Umbilical venous volume inflow and liver size in normal and abnormal fetal development

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Veneuze umbilicalis instroom en lever volume in de normale en gestoorde ontwikkeling van de humane foetus

Proefschrift

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"Nessuno diventa uomo innanzi di avere fatto una grande esperienza di se', la quale rivelando lui a lui medesimo, e determinando l'opinione sua intorno a se stesso, determina in qualche modo la fortuna e lo stato suo nella vita." Pensieri (G. Leopardi)

"Nobody becomes a man before learning from his own experience, which revealing him to himself, and defining his opinion about himself, determines in some way his own Fortune and his entire life." Pensieri (G. Leopardi)

A Luisa e Luigi

A Chiara e Martina

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Contents

Chapter 1

Human fetal umbilical venous inflow and fetal growth and size

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1.1 The fetal circulation with emphasis on umbilical venous inflow

1.1.1 Embryology and Anatomy

The fetal liver with its venous vasculature -umbilical and portal veins, ductus venosus and hepatic veins- and the inferior vena cava are the main areas of interest in the investigations of venous blood return to the fetal heart [1]. In the embryonic period the liver rudiment can be distinguished as early as the third week of conception, at stage 10 of the Carnegie classification and at an embryonic length of 2.0-3.5 mm . Blood from the seconday yolk sac is transported through the hepatic sinusoids via the vitelline vessels into the sinus venosus, whereas blood from the chorionic villi bypasses the liver to empty into the sinus via the right and left umbilical veins in stages 11-12 of the Carnegie classification and at an embryonic length of 2.5-5 mm [2]. At stage 19 of the Carnegie classification, at an embryonic length of 16-18 mm, the hepatic sinusoids become linked to the vitelline veins. The umbilical veins run on each side of the liver and carry well-oygenated blood from the placenta to the sinus venosus. As the liver develops, the umbilical veins lose their connection with the heart and empty into the liver, at stage 14-16 of the Carnegie classification. The persistent caudal part of the left umbilical vein becomes the umbilical vein, which carries all the blood from the placenta to the embryo. A large venous shunt-the ductus venosus-develops within the liver and connects the umbilical vein with the inferior vena cava [3]. During stage 14-16 the intra-abdominal part of the umbilical vein can be visualised as a vessel that ascends relatively steeply from the cord insertion into the inferior part of the falciform ligament. Then the vessel continues in a more horizontal and posterior direction and turns to the right to confluence with the transverse part of the left portal vein, which joins the right portal vein with its division into an anterior and posterior branch [4]. The ductus venosus originates from the umbilical vein before it turns to the right. The diameter of the ductus venosus measures approximately one-third that of the umbilical vein. It courses posteriorly and in a cephalic direction with increasing steepness in the same sagittal plane as the original direction of the umbilical vein and enters the inferior vena cava just below the diaphragm [5]. The three (left, middle, and right) hepatic veins reach the inferior vena cava in the same funnel-like structure as the ductus venosus. Huisman and co-workers in 1992 [6] have described this subdiaphragmatic venous vestibulum in anatomical studies.

1.1.2 Preload physiology

During intrauterine life it is the placenta that supplies the fetus with oxygen. Oxygenated placental blood is delivered to the fetus by the umbilical vein. Blood passes either through the liver circuit or through the ductus venosus to arrive in the inferior vena cava. Because of its fundamental importance the assessment of placental blood flow has represented a target of experimental [7-22] and clinical [23-50] research since fetal physiological studies were first undertaken. Blood flow, not velocity, determines oxygen delivery to the fetal tissues, hence its importance [51]. Indeed, many situations which result in cardiac compromise have their origin in an abnormal preload flow. Under normal conditions fetal cardiac output is mainly determined by the following variables [52].

(I) Amount of blood distending the ventricles immediately before contraction (*preload*)

(II) The combined resistance of the central and peripheral vascular beds (*afterload*)

(III) The intrinsic ability of the myocardial fibers to contract (*contractility*)

(IV) The rate of contraction (*heart rate*)

The effects of preload on ventricular output are described by the Frank-Starling mechanism, i.e. the strength of the contraction of the heart varies with end-diastolic filling, which has been demonstrated to be also applicable to the fetus [53]. The umbilical-placental unit, which absorbs much of the increase in circulating volume, limits changes in preload. Moreover, different sites of oxygenation imply different hemodynamics and also different venous blood pressures have significant implications regarding the physiology of venous inflow. Umbilical venous pressures have been measured through cordocentesis [54] and indirect calculations of central venous pressure based on Doppler velocimetry in the umbilical vein, ductus venosus or inferior vena cava were performed in our centre [55]. From this report it was demonstrated that fetal hemodynamics operate at very low blood pressure gradients. The functions of veins mainly depend on their specific physical properties. Since veins have a large cross-sectional area, blood flow experiences low resistance resulting in a relatively small pressure drop from venous capillaries to the right heart as compared with the reduction of blood pressure from the aorta towards the arterial capillaries [56]. Another important factor is the venous compliance. Increases in volume and pressure impose very little stretch on its thin-walled, elastic vessel structure. Because of its cross-section, compliance and length, the veins can contain a large proportion (60% to 80% in adults) of the circulating blood flow volume [52]. Their capacity and compliance are optimally utilised in the role of a variable blood reservoir that receives or releases blood volume with only small changes in pressure [56]. The functional role of the ductus venosus shunt has been reasonably well established. It has

been demonstrated [36-39] that the ductus venosus serves as a bypass of the hepatic microcirculation for well-oxygenated umbilical venous blood. Whereas in fetal lamb approximately 50% of umbilical blood is shunted through the ductus venosus [57], this appears to be less in the normally developing human fetus with a percentage of 40% at 20 weeks to as low as 15% at 38 weeks of gestation [39, 58]. Increased shunting has been established in the ductus venosus under hypoxaemic circumstances not only in the fetal lamb but also in the human fetus [59-60].

In human fetal hemodynamics, most Doppler ultrasound studies have sofar focused on flow velocity waveform analysis including venous inflow. Normal fetal growth depends on optimal fetal oxygenation and therefore adequate umbilical venous inflow. As quality of sonographic equipment improved, reports appeared on volume flow determinations in the arterial and venous fetal circulation. The principle problem of volume flow measurement lies in the determination of vessel size. Validation of any measuring technique of vessel size would therefore be necessary.

1.2 Fetal growth and size

Growth of the fetus is part of a more general change termed morphogenesis. Normal fetal growth depends on the appropriate increase in utero-placental and feto-placental blood flow. Since its introduction into obstetrics in the late 1950s, ultrasound has played an increasingly important role in the characterisation of normal fetal growth and the detection of fetal growth restriction. Improvements in image quality and scanning capability have permitted visualisation of greater anatomical detail which, in turn, has led to more sophisticated analyses of the growth process [61]. Using a primitive A-scan apparatus Donald was able to detect the strong reflections from the fetal skull and this technique rapidly led to accurate measurements of the fetal biparietal diameter (BPD). The definitive work on this measurements was done by Campbell [62] whose attention to the accuracy and detail enabled him to establish a normal range, which permitted the length of gestation to be calculated to within ± 5 days if a satisfactory measurement was obtained before 25 weeks of pregnancy. Most other ultrasound measurements have been developed with the objective of assessing the size of the fetal trunk and thereby obtaining more accurate information concerning fetal growth [63-64]. Thompson et al [65] who attempted to assess the cross-sectional area of the trunk, obtained the earliest recorded attempts at fetal trunk measurements. Moreover, trunk measurements have been developed further during the past years and many different techniques have been advocated. These include measurement of the thoracic diameters, abdominal circumference (AC) and femur length [66-67]. Although all these measurements have their advocates, measurement of the AC at the level of the fetal liver seems to hold the most promise and is currently considered as an indicator of intra-uterine fetal growth [68]. The rationale for this meaurement is that it corresponds most closely with the size of the fetal liver. The work started by Evans [69] using an animal model; this was subsequently confirmed by Gruenwald in the human fetus [70]. Using ultrasound, other authors [71-72] indicated that the fetal liver is the earliest organ to be affected when intra-uterine growth restriction occurs. An important condition in which we commonly see accelerated fetal growth is maternal insulin-dependent diabetes mellitus. In a different study [73-74] it was shown that fetal biparietal diameter (BPD) and head circumference (HC) measurements conformed to normal growth patters, but the AC growth was abnormally accelerated.

With modern sonographic technology, fetal weight can be estimated with reasonable accuracy [75-76]. Assessment of fetal growth has improved with advances in ultrasonography. A deeper understanding of fetal growth patterns was reached through customising the

birthweight standard according to physiological variables such as maternal booking weight, maternal height, parity, fetal sex and ethnic origin [77]. A significant improvement was established in the identification of infants who had failed to reach the expected birthweight and who were at increased risk of perinatal problems. Although ultrasound has been shown to be an invaluable tool for the assessment of fetal growth patterns the measurements currently employed are less than ideal, since mathematic formulas are necessary to convert them into weight or volumes. With the introduction of three-dimensional (3D) sonography, reproducible circumference and volumetric measurements have become feasible by simultaneous visualisation of three orthogonal fetal sections and volume calculation was simplified considerably [78-79].

Scant information is available regarding the relationship between umbilical venous inflow and fetal growth as expressed by fetal liver and brain size under normal or abnormal circumstances. Data on this relationship may assist us in our understanding of fetal oxygenation at different stages of abnormal growth.

1.3 Research objectives

In this thesis the following research objectives were addressed:

To calculate umbilical venous volume flow from cross-sectional area and flow velocity measurements with emphasis on: (i) the reproducibility of component measurements; (ii) normal and abnormal fetal development, the latter also relative to umbilical artery velocimetry.

To establish: (i) reproducibility and normal values for fetal liver volume as obtained by 3-dimensional ultrasound; (ii) its significance in identifying fetal growth restriction relative to head and upper abdominal circumference; (iii) its significance in identifying accelerated growth in fetuses of insulin dependent diabetic mothers.

To determine: (i) normal values for fetal brain/ liver volume ratio relative to gestational age; (ii) the relation between fetal brain/liver volume ratio and umbilical venous volume flow during normal and abnormal fetal development with emphasis on fetal growth restriction.

To establish: (i) fetal behavioural state dependency of umbilical venous volume flow in late third trimester normal pregnancy; (ii) the impact of maternal plasma expansion and antihypertensive treatment on umbilical venous volume flow in pre-eclampsia.

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Chapter 2

Fetal venous inflow and fetal size; a literature overview

2.1 Umbilical venous volume flow

Interest in umbilical venous volume flow as a provider of oxygen and nutrients to the developing fetus has been expressed for a long time. Before the introduction of two-dimensional real-time and pulsed Doppler ultrasound, human studies were restricted to indirect measuring techniques of umbilical blood flow. Most of the information was extracted from animal experiments, which often did not bear any resemblance with the human situation.

In this subchapter, data on umbilical venous volume flow are discussed both in human and moreover in animal experimental circumstances.

2.1.1 Human non-sonographic data

Indirect measurements of umbilical venous blood flow have been carried-out using different techniques (Table 2.1). All studies have in common that data were collected under non-physiological conditions varying from hysterotomy to the immediate post-partum period. The work of Odeblad [1] was the first attempt at calculating umbilical blood flow in the human. Most reports presented data on umbilical venous volume flow standardised for fetal weight. Of interest in that in four out of five studies carried-out at 38-40 weeks of gestation, rather similar estimates of umbilical venous volume flow were obtained, varying between 75 and 85 ml/min/kg. A higher figure of 110 ml/min/kg was reported by Assali et al [2] and Rudolph et al [3] earlier in gestation. It should be stated that the number of women in the studies by Odeblad [1] and Greenfield et al [4] were extremely small. No information on the number of women was available from the reports by Bartels et al [5] and Rooth and Sjostedt [6].

2.1.2 Animal experimental data

A desire for a better understanding of the fetal circulation including umbilical venous volume flow has been the driving force for much of the earlier work in animal preparations. The standard animal model used by most authors was the fetal lamb, since its circulation resembles that in man.

