 20 weeks to 7.6 ml at 40 weeks for the right ventricle (r = 0.87) and from 0.7 ml at 20 weeks to 5.2 ml at 40 weeks for the left ventricle (r = 0.91). Right (RVCO) and left (LVCO) ventricular cardiac outputs increased respectively (Kenny et al. 1986). During the second half of human gestation, RVCO was reported to increase from 100 ml/min to 1000 ml/min, while LVCO increased from 100 ml/min to 800 ml/min (Rasanen et al. 1996a). Right ventricular dominance persisted during the second half of gestation, and it even increased towards the term of gestation in appropriately grown human fetuses (Rasanen et al. 1996a). In FGR, a relative increase in LVCO associated with decreased RVCO has been demonstrated (al-Ghazali et al. 1989). Studies of progressively deteriorating growth-restricted fetuses have shown gradually decreasing cardiac output
(Rizzo & Arduini 1991). In addition, there is evidence of a temporal association between a drop in cardiac output and the onset of late heart rate decelerations (Rizzo & Arduini 1991).

The distribution of fetal CCO changes during gestation (Rudolph & Heymann 1970, Rasanen et al. 1996a). In studies on near-term fetal sheep, it has been demonstrated that approximately 2.5% of CCO is directed to the heart, 3.7% to the brain, 8.0% to the lungs, 31% to the carcass, and 45% to the placenta, respectively (Itskovitz et al. 1987). In late third trimester human fetuses, the proportion of right (RVCO%) ventricular cardiac output (60%) of CCO is significantly higher than the proportion of left (LVCO%) ventricular cardiac output (40%) of CCO (Rasanen et al. 1996a). The proportion of human fetal pulmonary volume blood flow (Qp) of CCO increased from 13% to 25% between 20 and 30 weeks of gestation, and after 30 weeks of gestation, it remained unchanged (Rasanen et al. 1996a). On the other hand, Sutton et al. found a four-fold increase in the absolute pulmonary volume blood flow, which comprised 22% of CCO between 18 and 38 weeks of gestation (Sutton et al. 1994). Maternal hyperoxygenation did not change the reactivity of the human fetal pulmonary circulation between 20 and 26 weeks. Between 31 and 36 weeks, the PI values of right and left pulmonary arteries decreased, the PI of DA increased. At the same time Qp increased while ductus arteriosus (QDA) and foramen ovale (QFO) volume blood flows decreased significantly with unchanged LVCO and RVCO (Rasanen et al. 1998). This suggests that fetal pulmonary circulation is under acquired vasoconstriction at least beyond 32 gestational weeks. The proportion of QDA remained stable during the second half of the gestation, varying between 32% and 40% of CCO. Between 20 and 30 weeks of gestation, the proportion of QFO has been demonstrated to decrease from 34% to 18% of CCO. After 30 weeks, QFO did not change (Rasanen et al. 1996a). Sutton et al. reported that blood flow through FO increased threefold between 18 and 38 weeks of gestation representing 31% to 17% of CCO (Sutton et al. 1994).

2.3.3.2 Systolic function

The right (RVeFo) and left (LVeFo) ventricular ejection forces are calculated by the formula: (1.055xCSAxTVIac)x(PSV/TTP), in which TVIac is the TVI during acceleration period in systole, PSV is the peak systolic velocity, and TTP is the time period from the onset of ejection to systolic peak velocity (Isaaz et al. 1989, Sutton et al. 1991b). Both RVeFo and LVeFo, which estimate the energy transferred from the ventricular myocardial shortening to work done by accelerating blood into the circulation, increase significantly during the second half of pregnancy with no difference between the ventricles (Sutton et al. 1991b). Ventricular ejection force calculation does not require estimation of ventricular volumes and is independent of ventricular configuration. Animal studies have suggested that early systolic flow is less affected by changes in afterload and preload than flow during late systole (Noble 1968). In addition, blood acceleration seems to be closely related to the force developed by the ventricle in early systole (Noble et al. 1966). In growth-restricted fetuses, ventricular ejection forces were reduced compared to normal fetuses. The degree of impairment seemed to be related to the severity of fetal
compromise (Rizzo et al. 1995a). In human fetuses with severe ductal constriction or occlusion, RVeFo was decreased, showing that a marked increase in the right ventricular afterload affects RVeFo development (Rasanen et al. 1997).

In the presence of mitral (MR) or tricuspid regurgitation (TR), ventricular contractility can be assessed from the upstroke of the regurgitation jet (dP/dT) (Fig. 3) (Tulzer et al. 1991b). It may have prognostic value in pregnancies complicated by nonimmune hydrops fetalis (Tulzer et al. 1991b).

The index of myocardial performance (IMP) describes the combined systolic and diastolic performance of the heart with the formula: $\text{IMP} = (\text{ICT} + \text{IRT}) / \text{ET}$, in which IRT and ICT are isovolumetric relaxation and contraction times, and ET is ejection time (Fig. 4) (Tei et al. 1995, Tsutsumi et al. 1999). The IMP of the fetal left and right ventricles decreases during the second half of the gestation (Tsutsumi et al. 1999). Tsutsumi et al. (Tsutsumi et al. 1999) found no difference in IMP values between 18 and 26 weeks of gestation in fetuses with FGR. However, from 27 to 40 weeks of gestation, IMP was significantly greater in fetuses with FGR than in the controls (Tsutsumi et al. 1999).

Fig. 3. Holosystolic tricuspid valve regurgitation.
Fig. 4. Schematic and ultrasonographic presentation of left ventricular time-intervals. ICT = isovolumetric contraction time, ET = ejection time, IRT = isovolumetric relaxation time, FT = filling time, early passive (E-wave) and atrial contraction (A-wave) waves of inflow waveform.

2.3.3.3 Diastolic function

The IRT is the time interval between closure of the semilunar valve and opening of the atrioventricular valve (Fig. 4). It represents the time period needed for the ventricle to actively decrease its pressure to the atrial level. The proportion (%) of IRT of the total cardiac cycle can be used to describe diastolic function of the heart. No change in fetal IRT% has been found in normal pregnancies during the second half of gestation (Tulzer et al. 1994). In growth-restricted fetuses, an increase in left ventricular IRT may reflect cardiac diastolic dysfunction (Tsyvian et al. 1995).

At the level of the atrioventricular valves, monophasic inflow pattern changes to biphasic after the 8th gestational week (van Splunder et al. 1995, van Splunder et al. 1996, Leiva et al. 1999). The biphasic inflow waveform contains two peaks representing early passive filling (E-wave) and atrial contraction (A-wave) during atrial systole (Fig. 4). Both E- and A-wave maximum velocities as well as total- and A-wave- TVI of both valves increased significantly with advancing gestation, and the A/E velocity-ratio decreased significantly (Tulzer et al. 1994, Veille et al. 1999). Normally, the maximum velocity of A-wave is higher than that of E-wave in human fetuses (Tulzer et al. 1994), and E- and A-wave velocities are higher in the tricuspid valve (TV) than in the mitral valve (MV), with no difference in A/E velocity ratio between the valves (Tulzer et al. 1994). The A-TVI/total TVI-ratio did not change significantly with advancing gestation, but this ratio was significantly higher at TV than at MV (Tulzer et al. 1994). The inflow waveforms are affected by preload of the heart and ventricular compliance. During the
first year of life, inflow velocities remain unchanged, but the ventricular filling shifts towards an early passive filling period (Veille et al. 1999). However, the E/A velocity- and TVI- ratios can also reflect atrial pressure. In growth-restricted fetuses, the TV and MV E/A velocity- ratio was significantly lower than in normal fetuses (Rizzo et al. 1988). However, no consistent differences were found in E/A velocity- ratios between compromised and noncompromised fetuses (Hecher et al. 1995a).

2.3.3.4 Afterload

The ratio of cardiac circumference to thoracic circumference (CC/TC) and their area ratio are used to evaluate the heart size. The ratio between the fetal cardiac and thoracic areas remains constant during the second half of pregnancy at 0.30 (0.05) (mean (SD)) (Respondek et al. 1992). In normal pregnancies, the CC/TC-ratio is found to be normal when less than 50% (Huhta et al. 2000). In cases with suspected cardiomegaly, including fetal hydrops, arrhythmias and increased septal thickness due to maternal diabetes, these ratios have been demonstrated to be significantly greater than in the normal group (Respondek et al. 1992).

In fetuses with normal cardiac anatomy, the prevalence of TR is 6.8% (Respondek et al. 1994). Tricuspid regurgitation has been classified to be trivial (nonholosystolic ≥72ms, maximum velocity <2m/s) or significant (holosystolic, maximum velocity >2m/s) (Fig. 3). Fetal TR can be a sign of elevated right ventricular pressure related to the conditions causing increased afterload, including the constriction of the DA and severe placental insufficiency. In addition, it can be related to the conditions causing increased preload of the heart, such as increased volume overload (Respondek et al. 1994). A change in the compliance of the right ventricle, for example, due to hypertrophy of the ventricular wall may lead to the development of TR. It has been speculated that TR is more uncommon in the fetus than in the newborn, because the fetal right ventricle functioning at systemic blood pressure level optimally supports the competent TV. The lower pressure in the right ventricle after birth results in nonsynchronous leaflet closure (Respondek et al. 1994).

Right (RVFS) and left (LVFS) ventricular fractional shortenings are calculated by the formula (ventricular fractional shortening (%) = [(inner diastolic diameter – inner systolic diameter)/inner diastolic diameter] x 100) (DeVore et al. 1984). They are obtained from M-mode recordings by placing the M-mode cursor perpendicularly towards the interventricular septum at the level of atroventricular valves in the four chamber-view of the heart (DeVore et al. 1984). In normal fetuses, the diameter of the right and left ventricles correlated with the biparietal diameter, and the right/left ratio of the ventricular diameters remained constant (1:1) throughout gestation. In addition, RVFS and LVFS were independent of gestational age, being between 28–40% during the second half of gestation (DeVore et al. 1984). An abnormal ventricular fractional shortening can reflect myocardial compromise or an increase in the ventricular workload (Huhta et al. 2000). In acute fetal ductal occlusion in lambs, the right ventricular systolic dimension increased and RVFS decreased due to an increase in the right ventricular afterload (Tulzer et al. 1991a). In fetuses with intrauterine distress, RVFS was found to be decreased and the
ratio of the right and left ventricular end-diastolic diameters was increased, while no difference in the left ventricular size and myocardial contractility was observed (Rasanen et al. 1989).

### 2.3.4 Fetal arterial circulation

Placental insufficiency may lead to inadequate tissue perfusion which can activate fetal compensatory mechanisms including increased oxygen extraction, redistribution of blood flow and blood volume augmentation.

Throughout the normal pregnancy, there is a continuous diastolic component in the blood velocity waveform patterns of cerebral arteries. In late gestational fetuses, the PI of MCA decreases (Woo et al. 1987, van den Wijngaard et al. 1989, Mari & Deter 1992). Animal studies have shown that during chronic hypoxemia, the fetus redistributes the blood flow to the brain and the myocardium at the expense of the lower body (Peeters et al. 1979). Accordingly, Doppler studies of growth-restricted and hypoxemic human fetuses have demonstrated reduced impedance and increased blood velocities in the internal carotid and middle cerebral arteries, and opposite changes in the DAo, the so-called brain-sparing effect (Jouppila & Kirkinen 1984, Wladimiroff et al. 1986, Vyas et al. 1990, Bilardo et al. 1990). The ratio between umbilical artery and fetal MCA PI values has been demonstrated to be the best predictor of adverse perinatal outcome (Wladimiroff et al. 1987). In addition, blood flow velocities in renal, adrenal and abdominal arteries have been investigated, but their clinical significance is unclear (Mari et al. 1995, Rizzo et al. 1995b, Tekay & Jouppila 2000).

During the second half of gestation, the blood velocity waveform pattern in DAo is normally continuous throughout the cardiac cycle due to low vascular resistance in placental circulation. It has been demonstrated that weight-indexed blood flow of DAo remains stable until 37 weeks of gestation, after which it slightly decreases (Marsal et al. 1987). In normal pregnancies, the total blood flow in the aorta has been demonstrated to correlate well with heart size and left ventricular output (Rasanen et al. 1988). In pregnancies complicated by hypertensive disorders, fetal aortic velocities were lower and the waveform indices were higher than in normal pregnancies, especially in FGR (Jouppila & Kirkinen 1984, Rasanen et al. 1988). In addition, fetuses in diabetic pregnancies had smaller volume blood flow in DAo than in the normal pregnancies (Rasanen et al. 1988). The ratio between DAo and MCA PI values has been used to describe redistribution of arterial circulation (Harrington et al. 1995).

The myocardium receives approximately 8% of the LVCO (Baschat et al. 1998). Technical development has enabled the visualization of the fetal coronary arteries. The right and left coronary arteries originate from the aortic sinuses. They supply the myocardium with highly oxygenated blood returning from the placenta via DV and FO to left ventricle. In appropriately grown fetuses with normal perinatal outcome, coronary artery blood flow was visualised in 10% of the fetuses after 31 gestational weeks with a median of 37 gestational weeks (Baschat et al. 1997). In FGR, the coronary artery blood flow was detected already at 27 gestational weeks in 20% of cases (heart-sparing effect). Baschat et al. (Baschat et al. 1997) also showed that visualization of coronary artery
blood flow was associated with a 50% mortality rate in FGR. Hypoxemia may cause vasodilatation of the coronaries and increase their blood flow to a demonstrable magnitude (Fig. 5) (Gembruch & Baschat 1996).

