Portal and umbilical venous distribution in the human fetus

A longitudinal ultrasound study

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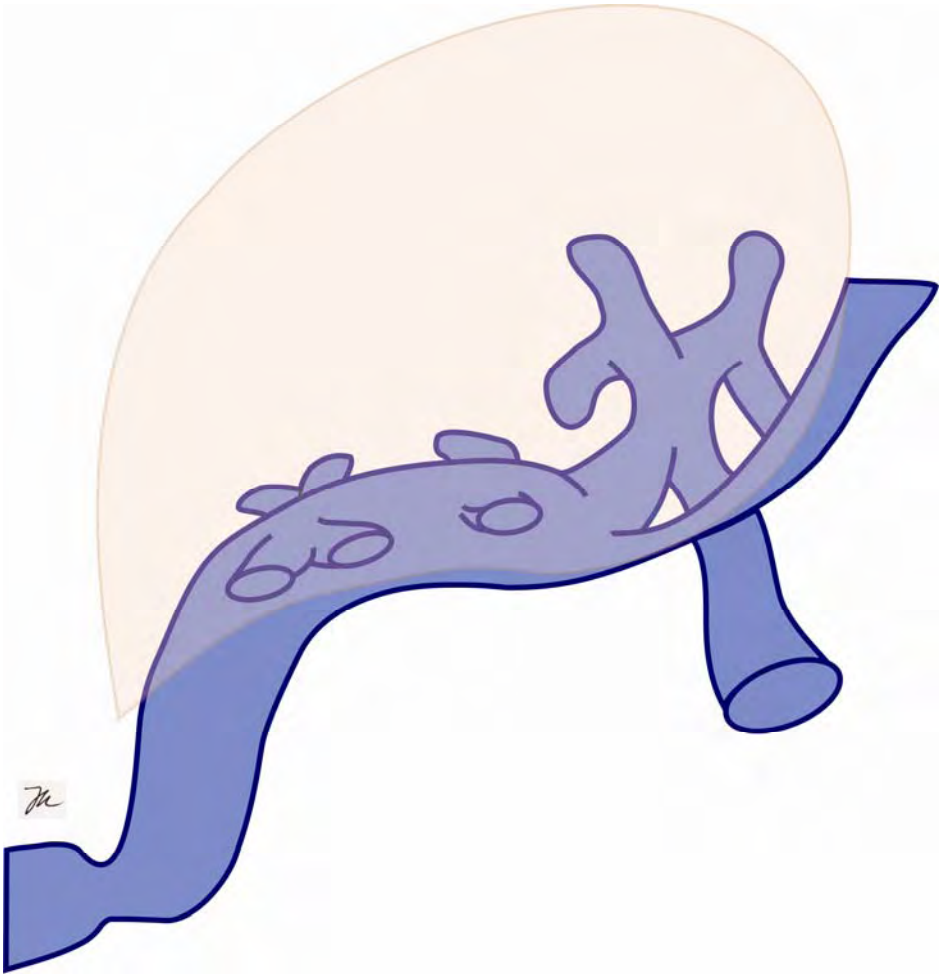

CONTENTS

1. ACKNOWLEDGEMENTS

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2. ABSTRACT

Knowledge of the venous perfusion of the human fetal liver is fragmentary and mainly limited to the umbilical circulation. Current ultrasound technology makes it possible to study blood flow non-invasively and to give new insights into the developmental changes during gestation.

AIMS: The general aim of the study was to map the hemodynamics of the venous supply to the fetal liver in humans. In detail: to establish longitudinal reference ranges for ductus venosus flow velocities and waveform indices (paper I), to establish a technique for direct flow measurement in the main portal stem and study its development during the second half of pregnancy (paper II), to assess the flow velocity pattern in the left portal vein (paper III), and to determine the distribution of venous blood supply within the liver and explore the impact of maternal and fetal factors (paper IV).

MATERIAL AND METHODS: After informed written consent, 160 women with low-risk pregnancies were recruited to a longitudinal study according to a protocol approved by the Regional Committee for Research Ethics (REK-Vest 04/3837). 4-5 ultrasound examinations, each lasting 60 minutes, were done at four weeks intervals between 20 and 40 weeks of gestation.

At each session the inner diameter was measured in: 1. the intraabdominal portion of the umbilical vein, 2. the ductus venosus, 3. the main portal stem, and 4. the left portal vein. In the same vessels, time-averaged maximum flow velocties were measured by Doppler ultrasound. Volume blood flow was calculated and normalised for fetal weight.

Mean and percentile curves were constructed using regression analysis and multi-level modelling. The impact of maternal and fetal factors on liver blood flow was investigated by deviance statistics.

RESULTS: The established longitudinal reference ranges for ductus venosus flow velocities and waveform indices allow the calculation of conditional percentiles, which are narrower and commonly shifted compared to those of the entire population (Paper I).

Blood flow in the main portal stem was pulsatile in 99%. Both diameter and flow velocities doubled during the observation period. Correspondingly, blood flow increased throughout gestation, and so did flow, normalised for fetal weight (Paper II).

Flow velocities in the left portal branch increased throughout gestation. We found pulsatile flow in 69%, usually directed towards the right lobe. However, intermittent flow reversal occurred during respiratory movements, and continuous reversal in 8% of the observations close to term (Paper III).

Total venous liver flow increased throughout gestation, while normalised flow decreased. Lobe specific flow distribution was stable during gestation directing 60% of the total venous liver flow to the left and 40% to the right lobe. The umbilical vein was the dominating venous blood source, but the portal fraction grew during the last trimester. Venous liver flow and its components were related to birthweight, while lobe specific flow and fractional flow distribution to the lobes were related to pregnancy weight gain (Paper IV).

CONCLUSION: We have established longitudinal reference ranges for all components of venous liver supply and the ductus venosus in the human fetus.

The reference ranges for ductus venosus velocimetry are appropriate for serial measurements, especially when conditional terms are applied (Paper I).

The present established technique of assessing flow in the main portal stem had a high success rate and could be used to show an increasing hemodynamic importance of the main portal stem towards term (Paper II).

The umbilico-portal watershed is usually situated in the right liver lobe, but may shift towards the left portal vein even in circulatory uncompromised fetuses. The time-averaged maximum flow velocity is suggested for the evaluation of the umbilico-portal watershed (Paper III).

The relationship between venous liver flow and birth weight may indicate a link between liver perfusion and fetal growth. The growing portal fraction of the total venous blood flow signifies the high circulatory priority given to the splanchnic circulation close to term. The relationship between low pregnancy weight gain and the distributional shift in favour of left liver lobe perfusion may be part of an adaptation to various intrauterine environments (Paper IV).

What is already known on this topic	What this study adds
Paper I	
Ductus venosus shunts 20 to 30% of umbilical venous blood. Ductus venosus velocimetry is used for hemodynamic assessment.	Longitudinal reference ranges for ductus venosus flow velocities and waveform indices including conditional terms for serial measurements in fetal assessment.
Paper II	
The main portal stem drains deoxygenated blood from the splanchnic circulation to the right liver lobe. Portal flow has not been directly measured in the human fetus, but has been estimated indirectly to account for 21% of the total venous supply at 36 weeks of gestation.	Standardized measurement technique and longitudinal reference ranges for portal vein diameter, flow velocities and blood flow. Blood flow in the main portal stem increases throughout gestation mainly explained by fetal gain. Normalised flow in the main portal stem increases during the last trimester.
Paper III	
Cases of hemodynamic compromise have shown reversed flow in the left portal vein. Left portal vein flow direction is suggested as a marker of fetal compromise. Flow velocities in the left portal vein have been measured at 36 weeks of gestation and shown orthograde flow towards the right liver lobe.	Normal development of orthograde flow in the left portal vein during the second half of pregnancy. Reference ranges for and terms for serial measurements of the time-averaged maximum blood velocity in the left portal vein suggested for the assessment of umbilico-portal watershed. Reversed flow is a normal phenomenon during fetal respiratory movements. Reversed flow may occur in normally developing fetuses, but only shortly before birth, and is probably rare.
Paper IV	
The total venous supply to the fetal liver and the umbilical venous blood flow are both related to abdominal circumference of the fetus. At 36 weeks, the umbilical vein accounts for 79% and the portal vein for 21% of the total venous liver flow, of which 60% are directed to the left and 40% to the right lobe. Maternal diet and body composition modify venous liver flow in late gestation.	Longitudinal reference ranges for total venous, left portal vein, ductus venosus and lobe specific blood flows. Longitudinal ranges for fractional flow distribution between the umbilical and portal veins and right and left liver lobes. The portal fraction of the total venous liver flow increases during the last trimester. The flow distribution between the left and right liver lobe is fairly stable throughout gestation. Venous liver flow and its components are related to birthweight. Low pregnancy weight gain is related to a distributional shift in venous liver flow in favour of the left lobe

3. LIST OF PAPERS

I: Kessler J, Rasmussen S, Hanson M, Kiserud T. **Longitudinal reference ranges for ductus venosus flow velocities and waveform indices.** Ultrasound Obstet Gynecol 2006;28: 890-898.

II: Kessler J, Rasmussen S, Kiserud T. **The fetal portal vein – normal blood flow development during the second half of the human pregnancy.** Ultrasound Obstet Gynecol; in press.

III: Kessler J, Rasmussen S, Kiserud T. **The left portal vein as a watershed of the fetal circulation: development during the second half of pregnancy and suggested method of evaluation.** Submitted

IV: Kessler J, Rasmussen S, Godfrey KM, Hanson M, Kiserud T. **Longitudinal study of umbilical and portal venous blood flow to the fetal liver: low pregnancy weight gain is associated with preferential supply to the left fetal liver lobe.** Submitted

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4. ABBREVIATIONS

CCO	Combined cardiac output
CI	Confidence interval
DA	Ductus arteriosus
DV	Ductus venosus
FGR	Fetal growth restriction
FO	Foramen ovale
IVC	Inferior vena cava
LeLL	Left liver lobe
LPV	Left portal vein
PV	Portal vein (main stem)
RiLL	Right liver lobe
RPV	Right portal vein
SD	Standard deviation
SE	Standard error
TAMXV	Time-averaged maximum flow velocity
UV	Umbilical vein

5. INTRODUCTION

5.1. Liver development

5.1.1. Organogenesis

The liver originates from the endoderm in a four step process: 1. Endoderm formation, 2. Endoderm patterning, 3. Liver induction, 4. Liver differentiation.

Immediately after gastrulation the endodermal layer forms the primitive gut tube which is divided into foregut, middle gut and hindgut. Already at this stage, genetic differences of the morphologically uniform endodermal cells are present. A thickened layer of endodermal cells located in the ventral foregut starts differentiating into hepatoblasts forming the liver bud. The hepatoblasts gradually invade the neighbouring mesenchyma of the septum transversum getting into contact with primitive endothelial cells. The process of differentiation and invasion is controlled by signalling molecules and transcription factors secreted by the endothelial, mesenchymal and hepatoblast cells. Similarly, a transcriptional cascade controls the later development of the bipotent hepatoblasts into hepatic lineages of hepatocytes and bile duct cells. The endothelial cells not only send early signals for differentiation and growth of the hepatoblasts, but also represent the cellular basis for the sinusoidal network of capillaries surrounding the hepatocytes (McLin et al. 2006; Zaret 2002).

5.1.2. Vascular development

In the fifth week after conception, three pairs of veins dominate the circulation of the embryo. The vitelline veins drain blood from the yolk sac, the umbilical veins (UV) transport blood from the chorium to the embryo and the cardinal veins collect the venous blood of the embryo itself. Normally the left UV remains patent throughout gestation while the right one atrophies. The vitelline veins, with a net of anastomoses around the duodenum, fuse to a single vessel and form the portal vein (PV) (Langman 1989).