Data presentation in the various papers ranges between graphic display of individual data relative to gestational age and only a mean value of umbilical venous volume flow standardised for fetal weight. A limiting factor in the analysis of animal data (Table 2.2) was the limited number of experiments carried-out in some studies ranging from 6 to 9 animals.

TABLE 2.1 Umbilical venous blood flow: non ultrasonic measurements in the human fetus

Authors	Year	N0 patients	Gestational Age (wks)	Method	Mean blood flow (ml/min)	Mean blood flow*kg fetus (ml/min/kg)
Odeblad[1]	1950	3	–	Electromagnetic flowmeter	–	144
Greenfield et al [4]	1951	1	13	Occlusion plethysmograph immediately after delivery	–	43.8
Assali et al [2]	1960	12	12 - 28	Electromagnetic microflowmeter and nitrous oxide (N_20) technique during hysterotomy	8.5-80	110
Bartels et al [5]	1962	–	40	Arteriovenous difference 0_2 in umbilical vessels and assumed 0_2 utilization by fetus (= 5ml/min/kg)	–	75
Rooth and Sjostedt [6]	1962	–	40	Comparison of fetal and maternal 0_2, and ratio of uteroplacental and fetoplacental circulation= 1.8 : 1.0	–	85
Stembera et al [7]	1965	17	38 - 40	Local thermodilution immediately after birth	246.3	75
Rudolph et al [3]	1971	33	10 - 20	Electromagnetic flowmeter; radioactive microsphere technique during abortion.	–	110
McCallum [8]	1977	52	39 - 40	Local thermodilution technique	171	75

TABLE 2.2 Umbilical venous blood flow: normal animal data

Authors	Year	Animal	Gestational age (days)	No animals	Methods	Range or mean blood flow (ml/min)	Range or mean blood flow *Kg fetus (ml/min/kg)
Cooper and Greenfield [9]	1949	lamb	60 - 143	16	Venous occlusion plethysmography	500	152 ± 43
Acheson et al [10]	1957	lamb	79 - 138	16	Venous occlusion Plethysmography	400	–
Dawes and Mott [11]	1964	lamb	87 - 141	30	Venous occlusion plethysmography	717	217±12 - 170 ± 14
Meschia et al [12]	1967	lamb	102 - 139	13	Steady state diffusion technique with urea as test substance	98 - 669	175 ± 20
Kirschbaum et al [18]	1967	lamb	136 -144	14	Electromagnetic flowmeter	–	183 ± 56
Rudolph and Heymann [19]	1967	lamb	110 - 147	9	Electromagnetic flowmeter and antipyrine method	–	Approx. 240
Makowski et al [13]	1968	lamb	90 -150	11	Steady state diffusion technique and radioactive microspheres	174 - 722	–
Novy and Metcalfe [20]	1970	lamb	–	8	Dye dilution principle	–	143 ± 62
Clapp et al [21]	1974	lamb	136 -144	7	Steady state diffusion technique and antypirine as test substance	–	278
Edelstone [22]	1980	lamb	116 - 145	31	Electromagnetic flowmeter; radioactive microsphere technique	–	216 ± 49
Wilkening et al [14]	1982	lamb	116 - 145	6	Steady state diffusion technique with antipyrine and ethanol as test substance	628	–
Bell et al [24]	1986	lamb	71 - 81	11	Steady state diffusion technique and $^3H_20$ with isotonic saline solution	468 ± 57	100 ± 12
Wilkening and Meschia [15]	1989	lamb	116 - 145	9	Steady state diffusion technique with antipyrine and ethanol as test substance	507± 89	–
Jensen et al [23]	1991	lamb	123 - 129	9	Electromagnetic flowmeter; radioactive microsphere technique	–	213 ± 55
Schmidt et al [17]	1991	lamb	126	12	Electromagnetic flowmeter; radioactive microsphere technique	450	–
Galan et al [16]	1999	lamb	124 - 136	6	Steady state diffusion technique and ethanol as test substance	603	208 ± 7.3

The first animal experimental report on umbilical venous volume flow dates back as early as 1949 [9]. The technique used to calculate umbilical venous volume flow was venous occlusion plethysmography, which later was also employed by Acheson et al [10] and Dawes and Mott [11]. They found mean umbilical venous volume flow values ranging between 410 and 717 ml/min.

Similar results were published applying other techniques such as the steady state technique based on the Fick principle [12-16] or electromagnetic flow meters [17]. Concerning the mean umbilical venous volume flow figures standardised for fetal weight, a reasonable similarity existed when taking the gestational age range into account. Most studies performed at approximately 100-145 days of gestation demonstrated a mean umbilical venous volume flow standardised for fetal weight ranging between 175 and 216 ml/min/kg. A lower mean value of 152 ml/min/kg at 60-143 days was reported by Cooper and Greenfield [9] and a much higher mean value of 468 ml/min/kg at 71-81 days of gestation was established by Bell et al [24]. In one rapport [20] no gestational age was given.

2.1.3 Human sonographic data

A considerable body of information on human umbilical venous volume flow is available from sonographics studies [25-43]. Before discussing the actual flow data, it is necessary to consider the methodological and biological aspects of these reports.

2.1.3.1 Methodological aspects

The two components for calculating volume flow are flow velocity and vessel size. Pulsed-wave Doppler ultrasound is employed for establishing flow velocities in fetal vessels. The velocity of an ultrasound reflector, i.e. an erythrocyte, is directly proportional with the change of the transmitted ultrasound frequency according to the next formula:

$$V = \frac{F_d \times c}{2 \times F_o \times \cos\alpha}$$

In which **V** is the velocity of the reflector, $\mathbf{F_d}$ is the change of the frequency, called the Doppler shift, $\mathbf{F_0}$ is the frequency of the transmitted sound, **c** is the velocity of ultrasound in the tissue and α is the angle between the insonating sound wave and the direction of the moving reflector.

Volume flow is calculated according to the following equation:

$$\text{Volume Flow} = \frac{V\,\text{mean} \times \pi \times d^2}{4} = V\,\text{mean} \times \text{vessel area}$$

In which $\mathbf{V_{mean}}$ is mean flow velocity over the vessel cross-sectional area and **d** is the vessel diameter. Based on this formula, there are a number of problems to be solved in order to collect information on volume flow e.g. the location of the vessel, the measurement of the insonation angle, mean flow velocity over the cross-sectional area and the vessel diameter [44]. The insonation angle preferably is zero degrees, which mean that one should attempt to align the Doppler beam as co-axial as possible to the vessel, which is the umbilical vein. The insonation angle should preferably be kept below 45 degrees and angle correction should be applied when the angle is larger than 10 degrees. The length of the Doppler sample volume should be adjusted with the view to ensuring measurement of flow velocities over the entire lumen of the vessel. Adjustment of the sample volume is achieved by listening to the Doppler signals and by visual analysis of the Doppler spectrum [44]. When considering the coiled shape of the cord, it should be realised that not only the angle between Doppler beam and the umbilical vein, but also the actual axial direction of the vein at the insonation point must be taken into account [45].

The component most subject to erroneous measurement is vessel size. It was demonstrated by Eik-nes et al [46] that volume flow calculation was susceptible to considerable errors when the vessel diameter was small. They found a SD of 0.26 mm for the interobserver variation of the umbilical vein diameter. Measuring the umbilical venous diameter 2-13 times in 163 women, Kiserud et al [47] found a mean within subject SD of 0.23 mm. It is known that an increasing number of measurements for calculation of a mean diameter substantially improves the precision of the vascular cross-sectional area assessment [47]. Barbera et al [41] found a coefficient of variation within subject around 3%, which is similar to observations done by Lees et al [40].

In our study we decided

Table 2.3 presents an overview of the methodology applied in umbilical venous volume measurements since the first studies by Gill in 1979 [25]. The total examination time needed to register flow velocity and vessel size was only documented by a few authors, and varied between 10 and 45 minutes. The transducer carrier frequency was recorded in nearly every instance, and ranged between 2 and 4 MHz. Whereas, in the majority of reports, the sample volume was reported to have covered the entire vessel width. Some investigators reported a sample volume of 4-10 mm, which should also have covered the entire vessel width taking the vessel size into account. A considerable variety existed regarding the insonation angle, which ranged widely between 0 and 20 degrees [38,41-43] and 30 and 65 degrees [27-28,31-32,34,37],

TABLE 2.3 Umbilical venous blood flow: methodological aspects in human sonographic studies

Authors	Year	PRF	Examination time (min)	Carrier Frequency (MHz)	Sample volume (mm)	Angle of insonation	Mean/max velocity	Vessel diameter/area	Intra abdominal part	Extra abdominal part
Gill [25]	1979	–	–	2	5 – 10	0° - 66°	Mean	Diameter	+	–
Eik-Nes et al [26]	1980	–	–	2	5 –10	45°	Mean	Diameter	+	–
Gill et al [27]	1981	–	10 - 20	3	Entire width of vessel	35° – 60°	Mean	Diameter	+	–
Jouppila et al [28]	1981	–	–	2	5 –10	50°	Mean	Diameter	+	–
Kurjak and Rajhvajn [29]	1982	–	< 15	3	Entire width of vessel	No exact angle given	Mean	Diameter	+	–
Griffin et al [30]	1983	–	30	2 - 3	Entire width of vessel	No exact angle given	Mean	Diameter	+	–
VanLierde et al [31]	1984	–	–	2	Entire width of vessel	30° – 65°	Mean	Diameter	+	–
Gill et al [32]	1984	–	10 - 30	1.5 - 3	4 – 10	30° – 65°	Mean	Diameter	+	–
Erskine and Ritchie [33]	1985	–	–	3	Entire width of vessel	No exact angle given	Mean	Diameter	+	–
Lingman et al [34]	1986	–	40	2	Entire width of vessel	45°	Mean	Diameter	+	–
Rasmussen [35]	1987	–	–	–	–	–	Mean	Diameter	+	–
Gerson et al [36]	1987	–	–	2.4	Entire width of vessel	–	Mean	Diameter	+	–
Laurin et al [37]	1987	–	–	2	Entire width of vessel	45°	Mean	Diameter	+	–
Sutton et al [38]	1990	–	–	3.5 - 5	Entire width of vessel	<20°	Mean	Diameter	–	+
Tchirikov et al [39]	1998	–	–	5 - 7	Entire width of vessel	< 60°	Mean	Diameter	+	–
Lees et al [40]	1999	–	3 - 10	3.5	Entire width of vessel	< 60°	Mean	Area	–	+
Barbera et al [41]	1999	–	10	2 - 5	Entire width of vessel	0° – < 20°	Mean	Diameter	–	+
Kiserud et al [42]	2000	–	45	4	Entire width of vessel	Mean 12°	Mean	Diameter	+	–
Ferrazzi et al [43]	2000	–	–	2 - 5	Entire width of vessel	0° – < 20°	Mean	Diameter	–	+

the latters being quite considerable and raising some concern about the accuracy of the velocity measurement. Of interest is the observation that nearly all reports were based on diameter measurements. Apart from the fact that the calliper placement was often not registered, conversion of vessel diameter measurements into cross-sectional vessel area data means enlarging its error. Only in one study the cross-sectional study vessel area was calculated [41]. In the majority of studies, the intra-abdominal part of the umbilical vein instead of the extra-abdominal free floating loop of the cord was selected for umbilical venous volume calculation. This was mainly based on the straight outline of the intra-abdominal part of the vein allowing easy and quick assessment of the insonation angle. Recently, it was pointed out that some kind of physiological stricture exists at the abdominal entrance of the umbilical vein, resulting in elevated flow velocities [48]. Umbilical venous volume flow measurements should, therefore, be carried out from some distance of the entrance of umbilical vein into the abdomen, either at intra-abdominal or extra-abdominal (free-floating loop) level [49].