![Coronary artery blood velocity waveform of a growth-restricted 32 week fetus (heart sparing effect).](image)

Two parallel circulatory systems (right and left ventricles) in the fetus are connected to each other by the aortic isthmus. Aortic isthmus blood velocity waveforms are recorded by placing the Doppler gate along the aortic arterial bridge between its junction with left subclavian arteria and DA (Fig. 6) (Fouron et al. 1994). Experimental studies have demonstrated that an increase in placental resistance causes changes in aortic isthmus blood flow profile before any significant change in umbilical artery Doppler blood velocity waveforms occurs (Bonnin et al. 1993). In normal human pregnancies, fetal aortic isthmus may reveal a short retrograde blood velocity waveform component during the last trimester which may indicate normal physiologic increase in the placental vascular resistance or in the fetal systemic vascular resistance (Fig. 6) (Fouron et al. 1994, Rasanen et al. 1996b). In addition, fetal lamb studies have suggested that the delivery of oxygen to the brain is preserved as long as the net blood flow in the fetal aortic isthmus is antegrade. In cases with retrograde aortic isthmus net blood flow, the delivery of oxygen has been shown to be diminished despite slightly increased volume blood flow to the cerebral arteries (Fouron et al. 1999).
2.3.5 Fetal venous circulation

Studies on fetal lambs have shown that oxygenated blood returning from the placenta via umbilical veins flows through the DV and left hepatic vein (LHV), and is mainly directed towards FO and the left atrium (Edelstone & Rudolph 1979). This has been confirmed also in human fetuses (Kiserud et al. 1991, Kiserud et al. 2000a). Compared to the 40–50% shunting of umbilical blood through DV found in animal experiments, the degree of shunting in the human fetus under physiologic conditions is reported to be less: 30–40% at 20 weeks and decreasing to 18% at 32 weeks, and to 15% at 38 weeks of gestation (Kiserud 2000b, Bellotti et al. 2000). The decrease in weight-indexed DV volume blood flow was from 60 ml/min/kg to 17 ml/min/kg (Bellotti et al. 2000). The right hepatic vein and IVC carry the lowest oxygen saturated blood mainly from the fetal lower body to the right atrium and across the tricuspid valve to the right ventricle (Rudolph 1985). The distribution of the venous return is designed to optimize adequate oxygen supply to the myocardium and the brain. It has been estimated in studies on fetal lamb that during hypoxemia or reduced umbilical flow, the blood shunted across DV increases and could reach as much as 70% of the umbilical blood flow (Behrman et al. 1970, Edelstone & Rudolph 1979). Active dilatation of DV and increased shunting has also been observed in human fetuses (Gennser 1992, Bellotti et al. 1998).
Pulsation in the umbilical vein is a normal finding during the first trimester of pregnancy, and normally pulsations have completely disappeared after 13 gestational weeks (Rizzo et al. 1992). Umbilical venous pulsations in ventricular systole are sometimes seen in oligohydramniotic fetuses and fetuses with an arteriovenous fistula. The systolic pulsation is thus transmitted directly to the local venous flow signal (Nakai et al. 1997). In severe placental insufficiency, pulsations in umbilical vein, increased reverse flow component in the fetal IVC and hepatic veins, and decreased or reversed flow component in DV during the atrial contraction have been observed (Fig. 7) (Hecher et al. 1995a). Umbilical venous pulsations during atrial systole are atrial contraction pressure waves which are transmitted from the right atrium back to the venous circulation (Huhta 1997). Elevated end-diastolic pressure in the right ventricle causes an increase in reversal of flow during atrial systole in IVC, hepatic veins, DV and umbilical vein. The changes in umbilical venous velocities originate in the fetal venous system and are transmitted to, rather than from, the placenta (Reed & Anderson 2000). The distance that these pressure waves are transmitted is proportional to the central venous pressure, the venous compliance and the force of atrial contraction (Reuss et al. 1983, Indik et al. 1991, Gudmundsson et al. 1991). Gudmunsson et al. (Gudmundsson et al. 1996) have also described umbilical venous pulsations in severe placental insufficiency (AEDV) with normal IVC and DV Doppler findings. They speculated that atrial pulsations transmitted through severely insufficient placenta could explain this finding. The volumetric blood flow values between umbilical arteries and umbilical vein seem to correspond with each other (Ferrazzi et al. 2000). Blood flow through the umbilical vein increases significantly between 20 and 38 weeks of gestation with no significant decrease (from 123 ml/min/kg to 109 ml/min/kg) in weight-indexed umbilical vein volume blood flow (Bellotti et al. 2000). Umbilical vein volumetric blood flow in fetuses with abnormal umbilical artery blood velocity waveforms (63–98 ml/min/kg) was significantly lower than in control fetuses (117–124 ml/min/kg) at any gestational age between 25 and 38 weeks (Ferrazzi et al. 2000).

Growth-restricted fetuses with abnormal blood velocity waveforms in their IVC had significantly increased ANP levels, indicating increased systemic venous pressure in these fetuses (Capponi et al. 1997). In addition, it has been demonstrated that growth-restricted fetuses with pathological umbilical venous pulsations have significantly lower pH and pO₂ values and higher pCO₂ values than those without pulsations (Rizzo et al. 1995c). Gudmundsson et al. (Gudmundsson et al. 1991) reported only four survivors out of 14 fetuses with nonimmune hydrops and umbilical venous pulsations, while all the fetuses without umbilical venous pulsations survived. With further widening of the ductus venosus and/or increased central venous pressure, the pulsating pattern in the umbilical vein may become biphasic. Double umbilical venous pulsations are associated with worse perinatal outcome than single pulsations (Hofstaetter et al. 2001).
2.4 Cardiac troponin T (cTnT)

2.4.1 Release and elimination of cTnT

Three troponin T genes have been described on the basis of molecular cloning in humans (Townsend et al. 1994). These are expressed in a tissue-specific manner and encode the troponin T isoforms expressed in cardiac muscle, slow skeletal muscle, and fast skeletal muscle, respectively. Multiple cTnT messenger RNAs have been shown to be present in the human heart, and a single-copy gene locus is mapped to the long-arm of chromosome 1 (Townsend et al. 1994). The human heart expresses four cTnT isoforms (cTnT1 through cTnT4) (Townsend et al. 1994, Saba et al. 1996). Cardiac troponin T is a thin filament protein which takes part in muscle contraction. Cardiac myocytes contain contractile proteins, which mainly form longitudinally running myofibrils. Each myofibril contains about 60 similar consecutive contractile sarcomeres. The sarcomeres are separated from each other by electron microscopically-detected Z-zones. Light fibers formed from actin fibers are linked to Z-zones. The light fibers contain the tropomyosin-troponin group. The troponin group consists of troponin C, troponin I and troponin T. The troponin complex on the actin filament regulates the force and the velocity of muscle contraction. Troponin
C functions as a calcium-receptor while troponin I prevents the adenosine triphosphatase activity when bound to actin. Troponin T fixes the troponin group to tropomyosin. During the relaxation period, the troponin group is bound to actin and tropomyosin, blocking the interaction of myosin and actin. The contraction of the sarcomere starts when calcium will be bound by troponin C, resulting in unlinkage of troponin I and actin. Troponin C is thus the protein which triggers heart muscle contraction. (Fig. 2). It has been shown in patients with congenital cardiac defects that cTnT is increased in hearts that are hemodynamically highly stressed (Saba et al. 1996).

After myocardial cell damage, troponins are released from the myocytes. The cTnT levels are detectable in 3–12 hours after the myocardial injury, and the concentration is in direct proportion to the extent of myocardial injury. Mean time to peak cTnT level is about 12–48 hours. The concentration returns to normal range after 5–14 days, which is four times longer than with creatine kinase myocardial band isoenzyme (CK-MB) fraction, probably due to sustained release of structurally bound protein from disintegrating myofibrils (Donnelly & Millar-Craig 1998). Cardiac troponin T is very cardiac specific, and it is not present in the serum following nonmyocardial muscle or other tissue damage (Gerhardt et al. 1991). In contrast to CK-MB-fraction, cTnT is more cardioselective, and persists longer and shows higher elevations relative to normal ranges (Karim et al. 1995). For these reasons, troponins are routinely used to diagnose acute myocardial ischemia.

### 2.4.2 Physiologic effects of cTnT

In a study on transgenic mice, the mutant human cTnT responsible for human hypertrophic cardiomyopathy leads to disarray, increased collagen synthesis in the heart, as well as diastolic dysfunction (Oberst et al. 1998). It has been proposed that impaired cardiac myocyte function leads to synthesis of a number of autocrine and paracrine factors, such as angiotensin II, that not only mediate the increased collagen synthesis but also cardiac hypertrophy. With current assays, a significant diagnostic difference does not appear to exist between cardiac troponin I and cTnT in patients with acute coronary artery syndromes (Adams 1999).

### 2.4.3 Cardiac troponin T in myocardial lesions

Analysis of cTnT has shown to be effective in detecting minor myocardial injury in human patients with acute coronary syndromes. The low cut-off concentration (0.10 ng/ml) is based on the fact that cTnT is not increased in patients with skeletal muscle disease or injury, resulting in low baseline levels of cTnT in the absence of active cardiac disease. In addition, cTnT has a higher myocardial tissue content relative to CK-MB fraction, thereby increasing its clinical sensitivity to irreversible injury. Cardiac troponin I and cTnT have been detected in adult patients with advanced congestive heart failure. Their
concentrations correlated with the severity of congestive heart failure and suggest an association with worse prognosis (Del Carlo & O'Connor 1999). In pediatric patients at risk of myocardial injury due to cardiovascular surgery or dosing of cardiotoxic doxorubicin for acute lymphoblastic leukemia, elevations in blood cTnT related to the severity of myocardial damage and predicted subsequent subclinical and clinical cardiac morbidity and mortality (Lipshultz et al. 1997).

2.4.4 Pregnancy and cTnT

The serum cTnT concentration in 15 neonates of pre-eclamptic mothers (0.70 ng/ml) was significantly higher than that in 17 control neonates (0.10 ng/ml). Neonates with increased cTnT demonstrated lower mitral A-wave maximum velocity (39 cm/s) than control fetuses (53 cm/s), and their mitral E/A peak velocity-ratio was higher (1.75) than that of control fetuses (1.23) (Narin et al. 1999). In addition, elevated umbilical artery and vein cTnT levels have been demonstrated to correlate with maternal magnesium sulfate exposure (Shelton et al. 1999). Elevated maternal cTnT levels have been associated with tocolytic therapy, while umbilical artery and vein cTnT concentrations did not correspond with maternal cTnT levels (Adamcova et al. 1999). Cord blood cTnT levels are reported to be unaffected by gestation, birth weight, sex, or mode of delivery (Clark et al. 2001). Cardiac troponin I and cTnT have been demonstrated to be useful biochemical markers for monitoring pregnant women for myocardial injury (Shivvers et al. 1999).

2.5 Atrial natriuretic peptide (ANP)

2.5.1 Synthesis and release of ANP

In 1981, de Bold and co-workers found that granule-enriched atrial extracts contained a substance which caused natriuresis and vasodilatation (de Bold et al. 1981). Atrial natriuretic peptide molecule was purified and sequenced two years later (Flynn et al. 1983). Soon after that B- (BNP) and C-type natriuretic peptides (CNP) were found. The main source of BNP is the cardiac ventricle, although it was initially found in porcine brain (Sudoh et al. 1988). C-type natriuretic peptide was first localised in the nervous system (Sudoh et al. 1990), but later found to be produced by the endothelial cells (Suga et al. 1992). The gene of ANP is located on chromosome 1 in humans. Transcription of the ANP gene yields a messenger RNA species that encodes a 151-amino acid pre-proANP precursor containing a 25-amino acid signal sequence. This signal peptide is important for the translocation of pre-proANP from the ribosome into the sarcoplasmatic reticulum. Pre-proANP is converted after cleavage of the signal peptide to a 126-amino acid proatrial natriuretic peptide, proANP1–126, which is the principal storage form of
ANP. The ProANP\textsubscript{1–126} is transported through the Golgi complex to secretory granules of atrial cardiocytes, and finally released by exocytosis to the extracellular space (Ruskoaho 1992). It is cleaved to the N-terminal fragment (proANP\textsubscript{1–98} = NT-proANP) and the major biologically active hormone (C-terminal peptide ANP\textsubscript{99–126}), which is more commonly marked as ANP\textsubscript{1–28} or ANP (Michener \textit{et al.} 1986, Sundsfjord \textit{et al.} 1988, Thibault \textit{et al.} 1988). Thus NT-proANP and ANP are produced in equimolar amounts. Cleavage of ProANP\textsubscript{1–126} into ANP and NT-proANP occurs in connection with exocytosis, presumably by the membrane-bound endonuclease Corin (Yan \textit{et al.} 2000). The circulating 28-amino acid human ANP is the biologically active form (Misono \textit{et al.} 1984).

The ANP gene is very actively expressed in fetal and neonatal ventricle (Sagnella 1998). Soon after birth, the ANP expression in the ventricles decreases to very low levels, but it can be re-induced by increased ventricular load. Elevation of the activity of ANP gene represents the return to fetal phenotype in response to ventricular load, together with the induction of genes of skeletal alpha actin, myosin light chain 1 and beta-tropomyosin (Nadal-Ginard & Mahdavi 1993). In addition, detectable levels of ANP messenger RNA have been found in central nervous system, lung, adrenal gland, kidney and vascular tissue. However, these levels are less than 1% of those detected in the atria, and therefore unlikely to have significant contribution to ANP plasma concentrations (Rosenzweig & Seidman 1991, Ruskoaho 1992).