Goussye et al. defined three main stages in the vascular development of the fetal liver based on the study of 51 human fetuses (Gouysse et al. 2002). From gestational week 5 to 10 only the precursors of the portal veins were visible, the

vessels retained their primitive uniform characteristics. Between the 10th and 25th gestational week the vascular architecture of the fetal liver was acquired through de-novo angiogenesis of the intra-hepatic arteries and growth and differentiation of the portal veins and hepatic sinusoids. This process is mainly controlled by the vascular endothelial growth factor exhibiting the maximal expression from week 10 to 25. After that time little changes in the vascular architecture and differentiation were observed.

5.2. Fetal circulation

For a long time, knowledge of the fetal circulation was mainly based on experimental data from animals. In particular, understanding the fetal circulation in human pregnancies has fundamentally been based on invasive studies in fetal sheep using radio labelled microsphere technique (Edelstone et al. 1978; Edelstone et al. 1980; Itskovitz et al. 1987; Rudolph 1985). However, different vascular and organ anatomy, higher temperature and lower haemoglobin in fetal lambs are likely to limit the direct use of experimental data in the human pregnancy (Kiserud 2005). The rapid development of diagnostic ultrasound has made it possible to study the human fetus non-invasively and the knowledge on the developmental changes during gestation grows continuously.

Specific features like the placental circuit, the position of the liver and the vascular shunts through the ductus venosus (DV), the foramen ovale (FO) and the ductus arteriosus (DA) constitute the difference between the fetal and the postnatal circulations.

5.2.1. Blood volume

The fetal blood volume is estimated to 10-12% of the body weight, which is higher than in adults (8%) (Brace 1983). This is explained by the placental blood pool, which constitutes nearly half the blood volume in fetal sheep at mid gestation and falls to 20% at term (Barcroft 1946). Changes in blood volume are rapidly compensated due to a high diffusion rate between the fetal compartments.

5.2.2. Arterial circulation

Both ventricles of the fetal heart pump blood in parallel to the systemic circulation. While the left ventricle perfuses the upper part of the body including the myocardium and the brain; apart from a small fraction to the lungs, the right ventricle directs blood through the DA to the lower part of the body and the placenta.

The combined cardiac output (CCO) of the human fetus increases throughout gestation, and depending on the measurement technique, a biventricular output of 1300 to 1900 ml*min^{-1} near term was calculated (Kiserud et al. 2006; Mielke et al. 2001; Rasanen et al. 1996). The CCO normalized for fetal weight is unchanged during pregnancy at approximately 400 ml*min^{-1}*kg^{-1} (Kiserud et al. 2006; Mielke et al. 2001). At mid gestation 30% of the CCO enters the placental circulation, decreasing to 20% after 32 weeks (Kiserud et al. 2006; Sutton et al. 1991). A consistent finding in all studies is the dominance of the right ventricle over the left with regard to output volume (Kiserud et al. 2006; Mielke et al. 2001; Simpson 2004). Depending on gestational age, 13-25% of the CCO is directed to the lunges, while 40% passes through the DA (Rasanen et al. 1996).

The DA is a short vessel that connects the pulmonary artery with the descending aorta, thus being part of the arterial outlet to the lower part of the body. The patency of the DA is mainly determined by circulating prostaglandin E_2 (Clyman et al. 1978).

Although often referred as one of the shunts in the fetal circulation, the vascular segment interposed (as a shunt) between the two parallel arterial circuits is rather the isthmus aortae than the DA (Fouron 2003). The blood flow pattern at the isthmus aortae is determined by the resistance of the brachiocephalic and subdiaphramatic circulations. Under physiological conditions, net flow at the isthmus is always antegrade, but an early diastolic flow reversal may occur in the last trimester due to decreasing cerebral resistance (Fouron et al. 1994).

The blood pressure in the human fetal heart was measured invasively between 16 and 29 gestational weeks (Johnson et al. 2000). In both ventricles the pressure increased throughout gestation with no significant differences between the left and

right sides. At 28 weeks the ventricular pressure was 35 / 7 mm Hg (Johnson et al. 2000). Compared with postnatal values the fetal arterial pressure is low, mainly due to the low resistance in the placental circulation (Simpson 2004).

5.2.3. Venous circulation

5.2.3.1. Circulatory arrangement

Oxygen and nutrient rich blood from the placenta returns to the fetus via the UV which first gives off branches to the left lobe of the liver. A fraction of the umbilical blood is then shunted through the DV, while the remainder is directed to the right liver lobe (RiLL) via the left portal vein (LPV).

Venous blood from the spleen, stomach, pancreas and intestine is drained into the portal vein, which perfuses the RiLL in fetal life under physiological conditions (Fig. 5).

At the level of the right atrium, the venous return from the superior and inferior vena cava (IVC) and the right hepatic vein is predominantly directed through the tricuspid valve into the right ventricle ("via dextra"). In contrast, most of the venous blood coming from the DV and left and medium hepatic veins is shunted through the FO over to the left atrium and ventricle ("via sinistra") (Barcroft 1946).

5.2.3.2. Oxygen saturation

The highest venous saturation is found in the UV and DV (80%), the lowest in the IVC (35%) and the PV (30%) (Bristow et al. 1981; Rudolph 1985). Thus, the left liver lobe (LeLL) is perfused by highly oxygenated umbilical blood, in contrast to the RiLL which is perfused by a mixture of umbilical and portal blood with a lower saturation. Since the oxygen extraction in the liver is modest (10-15%) (Rudolph 1985), the left and medium hepatic veins are important contributors of oxygenated venous blood to the "via sinistra".

Despite the preferential streaming of highly oxygenated venous blood from the DV and LeLL to the left heart and poorly oxygenated IVC and superior vena cava blood to the right ventricle, the actual difference in oxygen saturation between the left and right side is modest at 10-15% (Dawes et al. 1964; Rudolph 1985).

5.2.3.3. Blood pressure

The umbilical venous pressure in the human fetus was 4.5 mm Hg at mid gestation and increased to 6.5 mm Hg at term (Ville et al. 1994). Others found comparable values, but no significant increase with gestational age (Weiner et al. 1989). The pressure in both atria was constant at 3.5 mm Hg (Johnson et al. 2000), suggesting an umbilical or porto-caval pressure gradient of 1-3 mm Hg. This is in agreement with the calculation of the pressure gradient along the DV based on velocity measurement, which found a range from 0-2 mm Hg (Kiserud et al. 1994). This pressure gradient accelerates the umbilical blood through the DV at velocities sufficient to result in a fountain like blood stream that opens the FO (Kiserud et al. 1991).

5.2.3.4. Shunts and Flow distribution

Two of the shunts in the fetal circulation, the DV and the FO, are positioned on the venous side.

The first distributional unit for the umbilical venous blood is the DV. In the human fetus the DV is a slender, trumpet like vessel connecting the UV with the IVC. The length of the DV increases from 5 mm at 18 weeks to 15 mm at 34 weeks (Kiserud et al. 1994), the diameter at the inlet of the DV hardly exceeds 2 mm (Kiserud et al. 1992). Apart from one investigation (Tchirikov et al. 1998), human studies of low-risk pregnancies showed a shunt fraction of 30% at mid gestation and 20% near term (Bellotti et al. 2000; Haugen et al. 2004; Kiserud et al. 2000), which is considerably less than the shunt fraction of 50% in fetal sheep (Edelstone et al. 1978). Consequently, 70-80% of the umbilical venous return in human pregnancies perfuses the liver, which illustrates the high developmental priority given to this organ.

The portal fraction of the total venous liver flow has been measured in fetal sheep and indirectly calculated in human fetuses to be around 20%, while the UV is the dominating source of venous blood supply to the liver with a fraction of 80% (Edelstone et al. 1978; Haugen et al. 2004).

The second distributional unit is the FO. The anatomic arrangement of the FO flap, the Eustachian valve and the top of the interatrial septum, the crista dividens, facilitates the preferential streaming described above as the via dextra and sinistra concept (Kiserud et al. 1992). In human fetuses the shunt fraction through the FO was 34% at mid gestation, falling to 18% at 30 weeks and remaining stable until term (Rasanen et al. 1996). The limiting area for the shunt is in a horizontal plane between the FO flap and the crista dividens and does not increase after 30-32 weeks (Kiserud et al. 2001), which is in agreement with the stable shunt fraction until term.

5.2.3.5. Regulation of liver and ductus venosus flow

Fluid dynamics like viscosity and pressure are important regulators of liver and DV flow (Kiserud et al. 1997). The DV blood at high velocities performs as a Newtonian fluid (low viscosity), while the venous blood in the liver with its large capillary bed and low velocities is a non-Newtonian fluid (high viscosity). The non-Newtonian properties make the liver blood flow collapse when the perfusion pressure remains under the closing pressure of 1-4 mm Hg (Kiserud et al. 1997). Increasing viscosity (hematocrit) or decreasing perfusion pressure reduces liver flow more than DV flow, resulting in a higher shunt fraction.

Although the existence of an anatomical sphincter at the DV inlet is controversial (Tchirikov et al. 2006), the functional reactivity of the DV has been demonstrated in several studies (Adeagbo et al. 1982; Adeagbo et al. 1985; Adeagbo et al. 2004; Coceani et al. 1984; Kiserud et al. 2000; Tchirikov et al. 2003; Tchirikov et al. 2005). Stimulation by adrenergic substances and endothelin caused constriction, while adrenergic stimulation, NO and prostaglandins induced relaxation. Hypoxemia induces substantial dilatation of the DV and increases the shunt fraction (Edelstone et al. 1980).

Also the portal vascular bed is sensitive to catecholamines and prostaglandins (Paulick et al. 1990; Paulick et al. 1991) with a vasoconstrictive response stronger than in the DV (Tchirikov et al. 2003; Tchirikov et al. 2005), which may account for a circulatory shift towards more shunting through the DV (Tchirikov et al. 2006).

Respiratory movements, present 30% of the time in the near term fetus (Harding 1994), increase umbilical blood flow (Marsal et al. 1984) and may therefore affect venous liver flow.

Apart from local regulatory mechanisms, maternal diet and body composition also modify the fetal liver blood flow (Haugen et al. 2005).

5.2.3.6. Determinants of venous pulsation

The contraction of the atria and ventricles not only gives the blood kinetic energy to circulate, but also generates a pulse wave. The propagation of this wave follows so called transmission lines, which are typically the vessels in the arterial and venous circulations. One of these transmission lines starts in the right atrium, following the DV into the UV and the LPV, transmitting the pulse wave generated by atrial contraction (a-wave) (Kiserud 2003).

A higher amplitude of the pressure wave is generated by atrial distension (Frank Starling mechanism), when the atrioventricular valves are closed during atrial contraction (arrhythmias) or during adrenergic drive (Hasaart et al. 1986; Reuss et al. 1983) (Fig. 1). Furthermore, the amplitude of the a-wave is substantially increased during hypoxemia (Kiserud et al. 2001).