2.1.3.2 Biological variables

Not only methodological aspects but also biological variables should be considered when appreciating umbilical venous volume flow data. Fetal blood flow may be influenced by a number of intrinsic and extrinsic variables. Intrinsic are fetal breathing movements and fetal activity or behavioural states. There is a considerable body of data dealing with the impact of fetal breathing movements on arterial and venous flow velocity waveforms and vessel size [50-53]. This particularly applies to the venous circulation with a highly compliant venous vessel system [54]. Moreover, during the second half of the second trimester of pregnancy, the fetus is characterised by different activity states as expressed by low amplitude or high amplitude fetal heart rate variability patterns. As from 33-34 weeks of gestation well-defined fetal behavioural states can be identified with active and quiet sleep states. The most prominent behavioural patterns are expressed by the triad of high or low amplitude fetal heart rate variability, presence or absence of fetal eye movements and frequent or occasional fetal body movements [55]. It has been demonstrated in our centre that the fetal circulation is subject to behavioural states dependent changes [51, 53, 56-59]. Whereas no data are available on umbilical venous volume flow and fetal behavioural state, ductus venosus flow velocity waveforms analysis clearly suggests increased ductus venosus flow during the active sleep state [50].

TABLE 2.4 Umbilical venous blood flow: biological aspects in human sonographic studies

Authors	Year	Fetal breathing	Fetal behavioural states	Maternal drug administration
Gill [25]	1979	-	Only fetal quiescence	no
Eik-Nes et al [26]	1980	-	-	no
Gill et al [27]	1981	Fetal apnea	Only fetal quiescence	no
Jouppila et al [28]	1981	Fetal apnea	Only fetal quiescence	no
Kurjak and Rajhvajn [29]	1982	-	-	no
Griffin et al [30]	1983	Fetal apnea	-	no
Van Lierde et al [31]	1984	-	Only fetal quiescence	no
Gill et al [32]	1984	-	-	no
Erskine and Ritchie [33]	1985	-	Only fetal quiescence	no
Lingman et al [34]	1986	Fetal apnea	Only fetal quiescence	no
Rasmussen [35]	1987	-	-	no
Gerson et al [36]	1987	Fetal apnea	-	no
Laurin et al [37]	1987	Fetal apnea	Only fetal quiescence	no
Sutton et al [38]	1990	Fetal apnea	Only fetal quiescence	no
Tchirikov et al [39]	1998	Fetal apnea	Only fetal quiescence	no
Lees et al [40]	1999	Fetal apnea	Only fetal quiescence	no
Barbera et al [41]	1999	-	-	no
Kiserud [42]	2000	Fetal apnea	Only fetal quiescence	no
Ferrazzi et al [43]	2000	Fetal apnea	Only fetal quiescence	no

From Table 2.4 it can be seen that the majority of the studies on umbilical venous volume flow were performed during fetal apnoea and fetal quiescence. This is not surprising, sincefetal breathing and/or fetal activity may render accurate flow velocity and vessel size measurements impossible. Some studies, however, do not provide any information on fetal intrinsic variables.

Also, external variables -in particular maternal administration of certain medication- may affect umbilical venous volume flow. It has been documented that for instance dihydralazine is associated with an increased blood flow in the umbilical vein [60]. There was no mention of maternal drug administration in any of the reports presented in Table 2.4.

2.1.3.3 Clinical data

Clinical data on human fetal umbilical venous volume flow as obtained by combined 2-dimensional real time colour coded Doppler ultrasound and pulsed Doppler ultrasound are presented in Table 2.5. Most of the research on human fetal umbilical venous volume flow was carried-out during the eighties. Gill [25] was the first to determine umbilical venous volume flow using a combination of B-mode Ultrasound and Pulsed Doppler. Volume flow standardised for fetal weight measured at 25-40 weeks of gestation displayed a slight reduction from 112 to 104 ml/min/kg. Later studies comprising larger patient cohorts demonstrated similar results, i.e. a reduction from 110 to 90 ml/min/kg with advancing gestational age. Most other authors [28-29,38,41,61-62] also established a gestational age related reduction in weight related umbilical venous volume flow.

When considering absolute values, there appears to be a reasonable agreement with mean values ranging between 105 and 139 ml/min/kg. A somewhat higher mean value for weight related umbilical venous volume flow of 183.8 ml/min/kg was reported by Gerson et al [36]. No significant difference existed between intra-abdominal (Griffin et al [30]: 122 ml/min/kg) and extra-abdominal or intra-amniotic weight-related umbilical venous blood flow (Ferrazzi et al [43]: 108 ml/min/kg). Nevertheless, with the still existing variability in methodology as shown in Table 2.3, there is a clear need for standardised data of measuring techniques [63]. Finally, when comparing volume flow data obtained by means of ultrasound with those collected earlier by means indirect methods during hysterotomy or immediately post-partum, the latter seem to display lower values. This may be partly explained by the fact that the data obtained by means of indirect methods were collected at the very end of pregnancy which tend to be lower than earlier in pregnancy (see Tables 2.1 and 2.5).

TABLE 2.5 Umbilical venous blood flow in human sonographic studies

Authors	Year	Number of subjects	Gestational age (wks)	Range or mean volume flow (ml/min)	Range or mean volume flow*kg fetus (ml/min/kg)
Gill [25]	1979	12	25 - 40	99 – 334	112 – 104
Eik-nes et al [26]	1980	26	32 - 41	–	105 ± 26
Gill et al [27]	1981	47	22 - 40	300 at 36 wks	120 – 90
Jouppila et al [28]	1981	91	30 - 40	–	102.5 – 77.5
Kurjak and Rajhvajn [29]	1982	63	30 - 41	–	118 – 99
Griffin et al [30]	1983	45	28 - 30	–	122 ± 21
Van Lierde [31]	1984	20	37 - 40	366 ± 69	117 ± 16
Gill et al [32]	1984	118	22 - 40	300 at 36 wks	120 – 90
Erskine and Ritchie [33]	1985	15	28 - 40	–	125 ± 62
Lingman et al [34]	1986	21	27 - 40	160 – 210	138.7 – 65.2
Rasmussen [35]	1987	6	31 - 38	–	137 ± 9.5
Gerson et al [36]	1987	209	20 - 40	67 – 321	153.8 – 109
Laurin et al [37]	1987	21	32 - 41	–	105 ± 40.2
Sutton et al [38]	1990	74	19 - 42	50 – 500	130 (27 weeks) – 110
Tchirikov et al [39]	1998	55	21 - 41	214.9 ± 109.7	–
Lees et al [40]	1999	129	23 - 33	95 ± 64.8 – 303.3 ± 63.8	–
Barbera et al [41]	1999	70	20 - 38	97.3 – 529.1	128.7 – 104.2
Kiserud et al [42]	2000	196	20 - 40	20 – 230	130 – 70
Ferrazzi et al [43]	2000	109	30 - 35	–	104.5 ±26.4 – 124.0 ±30.3

2.1.3.4 Umbilical venous pulsations

Another observation regarding human fetal venous inflow is the appearance of umbilical venous pulsations. In literature end-diastolic venous pulsations corresponding to cardiac contractions were reported in fetuses with absent end-diastolic flow in the umbilical artery with severe bradycardia or tachycardia [64] and in fetuses with non-immune hydrops [65].

Umbilical venous pulsations reflect atrial pressure waves which are transmitted from the right atrium back to the inferior venous circulation [66]. End-diastolic umbilical venous pulsations have also been described in the human fetus with imminent asphyxia [34]. Nakai et al [67] reported umbilical pulsatile venous flow in hypoxic fetuses or in fetuses with atrio-ventricular valve anomalies. From these reports, umbilical venous pulsations have been considered a pathological finding. However the aetiology of pulsations seems to be different along gestation.

Umbilical venous pulsations in normally developing fetuses have been described as early as the first trimester of pregnancy [68-69]. Nakai et al [70] reported a gradual reduction in these pulsation until complete disappearance at approximately 23 weeks of gestation and attributed this pattern to a relative decrease in cardiac preload. Moreover, it has been reported that umbilical venous pulsations occur locally and transiently in normal fetuses during the third trimester of pregnancy, which may be caused by breathing episodes [71], or by local hypertorsion of the cord [70], but also during apnoea [72].

In summary, pulsating blood flow patterns appear to be of a physiological nature during the first trimester of pregnancy [68-69], probably due to the short anatomic distance between the umbilical vein and the heart, the pulsations of which are conducted to the umbilical vein. Later in pregnancy, umbilical venous pulsations may occur during fetal breathing movements [71], but also during fetal apnoea in the presence of normal fetal development [72]. Finally, umbilical venous pulsations have been established in relation to elevated right ventricular and diastolic pressure in different abnormal fetal conditions [34,64-65,67]. Here, venous pulsations are accompanied by increased late diastolic reversal of flow in the inferior vena cava and change of forward to zero to reversed late diastolic flow in the ductus venosus [72,73].

2.2 Fetal liver size

2.2.1 Methodological aspects

Liver size

During fetal life, the brain and liver are important organs in the evaluation of fetal growth and size. It is well known that during fetal hypoxaemia one of the first organs to be affected in growth is the fetal liver. Also in other aberrant fetal growth patterns, such as macrosomia, liver size may be affected. Up until recently, fetal biometry was limited to diameter, circumference and area measurements. Lately three-dimensional ultrasound has opened the possibility of determining organ volumes amongst which the fetal liver. Fetal liver length has been measured by several investigators [74-76] using the same measuring technique. The fetal aorta was identified in a longitudinal plane and then the transducer was moved parallel to this plane until both the right hemidiaphragm and the tip of the right liver lobe were visualised. This distance represented the liver length [77]. Fetal liver weight was estimated on the basis of longitudinal, antero-posterior and cephalo-caudal liver dimensions multiplied by a constant (K) of 0.42 which was obtained experimentally from adult liver studies. Baker et al [78] for the first time carried-out three-dimensional quantitative measurements in-utero for calculation of fetal brain and liver volumes using echo planar magnetic resonance. Fetal liver volume measurements using three-dimensional ultrasound were first introduced by Chang et al [79] and subsequently performed by Laudy et al [80].

Liver area measurements were also performed in newborn [81]. Scans were made in a vertical plane at the level of the liver at right angles to the sagittal axis of the newborn. Making parallel scans, the cross-section where the portal vein enters the liver was obtained. Subsequently, a photograph of the grey scale B scan ultrasonographs of this particular section of the liver was made and the liver area was calculated by a planimeter.

2.2.2 Clinical data

Vintzileos et al [74] investigating fetal liver length during the second half of pregnancy, found an increase from 28 mm to 55 mm demonstrating a different growth rate of the fetal liver length before and after 30 weeks of gestation. It was established that mean growth rate is 1.2 mm per week between 20 and 30 weeks and 1.7 mm per week between 31 and 41 weeks of gestation. Murao et al [82] also studying the second half of pregnancy considered the right hepatic lobe area to representing the entire liver size, which increased linearly with advancing gestation. The liver area is also closely correlated with such growth parameters

as biparietal diameter, femur length, and abdominal circumference. In a larger patient cohort, Murao et al [82] found that the right hepatic lobe increased linearly throughout gestation, whereas the left lobe increased linearly until approximately 32 weeks gestation, after which a slight slowing was observed. Moreover, Murao et al [83] attempted to use the ultrasound measurement of fetal liver length to detect growth-restricted fetuses proving that liver size of the latter was significantly smaller compared with that of normally growing fetuses. With the findings of Baker et al [78] using magnetic resonance imaging it was determined for the first time fetal liver volume during normally and abnormally fetal development. The same applied to fetal brain volume. Whereas in fetuses with a birthweight below the 10th centile, liver volume was below the lower limit of the normal range, this was not for brain volume. This suggested that the liver is the fetal organ more affected during fetal growth restriction whilst the brain size is spared during fetal malnutrition. Roberts et al [76] examined normal fetal liver measurements early in gestation starting from 15 weeks, confirming the data of Vintzileos et al [74] and Murao et al [82]. Chang [79] compared the intra-observer and inter-observer reproducibility between two dimensional ultrasound scanning and three dimensional ultrasound scanning, establishing a significant difference in liver volume measurements between the two techniques. Moreover, it was demonstrated that assessment of liver volume was more accurate using three-dimensional ultrasound scanning than the conventional two-dimensional ultrasound technique displaying no significant intra-observer and inter-observer differences when liver volume was assessed. Mean fetal liver volume ranged between 9.4 ml at 20 weeks and 62.0 ml at 32 weeks of gestation. Similar data were reported by Laudy et al [80] with values of 8 ml at 20 weeks and 116 ml at 38 weeks of gestation.