2.5.2 Regulation of ANP release

The predominant signal for ANP release is atrial wall stretch or atrial distension due to volume expansion (Lang \textit{et al.} 1985). Hypoxia is also a potent stimulus to ANP release (Lew & Baertschi 1989). Atrial stretch, increased heart rate, sympathetic stimulus and metabolic factors may mediate this effect (Ruskoaho 1992). Enhanced ANP release resulting from hyperosmolality with volume expansion has also been demonstrated (Arjamaa & Vuolteenaho 1985).

Endothelin-1, a potent vasoconstrictor of vascular smooth muscle, induces ANP secretion directly from the heart (Mantymaa \textit{et al.} 1990). Inhibition of endothelin-1 receptors decreases the ANP release induced by the volume load (Leskinen \textit{et al.} 1997). Endothelin-1 may also mediate atrial stretch-induced ANP release and effects of pressor hormones on the stress-activated release of ANP (Ruskoaho 1992). Endothelium- or endocardium-derived nitric-oxid may have an inhibitory effect on ANP secretion (Leskinen \textit{et al.} 1995). In addition, catecholamines (Ruskoaho 1992), acetylcholine (Ruskoaho \textit{et al.} 1985), angiotensin, arginine vasopressin, prostaglandins (Ruskoaho 1992) and both glucocorticoids and thyroid hormones increase circulating ANP levels (Rosenzweig & Seidman 1991).
2.5.3 Physiologic effects of ANP

Atrial natriuretic peptide exerts its effects by binding to specific membrane-bound receptors. Three natriuretic peptide receptors have been identified. The ANP\textsubscript{A} and ANP\textsubscript{B} receptors have guanylate cyclase activity and mediate the biological effects of the natriuretic peptides. The ANP\textsubscript{C} receptor functions mainly as a clearance receptor removing ANP from the circulation. All natriuretic peptides are bound by the ANP\textsubscript{C} receptor. Atrial natriuretic peptide and BNP act through the ANP\textsubscript{A} receptor and CNP through the ANP\textsubscript{B} receptor (Yandle 1994).

The main targets of ANP are kidneys and vascular smooth muscle. It decreases blood pressure due to a direct relaxation of vascular smooth muscle. In addition, it increases salt and water excretion, enhances capillary permeability, and inhibits the release or action of several hormones, such as aldosterone, angiotensin II, endothelin, renin and vasopressin (Ruskoaho 1992). The natriuretic effect results from a direct inhibition of sodium absorption in the renal collecting duct, increased glomerular infiltration and inhibited aldosterone production and secretion (Rosenzweig & Seidman 1991). Atrial natriuretic peptide therefore counteracts the renin-angiotensin-aldosterone system. Thus, increased adult ANP levels are detected in adult congestive heart failure, chronic renal failure and in severe essential hypertension (Ruskoaho 1992, Yandle 1994).

2.5.4 Elimination of ANP

The half-life of ANP is 2 to 5 minutes in humans and its metabolic clearance rate is about 14 to 25 ml/min/kg (Rosenzweig & Seidman 1991). The hormone is eliminated either enzymatically or through the clearance receptor. The ANP\textsubscript{C} receptor internalizes ANP and delivers it to lysosomes for degradation while the receptor itself is recycled. The most important enzyme regulating elimination of ANP is neutral endopeptidase 24.11. It is present in the glomerulus, in kidney smooth muscle cells, and at high levels in brush border membranes of the proximal tubule. It is also expressed in vascular tissue and vascular smooth muscle cells (Yandle 1994). Experimental and human studies have shown that inhibition of this enzyme potentiates the action of ANP (Kukkonen \textit{et al.} 1992). Inhibitors that inhibit both angiotensin converting enzyme and neutral endopeptidase 24.11 are more potent antihypertensive agents than pure angiotensin converting enzyme- inhibitors (Backlund \textit{et al.} 2001).

2.5.5 N-terminal peptide of proatrial natriuretic peptide (NT-proANP)

N-terminal peptide of proatrial natriuretic peptide (NT-proANP) is secreted in equimolar amounts with ANP (Itoh \textit{et al.} 1988). Its high plasma concentration relative to ANP is probably due to its longer half-life in the circulation (Thibault \textit{et al.} 1988). This is reflected in the proportionally larger increase in NT-proANP levels (15–20 fold).
compared with those of ANP (4–5 fold) in subjects with congestive heart failure and chronic renal failure (Yandle 1994). Elevated circulating NT-proANP levels in cases of congestive heart failure have been suggested to demonstrate symptomless left ventricular dysfunction (Lerman et al. 1993, Kettunen et al. 1994). The NT-proANP is eliminated by the kidney. Demonstration of biological activity of NT-proANP has failed, probably due to the absence of a specific receptor for the N-terminal fragment (Ruskoaho 1992). The NT-proANP assay can be used to characterize endogenous ANP secretion (Itoh et al. 1988).

### 2.5.6 Pregnancy and ANP

Conflicting data concerning the synthesis or storage of ANP in the placenta have been published (Inglis et al. 1993, McQueen et al. 1993). In experimental studies on rats, it has been shown that ANP does not cross the placenta (Mulay & Varma 1989). The lack of correlation between maternal and neonatal ANP concentrations supports the view that ANP does not cross the human placenta either (Shilo et al. 1989).

The fetus appears to produce its own atrial natriuretic factor (Hatjis et al. 1989). ANP has been detected as early as 16 gestational weeks in umbilical venous blood samples taken by cordocentesis (Ville et al. 1994). Fetuses with Rhesus isoimmunisation, characterized by long-term cardiac overload, showed significantly higher ANP levels compared to the controls (Kingdom et al. 1991, Ville et al. 1994, Walther et al. 2001). Fetal ANP levels correlated inversely with hematocrit, but not with gestational age. Fetal ANP levels showed a significant rise after transfusion, and this rise was related to the percentage of fetoplacental blood volume transfused. The changes in fetal ANP levels due to the volume expansion have been demonstrated as early as 21 weeks’ gestation (Panos et al. 1989). A weak, statistically non-significant, correlation was found between the change in fetal ANP levels and transient reductions in umbilical artery S/D-ratio (Kingdom et al. 1991). Increased neonatal ANP-levels as well as increased fetal umbilical venous ANP-levels have been reported in cases with growth restriction (Kingdom et al. 1992, Ville et al. 1994) and in fetuses born to patients with severe preeclampsia (Hatjis et al. 1989). In addition, studies on mice suggest that the maternal diabetes-induced increase in fetal ANP might be related to fetal myocardial hypertrophy (Mulay et al. 1995).

### 2.6 Validation of cTnT and NT-proANP assays

The performance of cTnT assay at low levels and the analytical limit of sensitivity have been established in children (Lipshultz et al. 1997). A precision study using serial dilutions of serum with elevated cTnT showed the linearity of the serum cTnT assay in the low range of 0.01 to 0.10 ng/ml (Lipshultz et al. 1997).

The sensitivity of the NT-proANP assay is 30 pmol/l with good reproducibility. The antiserum cross-reacts 100% with the purified human proatrial natriuretic peptide 1–126, whereas it does not recognize ANP itself, BNP or CNP (cross-reactivity less than 0.01%).
In the radioimmunoassay, the intra- and interassay coefficients of variation were less than 10% and 15%, respectively (Vuolteenaho et al. 1992). NT-proANP circulates in plasma in higher concentration, and it is more stable ex vivo than ANP. NT-proANP is shown to correlate well with ANP (Boomsma et al. 1996).
3 Hypothesis of the study

In placental insufficiency, several abnormalities in the fetal cardiovascular hemodynamics have been described by Doppler ultrasonography. The first hypothesis to be tested in this study is that there exists a relationship between Doppler ultrasonographic parameters and biochemical markers of fetal cardiac dysfunction and myocardial cell damage in pregnancies complicated by placental insufficiency and/or fetal growth restriction. Fetal lamb studies have revealed that oxygen content of the blood entering the cerebral circulation is maintained until aortic isthmus net blood flow becomes retrograde. The second hypothesis of this thesis is that retrograde aortic isthmus net blood flow is associated with changes in fetal cardiac function and peripheral arterial and venous circulations.

The specific aims of the research project are:

1. To determine the significance of atrial pulsations in the umbilical vein as regards to biochemical evidence of fetal cardiac dysfunction and cell damage (I–II).
2. To establish Doppler ultrasonographic parameters of human fetal central and peripheral circulations that are associated with biochemical evidence of cardiac dysfunction and myocardial cell damage (III).
3. To find the changes in fetal cardiac hemodynamics associated with diminished oxygen delivery to cerebral circulation in fetuses with signs of placental insufficiency (IV).
4. To demonstrate the changes in fetal peripheral arterial and venous circulations which are associated with retrograde net blood flow in the aortic isthmus in fetuses with placental insufficiency (V).
4 Material and Methods

4.1 Study populations

The subjects were recruited to the study protocols from the Department of Obstetrics and Gynecology at Oulu University Hospital in Oulu, Finland, during the years 1998–2001. All the study designs were prospective and cross-sectional. The gestational age was confirmed by ultrasonographic examination prior to 20 weeks of gestation in all cases. Cases with abnormal karyotype and major structural anomalies were excluded. The study protocols were approved by the local ethics committee and the subjects gave their informed consent before entering the study protocol. Table 1 describes the study groups and the Doppler ultrasonographic parameters and biochemical analyses used. In study III, the decision to break the study population into three subgroups based on umbilical arterial concentrations of fetal NT-proANP and cTnT was made prior to data analysis. In addition, in study III, the investigators obtaining and analyzing ultrasonographic data were blinded to the biochemical data. In studies I, II, IV and V, the control group consisted of subjects with uncomplicated pregnancy and labor studied between the same gestational weeks as the study populations. The neonatal birth weights in control group were between the 10th and 90th percentile. Diagnostic criteria of maternal hypertensive disorder followed American College of Obstetricians and Gynecologists guidelines (Committee on Technical Bulletins of the American College of Obstetricians and Gynecologists 1996). Placental insufficiency was defined as an abnormal umbilical artery blood velocity waveform profile (umbilical artery S/D ratio>3.5) (Forouzan et al. 1991). Fetal growth restriction was defined as a birth weight below the 10th percentile growth curve. In study II, the diagnostic criteria for fetal acidemia was umbilical artery pH< 7.10 at birth.
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<td>UA S/D - ratio</td>
<td>Pulsations in IA UV</td>
<td>CCO, LVCC, RVCO, RvFeo, LveFo, IRT, IMP</td>
<td>CC/TC - ratio</td>
</tr>
<tr>
<td></td>
<td>Pulsations in IA UV</td>
<td></td>
<td>TV and MV TVI</td>
<td>TV and MV TVI</td>
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<td>TR and MR</td>
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<td></td>
<td>RVFeo, LVFeo</td>
<td>RVFeo, LVFeo</td>
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<td></td>
<td>TV and MV TVI</td>
<td>TV and MV TVI</td>
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<td></td>
<td></td>
<td></td>
<td>IVC, LHV and DV PIV</td>
<td>IVC, LHV and DV PIV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pulsations in IA UV</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Characteristics of the material and methods used in the different studies of this research protocol. In studies II-IV, all except control fetuses st. jere from fetal growth restriction and/or placental insufficiency.
4.2 Ultrasonographic measurements

Image-directed pulsed and color Doppler ultrasonographic equipment (Acuson Sequoia 512, Mountain View, CA, USA) was used with a 4–8 MHz convex or a 5 MHz sector probe. The high pass filter was set at minimum. The acoustic output of the system was controlled according to ECMUS recommendations (European Committee for Medical Ultrasound Safety 1999a, European Committee for Medical Ultrasound Safety 1999b). All the ultrasonographic data were videotaped and analyzed afterwards, using the ultrasound equipment’s own cardiac measurement package. A Doppler waveform obtained at an angle of less than 15 degrees between the vessel and the Doppler beam was accepted for analysis. Three consecutive cardiac cycles were analyzed from Doppler velocity waveforms and their mean values were used for further analysis. For the volume blood flow calculations, the cross-sectional diameters of vessels were measured from frozen real-time images during systole by using the leading edge-to-leading edge method. Three separate measurements of vessel diameters were taken, and the mean value was used for further analysis. Calculations of CSAs of the arteries were based on the assumption that the cross-sections of the vessels were circular.

4.2.1 Uterine and umbilicoplacental hemodynamics

The main uterine arterial Doppler recordings were obtained from the placental side. If the placenta was centrally located, both uterine arteries were examined and the mean value was used in the analysis (I, II). Placental circulation was assessed by determining the PI-values of umbilical artery in free loops of the umbilical cord (I–V). Fetal heart rate was obtained from umbilical artery Doppler tracings (I–V).

4.2.2 Cardiac measurements

4.2.2.1 Volume blood flows

Volumetric blood flows across aortic (AoV) and pulmonary (PV) valves and DA were calculated directly (Q=CSA x TVI x FHR) (III–IV). The TVIs were obtained by planimetering the area underneath the Doppler spectrum. The LVCO equals the Q_AoV, the RVCO equals the Q_PV, and their sum is CCO (III, IV). The pulmonary volume blood flow (Q_P) was calculated indirectly by the formula: RVCO-Q_DA (IV). Foramen ovale volume blood flow (Q FO) was estimated as LVCO-Q_P (IV). The distribution (%) of CCO and weight-indexed CCO, LVCO, RVCO, Q_DA, Q_P and Q FO were calculated (III, IV). The actual birth weight was used for indexing purposes when the time interval between the last ultrasonographic examination and delivery was equal to or less than 4 days (III–V). In other cases, fetal weight estimation was based on the measurements of biparietal
diameter, head and abdominal circumferences and femur length, which is considered to be the most reliable method with 7.5% standard deviation (SD) (Hadlock et al. 1984) (IV–V).