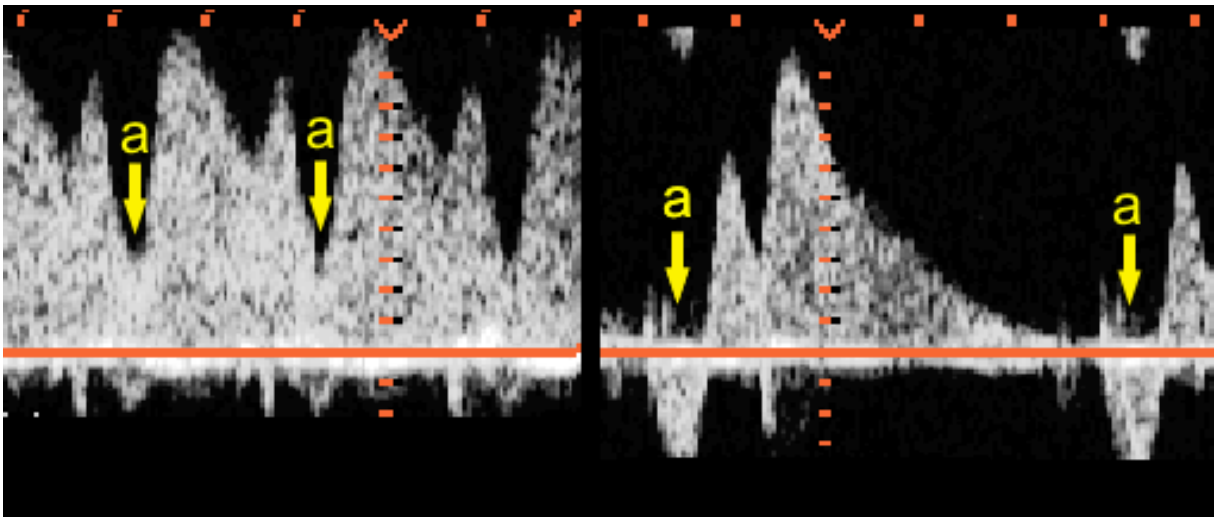

Figure 1: *Pulsed Doppler recording of the ductus venosus during normocardia (left) and bradycardia (right) at 24+6 weeks. Augmented a-wave during bradycardia due to distension of the atrium and the Frank Starling mechanism.*

The effect of the pulse wave on the flow velocities depends on the direction of the flow and pulse. A pulse wave travelling in opposite direction of flow causes a velocity deflection, while a velocity increment is observed when pulse and flow have the same direction (Kiserud 2003). This phenomenon has been described for DV and LPV flow of circulatory compromised fetuses (Kiserud et al. 2003), but is also present in healthy fetuses (Fig. 2).

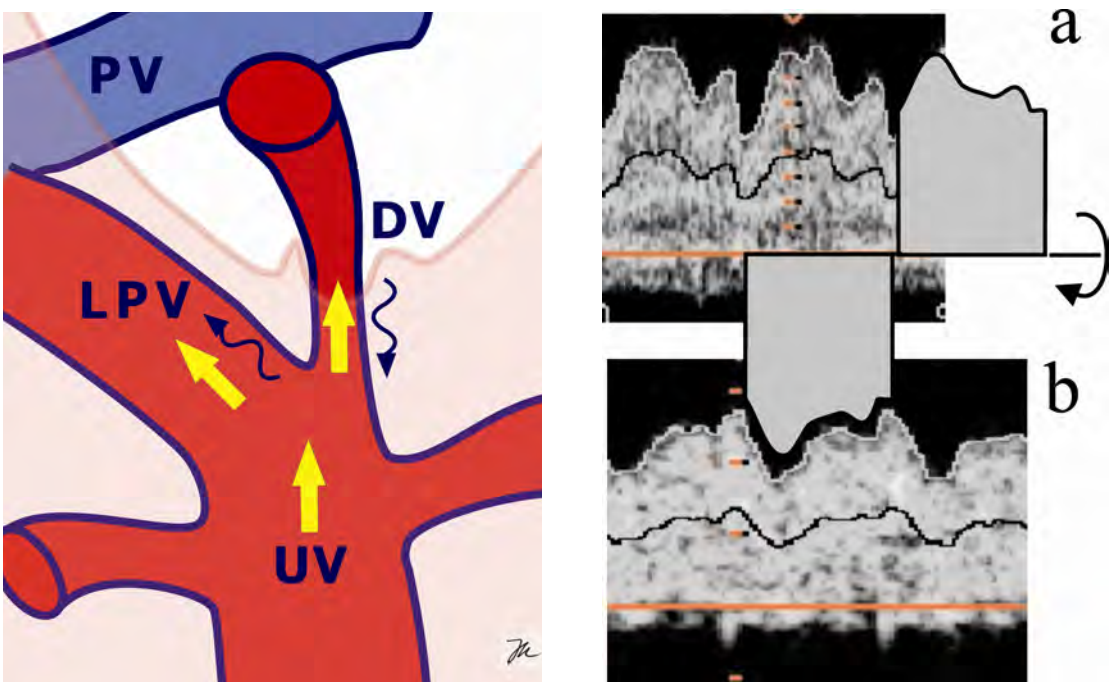

Figure 2: *<u>Left:</u> The atrial pressure wave (curved blue arrows) travels against the flow direction (straight yellow arrows) in the ductus venosus (DV), but after transmission into the left portal vein (LPV), it travels in the same direction as the blood flows. <u>Right:</u> The velocity deflection in the ductus venosus (a) corresponds therefore to a velocity increment in the left portal vein (b) and vice versa. UV: umbilical vein, PV: main portal stem. (28+5 weeks)*

Apart from the transmission of a pressure wave from the atrium into the venous system, pulse waves from neighbouring arteries may be transmitted to the venous system causing velocity increment (Kiserud 2003) (Fig. 3).

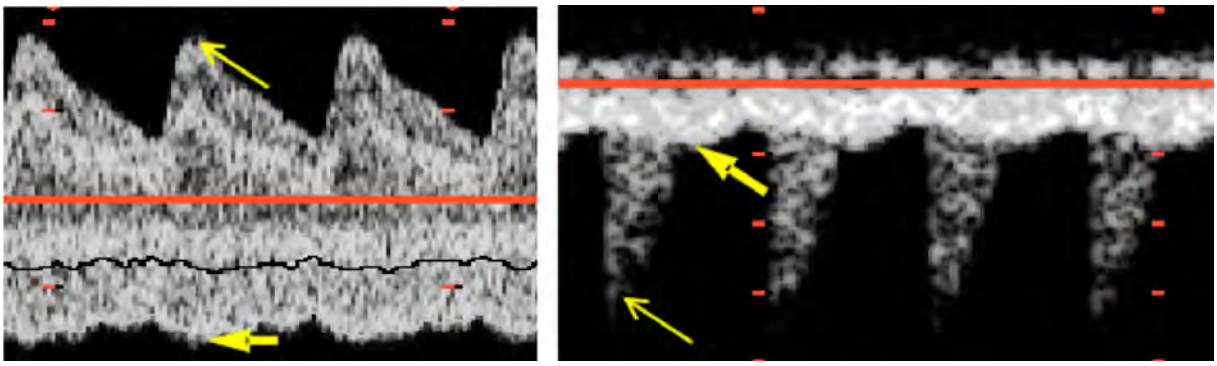

Figure 3: *Velocity increment in veins (bold arrow) due to pulse transmission from arteries (thin arrow). Left: umbilical vein and umbilical artery at the abdominal entrance (38+2 weeks); right: portal vein and hepatic artery (21+3 weeks).*

Analogously with light or sound waves, the emitted pressure wave is modified when travelling along the veins. Reflection of the wave is clinically important and occurs when the impedance along the transmission line changes. The most important determinant for the impedance is the vessel diameter. At the junction between the DV (small diameter/ high impedance) and the UV (large diameter/ low impedance) most of the pressure wave (a-wave) is reflected and therefore undetectable in the velocity pattern of the UV under physiological conditions (Kiserud 2003).

Another determinant of venous pulsation is the reservoir effect. The larger and more compliant a vessel is, the higher wave energy is required to cause a visible pulsation (Hellevik et al. 2000). The UV as a sizeable vessel represents such a reservoir, which further explains why the a-wave is usually not detectable in the UV.

5.2.3.7. Reproducibility and validity of flow measurements

When using ultrasound, calculation of volume blood flow is based on measurement of flow velocities and vessel diameter.

The reproducibility of venous Doppler recordings is acceptable (Barbera et al. 1999; Eik-Nes et al. 1984; Huisman et al. 1992; Kiserud et al. 1992; van Splunder et al. 1994). Calculation of normalized umbilical flow had a coefficient of variation of 7% (Rasmussen 1987). For DV velocities the coefficient of variation was 9-15% (Kiserud et al. 1992). Excellent agreement in UV flow calculation has been found comparing Doppler ultrasound and steady state diffusion technique (Barbera et al. 1999).

Since the diameter enters the formula for flow calculation in the second power, an erroneous measurement will introduce a substantial error into flow calculation. High accuracy in diameter measurement is therefore crucial for calculation of volume flow based on ultrasound. Validation of diameter measurements by ultrasound has been done in vitro and in fetal sheep, demonstrating that repeat measurements of the diameter reduce random error to an acceptable level (Kiserud et al. 1999). Following the concept of repeat measurement (six times), the intraobserver variation for calculated flow (vessel size 0.6 – 6 mm) was 1.5 -11% (Kiserud et al. 1999). Corresponding results were achieved in a clinical setting when five repeat diameter measurements resulted in a 95% confidence interval (CI) for the subject mean diameter of 0.2 mm (UV) and 0.15 mm (DV) (Kiserud et al. 1998).

5.2.4. Hemodynamic aspects of fetal growth restriction (FGR)

The present study examined a low-risk population. Since papers I and III present methods of assessment in circulatory compromised fetuses, it seems appropriate to address hemodynamic changes in fetal growth restriction.

The aetiology and pathogenesis of FGR is complex and not completely understood. However, morphological changes in the placenta are well documented and include a reduction in placental size, reduced number of villi, reduced intervillous space and a thickened trophoblastic epithelium (Mayhew et al. 2003). This results in a progressing impairment of placental function and an increase of resistance in the placental capillary bed, which is reflected by changes of the flow velocity pattern in the umbilical artery. As the capillary bed is reduced by more than 50%, enddiastolic blood flow in the umbilical artery becomes absent or reversed (Giles et al. 1985).

In spite of the increasing afterload due to high placental resistance, the growth restricted fetuses maintain the CCO per kg within the normal range. However, the blood fraction to the placenta decreases, leading to increasing re-circulation of deoxygenated blood in the fetus (Kiserud et al. 2006).

As a consequence of increased placental resistance, umbilical venous blood flow is reduced (Boito et al. 2002; Di Naro et al. 2002; Ferrazzi et al. 2000; Kiserud

et al. 2006; Tchirikov et al. 1998) in a graded fashion depending on the severity of placental compromise (Kiserud et al. 2006).

The vascular shunts on the venous and arterial sides enable the fetus to rearrange the circulation in order to compensate for the deficient nutrient and oxygen supply and to give priority to the vital organs: myocardium and brain.

The first distributional unit for the umbilical venous return is the DV-LPV system. The DV diameter is responsive to hypoxia (Kiserud et al. 2001; Kiserud et al. 2000) and increased shunting through the DV has been demonstrated in human FGR fetuses (Bellotti et al. 2004; Kiserud et al. 2006; Tchirikov et al. 1998) directing a greater proportion of oxygenated umbilical blood through the DV and FO to the myocardium and brain. However, DV shunting increases at the expense of the liver perfusion with potential short and long term consequences (Tchirikov et al. 2002). Furthermore, increased DV shunting may shift the umbilico-portal watershed towards the LeLL and finally reverse the blood flow direction in the LPV (Bellotti et al. 2004; Kilavuz et al. 2003; Kiserud et al. 2003).

At the level of the FO, an increased pulmonary vascular resistance (Rizzo et al. 1996) and the relatively higher right ventricular afterload due to high placental resistance facilitate increasing right to left shunting in FGR (Baschat 2006). However, when fetal deterioration progresses, FO shunting decreases (Mäkikallio et al. 2003) and smaller FO diameter relative to the right atrium size are found (Kiserud et al. 2004).

The third distributional unit is the DA with the aortic isthmus as the watershed between the brachiocephalic (brain) and subdiaphragmatic (placental) circulations. Net blood flow at the aortic isthmus is usually antegrade (Fouron et al. 1994) and strongly related to umbilical flow as demonstrated in fetal sheep (Bonnin et al. 1993). As long as the net flow at the isthmus of FGR fetuses is antegrade, the FO and left ventricular output fraction are relatively increased ensuring oxygen supply to the coronary and cerebral circulations (Mäkikallio et al. 2003). In contrast, growth restricted fetuses with retrograde aortic isthmus flow fail to rearrange the distribution of left and right ventricular output, which increases the risk of myocardial and cerebral hypoxemia (Mäkikallio et al. 2003) and is associated with non-optimal

postnatal neurodevelopment (Fouron et al. 2001). Monitoring of aortic isthmus blood flow in FGR is therefore suggested as an indirect method of assessing cerebral oxygenation and may refine timing of delivery (Fouron 2003).