2.3 Fetal brain/liver ratio

Intra-uterine growth restriction secondary to utero-placental insufficiency is usually asymmetric. Head growth, as assessed sonographically by means of measurements of the fetal biparietal diameter (BPD) or fetal head circumference (HC), may remain normal throughout pregnancy or may only drop below the normal growth curve late in gestation. Trunk growth, conversely, as indicated by abdominal circumference, may decrease earlier [85]. Because intra-uterine growth restriction follows from chronic uteroplacental insufficiency, fetal asphyxia may be the terminal event of this sequence. Oxygen and carbon dioxide exchange is flow-limited, therefore further flow reduction will eventually result in decreased placental respiratory competence and fetal hypoxaemia [85]. The asymmetry itself appears to represent a fetal adaptation to the uteroplacental insufficiency, and suggests preferential

redistribution of fetal cardiac output favouring brain development [8]. This explanation is partially supported by animal models showing that fetal hypoxia results in redistribution of cardiac output with maintenance of flow to the brain, heart, and adrenals, whilst flow is decreased to muscle, viscera, and carcass [87]. Campbell and Thoms [88] described the use of the sonographic *head-to-abdomen circumference ratio* (HC/AC) to differentiate fetuses into the subtypes "symmetrical" meaning proportionately small, and "asymmetrical", referring to those which present disproportionately lagging abdominal growth. These authors found that asymmetrical fetuses had relatively larger brains and were preferentially protected from the full effects of the growth-retarding stimulus. The same fetuses were at significantly greater risk for severe pre-eclampsia, fetal distress, operative intervention, and lower Apgar scores than their symmetrical counterparts. It has been considered that head and abdomen proportions in growth restricted infants indicate the timing and nature of the insult, with the assumption that extrinsic causes lead to asymmetric and intrinsic causes to symmetric growth restriction. Because placental insufficiency may result in diminished glucose transfer and hepatic storage [89], and considering that the liver comprises the bulk of the abdomen, fetal abdominal circumference, which reflects liver size, would be reduced. Moreover, it is proposed that there is preferential shunting of oxygen and nutrients to the brain, which allows normal brain and head growth [90-91]. This sequence of events can result in *asymmetrical growth restriction* with an abnormal relative increase in brain size, when compared with the small liver. Because the fetal brain is normally relatively large compared with the liver, the ratio of brain weight to liver weight usually is about 3:1 or more. The ratio may be increased to 5:1 or more during the last 12 weeks of pregnancy in the presence of fetal growth restriction [91-95]. Dashe et al [96] analysed head-to-abdominal circumference ratios in 8722 consecutive liveborn singletons that underwent ultrasound examinations within 4 weeks of delivery. Their results indicate that the prognosis of growth restricted infants with asymmetric growth is poorer than that of symmetrically grown infants and much worse than that of normally developing infants. Asymmetry of the fetal HC/AC indicates pronounced growth impairment. The increased morbidity of asymmetric SGA infants might reflect both earlier gestational age at delivery and lower weight for gestational age [96].

Likewise, from post-mortem studies it has been established that fetal growth restriction is associated with a raised brain/liver ratio [93]. Looking at the components of this ratio, reduction in fetal brain weight is less pronounced than fetal liver weight due to the so-called brain sparing effect, reflecting circulatory redistribution during fetal hypoxaemia [86].

It can be concluded that fetal liver volume can be reliably measured during the second half

of gestation using three-dimensional ultrasound. Introduction of a reliable method of mesuring fetal brain volume would allow determination of brain to liver volume ratio as a means of establishing more accurately normal and abnormal fetal growth patterns compared with the traditional head to abdominal circumference ratio. This is a subject of investigation which will presented in Chapter 3.

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Chapter 3

The present study: methodology

In this Chapter the methodology of umbilical venous volume flow determinations as well as of fetal brain and liver size is presented.

3.1 Umbilical venous volume flow

In the present study, flow velocity and vessel size components of volume flow were determined from both the intra-amniotic and intra-abdominal part of the umbilical vein. For the intra-amniotic study standard ultrasound equipment was used.

3.1.1 The measuring technique

Umbilical venous maximum flow velocity (mm/s) was measured using a combined two-dimensional real-time and colour-coded Doppler system (Toshiba SSA 140 A Toshiba Corp. Medical Systems Division, Tokyo, Japan) with a transducer carrier frequency of 5 MHz and 3.75 MHz, respectively. The system operates at output intensities of < 100 mW/cm² spatial peak temporal average in both imaging and Doppler mode. Due to the spiral shape of the umbilical vessels it is not possible to accurately determine the angle between the direction of the blood flow velocity and the ultrasonic beam of insonation. We therefore, used the colour Doppler mode to find the maximum velocity in the umbilical vein. Whilst scanning the umbilical cord under slightly different angles and different positions, the two dimensional colour Doppler presentation of the umbilical cord was carefully examined to find the colour spot with the maximum velocity, since the maximum velocity in the vein can only obtained with a zero angle of insonation. The sample volume was placed in this maximum velocity spot to obtain a high quality velocity recording during at least four seconds. Assuming a parabolic velocity profile in the umbilical vein, the mean velocity over the cross-sectional area can be calculated from the maximum envelope of the velocity recording, since the cross sectional mean velocity is half the maximum velocity under parabolic flow conditions. We decided to select a relative small sample length, between 0.2-0.3 cm, in comparison with the lumen of the umbilical vein to make sure that the waveforms obtained from the umbilical vein are not disturbed by velocity components from the umbilical artery under conditions of reversed flow velocity which can be found in growth-restricted fetuses. The high-pass filter was set at 100 Hz. Umbilical venous cross-sectional area (mm²) was expressed by the mean of three tracings of the inner edge of the vessel. The software program to achieve this was developed in our institution using Labview and Imaq Vision software, (National Instruments Austin,Tx). To obtain a two dimensional scan of the cross sectional area of the umbilical vein perpendicular to the direction of the flow velocity, the umbilical cord is scanned in a way that the umbilical arteries are presented as close as possible to a circular shape. To achieve

maximal accuracy we used the zoom facility of the Imaq vision programme. These pictures were read from the videotape with the Colour image acquisition board (NI-Imaq PCI-1411, National Instruments Austin, Tx) and stored on computer disk for further analysis. Umbilical venous cross-sectional areawas expressed by the mean of three tracings of the inner edge of the vessel. The software programme to achieve this was developed in our institution using Labview and Imaq Vision software, (National Instruments Austin, Tx).

Umbilical venous (UV) volume flow was calculated according to the formula: UV volume flow (ml/min) = 0.06 × time-averaged velocity (mm/s) × cross sectional vessel area (mm^2). Fetal biometry including biparietal diameter (cm), head and upper-abdominal circumference (cm) and femur length (cm) was performed following each Doppler flow velocity and vessel area determination. Fetal weight was estimated according to Hadlock's formula [1]. This was followed by calculation of umbilical venous volume flow per kilogram fetal weight. In the growth-restricted fetus, Doppler flow velocity waveforms were also recorded in the free-floating loop of the umbilical artery using the same methodology as described for the umbilical vein. All Doppler recordings and two-dimensional real-time images were stored on SVHS videotape in PAL format using a Panasonic AG 7350 (Matsushita Elect. Ind. Co., Japan) for off-line analysis.

3.2. Fetal brain and liver size

In order to determine the biometric relationship between fetal head and liver size, information on organ volume should be preferred to diameter data. In this subchapter the methodology will be presented for fetal brain volume estimates derived from head circumference measurements and for fetal liver volume determinations as established by means of three-dimensional (3D) ultrasound.

3.2.1. Fetal brain volume

Direct determination of fetal brain volume was not possible at the time of the study. Instead, an indirect estimate of fetal brain volume was made from head circumference measurements. Assuming the fetal head to approach the shape of a sphere, head volume (HV) was estimated according to the formula: HV (ml) = $1/6\ \pi \times d^3$, in which the diameter (d) which is the mean of the biparietal and fronto-occipital diameter, was determined from HC/π. As will be demonstrated in Chapter 6, fetal brain volume is approximately half of fetal head volume.

3.2.2. Fetal liver volume

The 5 MHz annular array transducer (VSW 3-5) of the Voluson 530 ultrasound machine (Kretz technik AG, Zipf, Austria) was used for volume scanning.

Organ volumes were determined by slicing through collected images and recording a truncated pyramidal volume. Depths as well as longitudinal and transverse dimensions of this volume are adjustable, with an initial area of 6.6 cm × 3.3 cm, up to a maximum area of 28 cm × 22 cm at a depth of 20 cm. This provides a maximum scanned volume of 5.9 l. The maximum resolution in the longitudinal direction is 512 ultrasound lines for each of the maximum of 500 slices in the transverse (sweep) direction.

The opening angle of each plane was 70 degrees and the angle of the volume sample was limited to a maximum of 60 degrees. The depth of the volume was restricted to 12-16 cm, with a resolution of 210 slices per volume. Following each scan; the volume is presented in three perpendicular planes on the monitor representing a frontal, sagittal and transverse cross-section of the fetus. Data are stored on hard-disc (Io. Mega Jaz Drive) for off-line analysis. In a first step towards fetal liver volume determination, a frontal cross-section of the liver anterior to the stomach is visualised. In a simultaneously demonstrated sagittal cross-section, the outline of the liver is traced manually in approximately 10 sagittal sections between the most lateral left and right points of the diaphragm in the frontal plane. The liver is measured from its upper limit at the diaphragm to its distal rim as the lower limit. The system automatically keeps track of the distances between the sections and calculated the total volume after each area measurement was completed.

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Chapter 4

Absolute umbilical venous volume flow

Introductory remarks

Until recently, the umbilical venous circulation has provoked only limited interest in favour of the umbilical artery circulation. Few data have appeared on volume flow due to the lack of precision of component measurements, notably cross-sectional vessel size. In this chapter, reproducibility, normal and abnormal data from umbilical venous blood flow are presented paying particular attention to absolute venous volume flow. By means of a method that allows accurate determination of umbilical venous cross-sectional vessel area it would be possible to obtain a full picture of the clinical significance of subsequent volume flow calculations in the human fetus. Moreover, to answer the question whether umbilical venous volume flow contributes to the prenatal detection of fetal growth restriction and whether umbilical venous volume flow can be affected during pre-eclampsia, information is needed on methodology, reproducibility of component measurements and normal data. It has been shown that behavioural state dependent changes occur in Doppler flow velocity waveforms at venous, cardiac and arterial level during the last few weeks of normal pregnancy. Information on possible behavioural state dependent changes in umbilical venous volume flow would provide us with a more complete picture of the normal fetal circulation in late pregnancy. Finally, the effect of maternal plasma expansion and anti-hypertensive treatment on utero-placental and feto-placental hemodynamics including umbilical venous volume flow in pre-eclampsia was studied. The next three research papers will discuss umbilical venous volume flow during (i) normal fetal growth and fetal growth restriction (4.1); (ii) different fetal behavioural states (4.2); (iii) and during maternal hemodynamic correction in pre-eclampsia (4.3).

4.1 Umbilical venous volume flow in the normally developing and growth restricted human fetus.

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Abstract

Objectives To determine reproducibility of umbilical venous volume flow components; to calculate umbilical venous volume flow in normal and growth restricted fetuses (SGA) in a cross-sectional study design.