### 4.2.2.2 Time-intervals

Left ventricular time-intervals were assessed by placing the Doppler gate into the left ventricle and obtaining simultaneously MV and AoV blood velocity waveforms (III, IV) (Fig. 4). The IRT was measured as the time period between the end of ejection and the onset of filling (Tulzer et al. 1994). The proportion (%) of IRT of the total cardiac cycle was calculated (III, IV). An index of myocardial performance (IMP) was calculated by the formula: \[ \text{IMP} = \frac{\text{ICT} + \text{IRT}}{\text{ET}} \], in which ICT is isovolumetric contraction time and ET is ejection time (Tei et al. 1995, Tsutsumi et al. 1999) (III, IV).

### 4.2.2.3 Ventricular ejection forces

Right (RVeFo) and left (LVeFo) ventricular ejection forces were calculated by the formula: \[ (1.055 \times \text{CSA} \times \text{TVI}_{ac}) \times \left( \frac{\text{PSV}}{\text{TTP}} \right) \], in which \( \text{TVI}_{ac} \) is the TVI during acceleration period in systole, PSV is the peak systolic velocity, and TTP is the time period from the onset of ejection to systolic peak velocity (Isaaz et al. 1989, Sutton et al. 1991b, Rizzo et al. 1995a) (III,IV). Both ventricular ejection forces were weight-indexed.

### 4.2.2.4 Inflow waveforms

Inflow blood velocity waveforms were recorded at the level of the tricuspid (TV) and mitral (MV) valves. The total TVI of TV and MV, as well as the TVI ratio of E- (early passive filling) and A- (atrial contraction) waves (E/A TVI), and A-wave TVI/total TVI-ratio of both valves were calculated (Tulzer et al. 1994) (III, IV).

### 4.2.2.5 Valvular regurgitation

The presence of tricuspid (TR) and mitral (MR) regurgitations were noted. Tricuspid regurgitation was classified as trivial (nonholosystolic ≤ 72ms) or holosystolic (Fig. 3) (Respondek et al. 1994)(III, V).
4.2.2.6 M-mode measurements

M-mode recordings were obtained by placing the M-mode cursor perpendicularly towards the interventricular septum at the level of atrioventricular valves in a four chamber-view of the heart. From M-mode recordings, right and left ventricular inner diameters (RVD, LVD) were measured during diastole and systole (III, V). Right and left ventricular fractional shortenings (RVFS, LVFS) were calculated (ventricular fractional shortening [%]=[(inner diastolic diameter-inner systolic diameter)/inner diastolic diameter]x100) (DeVore et al. 1984) (III, V).

4.2.3 Fetal arterial circulation

Middle cerebral artery blood velocity waveforms were obtained by placing the Doppler gate along the course of MCA (III, V). Blood flow in the DAo was assessed at the level of the diaphragm (III, V), and blood velocity waveforms of branch pulmonary artery (PPA) were recorded immediately after the bifurcation of the main pulmonary artery before the first branch of the right or left pulmonary artery (IV, V). Distribution of arterial circulation was obtained by using MCA, DAo and PPA PI values which describe vascular impedance in the arterial circulation (III–V). In addition, umbilical artery/MCA PI- and DAo/MCA PI- ratios were calculated (III, V). Aortic isthmus blood velocity waveforms were recorded (Fig. 6) (III–V). Time-velocity integrals of antegrade and retrograde components were measured and their ratio was calculated. Net blood flow was considered antegrade if the ratio was equal to or more than 1, and retrograde when the ratio was less than 1 (Fouron et al. 1999). In addition, the visualization rate of coronary artery circulation by color and pulsed Doppler ultrasonography demonstrating heart sparing effect was noted (Baschat et al. 1997) (Fig. 5) (III, V).

4.2.4 Fetal venous circulation

From fetal venous circulation, blood velocity waveforms of DV, LHV and IVC were assessed and their pulsatility index values for veins (PIV) were calculated (III, V). Pulsations occurring during atrial contraction in intra-abdominal umbilical vein and in free loops of umbilical vein were noted (Fig. 7.) (I–III, V).

4.2.5 Ultrasonographic parameters of the fetal heart

Absolute and weight-indexed cardiac outputs, Qp, QDA and QFO were calculated and the distribution of cardiac output was assessed. Systolic function of the heart was described by weight-indexed RVeFo and LVeFo. Right ventricular contractility was assessed by
using dP/dT values in the presence of TR. Diastolic function was assessed by using IRT% and the E/A TVI- and A TVI/total TVI- ratios of atrioventricular valves. The Index of myocardial performance was used to describe the combined systolic and diastolic performance of the heart. The CC/TC- ratio, RVFS, LVFS and both TR and MR were used to describe the afterload of the heart.

4.2.6 Analysis of cardiac biochemical markers

Immediately after delivery, at least 1 ml of blood was collected from the umbilical artery and centrifuged (I–III). In a subgroup (n=62) of subjects in studies I–II, blood samples of at least 1 ml from the umbilical vein and 5 ml from the maternal cubital vein were simultaneously taken. The serum samples were stored at −80°C until analyzed.

4.2.7 Cardiac troponin T

Serum cTnT concentrations were measured with commercially available enzyme-linked immunosorbent assay kits (Enzymum-Test Troponin T; Boehringer Diagnostics, Mannheim, Germany) according to manufacturer’s instructions.

4.2.8 N-terminal peptide of proatrial natriuretic peptide

The NT-proANP radioimmunoassay was carried out directly on unextracted plasma samples. Plasma samples in duplicates of 25 µL were incubated with rabbit antiserum and 125I-labelled human Tyrosineβ-proatrial natriuretic peptide79–98 overnight at 4°C. The bound and free fractions were separated with sheep antirabbit gammaglobulin antiserum in the presence of 8% polyethylene glycol 6000. Synthetic human proatrial natriuretic peptide79–98 was used as a standard.

4.3 Statistical analysis

Statistical analysis was performed by using the analysis of variance when comparisons were made between more than two groups and the data were normally distributed. If statistical significance was shown, the Scheffe F-test was used for further analysis. If the data were not normally distributed, the nonparametric Kruskal-Wallis test was chosen. Between two groups, comparisons were made by using Student t-test if the data were normally distributed. Otherwise, the Mann Whitney U-test was chosen. In study II, linear regression analysis was used to show the relationship between umbilical vein and artery.
and maternal cubital vein NT-proANP. The categorical data were compared using the chi-square test. Linear regression analysis was used to show the relationship of NT-proANP concentration to PIV values in venous circulation (III). A p value of 0.05 or less was selected as the level of statistical significance.
5 Results

5.1 Validation of methodology

5.1.1 Intra-observer variability of Doppler ultrasonographic measurements

The intra-observer variability in ultrasonographic measurements was tested in the different study populations by performing two ultrasonographic examinations. The second examination was performed 1–2 hours after the first examination. In study III, 17 subjects with uncomplicated pregnancy and 6 subjects with placental insufficiency were included in the analysis of the intra-observer variability. The mean intra-observer variability of RVCO and LVCO calculations varied from 5.4% to 6.3% (95% CI 4.7%–7.9%). The corresponding variability of ventricular ejection force calculations was 8.8% (95% CI 4.7%–12.8%). The mean intra-observer variabilities of MV and TV total TVI calculations ranged from 5.0% to 5.2% (95% CI 2.4%–7.8%). In time-interval calculations, the corresponding variability was from 8.0% to 9.7% (95% CI 5.4%–13.2%), respectively. In the umbilical artery, DAo and MCA PI calculations, the mean intra-observer variability ranged from 3.9% to 6.0% (95% CI 2.5%–9.5%). The mean intra-observer variability of DV, LHV and IVC PIV calculations ranged from 3.8% to 5.8% (95% CI 1.9%–7.5%).

Twelve fetuses in study IV showed that the mean intra-observer variability of volumetric blood flow measurements across AoV, PV and DA and direct QP calculation varied from 5.0% to 8.5% with 95% CI of 2.9%–11.1%. In addition, the correlation between indirect and direct QP in study IV was good (R= 0.93, p=0.0001). The mean intra-observer variability of MV and TV TVI calculations ranged from 1.9% to 10.9% (95% CI 0.6%–18.1%). In ventricular ejection force calculations, the mean variability was 7.3% (95% CI 3.5%–11.0%).
In study V, reproducibility and intra-observer variability of PIV calculations for the DV and IVC were analyzed in control group fetuses (n=31). The mean intra-observer variability of DV calculations was 9.3% (95% CI 6.2–12.4%), and in IVC calculations, it was 8.6% (95% CI 5.3–11.8%).

5.1.2 Assays of NT-proANP and cTnT

Umbilical artery and vein NT-proANP concentrations (n=62) demonstrated a significant correlation ($R=0.71$, $p<0.001$), while no correlation between umbilical artery NT-proANP concentrations and maternal cubital vein NT-proANP values (n=62) was found ($R=-0.07$, $p=0.75$) (II). There was no significant difference in NT-proANP concentrations of the umbilical artery and vein. The median (range) umbilical artery [580 pmol/l (226–3000 pmol/l)] and vein [679 pmol/l (210–2730 pmol/l)] NT-proANP concentrations were both higher ($p<0.02$) than in the maternal cubital vein [479 pmol/l (229–1359 pmol/l)] (II). No significant difference between umbilical vein and artery cTnT values was detected. Maternal vein cTnT concentrations were less than 0.10 ng/ml in every case (I). Mode of the delivery and gestational age did not affect NTproANP and cTnT concentrations, at least after 27 gestational weeks (I–III). In neonates born after uncomplicated pregnancy and labor, the +2SD level of umbilical artery NT-proANP was 1145 pmol/l (II), which was chosen to represent the cut-off level in study III. The cut-off level for umbilical artery cTnT was set at 0.10 ng/ml in studies I and III, which has been used as a level of clinically significant myocardial cell damage in adult patients.

5.2 Increased NT-proANP production with normal cTnT concentration

5.2.1 Clinical outcome

In 6 out of 12 fetuses with placental insufficiency and normal NT-proANP (<1145 pmol/l) concentrations, cesarean delivery was performed due to signs of fetal distress, while the corresponding number was 8 out of 11 cesarean deliveries among 25 fetuses with increased NT-proANP levels (>1145 pmol/l) (III). No differences were found in antenatal and perinatal data in pregnancies complicated by placental insufficiency with either normal or elevated NT-proANP levels (Table 2) (III). Newborns with uncomplicated pregnancy and fetal acidemia during labor had lower 5-minute Apgar scores and umbilical artery pH values than newborns with uncomplicated pregnancy and labor. In addition, newborns with acidemia during labor had lower pH values compared to newborns of pregnancies complicated by maternal hypertensive disorders.
Table 2. Perinatal data in the different studies cf this research protocol. In studies III–V, all except control fetuses showed from fetal growth restriction and/or placental insufficiency. All values are given as mean (SE) or median (range).

<table>
<thead>
<tr>
<th>Study</th>
<th>GA at study entry (weeks)</th>
<th>GA at delivery (weeks)</th>
<th>Birth weight (grams)</th>
<th>UA pH</th>
<th>5 min Apgar</th>
<th>NT-proANP (pmol/L)</th>
<th>cTnT (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Controls</td>
<td>40.0 (1.2)</td>
<td>3713 (408)↑</td>
<td>9 (7–10)</td>
<td>0 (0–0.14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No UV pulsation</td>
<td>37.6 (3.4)↑</td>
<td>2794 (588)↑</td>
<td>9 (3–10)</td>
<td>0 (0–0.16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV pulsations</td>
<td>29.8 (3.0)↑</td>
<td>666 (269)↑</td>
<td>6 (2–7)↑</td>
<td>0.18 (0.11–0.35)↑</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Study II</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Controls</td>
<td>39.9 (1.1)</td>
<td>3666 (384)↑</td>
<td>7.23 (0.68)</td>
<td>9 (7–10)</td>
<td>562 (228–1614)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No UV pulsation</td>
<td>37.8 (3.0)↑</td>
<td>2815 (944)↑</td>
<td>7.20 (0.66)</td>
<td>9 (5–10)</td>
<td>898 (326–3000)↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV pulsations</td>
<td>29.8 (2.9)↑</td>
<td>666 (269)↑</td>
<td>7.26 (0.69)</td>
<td>6 (2–7)↑</td>
<td>4625 (3516–16630)↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetal acidemia (pH&lt;7.10) during labor</td>
<td>40.2 (1.5)</td>
<td>3315 (510)↑</td>
<td>7.05 (0.66)↑</td>
<td>8 (5–10)*</td>
<td>925 (255–2430)↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study III</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Group 1</td>
<td>34.7 (3.7)</td>
<td>35.2 (3.1)</td>
<td>2086 (677)</td>
<td>7.26 (0.66)</td>
<td>9 (8–10)</td>
<td>737 (462–1161)</td>
<td>0.03 (0.03–0.69)</td>
</tr>
<tr>
<td>Group 2</td>
<td>32.9 (4.8)</td>
<td>33.7 (3.5)</td>
<td>1658 (578)</td>
<td>7.25 (0.65)</td>
<td>9 (3–10)</td>
<td>1652 (1166–7450)</td>
<td>0.03 (0.03–0.69)</td>
</tr>
<tr>
<td>Group 3</td>
<td>27.5 (4.6)**</td>
<td>28.1 (4.7)**</td>
<td>846 (471)</td>
<td>7.21 (0.10)</td>
<td>3 (1–9)↑</td>
<td>5179 (1755–16630)↑</td>
<td>0.35 (0.11–1.77)↑</td>
</tr>
<tr>
<td>Study IV</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Controls</td>
<td>30.5 (4.0)</td>
<td>39.5 (1.4)</td>
<td>3267 (444)</td>
<td>7.24 (0.65)</td>
<td>9 (2–9)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antegrade AOI</td>
<td>29.5 (5.9)</td>
<td>30.8 (4.0)↑</td>
<td>1308 (724↑↑)</td>
<td>7.24 (0.65)</td>
<td>9 (2–9)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrograde AOI</td>
<td>32.1 (3.8)</td>
<td>32.5 (3.9)↑</td>
<td>1252 (540↑↑)</td>
<td>7.26 (0.66)</td>
<td>8 (1–9)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study V</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>29.5 (4.5)</td>
<td>39.8 (1.4)</td>
<td>3487 (351)</td>
<td>7.24 (0.65)</td>
<td>9 (2–9)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antegrade AOI</td>
<td>29.5 (5.5)</td>
<td>30.8 (4.0)↑</td>
<td>1282 (674↑↑)</td>
<td>7.24 (0.65)</td>
<td>9 (2–9)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrograde AOI</td>
<td>32.1 (3.8)</td>
<td>32.5 (3.9)↑</td>
<td>1252 (540↑↑)</td>
<td>7.26 (0.66)</td>
<td>8 (1–9)**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05 vs controls (II, IV, V), ** p<0.01 vs groups 1 and 2 (III), vs controls (IV, V), † p<0.001 vs groups 1 and 2 (III), vs controls (I, II), ‡ p<0.0001 vs controls (I, IV, V).
5.2.2.1 Volumetric blood flows