Apart from redistribution at the level of the fetal shunts, local vascular autoregulatory adjustments modify the circulation in FGR, the common feature being increasing diastolic flow and decreasing pulsatility. Coronary and cerebral arterial vasodilatation in response to hypoxemia act synergistically with arterial and venous redistribution, facilitating blood supply to the myocardium and brain (Baschat 2006). Sparing the liver does not act synergistically with redistribution, but counteracts an organ steal effect caused by decreased venous supply (Baschat 2006). Furthermore sparing of the adrenal gland in FGR has been described (Mari et al. 1996).

In FGR before 32 gestational weeks a typical sequence of changes has been described starting with abnormal pulsatility in the umbilical artery and abnormal amniotic fluid index, followed by changes in the middle cerebral artery and aorta and finally affection of venous Doppler and short-time variation of the fetal heart rate (Hecher et al. 2001).

Arterial and venous redistribution as described above are dependent on a sufficient cardiac performance. As ventricular function declines the increasing cardiac preload facilitates an augmented a-wave in the precordial veins including DV which is predictive of acidemia (Baschat et al. 2004; Hecher et al. 1995) and an adverse neonatal outcome (Baschat et al. 2007; Hecher et al. 1995). Monitoring of DV blood flow velocities has therefore become a useful tool in timing delivery in early onset FGR.

5.3. Ultrasound examination

5.3.1. Gray scale ultrasound

Sound waves with a frequency above the audible level (16 Hz-20 kHz) are called ultrasound. Diagnostic ultrasound in obstetrics and gynaecology commonly uses frequencies of 3 – 7.5 MHz. Most of the transducers currently used are so called

array transducers, which are composed of a high number of transmitting units arranged in a curved linear shape.

When small structures like fetal vessels are measured by ultrasound, penetration and resolution of diagnostic ultrasound are important issues. After emission, the ultrasonic wave is partially absorbed due to friction forces depending on the density of the passed medium (impedance). The degree of attenuation (energy loss) of the ultrasonic wave is described by penetration. Apart from the impedance of the insonated tissue, penetration is directly dependent on the ultrasound frequency (Angelsen 2000).

The resolution along the direction of the beam is called radial resolution and is determined by the length of the emitted ultrasonic pulse. The resolution transverse to the beam is called lateral resolution and is determined by the width of the beam. An increase of the ultrasound frequency shortens the pulse length and narrows the beam width, which in turn increases resolution.

However, obstetric ultrasound is largely dependent on the use of low ultrasound frequencies (2.5-3.5 MHz) with relatively poor resolution, to ensure penetration of the fetal structures in the depth of the abdomen. Focussing the ultrasonic beam helps to escape the conflict between penetration and resolution (Angelsen 2000; Dudwiesus 1995).

The image quality of a structure also depends on the intensity of the reflected signals. On a smooth interface (e.g. vessel wall) the ultrasonic waves will largely be reflected with the angle of reflection equivalent to the angle of incidence. Consequently, the insonation should be nearly perpendicular to the surface in order to avoid substantial signal loss due to reflection (Dudwiesus 1995).

5.3.2. Doppler ultrasound

Doppler ultrasound is used to evaluate blood flow quantitatively and qualitatively. It is based on the Doppler principle, which describes the property of the ultrasonic wave of changing frequency when meeting a moving reflector. The frequency change, also called Doppler shift (F_d) is proportional to the velocity (V) of

the reflector met by the ultrasonic waves. Given a known ultrasound frequency (F_0), sound velocity (c) and angle of insonation () , the velocity of the moving reflector can be calculated as:

$$V = \frac{F_d \cdot c}{2 \cdot F_0 \cdot \cos\theta}$$

Different types of Doppler ultrasound are in clinical use: 1. Continuous-wave (CW) Doppler, 2. Power Doppler, 3. Colour Doppler, and 4. Pulsed Doppler (Maulik 2005).

CW Doppler ultrasound continuously transmits and receives ultrasonic signals. There is no range discrimination. CW Doppler ultrasound is mostly used in obstetrics for external fetal heart rate monitoring.

Colour Doppler transforms the measured Doppler shift into a colour coded map, which is superimposed on the gray scale image. Colour Doppler is able to discriminate flow direction and to give rough estimates of flow velocity (high/low) of a larger region of interest, while the temporal resolution is rather bad.

Power Doppler is based on the display of amplitude (intensity) data, reflecting the number of red blood cells scattering the ultrasonic beam. Power Doppler is able to detect low velocity flow independent of the angle of insonation and without Nyquist limit.

Pulsed or spectral Doppler is based on the repeat emission of ultrasonic wave pulses at a given sampling frequency (pulse repetition frequency, PRF [scale]). The Doppler shift of a small region of interest (sample volume) is transformed to a velocity spectrum that gives information on the distribution of velocities and enables high temporal resolution. An important limitation of pulsed Doppler ultrasound is expressed by the Nyquist limit: The maximum Doppler shift which can be recorded unambiguously is half the PRF and decreases with increasing range (depth) of the sample volume. It is also important to adapt the sample volume to the size of the vessel. A large sample volume may include velocities from neighbouring vessels leading to interference, while a small one may not cover the entire cross section of the vessel, thus missing part of the velocity spectrum (Maulik 2005).

A correct measurement of absolute velocities by Doppler ultrasound requires knowledge of the insonation angle. Following a cosinus function, the effect of the angle correction on the velocity measurement is small at insonation angles between 0-30 ° and becomes considerable when the angle exceeds this range (Fig. 4).

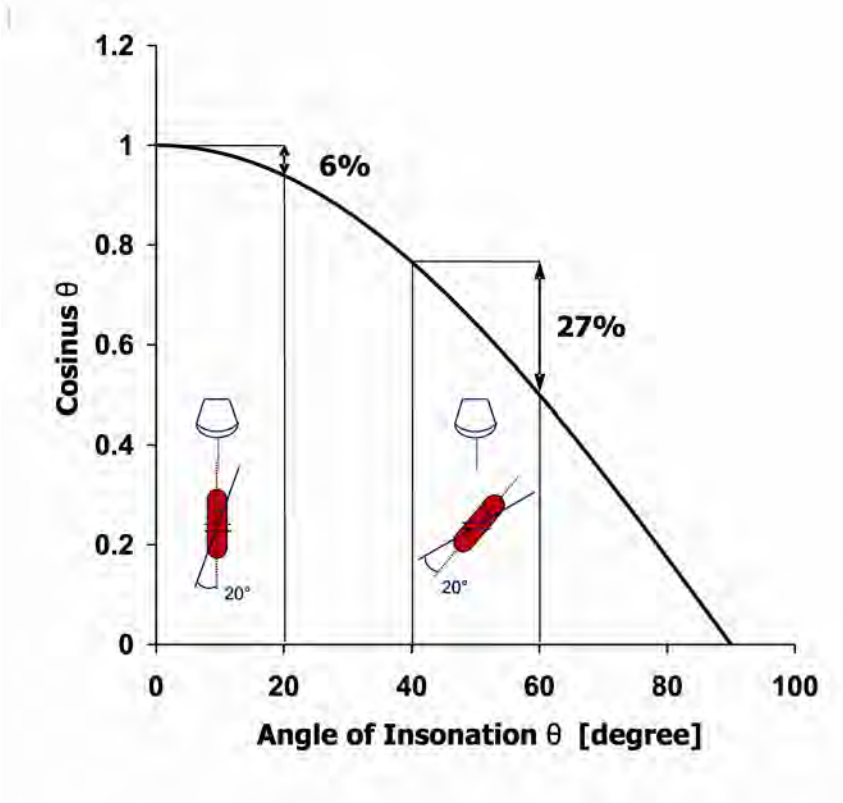

Figure 4: *Cosinus function. An erroneous angle correction of 20° imposes 6% error on the velocity measurement at insonation angles close to 0°, while the corresponding error is 27% at higher insonation angles.*

The filter setting of the ultrasound machine also has important implications for velocity measurement. High pass filters are implemented to reduce the disturbance level in the Doppler recording by excluding high amplitude – low frequency signals (e.g. vessel wall movement). Simultaneously, low velocity flow signals are also excluded, which has to be considered in the evaluation of the Doppler recording (Angelsen 2000).

5.3.3. Safety

There are three main biological effects of ultrasound: 1. temperature rise, 2. cavitation and 3. acoustic streaming.

Temperature rise is the result of a direct transformation of sound into heat energy, which has been demonstrated after ultrasound exposure of animals. A minimum temperature rise of 1.5 °C is considered necessary to cause damage (Jensh et al. 1999) and a rise up to 4.5 °C was observed in live guinea pig brains exposed continuously for two minutes to Doppler ultrasound (Horder et al. 1998). The temperature increase depends on the properties of both the ultrasound wave and the exposed tissue, with the highest absorption observed in mineralized bone (Barnett et al. 2001). The potential for damage is dependent on the stage of development, with the highest susceptibility during the 1st trimester (Barnett et al. 2001).

Cavitation is a non-thermal mechanism of formation, oscillation and collapse of bubbles (ter Haar et al. 1981). The existence of a tissue – gas interface is a prerequisite for this biological effect. Ultrasonically induced cavitation causes lung haemorrhage in newborn animals, but there is no evidence of such damage in the fluid filled fetal lungs (Barnett et al. 2001).

Acoustic streaming describes the bulk movement of fluid caused by the radiation force along the path of the ultrasonic wave (Barnett et al. 2001). The implications for health are uncertain (Duck 1998).

Since data on the direct biological effects of diagnostic ultrasound are exclusively derived from experimental studies, the investigation of potentially harmful effects has to rely on epidemiological studies. No association has been found between ultrasound exposure and birthweight, childhood malignancies or neurological development (Salvesen et al. 1999). However, the analysis of two randomized controlled trials (Kieler et al. 1998; Salvesen et al. 1993) and one cohort study (Kieler et al. 2001) showed a statistically significant association between left handedness among males and ultrasound exposure. A Cochrane review including these randomized trials presented no subgroup analysis according to sex, and concluded that there is no association between ultrasound and handedness (Neilson 2000). Based on the scientific evidence so far, there is no evidence that application of diagnostic ultrasound causes harm to the fetus (Abramowicz et al. 2000; Abramowicz 2002; Salvesen 2002). A prerequisite is the responsible use of the technology according to the international guidelines (Barnett et al. 2000; Barnett et al. 2001),

which emphasize the users responsibility according to the As Low As Reasonably Achievable (ALARA) principle.

The output display of the ultrasound machine enables the user to follow the potential bio effects of the applied ultrasonic energy. The mechanical index (MI) expresses the relative risk of mechanical effects (cavitation and streaming) occurring. The thermal index (TI) is an estimate of the temperature rise in °C induced by the output energy at a certain level. The index is displayed specifically to the insonated tissue, for example the thermal index for soft tissue (TIS). Although at present the output display is the best way of providing safety information, it gives only rough estimates of the possible biological effects (Abramowicz et al. 2000; Marsal 2005).

6. AIMS OF THE STUDY

The aim of the present study was to map the venous liver circulation of the human fetus during the second half of pregnancy. In detail:

- to establish longitudinal reference ranges for ductus venosus flow velocities and waveform indices,
- to develop a technique for diameter and velocity measurement of the main portal stem,
- to study the prenatal development of the main portal stem blood flow,
- to establish reference ranges for the fetal portal vein (main stem),
- to investigate the left portal branch as an indicator for the watershed between the umbilical and portal circulations,
- to suggest a method of evaluating the watershed phenomenon,
- to establish longitudinal reference ranges for this method,
- to describe the normal development of this parameter during pregnancy,
- to examine the sources of venous blood supply to the fetal liver and the distribution between the liver lobes,
- and to explore the impact of maternal and fetal parameters on venous liver flow.