Method Using Labview and Imaq-vision software, the cross-sectional inner vessel area was traced. Vessel area (mm^2) and time-averaged flow velocity (mm/s Doppler) were multiplied to calculate volume flow (ml/min) including flow/kg fetus. The coefficient of variation (CV) for vessel area and flow velocity scans and tracings was determined (n: 13; 26-35 wks). Normal charts for components and volume flow were constructed (n: 100; 20-36 wks) and related to data from growth restricted fetuses (birthweight < P5) (n:33; 22-36 wks).

Results Reproducibility: CV was 5.4% (vessel area) and 7.3% (time-averaged velocity) for scans and 6.6% and 10.5% for measurements, resulting in a CV of 8.1% (scans) and 11.9% (measurements) for volume flow. A gestational age related increase exists for vessel area, time-averaged flow velocity, and umbilical venous volume flow from 33.2 ± 15.2 (SD) ml/min at 20 weeks to 221.0 ± 32.8 (SD) ml/min at 36 weeks of gestation, but a reduction from 117.5 ± 33.6 (SD) ml/min to 78.3 ± 12.4 (SD) ml/min for volume flow per kilogram fetal weight. In SGA, the values were below the normal range in 31/33 for volume flow and in 21/33 cases for volume flow/kg fetus. Umbilical artery Pulsatility Index was significantly different between the subsets of normal and reduced volume flow/kg fetus.

Conclusion Measurements of umbilical venous vessel area and time-averaged velocity resulted in acceptable reproducibility of volume flow calculations, which show a 7-fold increase at 20-36 weeks. In growth restricted fetuses, volume flow is significantly reduced. When calculated per kg/fetus the values were reduced in 21 (63.6%) out of 33 cases.

Introduction

Since the introduction of Doppler technology for studying human fetal hemodynamics[1,2], most research has focussed on flow velocity waveform analysis. Few data have appeared on volume flow due to the lack of precision of component measurements, notably cross-sectional vessel size. For instance, the increase in vessel size is responsible for most of the rise in umbilical venous volume flow [3]. A method allowing accurate determination of umbilical venous cross-sectional vessel area would contribute to the clinical significance of subsequent volume flow calculations in the human fetus. A number of reports on sonographic assessment of umbilical venous volume flow have appeared in the past [4-10]. A comparative study concerning umbilical venous volume flow in human and ovine pregnancy has shown that volume flow is much lower in the former [3].

Recently, we developed a system for determining cross sectional vessel area from video frames. The objective of the present study was to calculate umbilical venous volume flow from cross-sectional area and flow velocity measurements with emphasis on: (i) the reproducibility of component measurements; (ii) normal and abnormal fetal development, the latter relative to umbilical artery velocimetry.

Material and Methods

During the period of 1 January – 1 November 2000, a total of 133 women with a singleton pregnancy from our outpatient unit consented to participate in the study following approval of the protocol by the Regional Ethics Review Board. Each woman was included in the study only once. Scanning was carried out by a single investigator to avoid variation in scanning technique. Pregnancy was uneventful, as expressed by an appropriate for gestational age fetus (AGA) (upper abdominal circumference above the 5th centile) [11], in 100 women resulting in the delivery of a healthy term infant with a birth weight between the 5th and 95th centile according to the Kloosterman tables [12]. A small for gestational age fetus (SGA) defined by an upper-abdominal circumference below the 5th centile [11] and a birth weight below the 5th centile for weight of gestation according to Kloosterman tables was established in 33 women. Pregnancy duration which was determined from the last menstrual period and confirmed by ultrasound before 20 weeks of gestation varied between 20 and 36 weeks (median: 29 weeks) in uncomplicated pregnancies and between 22 and 36 weeks (median: 29 weeks) in the SGA subset. Maternal age in both subsets ranged between 18 and 42 years (median: 30 years). Umbilical venous maximum flow velocity (mm/s) was measured using a combined two-dimensional real-time and colour-coded Doppler system (Toshiba SSA 140 A Toshiba Corp. Medical Systems Division, Tokyo, Japan) with a transducer carrier frequency of 5 MHz and 3.75 MHz, respectively. The system operates at output intensities of < 100 mW/cm² spatial peak temporal average

in both imaging and Doppler mode. Due to the spiral shape of the umbilical vessels it is not possible to accurately determine the angle between the direction of the blood flow velocity and the ultrasonic beam of insonation. We therefore, used the colour Doppler mode to find the maximum velocity in the umbilical vein. Whilst scanning the umbilical cord under slightly different angles and different positions, the two dimensional colour Doppler presentation of the umbilical cord was carefully examined to find the colour spot with the maximum velocity, since the maximum velocity in the vein can only be obtained with a zero angle of insonation. The sample volume was placed in this maximum velocity spot to obtain a high quality velocity recording during at least four seconds (Fig.1). Assuming a parabolic velocity profile in the umbilical vein, the mean velocity over the cross-sectional area can be calculated from the maximum envelope of the velocity recording, since the cross sectional mean velocity is half the maximum velocity under parabolic flow conditions. We decided to select a relative small sample volume length, between 0.2-0.3 cm, in comparison with the lumen of the umbilical vein to make sure that the waveforms obtained from the umbilical vein are not disturbed by velocity components from the umbilical artery under conditions of reversed flow velocity which can be found in growth-restricted fetuses. The high-pass filter was set at 100 Hz.

Umbilical venous cross-sectional area (mm^2) was expressed by the mean of three tracings of the inner edge of the vessel. The software program to achieve this was developed in our institution using Labview and Imaq Vision software, (National Instruments Austin,Tx). To obtain a two dimensional scan of the cross sectional area of the umbilical vein perpendicular to the direction of the flow velocity, the umbilical cord is scanned in a way that the umbilical arteries are presented as close as possible to a circular shape (Fig.2). To achieve maximal accuracy we used the zoom facility of the Imaq vision program. These pictures were read from the videotape with the Colour image acquisition board (NI-Imaq PCI-1411, National Instruments Austin, Tx) and stored on computer disk for further analysis. Umbilical venous (UV) volume flow was calculated according to the formula: UV volume flow (ml/min) = 0.06 × (time-averaged velocity (mm/s) × cross sectional vessel area (mm^2). Fetal biometry including biparietal diameter (cm), head and upper-abdominal circumference (cm) and femur length (cm) was performed following each Doppler flow velocity and vessel area determination. Fetal weight was estimated according to Hadlock's formula [13]. This was followed by calculation of umbilical venous volume flow per kilogram fetal weight. In the SGA fetus, Doppler flow velocity waveforms were also recorded in the free-floating loop of the umbilical artery using the same methodology as described for the umbilical vein. All Doppler recordings and two-dimensional real-time images were stored on SVHS videotape in PAL format using a Panasonic AG 7350 (Matsushita Elect. Ind. Co., Japan) for off-line analysis.

Reproducibility

In 13 uncomplicated singleton pregnancies varying between 26 and 35 weeks of gestation, umbilical venous cross-sectional vessel area recordings were collected on t=0 min and t=5 min on day 1 and day 2 (=four recordings). Umbilical venous vessel area was measured at three different levels: close to the fetus or placenta and in the centre of the free-floating loop of the cord, every level was measured twice at 5-minute time intervals. The measurements were repeated the following day. Thus, 12 recordings (3×2×2) were available from each pregnancy for determination of the intra-observer variability as expressed by the coefficient of variation (CV) for umbilical venous cross-sectional vessel area recordings. To establish the CV for vessel area tracing, these were carried out twice during the same examination for each vessel area recording. Similarly, umbilical venous maximum velocity waveforms were recorded on t=0 min and t=5 min on day 1 and day 2, but only on one location in the free-floating loop of the umbilical cord in the same 13 pregnancies, resulting in four recordings from each pregnancy for establishing the CV for umbilical venous time-averaged flow velocity. From umbilical venous cross-sectional vessel area and time-averaged velocity data, the CV for umbilical venous volume flow was determined. Analysis of variance was used to calculate the variance within patients split up into recordings and tracings. The CV was calculated by dividing this by the overall mean of the measurements.

Statistics

Coefficients of variation were calculated for intra-observer variability regarding umbilical venous cross-sectional vessel area (mm^2) and time-averaged velocity (mm/s) and resulting absolute volume flow (ml/min). We modelled the relationship between absolute umbilical venous volume flow, umbilical venous volume flow per kilogram fetal weight and volume flow components (cross-sectional vessel area, time-averaged velocity) and gestational age by a cubic polynomial. In case the quadratic term was left out, it was because of too low a tolerance (SPSS Version 8.0). To present variability around the estimated regression curves, centile curves (p5 and p95) were computed with the method described by Altmann [14]. In fetal growth retardation, differences in umbilical artery Doppler velocimetry between subsets of normal and abnormal umbilical venous volume flow per estimated fetal weight were assessed by the Student t-test. The level of statistical significance was set at 0.05.

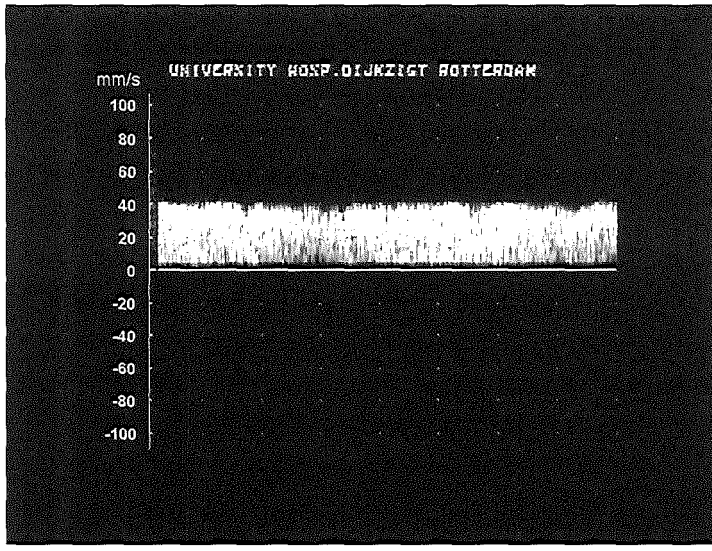

Figure 1. *Doppler ultrasound recording of blood flow velocity waveforms from the umbilical vein at 30 weeks of gestation.*

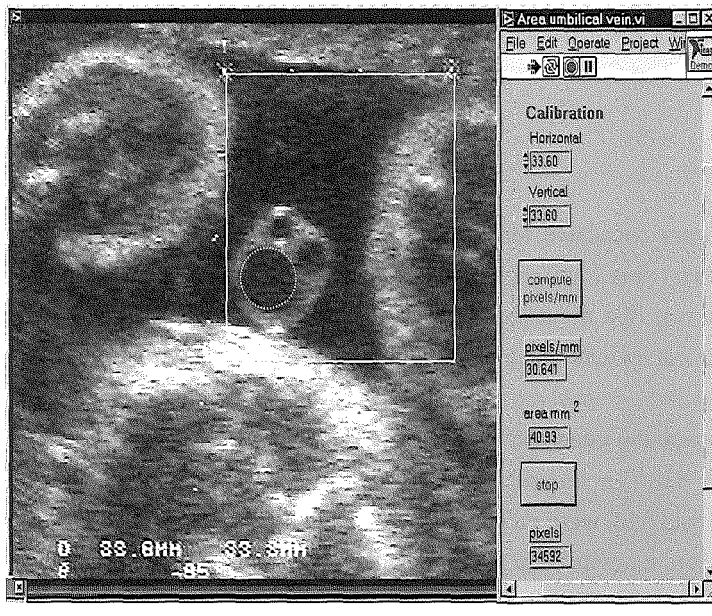

Figure 2. *A ultrasound B-mode image of the cross sectional area of the umbilical vein from the same woman, the umbilical cord is scanned in a way that the umbilical vessels are presented as close as possible to a circular shape.*

Results

No significant difference was found between umbilical venous cross-sectional areas measured on three different locations of the umbilical cord. Data were therefore lumped together for further analysis. Total coefficient of variation for umbilical venous cross-sectional area was 9.1% (recordings: 5.4% and measurements: 7.3%) and for time-averaged velocity 12% (recordings: 7.3% and measurements: 10.5%), resulting in a coefficient of variation for umbilical venous volume flow of 8.1% between recordings and 11.9% between measurements, respectively. In uncomplicated pregnancy, mean umbilical venous cross-sectional area (Fig. 3) increased from 9.9 ± 3.5(SD) mm^2 at 20 weeks to 51.4 ± 7.1 (SD) mm^2 at 36 weeks of gestation. At the same time, mean umbilical venous time-averaged (TA) velocity (Fig. 4) raised from 52.6 ± 12.0 (SD) mm/s to 71.3 ± 8.7(SD) mm/s; this difference was not statistically significant. As a result, mean umbilical venous volume flow (Fig. 5) increased significantly from 33.2 ± 15.2 (SD) ml/min at 20 weeks to 221.0 ± 32.8 (SD) ml/min at 36 weeks of gestation, but reduced significantly from 117.5 ± 33.6 (SD) ml/min to 78.3 ±12.4 (SD) ml/min when expressed as volume flow per kilogram fetal weight (Fig. 6).