Weight-indexed RVCO, LVCO and CCO, and RVCO% and LVCO% of CCO in fetuses with placental insufficiency and increased NT-proANP levels did not differ from those fetuses who suffered from placental insufficiency but had normal NT-proANP levels (Table 3).

5.2.2.2 Systolic and diastolic function of fetal heart

Weight-indexed RVeFo and LVeFo did not differ between fetuses with placental insufficiency and with either normal or elevated NT-proANP levels (Table 3). Right ventricular contractility as assessed by TR dP/dT was similar among these fetuses, with a mean value of 717 mmHg/s and 820 mmHg/s in fetuses with normal and elevated NT-proANP levels, respectively.

The left ventricular IRT%, the total TVIs of TV and MV, and their E/A TVI- and A TVI/total TVI- ratios did not differ between the groups. The index of myocardial performance was similar between the groups (Table 3).

5.2.2.3 Afterload

The CC/TC ratio, RVFS and LVFS did not differ between fetuses with normal and elevated NT-proANP levels. The detection rate of TR was similar in both groups (Table 3).
Table 3. Results of ultrasonographic measurements of cardiac function in fetuses with placental insufficiency, and a) normal NT-proANP and cTnT, b) increased NT-proANP and normal cTnT, and c) increased cTnT levels.

<table>
<thead>
<tr>
<th></th>
<th>Normal NT-proANP (≤1145 pmol/l) (n=12)</th>
<th>Increased NT-proANP (&gt;1145 pmol/l) (n=25)</th>
<th>Increased cTnT (&gt;0.10 ng/ml) (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volumetric blood flow</strong></td>
<td></td>
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</tr>
<tr>
<td>CCO (ml/min/kg)</td>
<td>523 (72)</td>
<td>548 (121)</td>
<td>499 (189)</td>
</tr>
<tr>
<td>RVCO (ml/min/kg)</td>
<td>299 (40)</td>
<td>327 (90)</td>
<td>272 (129)</td>
</tr>
<tr>
<td>LVCO (ml/min/kg)</td>
<td>223 (51)</td>
<td>221 (42)</td>
<td>226 (77)</td>
</tr>
<tr>
<td>RVCO%</td>
<td>57.2 (5.2)</td>
<td>59.1 (4.5)</td>
<td>53.3 (13.1)*</td>
</tr>
<tr>
<td>LVCO%</td>
<td>42.8 (5.2)</td>
<td>40.9 (4.5)</td>
<td>46.7 (13.0)*</td>
</tr>
<tr>
<td><strong>Systolic function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVeFo (mN/kg)</td>
<td>6.56 (1.57)</td>
<td>7.35 (2.91)</td>
<td>5.40 (3.50)</td>
</tr>
<tr>
<td>LVeFo (mN/kg)</td>
<td>6.18 (1.94)</td>
<td>6.12 (1.88)</td>
<td>5.96 (2.75)</td>
</tr>
<tr>
<td><strong>Diastolic function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRT%</td>
<td>13.2 (2.1)</td>
<td>12.6 (1.9)</td>
<td>13.4 (2.8)</td>
</tr>
<tr>
<td><strong>Tricuspid valve</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total TVI (cm)</td>
<td>5.8 (0.9)</td>
<td>5.5 (1.3)</td>
<td>5.0 (1.0)</td>
</tr>
<tr>
<td>E/A TVI</td>
<td>0.61 (0.29)</td>
<td>0.62 (0.27)</td>
<td>0.62 (0.35)</td>
</tr>
<tr>
<td>A TVI/total TVI</td>
<td>0.63 (0.13)</td>
<td>0.63 (0.14)</td>
<td>0.67 (0.18)</td>
</tr>
<tr>
<td><strong>Mitrval valve</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total TVI (cm)</td>
<td>5.6 (1.1)</td>
<td>5.0 (1.1)</td>
<td>4.7 (1.5)</td>
</tr>
<tr>
<td>E/A TVI</td>
<td>0.79 (0.22)</td>
<td>0.80 (0.27)</td>
<td>0.88 (0.35)</td>
</tr>
<tr>
<td>A TVI/total TVI</td>
<td>0.56 (0.08)</td>
<td>0.57 (0.08)</td>
<td>0.55 (0.09)</td>
</tr>
<tr>
<td>IMP</td>
<td>0.59 (0.11)</td>
<td>0.54 (0.09)</td>
<td>0.60 (0.13)</td>
</tr>
<tr>
<td><strong>Afterload</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC/TC (%)</td>
<td>57.0 (4.4)</td>
<td>57.5 (3.3)</td>
<td>61.3 (6.1)*</td>
</tr>
<tr>
<td>RVFS (%)</td>
<td>22.6 (7.6)</td>
<td>26.2 (5.0)</td>
<td>13.2 (11.4)*</td>
</tr>
<tr>
<td>LVFS (%)</td>
<td>29.0 (7.3)</td>
<td>32.8 (6.7)</td>
<td>26.2 (12.0)</td>
</tr>
<tr>
<td>TR</td>
<td>3/12 (25%)</td>
<td>3/25 (12%)</td>
<td>6/11 (55%)*</td>
</tr>
</tbody>
</table>

*p<0.05 vs Increased NT-proANP

5.2.3 Fetal arterial circulation

In pregnancies complicated by preeclampsia, fetuses with abnormal umbilical artery blood velocity waveforms and normal umbilical vein blood flow profile had significantly higher umbilical artery NT-proANP concentrations than fetuses with normal umbilical artery blood velocity waveforms (Table 2) (II). When fetuses suffering from placental
insufficiency were divided into the subgroups using umbilical artery NT-proANP cut-off value of 1145 pmol/l, no difference in PI values of umbilical artery, MCA and DAo was found between fetuses with normal and elevated NT-proANP levels (Table 4) (III). Neither did the DAo/ MCA PI- ratio differ between these fetuses (III). The detection rate of retrograde net blood flow in the aortic isthmus was similar among these fetuses (III). In addition, no difference between the groups was found in the visualization rate of coronary artery circulation (Table 4) (III).

5.2.4 Fetal venous circulation

In study III, PIV values in DV, LHV and IVC did not differ significantly between fetuses with normal NT-proANP concentrations and fetuses with elevated NT-proANP concentrations without myocardial cell damage (Table 4). No difference in the incidence of atrial pulsations in the umbilical vein was detected between these two groups (Table 4) (III).

Table 4. Ultrasonographic parameters describing arterial and venous circulations in fetuses with placental insufficiency, and a) normal NT-proANP and cTnT, b) increased NT-proANP and normal cTnT, and c) increased cTnT levels.

<table>
<thead>
<tr>
<th></th>
<th>Normal NT-proANP (≤1145 pmol/l) (n=12)</th>
<th>Increased NT-proANP (&gt;1145 pmol/l) (n=25)</th>
<th>Increased cTnT (&gt;0.10 ng/ml), (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial circulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA PI</td>
<td>1.34 (0.42)</td>
<td>1.70 (0.88)</td>
<td>3.13 (1.45)**</td>
</tr>
<tr>
<td>MCA PI</td>
<td>1.43 (0.43)</td>
<td>1.42 (0.36)</td>
<td>1.52 (0.45)</td>
</tr>
<tr>
<td>DAo PI</td>
<td>2.37 (0.50)</td>
<td>2.58 (0.51)</td>
<td>2.77 (0.86)</td>
</tr>
<tr>
<td>DAo/MCA PI</td>
<td>1.76 (0.56)</td>
<td>1.95 (0.65)</td>
<td>2.19 (1.32)</td>
</tr>
<tr>
<td>Retrograde AOI</td>
<td>4/11 (36%)</td>
<td>7/20 (35%)</td>
<td>5/10 (50%)</td>
</tr>
<tr>
<td>Coronary artery visualization</td>
<td>4/12 (33%)</td>
<td>4/25 (16%)</td>
<td>3/11 (27%)</td>
</tr>
<tr>
<td>Venous circulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVC PIV</td>
<td>2.32 (1.60-4.36)</td>
<td>2.33 (1.02-5.66)</td>
<td>4.95 (2.46-6.45)*</td>
</tr>
<tr>
<td>LHV PIV</td>
<td>2.50 (1.06-7.28)</td>
<td>3.86 (1.76-9.37)</td>
<td>5.84 (3.29-46.59)*</td>
</tr>
<tr>
<td>DV PIV</td>
<td>0.59 (0.34-1.14)</td>
<td>0.55 (0.26-1.24)</td>
<td>1.17 (0.57-3.81)**</td>
</tr>
<tr>
<td>IA UV pulsations</td>
<td>2/12 (17%)</td>
<td>4/25 (16%)</td>
<td>9/11 (82%)*</td>
</tr>
</tbody>
</table>

*p<0.01 vs groups 1 and 2, ** p<0.001 vs groups 1 and 2
5.2.5 NT-proANP and acidemia during the labor

Newborns with uneventful pregnancy and acidemia during labor had significantly (p<0.001) higher NT-proANP concentration than newborns with uncomplicated pregnancy and labor (Table 2) (II). The umbilical artery NT-proANP concentrations in newborns with acidemia during labor did not differ from those detected in newborns of hypertensive pregnancies with normal nonpulsatile umbilical vein blood velocity waveform profile (Table 2) (II).

5.3 Increased fetal cTnT concentration

5.3.1 Clinical outcome

Gestational age at delivery, birth weights and 5-minute Apgar scores were significantly lower in fetuses with elevated cTnT (>0.10 ng/ml) levels than in fetuses with normal cTnT values (<0.10 ng/ml) (Table 2). The umbilical artery pH values of the newborns did not differ between the groups (Table 2). Cesarean delivery was performed due to signs of fetal distress in 8 out of 11 cases (III).

5.3.2 Fetal heart

Fetuses with myocardial cell damage had significantly higher neonatal umbilical artery NT-proANP concentrations than fetuses with normal umbilical artery cTnT concentrations (Table 2) (III).

5.3.2.1 Volumetric blood flows

No difference in weight-indexed RVCO, LVCO and CCO was found between fetuses with placental insufficiency and either elevated or normal cTnT levels (Table 3) (III). However, in fetuses with elevated cTnT levels, the LVCO% was greater (p<0.05) and the RVCO% was less (p<0.05) than in fetuses with only increased NT-proANP levels (Table 3) (III).
5.3.2.2 Systolic and diastolic function of fetal heart

Weight-indexed RVeFo and LVeFo of fetuses with elevated cTnT levels did not differ from those of fetuses with normal cTnT concentrations (Table 3) (III). In addition, right ventricular contractility assessed by TR dP/dT was similar in fetuses with myocardial cell damage (605 mmHg/s) to fetuses with normal cTnT levels (III).

No difference in the left ventricular IRT%, the total TVIs of TV and MV, and their E/A TVI- and A TVI/total TVI- ratios was found between the groups (Table 3) (III). In addition, IMP was similar among these fetuses (Table 3) (III).

5.3.2.3 Afterload

Fetuses with myocardial cell damage had a greater (p<0.05) CC/TC ratio than fetuses with normal cTnT levels (Table 3) (III). The presence of TR was more common (p<0.03) in fetuses with a rise in cTnT concentrations (55%) than in fetuses with only increased NT-proANP levels (12%) (Table 3) (III). Three out of 11 fetuses with myocardial cell damage had MR (III). The RVFS of fetuses with myocardial cell damage was less than that in fetuses with only increased NT-proANP level (p<0.05), while LVFS did not differ between the groups (Table 3) (III).