7. MATERIAL AND METHODS

7.1. Study population

The study protocol was approved by the Regional Committee for Research Ethics (REK-Vest 04/3837) and included a pilot and a main study.

Women attending the ultrasound department for the routine scan between 17 and 20 weeks of gestation were invited to the present study. A total of 40 women were recruited to a pilot study from May until August 2004. After that, a total of 160 healthy pregnant women consecutively entered the study during the period of August 2004 – July 2005. Gestational age was assessed by ultrasound in the second trimester (Johnsen et al. 2004). Reasons for exclusion were twins, fetuses with a malformation or chromosomal aberration, chronic maternal diseases (i.e. hypertension, diabetes, rheumatic or autoimmune diseases, dyslipidemia) or complicated obstetric history (i.e. preeclampsia, growth restriction, placental abruption, gestational diabetes, delivery < 37 weeks of gestation).

The study protocol aimed at four or five examinations per participant at four weeks intervals starting at 20-22 weeks of gestation. Participants in the pilot study were examined once. Each session lasted 60 minutes.

After birth, information on gender, weight, length, Apgar score, mode of delivery, transfer to the neonatal intensive care unit and neonatal complications were collected from the medical records.

7.2. Measurement

A Sonos 7500 ultrasound machine (Philips, Seattle, USA) with a 3.5 MHz (2-6 MHz) curved linear transducer including colour Doppler (2.5 MHz) and pulsed Doppler (3 MHz) facilities was used for the study. The high-pass filter was set at 50 Hz. The mechanical index (MI) and the thermal index for soft tissue (TIS) were usually at the level of 1.1 or below. In a few obese participants higher energy output was occasionally needed, increasing TIS and MI to 1.4. Pulsed Doppler ultrasound as the modality with highest energy transfer was used restrictively (Tab. 1).

Image	*Mode of ultrsaound*	*Time [min]*	*[%]*
Frozen		14:33	25
Unfrozen	2 D	20:42	35
	Colour Doppler	21:29	36
	Pulsed Doppler	2:36	4
Total		59:20	100

Table 1: *Use of the different ultrasound modalities during the examination. Analysis of one video taped examination.*

Vessel diameter and flow velocities were measured at: 1. the intraabdominal portion of the UV, 2. the inlet of the DV, 3. the LPV, and 4. the PV (Fig. 5).

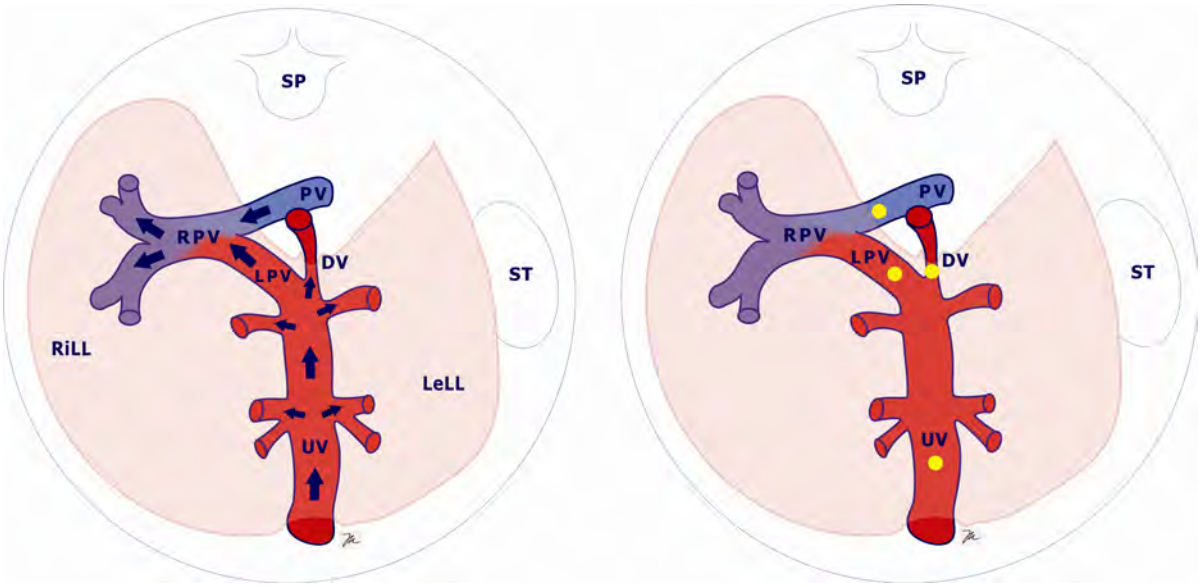

Figure 5: *Venous blood supply of the fetal liver with level of oxygenation, flow direction (blue arrows) and measure sites (yellow circles). Red colour: high oxygen saturation, blue colour: low oxygen saturation, purple colour: mixture of blood with high and low saturation. UV: umbilical vein, DV: ductus venosus, LPV: left portal vein, RPV: right portal vein, PV: main stem of the portal vein, LeLL: left liver lobe, RiLL: right liver lobe, ST: stomach, SP: spine.*

In general, the vessel diameter (D) was measured between the inner edges in a perpendicular insonation to the vessel wall. Diameter was measured repeatedly (UV, LPV, PV: median 4 times , DV: median 5) to reduce random error (Kiserud et al. 1998) and the individual mean diameter was used for further analysis. At the same

site, with an insonation along the vessel axis, time-averaged maximum flow velocity (TAMXV) was measured using pulsed Doppler. The angle of insonation was kept as low as possible and always ≤ 30°.

The volume blood flow (Q) was calculated as:

$$Q = \frac{\pi}{4} \cdot D^2 \cdot h \cdot TAMXV$$

h: velocity profile factor, h=0.5 for UV, LPV and PV, h=0.7 for DV

For the umbilical vein a parabolic distribution of the velocities in the vessel with *h*=0.5 was suggested (Kiserud et al. 1994) and an excellent correlation between 0.5 TAMXV and the intensity weighted mean velocity was shown in a recent study (Acharya et al. 2005). A different velocity profile was experimentally proven for the DV with a partially blunted flow expressed by *h*=0.7 (Kiserud et al. 1998; Pennati et al. 1996).

The maternal pre-pregnancy body mass index (BMI) was calculated as:

$$BMI = \frac{weight(kg)}{height(m)^2}$$

The ponderal index of the neonate (Rohrer 1908) was calculated as:

$$Ponderal\ index = \frac{weight(kg)}{length(m)^3}$$

7.3. Statistics

7.3.1. Power calculation

Anticipating that a cross-sectional study needs a certain number of participants (Nc), Royston et al.(Royston et al. 1995) calculated the corresponding number in a longitudinal study (N_l) for the same purpose as: $N_l = Nc/2.3$. According to previous experience in cross-sectional studies (Altman et al. 1997; Kiserud et al. 2000; Skulstad et al. 2002), approximately 15 observations per gestational week are necessary for the calculation of reliable percentiles. An observation period of 20 weeks corresponds to Nc = 300 and N_l = 130 according to the above formula. An anticipated success rate of 80% increases the necessary study population to 160 participants.

7.3.2. Construction of mean and percentile curves

The construction of mean and percentile curves followed the principles described by Royston (Royston 1995; Royston et al. 1995; Royston et al. 1998). Statistical analysis was performed using SPSS (SPSS Inc, Chicago, IL, USA). Multilevel modelling (level 1: measurement, level 2: fetus) was used to construct mean and percentile curves for each variable according to gestational age using the MlWin program (MlWin, Centre for Multilevel Modelling, University of Bristol, UK). The assumption of normal distribution was checked for the outcome variables, and the power in which the dependent variables were transformed was found using Box-Cox transformation. Fractional polynomials were specified as two components, one fixed and one random.

The 2.5^{th}, 5^{th}, 10^{th} and 25^{th} percentiles were calculated by subtracting 1.96 standard deviation (SD), 1.645 SD, 1.282 SD and 0.674 SD from the mean. The 97.5^{th}, 95^{th}, 90^{th} and 75^{th} percentiles were estimated by adding the respective multiples of the SD to the mean. The CI of the mean was obtained from the standard error (SE) of the mean using the MlWin program. To obtain an approximate SE of percentiles, transformed observations ± 1.645 SD were regressed against gestational age. The SE around the regression lines, which equalled the 5^{th} and 95^{th} percentiles, was used to obtain 95% CIs around these percentiles.

The longitudinal design permitted estimation of conditional reference values, i.e. given an observation at one examination, we calculated a conditional value at a later gestational age.

7.3.3. Deviance analysis

To assess the possible effect of maternal and fetal variables (fetal gender and tertiles of maternal birth weight, pre-pregnancy BMI, pregnancy weight gain, birth weight, and newborn's ponderal index) on the flow variables, we included these possible determinants as indicator variables in the multilevel regression models that describe the mean curves. Indicator variables with significant improvement in the

goodness of fit to the models, as assessed by the deviance statistics (2 with $p < 0.05$), were considered to influence the flow significantly.

7.3.4. Reproducibility

The inter and intra observer variation for the measurement of the DV flow velocities and waveform indices, as well as portal diameter and velocity measurements, were calculated as limits of agreement according to Bland and Altman (Bland et al. 1986). The same method was applied when comparing two approaches for calculation of RiLL flow.

8. RESULTS AND COMMENTS

8.1. General characteristics

8.1.1. Study population

Characteristics of the participating women, the course of pregnancy and the neonatal outcome are presented in Table 2. The study aimed at recruitment of low-risk pregnancies. Since some of the results were used to establish reference ranges for clinical use (Paper I and III), the selection of the study population is an important issue. The exclusion of participants developing pregnancy complications has been criticised for producing super normal reference ranges. We decided not to exclude participants after enrolment to the study, regardless of the further pregnancy course. A certain degree of selection bias is still possible, since the voluntarily participating women tend to be healthier than those declining to enter the study. However, it was reassuring, that the mean birth weight in the study was similar to that of the background population (Skjaerven et al. 2000), and the cesarean section rate of 9.4% did not differ much from that in the general population (11%).

Participants			
Age	(years)	29	20 - 43
Para 0	(N)	93	58%
Pre-pregnancy BMI	($kg*m^{-2}$)	23.4	± 3.8
Pregnancy weight gain	($kg*week^{-1}$)	0.36	± 0.14
Maternal birth weight	(g)	3360	± 520
Delivery			
Gestational age at delivery	(weeks)	40+3	33+3 - 42+4
Cesarean section	(N)	15	9.4%
Neonates			
Male	(N)	82	51%
Female	(N)	78	49%
Birth weight	(g)	3550	2100 - 4700
Ponderal index	($kg*m^{-3}$)	27.7	± 2.3
Transfer to NICU	(N)	7	4.4%

Table 2: *Characteristics of the study population (N=160). Data presented as median – range, mean ± SD and number – percent. NICU: neonatal intensive care unit.*

8.1.2. Success rate

A total of 593 examinations were done in 160 participants (median: 4 per participant, range 1-5). The success rate was highest for calculation of UV flow (99%) and lowest for calculation of the liver lobe fractions (84%) (Tab. 3).

	Measurement							
	Diameter		*Flow velocity*		*Volume flow*		*Fraction*	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Umbilical vein	588	99	579	98	574	97		
Ductus venosus	580	98	547	93	543	92		
Left portal vein	572	96	554	93	542	91		
Main portal stem	573	97	576	97	559	94		
Total liver flow					525	89		
Left liver lobe flow					508	86		
Right liver lobe flow					529	89		
Ductus venosus shunt							534	90
Portal/umbilical fraction of venous liver flow							522	88
Left/right liver lobe fraction							497	84

Table 3: *Number of measurements (N) and success rate (%) related to the total number of examinations (N=593).*

8.2. Umbilical vein

The UV transports oxygen and nutrient rich blood from the placenta to the fetus. Blood flow in the UV has been studied in the free loop of the umbilical cord (Barbera et al. 1999; Boito et al. 2002) as well as in the intra abdominal portion of the vein (Acharya et al. 2005; Bellotti et al. 2000; Gill et al. 1984; Kiserud et al. 2000). All studies showed increasing umbilical blood flow throughout gestation, but they differ somewhat in the calculated values.