In fetal growth restriction, mean gestational age at delivery was 32 ± 4.3 (SD) weeks and the mean lag time between sonographic examinations and delivery was 2.3 ± 1.3 (SD) days. Mean umbilical venous volume flow (Fig. 5) was situated below the 5^{th} centile of the normal range in 32 (97%) out of 33 cases, which was mainly determined by a reduction in umbilical venous cross-sectional vessel area (45.1% of the expected p50 values). However, when expressed per kilogram fetal weight, umbilical venous volume flow was reduced (<P5) in only 21 (63.6%) out of 33 cases (Fig. 6). A statistically significant difference (1.7 ± 0.9 (SD) vs. 3.2 ± 2.2(SD)) for mean umbilical artery PI was established between the fetal SGA subsets of normal and reduced umbilical venous volume flow per kilogram fetal weight.

Mean head to abdominal circumference ratio was not statistically significantly different (1.21 ± 0.08 (SD) vs. 1.19 ± 0.08 (SD)) between the two SGA subsets. When looking at the outcome in live-born fetuses, mean umbilical cord pH (7.17 ± 0.2 (SD) vs. 7.20 ± 0.1 (SD)) between the two subsets was not significantly different. The same applies to mean fetal birthweight (1247g. ± 720 (SD) vs. 1009 g ± 463 (SD)). In the SGA group with normal umbilical venous flow per kilogram fetal weight, 9/12 (75%) fetuses survived, the remaining three resulted in intra uterine death. In the SGA subset with reduced umbilical venous flow per kilogram fetal weight, 15/21 (71.4%) fetuses survived. There were five cases of intra-uterine death and one case of post partum death.

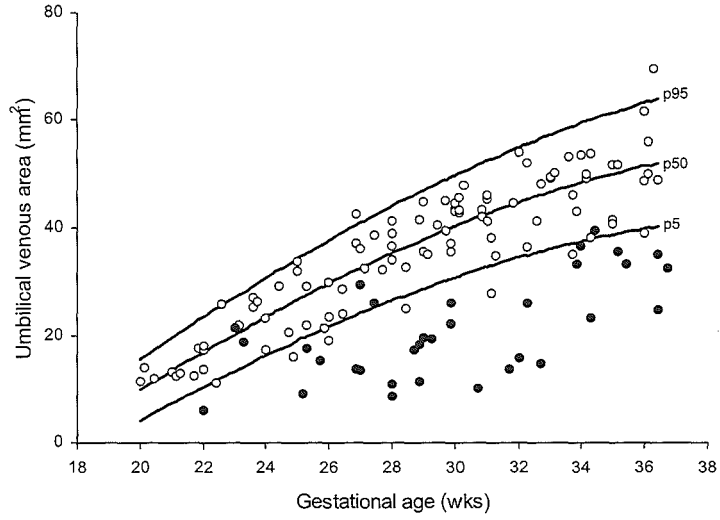

Figure 3. *Umbilical venous cross-sectional area (mm^2) relative to gestational age (GA). Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. Closed circles (•) represent SGA fetuses.*

P50: cubic fit = $-0.000862 \times GA^3 + 4.679124 \times GA - 76.775428$.

P5-P95: $P50 \pm 1.64 (0.178656 \times GA - 0.765755)$

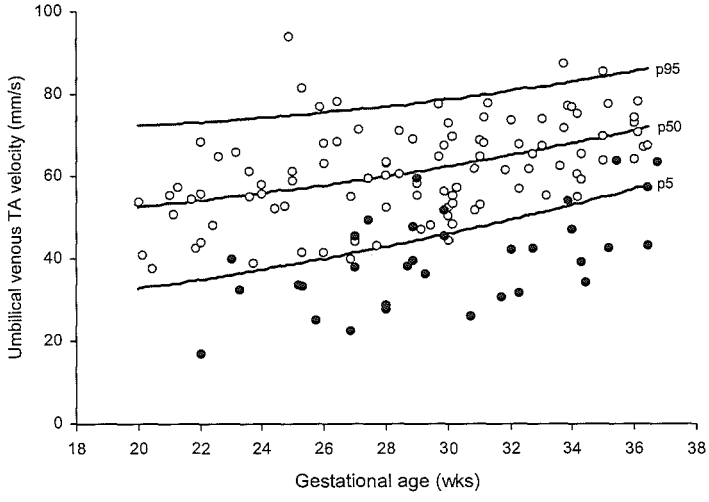

Figure 4. *Umbilical venous time-averaged (TA) maximum envelope velocity (mm/sec) relative to gestational age (GA). Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. Closed circles (•) represent SGA fetuses.*

P50: cubic fit = $0.000216 \times GA^3 + .0.011526 \times GA^2 + 46.271224\ GA - 0.205664$.

P5-P95: $P50 \pm 1.64 (-0.164138 \times GA + 12.874632)$

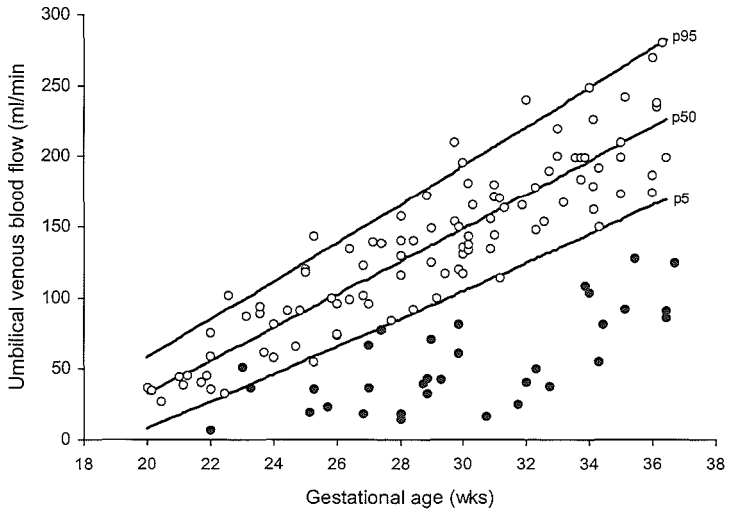

Figure 5. *Umbilical venous volume flow (ml/min) relative to gestational age (GA). Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. Closed circles (•) represent SGA fetuses.*

P50: cubic fit = 0.000328 × GA^3 +10.944931 × GA – 188.288068

P5-P95: P50 ± 1.64 (0.928218 × GA – 6.422451)

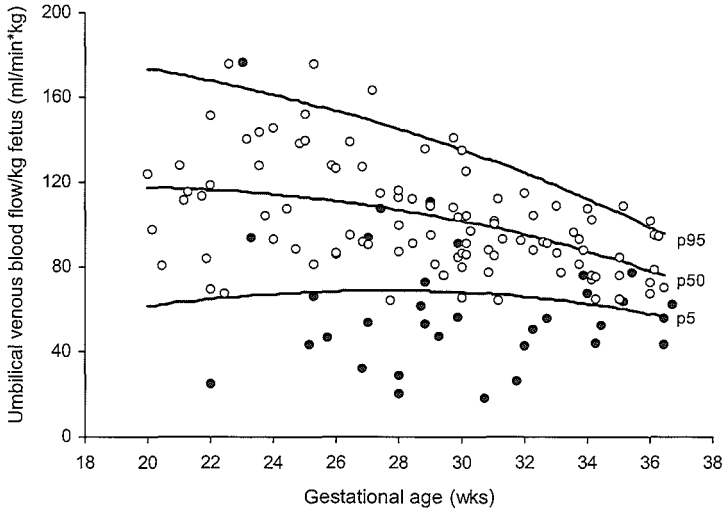

Figure 6. *Umbilical venous volume flow/ Kg estimated fetal weight (ml/min/kg) relative to gestational age (GA). Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. Closed circles (•) represent SGA fetuses. P50: cubic fit = -0.001670 GA^3 + 1.579665xGA + 99.293341. P5-P95: P50 ± 1.64 (1.076244 × GA + 48.623154).*

Discussion

The present study provides data on umbilical venous volume flow and its components in the appropriate for gestational age (AGA) and small for gestational age (SGA) fetus.

An acceptable accuracy and reproducibility of volume flow measurements are a prerequisite for a meaningful use of fetal volume flow measurements in a clinical setting. Accuracy determination was not an objective of our study. Previous work, however, has demonstrated an excellent agreement between combined 2-D real time and pulsed Doppler and invasive measurements of volume flow in the human [15,16] and animals [17-18]. Volume flow measurement in the umbilical vein requires that the angle of vessel interrogation and vessel size are meticulously established. Small errors in the volume flow components will result in larger errors of volume flow calculation [1,2]. In this study the maximum frequency method is used to determine the mean umbilical venous velocity. Other studies have stated that this method has a clear advantage, since it is less susceptible to the effects of noise [19,20]. However this method assumes a parabolic flow profile. Since the umbilical vein can be modelled as a helicoidal tube, instead of an axially symmetrical parabolic profile, a deformed asymmetrical profile can be expected [21]. This may lead to an overestimation of flow velocity. On the other hand, since a departure from zero angle of vessel interrogation always leads to an underestimation of the velocity, we assume that these two opposite deviations will cancel each other out. Measurement of the largest vessel diameter during systole may lead to an overestimation of volume flow in the descending aorta [22]. In the present study this was not an issue, since no pulsatility changes of vessel diameter occurred in the umbilical vein except for a very few SGA fetuses.

Reproducibility for umbilical venous cross-sectional area recordings and tracings was 9% and for umbilical venous time-average velocity recordings and measurements 12%. Acceptable reproducibility was thus achieved for volume flow (recordings: 8.1%; measurements: 11.9%).

In AGA fetuses, increase in umbilical venous volume flow was as much as 7-fold during the second half of pregnancy. This increase was mainly determined by a 5-fold increase in cross-sectional vessel area and to only a minor extent by a rise in time-averaged velocity, which is in agreement with previous studies [3,7,23].

Absolute volume flow results from our study are lower (mean: 230 ml/min at 36 weeks) than those reported by Sutton et al [10,23] and Barbera et al [3] with a mean volume of 300 ml/min at 36 weeks. Similarly, for umbilical venous volume flow per kg fetal weight, our data (mean: 78 ml/min/kg fetal weight) are lower then those from the other two study groups (mean: 110 ml/min/kg fetal weight) at 36 weeks. This discrepancy seems to be mainly determined by a difference in time-averaged velocity calculation. Other earlier

human studies indicate results similar or higher to ours with fetal weight related volume flow during the last ten weeks of gestation varying between 70 and 110 ml/min [5,7,9,24].