5.3.3 Fetal arterial circulation

Fetuses with myocardial cell damage had higher (p<0.001) umbilical artery PI values than fetuses with normal cTnT levels (Table 4) (III). No differences in PI values of MCA and DAo, and the DAo/ MCA PI-ratio were found between the groups (Table 4) (III). The detection rate of retrograde net blood flow in the aortic isthmus was similar among the groups (Table 4) (III). In addition, no difference between the groups was found in the visualization rate of coronary artery circulation (Table 4) (III).

5.3.4 Fetal venous circulation

In venous circulation, PIV values in DV, LHV and IVC were higher (p<0.01) in fetuses with myocardial cell damage than in fetuses with normal cTnT values (Table 4) (III). When all the fetuses in study III were combined (n= 48), DV, LHV and IVC PIV values demonstrated a significant positive correlation with umbilical artery NT-proANP concentrations (Fig. 8). Fetuses with myocardial cell damage demonstrated atrial pulsations in umbilical vein more often than fetuses with normal cTnT values (p<0.01) (Table 4) (I, III).
Fig. 8. Correlation of DV, LHV and IVC PIV values with umbilical artery NT-proANP concentrations in fetuses with placental insufficiency. Normal NT-proANP and cTnT=group 1, increased NT-proANP and normal cTnT=group 2, and increased cTnT=group 3. (DV PIV=0.00015xNT-proANP+0.42, R=0.76, p=0.0001; LHV PIV=0.0020xNT-proANP+0.76, R=0.76, p=0.0001; IVC PIV=0.00023xNT-proANP+2.34, R=0.57, p=0.001).
5.4 Retrograde net blood flow in the aortic isthmus

5.4.1 Clinical outcome

Fetuses with placental insufficiency and either antegrade or retrograde aortic isthmus net blood flow were delivered earlier (p<0.0001) and with lower birth weight (p<0.0001) than control fetuses with no difference as regards to the direction of aortic isthmus net blood flow (Table 2) (IV, V). Apgar scores at 5-minute age did not differ between the study groups but were significantly lower than in the control group (IV, V). Fetuses with retrograde aortic isthmus net blood flow were delivered via cesarean route more often (p<0.01) due to signs of fetal distress than fetuses with antegrade net blood flow (IV, V). No difference in umbilical artery pH and pO₂ was detected between the groups (Table 2) (IV, V).

5.4.2 Fetal heart

5.4.2.1 Volumetric blood flows

Fetuses with placental insufficiency and either antegrade or retrograde aortic isthmus net blood flow had lower weight-indexed CCO, LVCO, RVCO (p<0.05) compared to the control group (Table 5) (IV). Weight-indexed Qp was lower (p<0.01) in fetuses with antegrade aortic isthmus net blood flow than in the control group fetuses, while weight-indexed QFO was higher (p<0.05) than in fetuses with retrograde aortic isthmus net blood flow (Table 5, Fig. 9) (IV). The LVCO% and RVCO% did not differ significantly between the study groups (Table 5, Fig. 9) (IV). Fetuses with antegrade aortic isthmus net blood flow had greater QDA% (p<0.05), and QP% was less (p<0.05) than in the control group (IV). Furthermore, in fetuses with antegrade aortic isthmus net blood flow, QFO% of CCO was higher (p<0.01) than in fetuses with retrograde aortic isthmus net blood flow and in the control group (Table 5, Fig. 9) (IV). The proportion of QFO of LVCO was greater (p<0.05) in fetuses with antegrade aortic isthmus net blood flow (71%) than in the control group (49%) or in the fetuses with retrograde aortic isthmus net blood flow (48%) (IV).
Fig. 9. Distribution of fetal cardiac output in the control group fetuses (Normal), in fetuses with antegrade aortic isthmus net blood flow (Group 1), and in fetuses with retrograde aortic isthmus net blood flow (Group 2).
Table 5. Cardiac volumetric blood flow and their proportions (%) of combined cardiac output in control fetuses and in fetuses with placental insufficiency and either antegrade or retrograde aortic isthmus (AOI) net blood flow.

<table>
<thead>
<tr>
<th>Volumetric blood flow</th>
<th>Controls</th>
<th>Antegrade AOI</th>
<th>Retrograde AOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCO (ml/min/kg)</td>
<td>647 (113)</td>
<td>529 (141)*</td>
<td>549 (153)*</td>
</tr>
<tr>
<td>RVCO (ml/min/kg)</td>
<td>391 (74)</td>
<td>287 (57)*</td>
<td>317 (106)*</td>
</tr>
<tr>
<td>LVCO (ml/min/kg)</td>
<td>298 (67)</td>
<td>242 (94)*</td>
<td>231 (59)*</td>
</tr>
<tr>
<td>Qp (ml/min/kg)</td>
<td>150 (63)</td>
<td>99 (48)†</td>
<td>125 (74)</td>
</tr>
<tr>
<td>QDA (ml/min/kg)</td>
<td>244 (50)</td>
<td>208 (49)</td>
<td>208 (77)</td>
</tr>
<tr>
<td>QFO (ml/min/kg)</td>
<td>153 (74)</td>
<td>163 (79)‡</td>
<td>105 (56)</td>
</tr>
</tbody>
</table>

| Proportions (%) of CCO | | |
|------------------------| | |
| RVCO%                  | 56.8 (4.7)| 55.1 (5.2)| 57.2 (6.3)| |
| LVCO%                  | 43.2 (4.7)| 44.9 (5.2)| 42.8 (6.3)| |
| Qp %                   | 23.4 (7.7)| 17.8 (6.8)* | 21.4 (10.5)| |
| QDA %                  | 35.1 (6.8)| 42.2 (18.3)* | 37.4 (11.5)| |
| QFO %                  | 23.5 (11.1)| 32.0 (16.9)** | 19.7 (10.8)| |

| Systolic function | | |
|-------------------| | |
| RVeFo (mN/kg)     | 7.1 (2.1)| 5.5 (1.6)| 6.4 (3.0)| |
| LVeFo (mN/kg)     | 7.0 (2.4)| 6.4 (3.7)| 5.9 (2.3)| |

| Diastolic function | | |
|--------------------| | |
| IRT %              | 8.8 (1.2)| 13.4 (1.8)† | 13.2 (1.9)† |

| Tricuspid valve | | |
|-----------------| | |
| total TVI (cm)  | 6.5 (1.1) | 4.9 (0.8)† | 4.9 (0.8)† |
| E/A TVI         | 0.61 (0.17)| 0.69 (0.24)| 0.84 (0.87)| |

| Mitral valve | | |
|--------------| | |
| Total TVI (cm)| 6.5 (1.1)| 4.4 (0.8)† | 4.9 (0.1)† |
| E/A TVI      | 0.71 (0.15)| 0.76 (0.17)| 1.08 (0.75)* § |
| IMP          | 0.35 (0.06)| 0.56 (0.09)† | 0.57 (0.07)† |

| Afterload | | |
|-----------| | |
| CC/TC (%) | 50.0 (1.9)| 57.6 (3.7)† | 60.8 (4.5)* § |
| RVFS %    | 30.1 (7.0)| 17.7 (8.0)§ |
| LVFS %    | 30.4 (5.1)| 28.2 (5.2) |
| TR         | 2/18 (11%)| 7/11 (64%)§ |

*p < 0.05 vs control group, † p < 0.01 vs control group, ‡ p < 0.05 vs retrograde AOI
** p < 0.01 vs control group and retrograde AOI, § p < 0.05 vs antegrade AOI
5.4.2.2 Systolic and diastolic function of fetal heart

Weight-indexed RVeFo and LVeFo did not differ between the control group and fetuses with placental insufficiency and either antegrade or retrograde aortic isthmus net blood flow (Table 5) (IV). In addition, the ejection forces did not differ between the right and left ventricles in any of the groups. Fetuses with either antegrade or retrograde aortic isthmus net blood flow demonstrated greater left ventricular IRT% (p<0.01) than did control group fetuses (Table 5) (IV).

In fetuses with placental insufficiency and either antegrade or retrograde aortic isthmus net blood flow, the total TVIs of TV and MV were less (p<0.001) than in the control group (Table 5) (IV). Fetuses with retrograde aortic isthmus net blood flow demonstrated greater (p<0.05) E/A TVI ratio of MV compared to control group fetuses and fetuses with antegrade aortic isthmus net blood flow (IV). No difference in E/A TVI ratio of TV was found between these groups (IV). Fetuses with placental insufficiency and either antegrade or retrograde aortic isthmus net blood flow demonstrated increased IMP values (p<0.0001) compared to the control group (Table 5) (IV).

5.4.2.3 Afterload

The mean (SD) CC/TC ratio of fetuses with placental insufficiency and retrograde aortic isthmus net blood flow (60.8% (4.5%)) was greater than that of normal fetuses (50.0% (1.9%), p<0.001) and fetuses with placental insufficiency and antegrade aortic isthmus net blood flow (57.6% (3.7%), p<0.05) (V). Fetuses with retrograde aortic isthmus net blood flow had lower RVFS values than fetuses with antegrade aortic isthmus net blood flow (p<0.01), while no difference in LVFS values was noticed (Table 5) (V). Tricuspid regurgitation was present in seven of 11 (64%) fetuses with retrograde aortic isthmus net blood flow, and in two of 18 (11%) fetuses with antegrade aortic isthmus net blood flow (p<0.003) (Table 5) (V).

5.4.3 Fetal arterial circulation

Umbilical artery, DAo and DA PI values were significantly higher, and the PIs of the MCA were significantly lower in fetuses with placental insufficiency with no difference as regards to the direction of aortic isthmus net blood flow than in the control fetuses (Table 6) (V). Fetuses with retrograde aortic isthmus net blood flow had greater PI of the PPA (p<0.001) than the control fetuses and fetuses with antegrade aortic isthmus net blood flow (Table 6) (V). The DAo/MCA PI ratios were higher (p<0.0001) in fetuses with placental insufficiency (Table 6) (V). However, no difference between fetuses with antegrade and retrograde aortic isthmus net blood flow was observed (V). Coronary
artery blood velocity waveforms were visualized in seven of 11 (64%) fetuses with retrograde aortic isthmus net blood flow, and in four of 18 (22%) fetuses with antegrade aortic isthmus net blood flow (p<0.003) (Table 6) (V).

5.4.4 Fetal venous circulation

The PIV of the DV was significantly higher in fetuses with retrograde aortic isthmus net blood flow than in the control fetuses and in fetuses with antegrade aortic isthmus net blood flow (Table 6) (V). In IVC blood velocity waveforms, PIV values did not differ between the groups (Table 6) (V). Atrial pulsations in the intra-abdominal umbilical vein were noted in seven of 11 (64%) fetuses with retrograde aortic isthmus net blood flow, and in seven of 18 (39%) fetuses with antegrade aortic isthmus net blood flow (p=0.20) (Table 6) (V).

Table 6. Arterial and venous circulations in the control fetuses and fetuses with placental insufficiency and either antegrade or retrograde aortic isthmus net blood flow.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Antegrade AOI</th>
<th>Retrograde AOI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial circulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA PI</td>
<td>1.01 (0.18)</td>
<td>2.57 (1.44)†</td>
<td>2.80 (1.91)†</td>
</tr>
<tr>
<td>MCA PI</td>
<td>1.96 (0.29)</td>
<td>1.44 (0.43)‡</td>
<td>1.33 (0.25)‡</td>
</tr>
<tr>
<td>PPA PI</td>
<td>3.33 (0.26)</td>
<td>4.51 (1.44) *</td>
<td>7.55 (3.87)†,§</td>
</tr>
<tr>
<td>DA PI</td>
<td>2.67 (0.29)</td>
<td>3.05 (0.42)†</td>
<td>3.15 (0.72)†</td>
</tr>
<tr>
<td>DAo PI</td>
<td>2.03 (0.22)</td>
<td>2.67 (0.60) *</td>
<td>3.13 (1.08)†</td>
</tr>
<tr>
<td>DAo/MCA PI</td>
<td>1.05 (0.17)</td>
<td>2.06 (0.90)‡</td>
<td>2.36 (0.63)‡</td>
</tr>
<tr>
<td>Coronary artery visualization</td>
<td>0/31 (0%)</td>
<td>4/18 (22%)</td>
<td>7/11 (64%)§</td>
</tr>
<tr>
<td><strong>Venous circulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVC PIV</td>
<td>2.15 (1.13-2.89)</td>
<td>2.46 (1.02-6.19)</td>
<td>3.30 (1.76-11.94)</td>
</tr>
<tr>
<td>DV PIV</td>
<td>0.56 (0.30-0.71)</td>
<td>0.60 (0.26-1.24)</td>
<td>1.10 (0.33-3.81)†,§</td>
</tr>
<tr>
<td>IA UV pulsations</td>
<td>0/31 (0%)</td>
<td>7/18 (39%)</td>
<td>7/11 (64%)</td>
</tr>
</tbody>
</table>

* p< 0.05 vs control group, † p< 0.01 vs control group, ‡ p< 0.001 vs control group, § p< 0.001 vs group 1
6 Discussion

6.1 Validation of methodology

6.1.1 Ultrasonographic parameters

In a sheep model, volumetric blood flow measurements through the abdominal aorta and umbilical vein obtained by Doppler ultrasonography have been shown to be valid in different flow rates by using radionuclide-labeled microspheres and electromagnetic flow transducers (Schmidt et al. 1991). Meijboom et al. demonstrated that the intra- and interobserver variabilities in Doppler ultrasonographic measurements of volume blood flow across TV were less than 5% in invasively monitored dogs and in children undergoing cardiac catheterization (Meijboom et al. 1985). Previously, it has been shown that Doppler-determined maximal velocity tracings across cardiac valves vary by less than 10% (Reed et al. 1986). In a randomized blinded study on human fetuses, intra-observer variability in volumetric blood flow measurements of RVCO, LVCO, QDA and Qp has been shown to be less than 9% (Rasanen et al. 1998). In addition, an excellent correlation between two independent calculations of RVCO from PV and Qp+QDA has been shown in human fetuses (Rasanen et al. 1998). In the present study, the angle between the Doppler beam and the vessel was kept at <15 degrees to minimize methodological errors in volume blood flow calculations. Vessel diameters were measured at least three times by using the well-established leading edge-to-leading edge method. The mean intra-observer variabilities of volumetric blood flow calculations of RVCO, LVCO, QDA and Qp were comparable with previous reports. In this study, indirect estimation of Qp was also found to correlate significantly with direct Qp calculation.