We found a blood flow of 44 ml*min^{-1} at 21 weeks increasing to 201 ml*min^{-1} at 36 weeks with no further significant change until term. Our flow quantification is in line with some previous studies (Acharya et al. 2005; Boito et al. 2002; Kiserud et al. 2000), while others found higher flow values (Barbera et al. 1999; Bellotti et al.

2000; Gill et al. 1984), probably due to different study size, measure site and method of flow calculation.

The normalized umbilical flow decreased during the second half of pregnancy confirming previous results (Acharya et al. 2005; Barbera et al. 1999; Kiserud et al. 2000). In contrast, the normalized fetal cardiac output is reasonably constant, resulting in a decreasing fraction of cardiac output perfusing the placenta as gestation advances (Kiserud et al. 2006).

8.3. Ductus venosus

The DV represents one of the shunts specific for the fetal circulation. It connects the UV with the IVC and the right atrium. The investigation of the human DV by Doppler ultrasound was first described 1991 (Kiserud et al. 1991) and has since become a useful tool of monitoring fetuses with growth restriction (Baschat et al. 2007; Baschat et al. 2004; Ferrazzi et al. 2002; Hecher et al. 2001; Hecher et al. 1995; Kiserud et al. 1994; Rizzo et al. 1994) and congestive heart disease (Baez et al. 2005; Hofstaetter et al. 2006; Kiserud et al. 1993). The published reference data are mainly based on cross-sectional studies (Axt-Fliedner et al. 2004; Bahlmann et al. 2000; Hecher et al. 1994), which are not necessarily appropriate for the serial measurements commonly requested in fetal monitoring.

We therefore established longitudinal reference ranges for DV flow velocities and waveform indices (Fig. 6, left). The longitudinal study design allows the calculation of conditional percentiles, i.e. the reference ranges of a given measurement are based on a former measurement in the same fetus. This approach takes into account the physiological variation of the flow variable by adapting the reference ranges to the individual biological capacity of the fetus (Fig. 6, right).

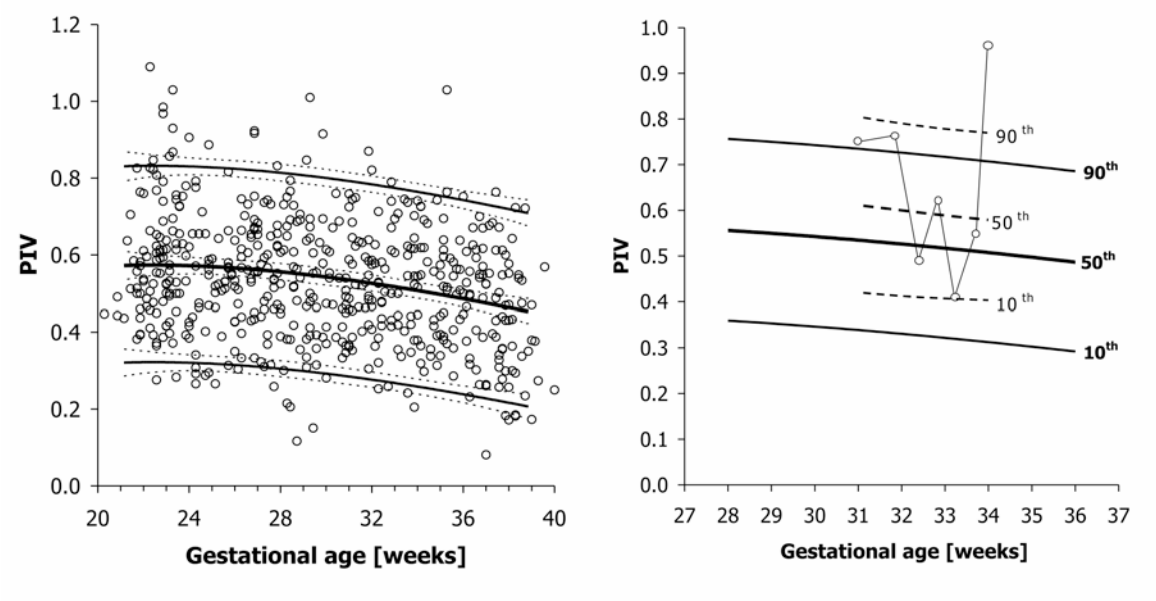

Figure 6: *<u>Left:</u> Longitudinal reference ranges for the pulsatility index for veins (PIV) of the ductus venosus based on 547 observations in 160 low-risk pregnancies. The 5th, 50th and 95th percentile are presented (solid lines) with 95% confidence interval (dashed lines).*
<u>Right:</u> Serial measurements of the PIV of the ductus venosus in a growth restricted fetus until delivery at 34 weeks. Conditional percentiles (dashed lines) are based on the first measurement. Reference ranges are indicated (solid lines).

Since the DV plays a crucial role in distributing the umbilical blood either to the liver or directly to the right atrium, the estimation of blood flow in the DV is an important physiological variable in the fetal circulation. In our study the DV volume flow increased from 13 ml*min^{-1} at 21 weeks to 45 ml*min^{-1} at 39 weeks. Although the diameter measurement in the vessel operates at the limits of resolution, these flow values confirm the majority of the previous studies (Bellotti et al. 2000; Haugen et al. 2004; Kiserud et al. 2000), but are considerably lower than data from Tchirikov et al. (Tchirikov et al. 1998). This difference is due to a larger DV inlet diameter measured in the latter study.

The normalized DV flow decreased during the second half of pregnancy, which is also in agreement with previous studies (Bellotti et al. 2000; Kiserud et al. 2000).

8.4. Portal vein

Apart from the UV, the fetal liver receives venous blood from the splanchnic circulation via the portal vein. Previous studies on the venous liver perfusion of human fetuses lacked data on portal blood flow (Bellotti et al. 2000), giving limited conclusions on total venous liver flow and lobe distribution.

In order to study the blood flow development in the PV of the fetus, we established a standardized technique for diameter and velocity measurement. The blood flow was pulsatile in all but six measurements (Fig. 7, left). Volume blood flow was estimated to 5 $ml*min^{-1}$ at mid gestation, and increased to 40 $ml*min^{-1}$ at term due to both growth of the vessel and increase of flow velocities. In contrast to the UV, normalized blood flow in the PV also increased during the last trimester, which signifies the importance of the splanchnic circulation for the venous liver perfusion near term (Fig. 7, right). Although the weighted mean flow velocity in late gestation is approximately half that in adult life (7.6 vs. 15 $cm*s^{-1}$) (Barbaro et al. 1999; Moriyasu et al. 1986), normalised blood flow near term and in adult life are almost equivalent (13 vs. 16 $ml*min^{-1}*kg^{-1}$) (Moriyasu et al. 1986).

The present technique makes it possible to study global venous liver supply in the fetus under physiological conditions and during circulatory compromise.

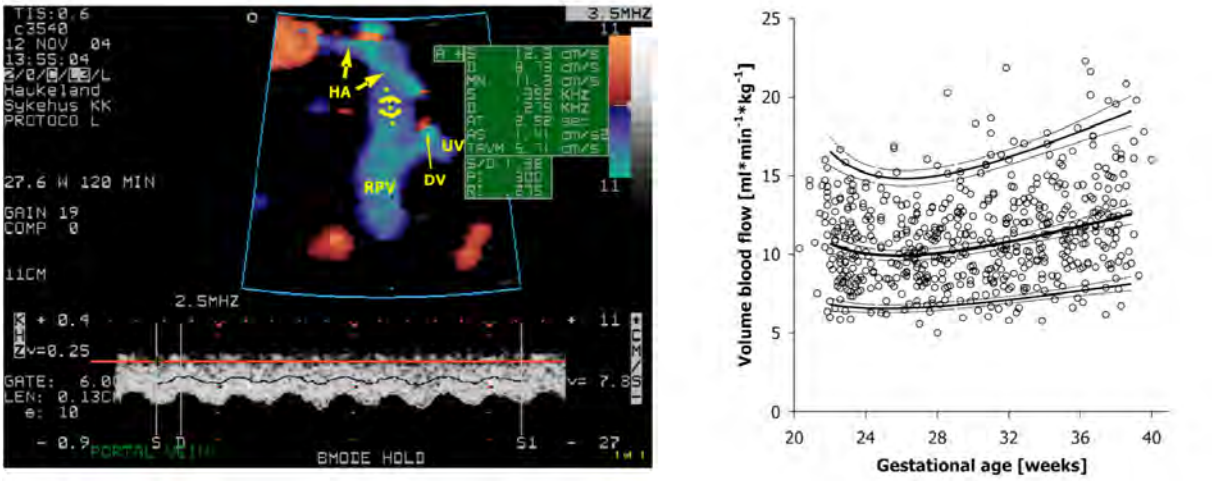

Figure 7: *<u>Left:</u> Pulsed Doppler recording at the main stem of the portal vein with the typical monophasic pulsatile velocity pattern at 27+6 weeks of gestation. The crossing arteria hepatica propria (HA, bold arrows) and the ductus venosus (DV, thin arrow) serve as anatomical landmarks. RPV: Right portal branch, UV: Umbilical vein*
<u>Right:</u> The development of volume flow in the main stem of the fetal portal vein normalised for fetal weight and based on 558 longitudinal observations in 160 low-risk pregnancies. The 5, 50 and 95 percentiles (solid lines) are presented with 95% confidence intervals (dashed lines). Flow calculation was based on 0.5 time-averaged maximum velocity.

8.5. Left portal vein as an indicator of the watershed between the umbilical and portal circulations

The LPV connects the umbilical with the portal circulation and was suggested as one of the watershed areas of the fetal circulation based on observations in circulatory compromised fetuses (Kilavuz et al. 2003; Kiserud et al. 2003). A previous cross-sectional study on late gestation human fetuses found exclusively forward flow in the LPV (Haugen et al. 2004), suggesting that the watershed between the portal and umbilical circulation was situated in the RiLL.

We systematically investigated the blood flow development in the LPV of healthy fetuses. Flow velocity increased up to 31 weeks, remained stable until 37 weeks and decreased slightly towards term. Intermittent flow reversal in the LPV occurred during respiratory movements (Fig. 8, right) and permanent flow reversal in 2/24 observations (8%) close to term, which demonstrates that the watershed region may shift towards the LPV even in uncomplicated pregnancies.

We suggested the use of TAMXV in the LPV for quantification of the watershed phenomenon and provided longitudinal reference ranges for the second half of pregnancy (Fig. 8, left).

Similar to the UV, volume blood flow in the LPV increased during the observation period, while normalised blood flow decreased.