When taking into account the entire second half of pregnancy, we observed a significant decrease in fetal weight related umbilical venous volume flow. Also, here our data are at variance with data from the groups of Sutton et al [10] and Barbera et al [3], who found a non significant reduction in weight related volume flow. However, a significant reduction was established by Lingman et al [25]. The latter proposed that this weight related reduction in umbilical venous volume flow is associated with an increase in both visceral flow and flow to the lower extremities as pregnancy progresses. This redistribution of blood flow was also found in other species and may be due to a discordant growth of placenta and fetal body during the third trimester of pregnancy [26-27].

Umbilical venous volume flow data were significantly reduced in the SGA fetuses, which is in agreement with previous reports [5,24,28,29]. As for the volume flow components, time-averaged velocity was reduced in 66% (22/33) and vessel area was reduced in even 84% (28/33) of SGA fetuses. A different distribution was found for fetal weight related volume flow data with 37% (12/33) still situated in the normal range.

Umbilical artery PI is an important parameter for downstream impedance and indirectly for fetal-placental perfusion. Raised umbilical artery PI as a result of reduced end-diastolic flow velocity is associated with some degree of fetal hypoxaemia [30]. Umbilical artery PI was significantly raised in SGA fetuses with reduced weight-related umbilical venous volume flow, suggesting a more pronounced state of fetal hypoxaemia compared with SGA fetuses associated with normal weight-related umbilical venous volume flow. However, this is not reflected in fetal outcome as expressed by survival-rate and umbilical cord pH. Also birthweight is not significantly different between the two subsets.

It is concluded that umbilical venous volume flow measurements demonstrate an acceptable reproducibility. Normal volume flow increases 7-fold during the second half of pregnancy, but when expressed per kg fetal weight depicts a significant reduction during the last trimester. Umbilical venous volume flow is reduced in the SGA fetus, but when related to fetal weight both normal and reduced values were obtained. Whereas umbilical artery PI was significantly raised in the latter, fetal outcome was not essentially different between the two subsets.

4.2 Umbilical venous volume flow and fetal behavioural states in the normally developing fetus.

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Abstract

Objectives: To determine the relationship between umbilical venous (UV) volume flow and fetal behavioural states 1F (quite sleep) and 2F (active sleep) in normal pregnancies at 36-40 weeks of gestation.

Method: Fetal behavioural states were established in 17 normal pregnancies by means of combined assessment of fetal heart rate (FHR), fetal eye and body movements. Umbilical venous (UV) vessel area (mm^2) as obtained by tracing the inner vessel area using Labview and Imaq-vision software and umbilical venous (UV) time-averaged flow velocity (mm/s Doppler) were multiplied to calculate UV volume flow (ml/min), including flow/kg fetus. PI in the umbilical artery was also determined. In each woman, all parameters were measured between 3-5 times in each behavioural state. Data are reported as mean ± 1 SD and analysed by paired t test.

Results: No statistically significant behavioural-state-related changes were observed for UV time-average velocity and UV volume flow, resulting in UV volume flow per kg fetus of 69.1 ± 14.9 ml/min/kg at 1F and 71.6 ± 12.1ml/min/kg at 2F (ns). A statistically significant increase (p=0.02) was established for UV cross-sectional area (46.4 ± 8.6 mm^2 vs 49.0 ± 10.1 mm^2) and for FHR from 134.2 ± 10.3 bpm in 1F to 144.2 ± 7 bpm in 2F. Umbilical artery PI was not significantly different between the two behavioural states.

Conclusion: It can be concluded that on the basis of high venous vessel wall compliance, the significant increase in UV cross-sectional area during fetal behavioural state 2F may be determined by a rise in mean venous pressure. The significant rise in FHR may reflect elevated fetal cardiac output during this behavioural state. This is further supported in part by a mild increase in UV volume flow.

Introduction

It has been shown that variables such as fetal eye movements and fetal heart rate (FHR) pattern are closely related to the neurological condition of the fetus. A classification of behavioural states in the full-term newborn was introduced by Prechtl and Beintema [31] on state criteria such as opened or closed eyes, regular or irregular respiration and present or absent body movements.

With the introduction of high-resolution ultrasound, well-defined behavioural states could also be identified in the human fetus in late pregnancy. Nijhuis et al [32] defined four fetal behavioural states with fetal behavioural state 1F (quiet sleep) and 2F (active sleep) being most prevalent.

Doppler ultrasound studies have demonstrated that changes in fetal behavioural state are associated with specific alterations in fetal hemodynamics [33]. From flow velocity waveform changes in the fetal descending aorta [34] and internal carotid artery [35] increased blood flow to the left heart was proposed with the purpose of meeting raised energy demands during the active sleep state. This was supported by increased time-averaged flow velocities in the ductus venosus [36] and at the level of the forame ovale [37], suggesting elevated venous inflow during this behavioural state.

The question arises as to whether the state dependency of venous inflow also exists at the level of the umbilical vein. We hypothesise that the assumed increase of venous inflow into the left heart is based on preferential blood flow through the placental-umbilical circulation.

To test this we determined volume flow in the umbilical vein according to a recently reported combined 2-D real-time and Doppler ultrasound technique [38] during behavioural state 1F (quiet sleep) and 2F (active sleep) in 20 normally developing fetuses at 35-40 weeks of gestation.

Methods

Study design

During the period of March-November 2002, twenty women with a normal singleton pregnancy recruited from the antenatal clinic consented to participate in the study. The study was approved by the Hospital Ethics Committee. Gestational age which was determined from the last menstrual period and confirmed by ultrasound measurements of fetal crown rump length (10-12 weeks) or fetal biparietal diameter (13-20 weeks). Gestational age ranged between 35 and 40 weeks. Maternal age varied between 25 and 39 years (median 30 yrs). All participants were non-smokers and, except for iron tablets, were receiving no medications. Fetal birthweight was situated between the 10th and 90th

centile according to the Kloosterman Tables corrected for maternal parity and fetal sex [12]. There were no structural anomalies.

Recording technique

A Toshiba SSH 140A (Toshiba Corporation, Medical Systems Division, Tokyo, Japan) unit was used for all Doppler and 2 D real-time ultrasound recordings. Umbilical venous cross-sectional area (mm^2) was expressed by the mean of three tracings of the inner edge of the vessel [38]. The software programme to obtain these tracings was developed in our Department using Labview and Imaq Vision software (National Instruments Austin, Tx). Umbilical venous maximum flow velocity (mm/s) was measured using a combined two-dimensional real-time and colour-coded Doppler system with a transducer carrier frequency of 5 MHz and 3.75 MHz, respectively. The angle of insonation was less than 15 degrees. The system operates at output intensities of < 100 mW/cm^2 spatial peak temporal average in both imaging and Doppler mode. Sample volume length for all flow velocity waveforms ranged between 0.2-0.3 cm; the high-pass wall filter was set at 100 Hz. All ultrasound studies were carried-out with the women being in a semirecumbant position.

A more detailed description of both vessel area and umbilical venous flow velocity measuring techniques for calculation of volume flow have been described in detail previously [38]. In brief, umbilical venous (UV) volume flow was calculated according to the formula: UV volume flow (ml/min) = 0.06 × time-averaged velocity (mm/s) × cross sectional vessel area (mm^2). Doppler recordings of the umbilical artery flow velocity waveform for calculation of the Pulsatility Index were obtained from the free loop of the umbilical cord. In each instance, Doppler recordings was followed by measurement of fetal biparietal diameter (BPD, cm) head (HC, cm) and upper abdominal circumference (AC, cm) and femur length (FL, cm). Fetal weight was estimated from these parameters according to Hadlock' formula [13]. This was followed by calculation of umbilical venous volume flow per kilogram fetal weight. Scanning was performed by one examiner (SB).

Fetal behavioural states

In order to establish fetal behavioural states 1F and 2F, we recorded the following variables:

(i) fetal heart rate (FHR), which was obtained from a Cardiotocograph (8040A Hewlett-Packard GMBH, Germany)

(ii) Fetal eye movements that were studied by ultrasonic visualisation of the fetal eye lens in a transverse scanning plane through the fetal orbits.

(iii) Fetal body movements which were established from a sagittal cross-section of the fetal trunk.

Fetal behavioural state 1F was characterised by the combination of complete absence of fetal eye movements, quiescence of fetal body movements, with occasional startles and a stable heart rate pattern with a small oscillation bandwidth. Isolated accelerations do occur, but these are strictly related to movements. Fetal behaviour state 2F was defined as the continuous presence of fetal eye movement, frequent periods of gross fetal body movements and a heart rate pattern with a wider oscillation bandwidth than in state 1F and frequent accelerations during movements. Umbilical venous time-averaged velocity and umbilical venous cross-sectional area for calculation of volume flow and umbilical artery waveforms were recorded during fetal apnoea when a clear fetal behaviour state had been identified and when this state had been present for at least three minutes [35]. Three consecutive flow velocity waveforms were used to calculate umbilical artery PI and at least 5 seconds of recordings for continuous umbilical vein blood flow velocity to calculate time average velocity. Measurements were performed between three and five times during each behavioural state in each fetus.

Statistical analysis

Repeated measurements within a state within a child were averaged before the statistical analysis was done. Haemodynamic parameters were collected in every fetus during each behavioural state. Calculations were performed with SPSS 10 software package (SPSS Inc., Chicago, IL). Data are reported as mean and standard deviation. The paired t-test was used to compare differences between each fetal behavioural state in the same fetus. The level of statistical significance was set at $p \leq 0.05$.

Results

Mean and standard deviation of hemodynamic parameters from both behavioural states are presented in Table 1. Technically acceptable Doppler signals could not be obtained in three women, leaving 17 women for further analysis. Recording failures were mainly due to fetal breathing movements both in behavioural states 1F and 2F. No statistically significant difference existed between fetal behavioural state 1F and 2F for umbilical venous time-average velocity (Figure 1), and umbilical venous volume flow (Figure 3). However, a statistically significant difference ($p=0.02$) was found for umbilical venous cross-sectional area (Figure 2) between the two behavioural states. Fetal heart rate was significant higher ($p=0.001$) in behavioural state 2F (Figure 4). Umbilical artery PI was not significantly different between the two behavioural states (Figure 5). Mean gestational age at delivery was 39.9 ± 1.2 (SD) weeks, mean fetal birth weight was 3410 ± 375 (SD) g and mean lag time between measurement and delivery was 1.9 ± 1.6 (SD) weeks.