In human fetal studies, intra-observer variability in RVeFo and LVeFo calculations between 18 and 38 weeks of gestation has been less than 10% (Rizzo et al. 1995a, Rasanen et al. 1997), and the corresponding interobserver variability less than 13% (Rizzo et al. 1995a). In this study, intra-observer variability of fetal ventricular ejection force calculations was 7 – 8%, and thus comparable with previous reports.
The intra-observer variability as regards PI calculations in the human fetal arterial circulation has been shown to be less than 4% (Rasanen et al. 1998). In the present study, PI and PIV calculations showed intra-observer variability of less than 6%, which is comparable with previous reports.

Other potential limitation of this study could be that some of the ultrasonographically obtained cardiovascular parameters change with advancing gestation in normal uncomplicated pregnancies (Rizzo et al. 1988, Tulzer et al. 1994, Rizzo et al. 1995a, Tsutsumi et al. 1999). However, in study III, in which all the pregnancies were complicated by placental insufficiency, cardiovascular parameters obtained by Doppler ultrasonography did not correlate with gestational age, which is in agreement with earlier studies (Rizzo et al. 1988, Rizzo et al. 1995a, Tsutsumi et al. 1999). In studies IV and V, the gestational age at study entry was similar among the studied groups.

### 6.1.2 Cardiac biochemical markers

The sensitivity of the NT-proANP assay is 30 pmol/l. The intra-assay and interassay coefficients of variation are < 10% and < 15%, respectively (Vuolteenaho et al. 1992). In experimental studies on rats, it has been shown that ANP does not cross the placenta (Mulay & Varma 1989). Shilo et al. found no correlation between maternal and neonatal ANP concentrations, which supports the view that it does not cross the human placenta (Shilo et al. 1989). The molecular size of NT-proANP (98 amino acids) is several times greater than that of ANP (28 amino acids), making it very likely that it does not cross the placenta either. The mode of delivery does not influence umbilical artery or vein plasma ANP concentrations (Kingdom et al. 1992). All these findings are in agreement with the results in study II. When umbilical artery and vein blood samples were compared, NT-proANP concentrations did not differ significantly. However, maternal cubital vein NT-proANP concentrations were significantly lower than corresponding umbilical artery and vein concentrations. In addition, maternal cubital vein NT-proANP values showed no correlation to newborn umbilical artery NT-proANP, while a significant correlation was found between newborn umbilical artery and vein concentrations. These results demonstrate that umbilical artery NT-proANP was of fetal origin. In addition, it has been shown previously that fetal ANP concentrations do not change after 16 weeks of gestation (Ville et al. 1994).

The cTnT assay has shown excellent reproducibility and a statistically significant ability to distinguish between values differing in the low range by 0.01 ng/ml (Lipshultz et al. 1997). The cTnT was measurable in children of all ages with myocyte damage and the smallest detectable troponin T elevations were 0.03 ng/ml (Lipshultz et al. 1997). This minimum detectable level was based on validation of the performance of the cTnT assay in the submyocardial infarction range of 0 to 0.10 ng/ml. A cTnT concentration equal to or more than 0.10 ng/ml was selected as the level of a clinically significant increase in this study, as used earlier in adults (Ohman et al. 1996). The measuring range of the used cTnT kit varies from 0 to 15 ng/ml, and the lower detection limit is 0.02 ng/ml. The monoclonal antibodies used are highly specific for cTnT. No measurable cross-reaction has been observed with skeletal muscle TnT or other myocardial filamentary proteins.
Cardiac troponin T concentrations start to rise within 4 to 12 hours after myocardial cell destruction and they remain high for over a week after the injury (Katus et al. 1991). Maternal cTnT concentrations were normal in our series, even in cases with increased newborn cTnT levels, demonstrating that umbilical artery cTnT measured in newborns was not of maternal origin. In addition, our results show that gestational age at delivery does not appear to affect newborn cTnT levels, at least after 27 weeks of gestation. It has been shown that during and after delivery, maternal troponin I levels remain undetectable (Shivvers et al. 1999). Troponin I levels were not affected by obstetric anesthesia, prolonged labor, or operative delivery. As regards cTnT, it was shown in study I that mode of delivery did not affect newborn cTnT levels.

6.2 Increased fetal NT-proANP levels without myocardial cell damage (II, III)

Newborns with acidemia during labor had higher umbilical artery NT-proANP levels than neonates after uncomplicated pregnancy and labor, although the course of pregnancy was uneventful, the birth weights were within the normal reference range and fetal heart rate tracings were normal when the subjects were admitted to the labor unit. These findings show that relatively acute changes in fetal acid/base status are reflected in the cardiovascular system as an increase in the release of NT-proANP. Previously, inversely related newborn umbilical vein ANP concentrations to umbilical artery pH values have been thought to indicate fetal cardiac response to the stress of acidosis (Kingdom et al. 1992). Studies on animals have shown that ANP release and production in the fetus are increased by acute hypoxemia and a rise in atrial pressure. Immunohistochemical studies have shown that ANP is present in the atria, the ventricular impulse-conducting system, and also in working ventricular cardiocytes (Jougasaki et al. 1989). Similarly, pro-ANP is expressed in human fetal ventricles, in which the number of myofibers containing ANP granules is greater in the subendocardial than in the subepicardial region (Tsuchimochi et al. 1988). In human fetuses, the connection between acute hypoxemia and increased ANP production is not so evident. Newborn pO2 did not show any independent relationship with ANP concentrations (Kingdom et al. 1992). According to our study, acute fetal distress with acidemia during labor is capable of triggering fetal cardiac dysfunction indicated by a rise in a release of NT-proANP.

Newborns with abnormal umbilical artery blood velocity waveform pattern during fetal life had higher NT-proANP levels than those with a normal umbilical artery blood flow velocity pattern, indicating an activated ANP system in fetuses suffering from placental insufficiency. In placental insufficiency, the afterload faced by the fetal right ventricle is increased, and these fetuses tend to be hypertensive (Stale et al. 1991). Thus, ANP secretion seems to be activated in response to an increase in afterload. It has been shown during intravascular red cell transfusion in utero that human fetal ANP concentrations increase promptly in response to an acute vascular volume expansion and an acute rise in the afterload (Panos et al. 1989). The NT-proANP levels in studies II and III show that in hypertensive pregnancies, atrial wall stretch of the fetal heart is increased,
and it rises with further deterioration of placental function. Capponi et al. found no difference in ANP levels between appropriately grown fetuses and growth-restricted fetuses with abnormal umbilical artery and normal inferior vena cava Doppler ultrasonographic findings (Capponi et al. 1997). This is not contradictory to our findings. The half-life of NT-proANP, which is released in equimolar amounts with ANP, is longer than that of ANP, and thus it is reflected in a larger increase in the NT-proANP level compared with that of ANP.

Fetuses with increased umbilical artery NT-proANP concentrations had similar weight-indexed LVCO, RVCO and CCO compared to fetuses with normal NT-proANP levels. In addition, the distribution of cardiac output did not differ between these two groups. No differences were observed in parameters describing systolic and diastolic functions of the fetal heart. Significantly increased neonatal and umbilical venous ANP levels have been reported in anemic, acidemic, hydropic and growth-restricted fetuses (Hatjis et al. 1989, Panos et al. 1989, Kingdom et al. 1992, Ville et al. 1994). However, no significant correlation was found between fetal ANP levels and reductions in the umbilical artery S/D-ratio (Kingdom et al. 1991). Our Doppler findings of fetuses with increased NT-proANP levels demonstrate the large functional capacity of the fetal heart in pregnancies complicated by placental insufficiency.

6.3 Fetal myocardial cell damage (I, III)

In normal pregnancies with uncomplicated deliveries, newborn cTnT was not clinically significantly (> 0.10 ng/ml) increased. Maternal hypertensive disorders with or without signs of placental insufficiency and with normal umbilical venous return are not associated with significant fetal myocardial cell damage.

Based on increased umbilical artery PI values, decreased RVFS, more frequent visualization of TR and increased cardiac size, it seems that the right ventricular afterload is increased in fetuses with elevated cTnT levels. The right ventricle mainly reflects the circulation and resistance in the fetal lower body, placenta and pulmonary bed, while the left ventricle is responsible for the circulation in the coronary and cerebral arteries and the fetal upper body. In acute experiments on fetal lambs, increase in right ventricular afterload by means of ductal occlusion significantly decreased RVFS, and TR was immediately present. All these changes resolved after ductal occlusion was released (Tulzer et al. 1991a). In human fetuses with increased vascular impedance in the descending aorta, RVFS was found to be decreased with no change in the left ventricle (Rasanen et al. 1989). In addition, in our study III, the RVCO% was less and LVCO% was greater in fetuses with placental insufficiency and increased cTnT levels than in fetuses with only a rise in NT-proANP production. This suggests that fetuses with increased right ventricular afterload shift their cardiac output from the right to the left ventricle. However, no difference in weight-indexed CCO was found. This finding is in agreement with the results of al-Ghazali et al., who reported that the RVCO% was about 58% in growth restricted fetuses with relatively mild placental insufficiency, but it was significantly lower in fetuses with more severe placental insufficiency and FGR (al-Ghazali et al. 1989). In study III, the increased right ventricular afterload seemed to
originate from the placenta. Fetuses with elevated cTnT levels demonstrated significantly higher incidence of TR (54.5%) than reported previously during the second half of gestation (6.8%) (Respondek et al. 1994). However, similar TR dP/dT between fetuses with increased cTnT levels and fetuses with only increased NT-proANP concentrations suggest that the right ventricle is able to maintain its contractility despite the increase in right ventricular afterload.

In animal studies, it has been previously shown that ejection forces are not affected by afterload (Noble et al. 1966). During the second half of gestation, human fetal ejection forces of both ventricles increase with no difference between the ventricles (Rasanen et al. 1997). However, fetuses with severe ductal constriction or occlusion demonstrated lower RVeFo values than fetuses with patent DA (Rasanen et al. 1997). Based on similar weight-indexed ventricular ejection forces, fetuses with elevated cTnT levels in our series seem to be able to maintain adequate systolic function of the heart. The diastolic function of the fetal heart described by the IRT% was also similar between fetuses with only elevated NTpro-ANP levels and fetuses with increased cTnT concentrations. Thus, it seems that global myocardial function and ventricular compliance are not changed even in conditions with significant fetal myocardial cell damage.

Redistribution of fetal arterial circulation, as determined by the DAo/MCA PI-ratio, the incidences of retrograde net blood flow in the aortic isthmus and visualization of coronary artery blood flow, did not differ between fetuses with elevated cTnT levels and fetuses who had only increased NT-proANP levels. Retrograde net blood flow in the aortic isthmus has been associated with diminished oxygen delivery to the cerebral circulation even though the volume blood flow is maintained (Fouron et al. 1999). Growth-restricted fetuses with easily visualized coronary artery blood flow have been suggested to be at a high risk of intrauterine death and postpartum circulatory failure (Baschat et al. 1998). Fetuses with elevated cTnT levels in study III, demonstrated increased right ventricular afterload. A rise in pressure causes a greater demand for oxygen in the myocardium than an increase in volume load. A rise in right ventricular afterload increases the wall stress, thus increasing oxygen consumption by the myocardium. This is also supported by the fact that the right ventricular systolic diameter remained proportionally greater in fetuses with elevated cTnT levels. However, it seems that fetal compensatory mechanisms, including coronary artery vasodilatation and increased blood flow, are able to meet the increased oxygen demand because the incidences of demonstrable coronary artery circulation were similar in fetuses with elevated cTnT levels and fetuses with only increased NT-proANP concentrations. Studies on fetal lambs have also shown large coronary vascular reserves in response to diminished oxygen content of the blood (Reller et al. 1995). Increased right ventricular afterload and similarity in demonstrable coronary artery circulation in fetuses with elevated cTnT levels suggest that fetal myocardial cell damage is more related to increased afterload and pressure than diminished oxygen delivery to the myocardium. The increase in wall tension is greater in the subendocardial than in the subepicardial region. In the coronary circulation, the reserve of the subendocardial region is less than that of more superficial layers. Thus, the subendocardial area could be more vulnerable to hypoxemia in the human fetus.
Fetuses with myocardial cell damage had increased pulsatility in the blood velocity waveforms of the DV, LHV and IVC. Their NT-proANP concentrations were higher than in fetuses with no signs of myocardial cell damage. The PIV values in DV, LHV and IVC had a significant positive correlation with umbilical artery NT-proANP concentrations demonstrating that DV, LHV and IVC PIV values reflect fetal systemic venous pressure. Studies on fetal lambs have shown that a rise in systemic venous pressure is associated with increased pulsatility in the systemic venous blood velocity waveforms (Gudmundsson et al. 1999). It has also been shown that changes in umbilical venous velocities originate in the fetal venous system and are transmitted towards the placenta (Reed & Anderson 2000). Fouron et al. showed in a lamb model that an increase in fetal right ventricular pressure is associated with a marked accumulation of products of reactive O₂ species generation in the right ventricular myocardium (Fouron et al. 2001a). The findings in studies I and III demonstrate that the presence of increased pulsatility in systemic veins and atrial pulsations in the fetal intra-abdominal umbilical vein are associated with biochemically detectable myocardial cell damage. In study I, serum cTnT concentrations were increased above the clinically significant level in every newborn who experienced atrial pulsations in the intra-abdominal umbilical vein during fetal life.