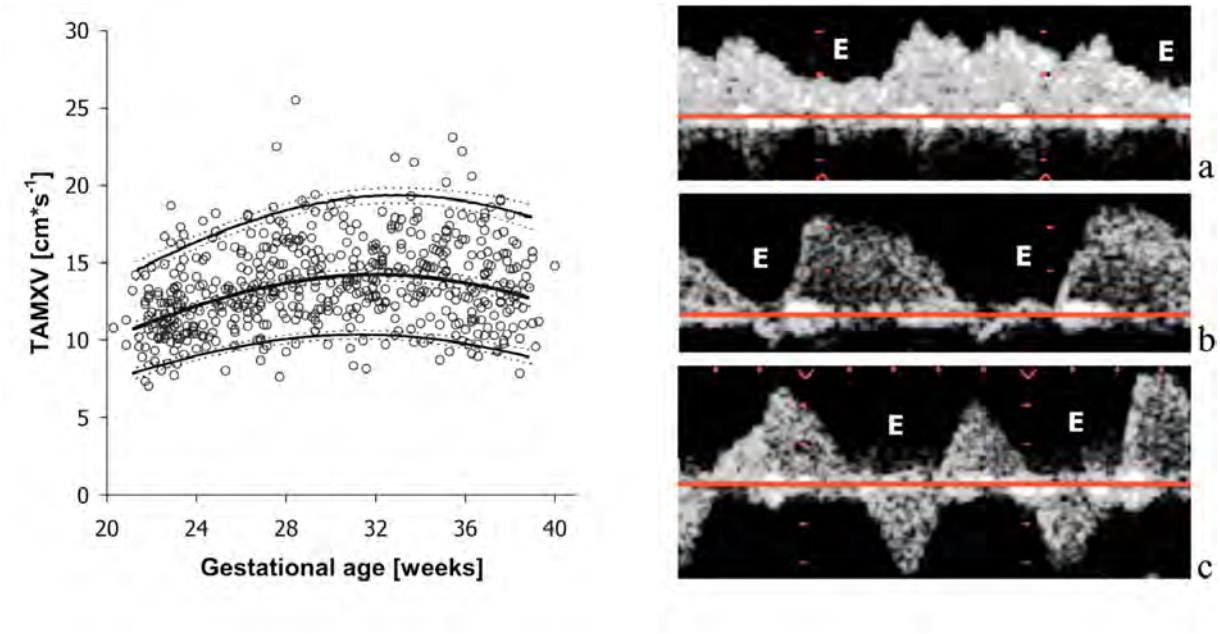

Figure 8: *<u>Left:</u> Time-averaged maximum velocity (TAMXV) in the left portal vein. 554 longitudinal observations in 160 low-risk pregnancies. 5, 50 and 95 percentile (solid lines) with 95 % confidence interval (dashed lines). Not shown on the graph: measurement at 39+2 weeks: - 13.7 cm/s (case 1). Orthograde flow velocity is shown for the case with both orthograde and retrograde flow velocity during one examination (case 2). Further information on case 1 and 2 in paper III.*
<u>Right:</u> Blood flow pattern in the left portal vein during respiratory movements of the fetus. Expiration (E) causes a reduction (a,b) or reversal (c) of the blood flow.

8.6. Lobe specific and total venous liver flow

The total venous liver flow increased during the second half of pregnancy, while normalised flow decreased (Fig. 9). A similar pattern was found for flow development in the left and the right liver lobes. That means that the flow increase is explained by fetal growth, thus confirming previous studies which found a correlation between fetal abdominal circumference and venous liver flow (Bellotti et al. 2000; Haugen et al. 2004).

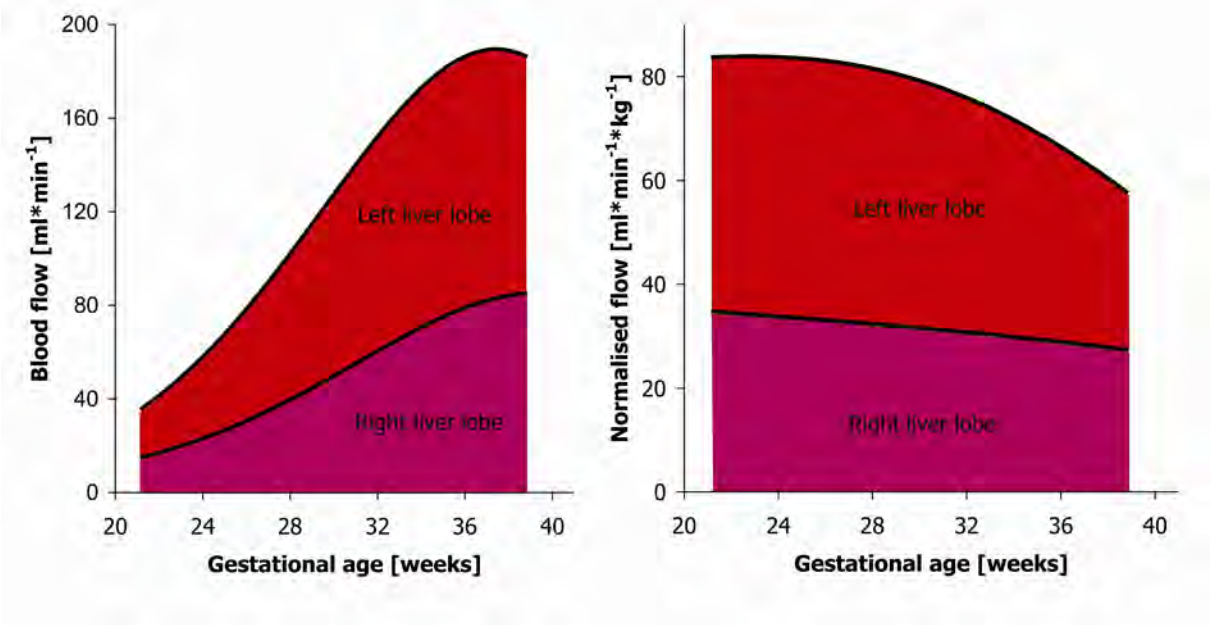

Figure 9: *Total venous liver flow and lobe specific flow in absolute terms (left) and normalised for fetal weight (right). Data are presented as mean curves (solid lines) based on 525 and 529 observations for total venous and right liver flow respectively.*

8.7. Distribution of umbilical and portal flow

The UV dominated the venous blood supply of the liver. However, the portal fraction increased during the observation period, in particular near term (Fig. 10, right). We interpret this finding as a gradual approximation to the postnatal circulation.

The flow distribution within the fetal liver was fairly stable as 60% of the venous blood was directed to the LeLL and 40% to the RiLL (Fig. 10, left). These figures are in agreement with a previous cross-sectional study at 36 weeks (Haugen et al. 2004). In contrast, the lobe distribution in adult life is opposite, directing 32% of portal flow to the left lobe and 68% to the right lobe (Barbaro et al. 1999). The time course of this circulatory shift is not clear, but cessation of the umbilical circulation at birth probably represents a step during this development associated with degenerative changes in the LeLL (Emery 1952).

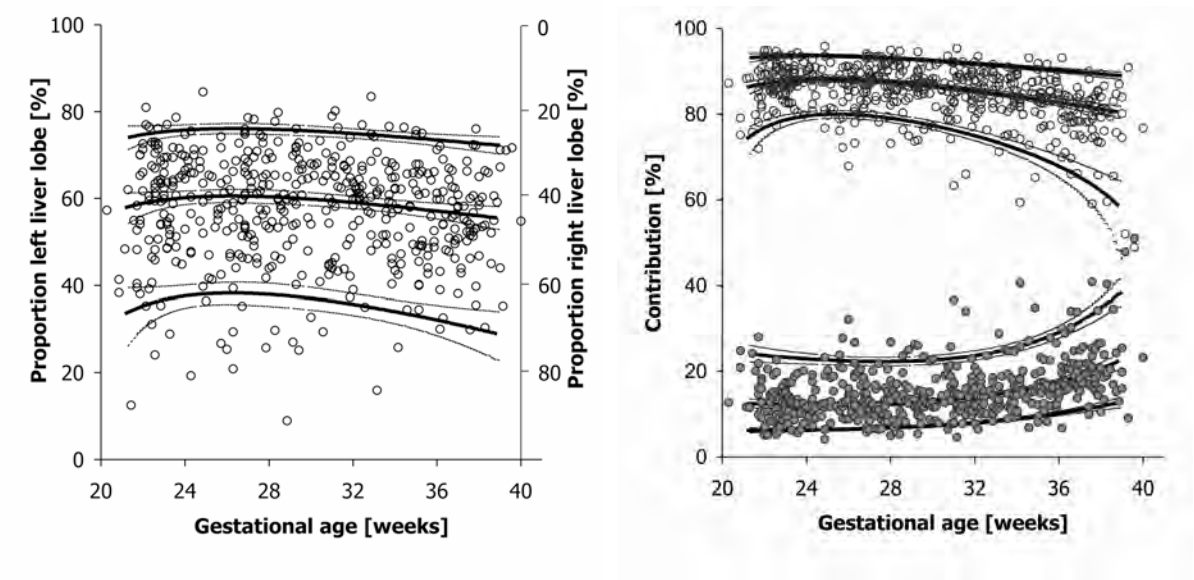

Figure 10: *<u>Left:</u> Proportion of total venous blood supply directed to the left and right liver lobes, based on 497 observations in 160 low-risk pregnancies.*
<u>Right:</u> Contribution of the main portal stem (filled circles) and the umbilical vein (open circles) to the total venous blood supply of the fetal liver, based on 522 observations in 160 low-risk pregnancies.

8.8. Influence of fetal and maternal factors on venous liver circulation

8.8.1. Influence of birthweight

Experiments in fetal sheep demonstrated a link between venous liver flow and fetal growth (Tchirikov et al. 2001; Tchirikov et al. 2002) and recent human studies found a relationship between abdominal circumference and venous liver flow (Bellotti et al. 2000; Haugen et al. 2004).

In the present study, total venous liver flow (Fig. 11), lobe specific flow, UV, LPV and PV flow were all positively related to birthweight. These findings confirm previous results and support the concept of a link between liver perfusion and fetal growth.

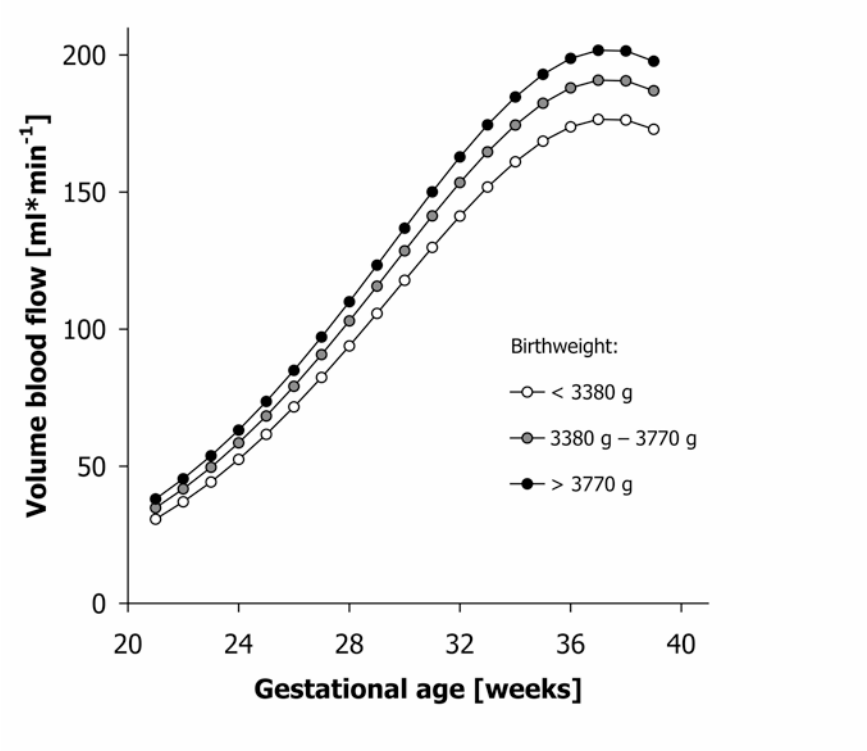

Figure 11: *Effect of birthweight on total venous liver flow based on deviance statistics ($p < 0.001$).*

8.8.2. Influence of BMI and pregnancy weight gain

It was shown in a previous study (Haugen et al. 2005) that fetal liver flow is modulated by the maternal body composition and diet.

Although maternal BMI had no overall effect on venous liver flow, the DV shunt fraction in late gestation (35-40 weeks) was positively related to BMI ($p<0.05$) which confirms earlier findings (Haugen et al. 2005).