6.4 Retrograde aortic isthmus net blood flow (IV, V)

In placental insufficiency, the parallel circulatory system of the fetal heart ensures a highly oxygenated blood supply to the coronary and cerebral circulations: well-oxygenated blood entering from the placenta flows through the ductus venosus and left hepatic vein via the foramen ovale to the left atrium and ventricle (Kiserud et al. 1991) and blood from the fetal lower body enters the right atrium and ventricle through the inferior vena cava and other hepatic veins (Fig. 1). The aortic isthmus has a dynamic role in connecting these two parallel circulatory systems in the fetus. Based on the findings in an acute fetal lamb model, Fouron et al. showed that oxygen delivery to the brain does not decrease until the net blood flow through the aortic isthmus becomes retrograde (Fouron et al. 1999). The volume blood flow in the cerebral circulation is maintained even in the presence of retrograde aortic isthmus net blood flow.

In our study population, placental function, as assessed by placental vascular impedance, seemed to be equally impaired, and umbilical artery pH and PO₂ values at birth were similar in fetuses with either antegrade or retrograde net blood flow in the aortic isthmus. It is important to note that retrograde aortic isthmus net blood flow could be detected in the presence of normal looking umbilical artery blood velocity waveform profiles. This is in agreement with the results of studies on fetal lambs, which have shown that the aortic isthmus blood flow profile can change prior to deterioration of the umbilical artery blood velocity waveform pattern (Bonnin et al. 1993).

In pregnancies complicated by placental insufficiency, fetal weight-indexed RVCO and LVCO were significantly reduced with no difference as regards to direction of aortic isthmus net blood flow. The RVCO% and LVCO% were similar in fetuses with either retrograde or antegrade aortic isthmus net blood flow. However, in fetuses with antegrade aortic isthmus net blood flow, the QDₐ% was increased, and the QP% was decreased. This
demonstrates a shift in the distribution of RVCO from the pulmonary to the systemic circulation. Human fetal studies have shown that the $Q_F^\%$ increases and the $Q_{DA}^\%$ decreases significantly with no change in the $Q_{DA}^\%$ during the second half of gestation (Rasanen et al. 1996a). Furthermore, the proportion of umbilical volume blood flow shunted through the DV has been shown to decrease significantly from 40% to 15% between 20 and 38 weeks of gestation (Bellotti et al. 2000). In addition, animal and human studies have demonstrated that during the last trimester of pregnancy, fetal pulmonary circulation is under acquired vasoconstriction which is affected by fetal oxygen tension (Lewis et al. 1976, Morin et al. 1988, Rasanen et al. 1998). In placental insufficiency, oxygen delivery from the placenta can be impaired, leading to lower oxygen content of the fetal blood. As a result, vasoconstriction of the pulmonary arterial bed and a drop in pulmonary volume blood flow could occur. In addition, the $Q_{FO}^\%$ was greater in fetuses with antegrade aortic isthmus net blood flow compared with fetuses with retrograde aortic isthmus net blood flow, thus increasing its role in making up left ventricular cardiac output. In this way, in placental insufficiency, fetuses with antegrade net blood flow in the aortic isthmus are able to ensure highly oxygenated blood to the coronary and cerebral circulations. Fetuses with retrograde net blood flow in the aortic isthmus failed to reorganize the distribution of right and left ventricular cardiac outputs, and thus increase volume blood flow through the foramen ovale to the same magnitude as fetuses with antegrade net blood flow. In fetuses with retrograde aortic isthmus net blood flow, oxygen delivery to the coronary and cerebral circulations is diminished compared to fetuses with antegrade aortic isthmus net blood flow, even in the presence of similar pO2 in the umbilical vein. This may predispose these fetuses to coronary and cerebral hypoxemia. This is supported by the fact that fetuses with retrograde aortic isthmus net blood flow were delivered more frequently by cesarean section due to signs of fetal distress.

The weight-indexed RVeFo and LVeFo in fetuses with placental insufficiency and either antegrade or retrograde net blood flow in the aortic isthmus did not differ from those measured in the control fetuses. Rizzo et al. demonstrated that the ejection forces of both ventricles were significantly and symmetrically decreased in growth-restricted fetuses. Adverse obstetric outcome was observed in growth-restricted fetuses in which the ejection forces of both ventricles were below the 5th centile of the normal limits for gestation. In addition, a relationship between the severity of acidosis and diminished RVeFo and LVeFo was demonstrated. The differences between these studies can be explained by a different study design. In this study, weight-indexed ejection force values were used because fetuses with placental insufficiency tend to be of a lower weight. In addition, umbilical artery pH values at delivery were similar among the groups, suggesting that fetuses in our study were delivered prior to development of severe fetal acidosis. Consequently, these findings show that the fetal heart is able to preserve its systolic function even in the presence of retrograde net blood flow in the aortic isthmus.

The proportion of IRT of the total cardiac cycle was greater in fetuses with placental insufficiency than in control fetuses with no difference as regards to the direction of aortic isthmus net blood flow. This suggests impaired diastolic function of the fetal heart in placental insufficiency. Increased pressure gradient between systemic and atrial levels might also explain the longer IRT proportion of the cardiac cycle, because fetuses with placental insufficiency tend to be hypertensive (Stale et al. 1991). However, significantly
higher IMP values suggest impaired global cardiac function in these fetuses compared to
the control group, as shown earlier by Tsutsumi et al. (Tsutsumi et al. 1999). Lower total
TVIs of the TV and MV in fetuses with placental insufficiency might be a consequence of
lower cardiac output in these fetuses. The tricuspid valve E/A TVI- ratio did not differ
between fetuses with either retrograde or antegrade aortic isthmus net blood flow.
However, the MV E/A TVI- ratio demonstrated a shift towards the early passive filling
period of the ventricle in fetuses with retrograde aortic isthmus net blood flow. The E/A
TVI- ratios of TV and MV increase with advancing gestation, which is thought to reflect
improvement in the diastolic function of the fetal heart (Rizzo et al. 1988). However,
loading conditions of the heart may affect E/A TVI- ratios. Elevation in atrial pressure
increases the E- component of the inflow velocity waveform. Thus, the greater E/A TVI-
ratio of MV in fetuses with retrograde aortic isthmus net blood flow may indicate
increased left atrial pressure. Increased left atrial pressure and impaired left ventricular
diastolic function could also reduce the blood stream through the foramen ovale.

Fetuses with retrograde net blood flow in the aortic isthmus had higher right
ventricular afterload than fetuses with antegrade net blood flow. The greatest CC/TC-ratio
in fetuses with placental insufficiency and retrograde net blood flow in the aortic isthmus
demonstrates the increase in relative heart size. Fetal right ventricular afterload is mainly
a result of vascular resistance in the fetal lower body, placenta and pulmonary arterial
bed. No difference in vascular impedances in the umbilical artery and DAO was found,
and DA blood velocity waveform profiles showed no signs of ductal constriction in
fetuses with either retrograde or antegrade aortic isthmus net blood flow. In fetuses with
retrograde net blood flow in the aortic isthmus, RVFS was decreased and the incidence of
TR was the highest. This indicates altered loading conditions of the right ventricle and
suggests that the pulmonary arterial circulation has an important role in the regulation of
right ventricular afterload. Consequently, increased cardiac afterload, and, thus, increased
ventricular pressure and wall tension, may lead to an increased oxygen demand of the
myocardium.

Redistribution of fetal arterial circulation, as determined by the DAo/MCA PI ratio,
was similar regardless of the direction of aortic isthmus net blood flow. This finding
demonstrates that there is already a maximal redistribution of cerebral circulation in the
presence of antegrade aortic isthmus net blood flow, which does not further increase in
fetuses with retrograde aortic isthmus net blood flow. Thus, in fetuses with placental
insufficiency, vascular impedance in the middle cerebral artery does not reflect the
direction of aortic isthmus net blood flow and the oxygen content of the blood entering
the cerebral circulation. However, fetuses with retrograde aortic isthmus net blood flow
demonstrated higher proximal pulmonary artery PI values than fetuses with antegrade net
blood flow. Circulation in the fetal pulmonary arterial bed is regulated by fetal oxygen
tension (Rasanen et al. 1998). Hypoxemia causes vasoconstriction and increases
pulmonary arterial vascular resistance, and the effects of hyperoxemia are opposite
(Morin et al. 1988). In addition, an increase in pulmonary arterial pressure is capable of
inducing vasoconstriction of the pulmonary arterial bed after 26 weeks of gestation
(Rasanen et al. 1999). In placental insufficiency, impaired oxygen delivery from the
placenta to the fetus could lower the oxygen content of the fetal blood, and lead to
vasoconstriction of the pulmonary arterial circulation. In addition, these fetuses tend to be hypertensive and their pulmonary arterial pressure may be increased, which could lead to vasoconstriction of the pulmonary arterial bed.

Fetuses with retrograde net blood flow in the aortic isthmus demonstrated increased pulsatility in DV compared with fetuses with antegrade net blood flow. Pulsatility in IVC did not differ between the groups. The finding that fetuses with retrograde net blood flow in the aortic isthmus are unable to increase $Q_{Fo}$ could explain the increased pulsatility in the DV blood velocity waveforms. In addition, fetuses with retrograde net blood flow had signs of elevated left atrial pressure. Furthermore, diminished placental volume blood flow could make the DV blood flow profile more sensitive to changes in atrial pressure.

These results suggest that the oxygen content of the blood entering the left ventricle is diminished in fetuses with retrograde aortic isthmus net blood flow compared with fetuses with antegrade net blood flow, even in the presence of similar umbilical vein pO$_2$ values. This is also supported by the fact that visualization of coronary artery blood flow, described earlier as a heart-sparing effect (Baschat et al. 1997), was significantly more common in fetuses with retrograde net blood flow in the aortic isthmus, suggesting diminished oxygen delivery to the myocardium and, thus vasodilatation of the coronary arteries. This may cause subtle changes in myocardial performance and an increase in left atrial pressure. In addition, oxygen consumption of the fetal heart could be increased in pregnancies complicated by placental insufficiency.

### 6.5 Clinical implications

In pregnancies complicated by placental insufficiency, the optimal timing of delivery is still an issue to be resolved. In a longitudinal study with growth-restricted fetuses after 24 weeks of gestation, the DV pulsatility index and short-term variation of fetal heart rate were important indicators for the optimal timing of delivery prior to 32 weeks of gestation. Delivery should be considered if one of these parameters becomes persistently abnormal (Hecher et al. 2001). The results of the present study demonstrate that in the presence of abnormal systemic venous blood velocity waveform patterns, the fetus already has biochemically detectable myocardial cell damage. The clinical significance of the fetal myocardial cell damage must be addressed in future studies. Based on our findings, the most optimal timing of the delivery in pregnancies complicated by placental insufficiency might be before the systemic venous blood flow patterns become abnormal.

The first report of the clinical significance of the direction of aortic isthmus net blood flow suggests that children who had retrograde aortic isthmus net blood flow during the fetal period have increased risk for neurodevelopmental deficit at least at the ages of 2 and 4 years (Fouron et al. 2001b). Our results show that fetuses with retrograde aortic isthmus net blood flow are delivered more often by cesarean section because of signs of fetal distress. In addition, the oxygen content of the blood entering the coronary and cerebral circulations is less than in fetuses with antegrade net blood flow in the aortic isthmus. Doppler examination of cerebral circulation does not reveal the direction of
aortic isthmus net blood flow. The investigation of aortic isthmus net blood flow should be incorporated in the evaluation of fetal well-being in pregnancies complicated by placental insufficiency.
7 Conclusions

1. Fetuses with placental insufficiency and atrial pulsations in their umbilical vein have increased umbilical artery NT-proANP and cTnT levels, demonstrating that atrial pulsations in the umbilical vein reflect increased systemic venous pressure and myocardial cell damage (I-II).

2. In placental insufficiency, umbilical artery NT-proANP levels correlate significantly with pulsatility index values for veins in the ductus venosus, left hepatic vein and inferior vena cava (III). Fetal myocardial cell damage, as regards to elevated umbilical artery cTnT levels, is associated with a shift in cardiac output in favor of the left ventricle, an increase in right ventricular afterload and increased pulsatility in the systemic venous blood velocity waveforms, the latter indicating a rise in systemic venous pressure, (III).

3. Fetuses with placental insufficiency and antegrade aortic isthmus net blood flow demonstrate a shift in their right ventricular cardiac output from the pulmonary to the systemic circulation, and foramen ovale volume blood flow makes up the majority of their left ventricular output. Fetuses with retrograde aortic isthmus net blood flow fail to demonstrate these changes, and they have signs of increased left atrial pressure (IV).

4. Fetuses with retrograde aortic isthmus net blood flow demonstrate a rise in right ventricular afterload and increased pulsatility in ductus venosus blood velocity waveforms with no difference in the redistribution of the arterial circulation compared with fetuses with antegrade aortic isthmus net blood flow (V).
8 References