Pregnancy weight gain strongly affected venous liver flow. Low pregnancy weight gain was associated with a distributional shift from the RiLL to the LeLL (Fig. 12). A recent study revealed functional differences between the liver lobes in fetal baboons, where for example genes related to cell biogenesis, protein biosynthesis and phospholipid metabolism were up-regulated in the left lobe (Cox et al. 2006). Left liver sparing is therefore likely to affect liver function and metabolism and may be part of an adaptation to a changed intrauterine environment. Subtle functional differences between the two lobes were found in the adult human liver (Barbaro et al. 1999; Jacobsson et al. 2005). A shifted balance of venous perfusion

within the fetal liver may therefore have long-term consequences for liver function and link intrauterine environment with health and disease in adulthood.

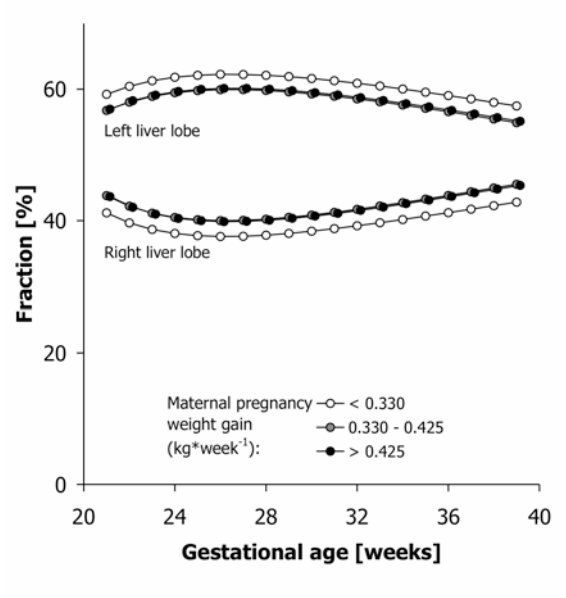

Figure 12: *Effect of pregnancy weight gain on the distribution of venous blood flow to the liver lobes based on deviance statistics ($p<0.0001$).*

8.9. Validation of right liver flow measurement

The quantification of total venous liver flow and lobe specific flows is complex and depends on velocity and diameter measurements at different measure sites.

Two approaches are feasible for measurement of RiLL flow, either the measurement of flow in the right portal vein (RPV) or the summation of flow in the LPV and PV (Fig. 13).

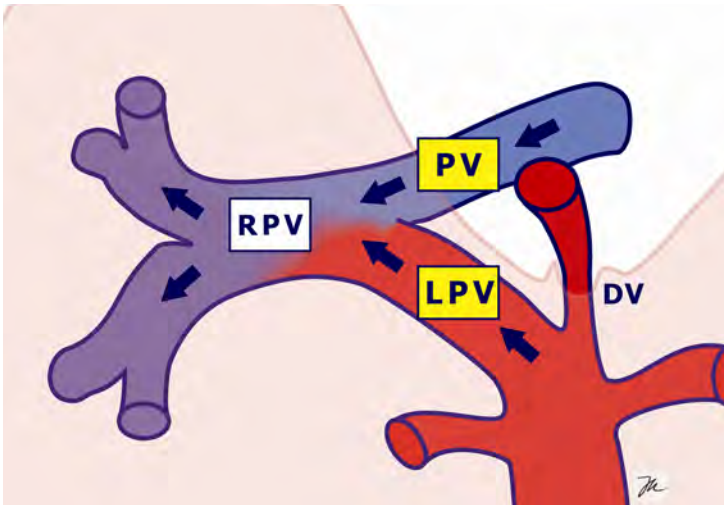

Figure 13: *Blood flows of the left portal vein (LPV) and main portal stem (PV) becomes blended in the right portal vein (RPV). The blood flow to the right liver lobe is equivalent to the flow in the right portal vein and the sum of flows in the left portal vein and the main portal stem. DV: ductus venosus.*

In 28 fetuses flow measurement of the RPV was done, in addition to calculation of flow in the LPV and PV, which made it possible to compare the two methods for right liver flow calculation. There was a fairly good correlation between the two methods (Fig. 14, left). The 95% limits of agreement, however, are wide, but mainly attributed to four outliers (Fig. 14, right). The large difference between the methods in some cases may be due to measurement error or a biological flow variation during the time of examination.

While the calculation of right liver flow in the present study is based on the summation of LPV and PV flow, Haugen et al. measured RPV flow and calculated PV flow subtracting LPV flow from RPV flow. Despite the different approach to calculation of RiLL flow, the present study confirms the results of this previous human study (Haugen et al. 2004), which was reassuring for the methods applied in both studies (Tab. 4).

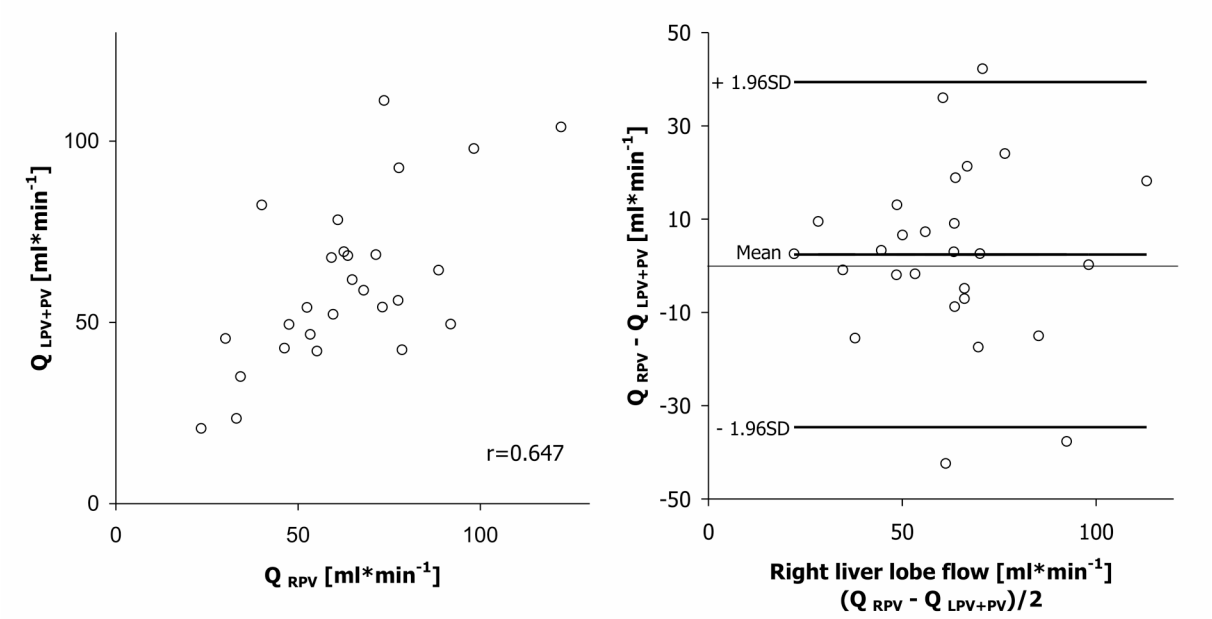

Figure 14: *<u>Left:</u> Scatterplot of right portal flow (Q_{RPV}) and the sum of left portal and portal main stem flow (Q_{LPV+PV}), measured in the same fetuses during one examination. r: coefficient of correlation. <u>Right:</u> Difference against mean plot with limits of agreement between Q_{RPV} and Q_{LPV+PV}.*

*Flow [ml*min^{-1}]*	*Present study*	*Haugen et al.*
Total venous liver	186 (124-286)	185 (114-277)
Right liver lobe	79 (55-110)	78 (49-120)
Left liver lobe	104 (59-167)	104 (45-177)
Left portal vein	44 (26 - 75)	42 (17 - 68)
Main portal stem	32 (22 - 46)	37 (16 - 65)

Table 4: *Data on total venous and lobe specific flow and comparison with a previous human study (Haugen et al. 2004). Presented as mean with 10th and 90th percentile in brackets.*

9. CONCLUSIONS

We have constructed longitudinal reference ranges for ductus venosus flow velocities and waveform indices, which reflect the development during the second half of pregnancy. Our reference ranges differ slightly from those published earlier with cross sectional design. The presented reference ranges allow the calculation of conditional percentiles and are thus appropriate for serial measurements in the compromised fetus.

We have established a standardised technique for direct flow measurement in the fetal main portal stem, provided reference ranges and studied blood flow development during the second half of pregnancy with a high success rate. Both vessel diameter and flow velocities doubled during in the second half of pregnancy. Consequently, volume blood flow increased, which was mainly explained by fetal weight gain. In contrast to a general reduction of normalised venous flow with gestation, the normalised flow in the main portal stem still showed an increase signifying the importance of the splanchnic circulation in venous liver flow, particularly shortly before birth.

We have investigated the blood flow development in the left portal vein during the second half of pregnancy and usually found forward flow indicating that the watershed between the umbilical and portal circulation is situated in the right liver lobe. A reversal of blood flow in the left portal vein during standard recording conditions was found to be rare (8%) and only shortly before birth, while intermittent flow reversal occurred during respiratory movements. These findings indicate that a shift of the umbilico-portal watershed towards the left may also occur in circulatory uncompromised fetuses. We suggested the time-averaged maximum velocity in the left portal vein for the evaluation of the watershed between the umbilical and portal circulations, and provided longitudinal reference ranges including terms for calculating conditional percentiles for serial measurements.

We studied the components of fetal venous liver flow, their distribution within the liver, and provided longitudinal reference ranges for the second half of the human

pregnancy. Confirming previous human studies, we found, that approximately 31% of the umbilical blood was shunted through the ductus venosus at mid gestation decreasing to 24% near term. The present study adds that total venous liver flow increased throughout gestation, while normalised flow decreased. This reflects the flow development of the umbilical vein, which dominated the venous blood supply of the liver with a fraction of 80-85%, while the portal vein accounted for 10-15%. However, the growing portal blood fraction during the last weeks of gestation signifies the increasing importance of the splanchnic circulation. The distribution of global venous blood flow was fairly stable directing 60% to the left and 40% to the right lobe.

We found that birthweight was positively related to total venous liver flow, lobe specific flow and flow in the umbilical, left portal and portal veins. These findings support the concept of a link between liver perfusion and fetal growth. Low pregnancy weight gain was associated with left liver sparing which may be part of adaptation to various intrauterine environments with possible short and long term implication for liver function and metabolism.

10. FUTURE ASPECTS

In the present study we established reference ranges for fetal venous liver flow in a low-risk population. Our hypothesis was that the venous liver flow and its distribution are changed by growth disturbances. The relationship between birth weight and liver flow supports this concept. Parallel to the present study, we therefore investigated venous liver flow in 40 macrosomic and 40 growth restricted fetuses. The analysis of these data will hopefully give more detailed information about the effect of accelerated and restricted fetal growth on venous liver perfusion.

The strong effect of maternal weight gain on venous liver flow in the present study prompts the question of other nutritional or behavioural maternal factors which may modulate the fetal liver circulation. It is thus promising that approximately half the women recruited to the present study are also participating in the Norwegian Mother Child Cohort study, which includes detailed and validated questionnaires on nutrition before and during pregnancy and collection of maternal blood samples. Thus, it would be feasible to link fetal blood flow data with information on maternal nutrition and metabolism extending the knowledge on regulation of fetal growth and development.

The longitudinal reference ranges and conditional terms are expected to improve precision in the use of DV velocimetry as a diagnostic test in serial measurements. A next phase of studies to test this is awaited.

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12. ERRATA

The following typographical and grammatical corrections have been made after submission of the thesis:

Page 25, para 1, line 7: “,” inserted after “exposed tissue”
Page 33, para 1, last line: “the” inserted before “general population”

13. ORIGINAL PAPERS