Table 1

	FBS1		FBS2				
	Mean	SD	Mean	SD	p-value	95% CI	
Umbilical venous vessel area (mm^2)	46.4	8.6	49.0	10.1	0.02 *	+4.95	+0.37
Umbilical venous TA velocity (mm/s)	69.1	14.9	71.6	12.1	0.3	+7.85	-2.68
Umbilical venous volume flow (ml/min)	192.7	49.6	211.4	57.4	0.1	+42.79	-5.43
Umbilical venous volume flow/kg (ml/min/kg)	64.4	19.4	69.6	17.8	0.1	+12.42	-1.95
Umbilical artery Pulsatility Index	0.86	0.1	0.84	0.1	0.4	+0.034 -	0.083
Fetal Heart Rate (bpm)	134.2	10.3	144.2	7.0	0.001 *	+13.48	+6.65

TA: time-averaged, *: p-value statistically significant

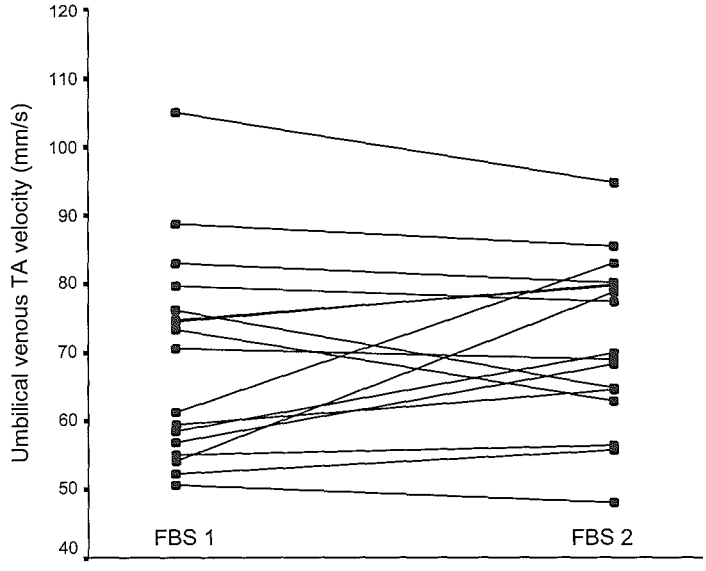

Figure 1. *Umbilical venous time-averaged (TA) velocity (mm/sec) during fetal behavioural state 1F (FBS 1) and 2 F (FBS 2) in 17 normally developing term fetuses.*

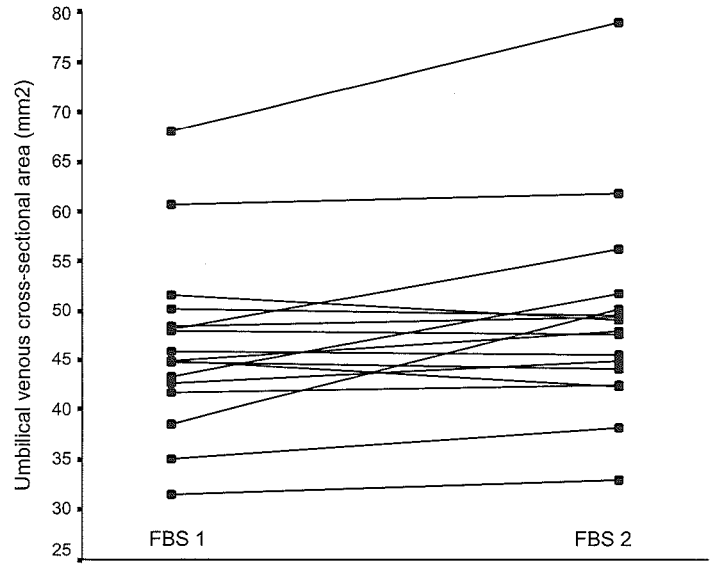

Figure 2. *Umbilical venous cross-sectional area (mm^2) during fetal behavioural state 1F (FBS 1) and 2 F (FBS 2) in 17 normally developing term fetuses.*

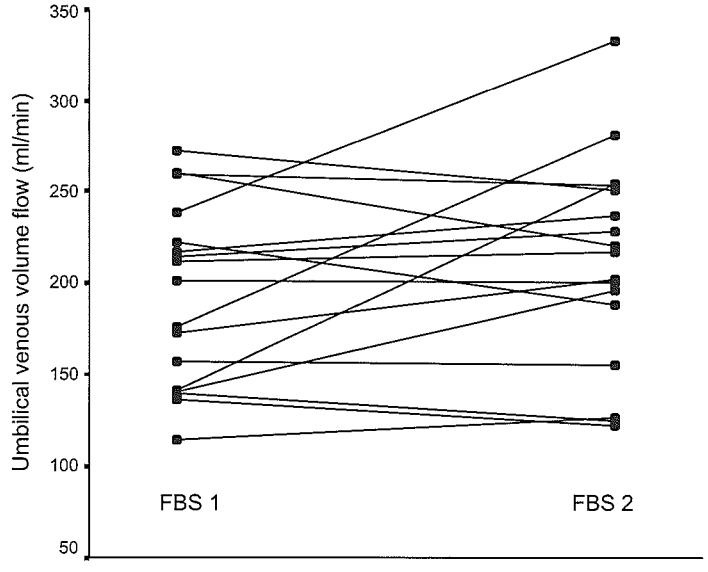

Figure 3. *Umbilical venous volume flow (ml/min) during fetal behavioural state 1F (FBS 1) and 2 F (FBS 2) in 17 normally developing term fetuses.*

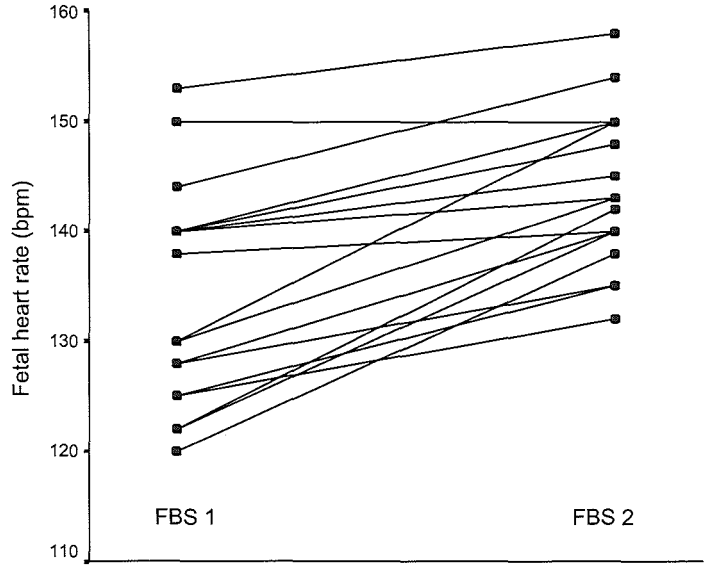

Figure 4. *Fetal heart rate (FHR) (bpm) during fetal behavioural state 1F (FBS 1) and 2 F (FBS 2) in 17 normally developing term fetuses.*

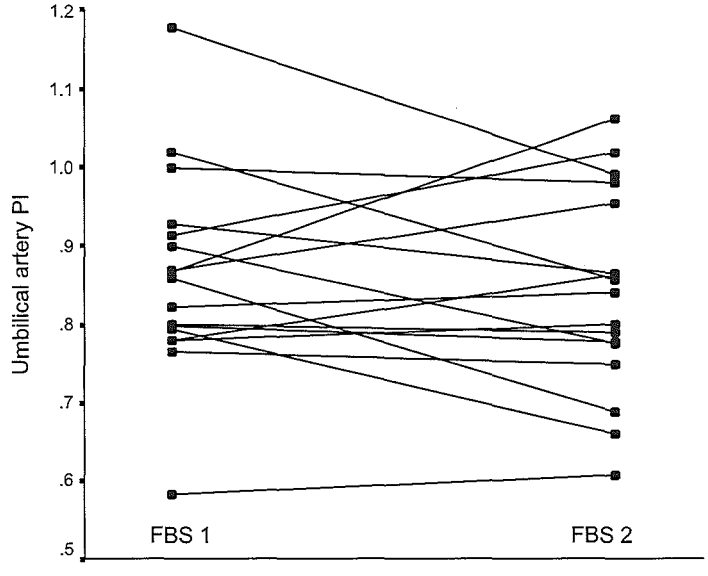

Figure 5. *Umbilical artery PI values during fetal behavioural state 1F (FBS 1) and 2 F (FBS 2) in 17 normally developing term fetuses.*

Discussion

In normal pregnancy fetal neurologic development is expressed by the emergence of fetal activity and the development of specific movement patterns. This can be followed throughout pregnancy resulting in well-defined fetal behaviour states in the third trimester of gestation [32,39]. Existence of activity or behavioural states near term, that has correspondence with postnatal sleep states, is well documented [32]. The presence of different state behaviour was first established in fetal animal, mainly sheep, using chronic catheterization techniques [40-44]. With the introduction of high-resolution real-time ultrasound equipment, it became feasible to undertake studies on the human fetus thus enabling detection and recording of such fetal activities as hiccups, swallowing, micturition and eye movements. The latter fetal activity was used together with fetal body movements, and fetal heart rate for the identification of fetal behavioural states. With a pulsed Doppler ultrasound system, it was demonstrated that in late pregnancy fetal behavioural states are associated with specific haemodynamic adaptations [33,45]. From our centre behavioural state dependent changes have been reported in flow velocity waveforms at systemic arterial [35], cardiac [46,47] and pulmonary levels [48] during normal fetal development. At systemic arterial level a reduced pulsatility index exists in the descending aorta [34] and intracerebral arteries [35] reflecting a reduced downstream impedance, probably as a result of an increased energy demand and therefore increased blood flow to the fetal trunk and cerebrum. This is further supported by a shift from right to left ventricular cardiac output [49] as well as a 7.4 % rise in fetal heart rate observed in the present study suggesting elevated left ventricular cardiac output during fetal behavioural state 2F. Increased cardiac output should be balanced by a raised cardiac inflow and this is suggested by elevated ductus venosus flow velocities reported earlier [46] and the slightly increased (9.7 %) umbilical venous volume flow during fetal behavioural state 2F.

A significant increase in umbilical venous cross-sectional vessel area of 5.6% was found during the behavioural state 2F. The observed increase in umbilical venous vessel size may reflect a rise in mean pressure in this vessel based on a highly compliant venous vessel wall [50,51] which either is the result of elevated umbilical venous volume flow or a raised resistance in the hepato-ductal system [52]. The latter, however, is unlikely.

Controversial data on fetal behavioural state related changes in umbilical venous volume flow have been reported from animal experimental work. Clapp [53] detected an increase in umbilical venous blood flow during high voltage electrocortical activity in the near term lamb fetus, which corresponds to state 1F in the human fetus. Conversely, Walker [54] and Slotten [55] observed some increase in umbilical blood flow during low voltage electrocortical activity in the same animal which corresponds to state 2F in the human fetus, whilst Jensen [56] reported no changes. In the light of these discrepancies in animal

studies, which may be due to different techniques and study designs, our results proved a slight increase in umbilical venous volume flow during behavioural state 2F in line with the reports of Walker [54] and Slotten [55]. In agreement with an earlier study from our centre [35] no significant difference in umbilical artery PI was found, indicating behavioural state independancy of feto-placental arterial down stream impedance.

It can be concluded that on the basis of high venous vessel wall compliance, the significant increase in umbilical venous cross-sectional area during fetal behavioural state 2F may be determined by a rise in mean venous pressure. The significant rise in fetal heart rate may reflect elevated fetal cardiac output during this behavioural state. This is further supported in part by a mild increase in umbilical venous volume flow.

4.3 The impact of maternal volume plasma expansion and antihypertensive treatment with intravenous dihydralazine on fetal and maternal hemodynamics during pre-eclampsia. A clinical, echo-Doppler and viscometric study.

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Submitted to: Ultrasound Obstet Gynecol.

Abstract

Objectives: To establish the effects of plasma volume expansion (PVE) followed by intravenous dihydralazine (DH) administration on maternal whole blood viscosity (WBV) and hematocrit (Ht), utero-placental and feto-placental downstream impedance and umbilical venous (UV) volume flow in pre-eclampsia .

Method: In 13 pre-eclamptic women maternal and fetal haemodynamics were established by means of combined measurement of maternal arterial blood pressure, WBV, Ht and uterine artery resistance index (RI) as well as umbilical artery pulsatility index (PI) and UV volume flow obtained from UV vessel area and UV time-averaged flow velocity. In each woman, all parameters were measured 4 times at baseline, after PVE, after DH and 24 hours after the start of treatment.

Results: Maternal diastolic BP, Ht and WBV display a significant reduction after administration of PVE. In the fetus umbilical artery PI decreases significantly whereas a significant increase in UV cross-sectional area was detected. After maternal DH administration, arterial systolic and diastolic BP and umbilical artery PI show a significant decrease compared with the measurements following PVE. At 24 hours, only maternal systolic and diastolic blood pressures display a significant further decrease. No significant changes were established for the uterine artery RI, UV time-averaged velocity and UV volume flow during the entire study period.

Conclusion: During pre-eclampsia, maternal PVE followed by DH administration, results in a significant reduction in maternal diastolic blood pressure, maternal Ht and WBV. Maternal PVE is associated with a significant increase in UV cross-sectional area and a non-significant rise of 11% in UV volume flow. Maternal DH administration does not result in any change in UV cross-sectional area. However umbilical artery PI decreases significantly after both PVE and DH treatment.

Introduction

In pre-eclampsia, maternal haemodynamics are characterised by relative hypovolemia, raised total peripheral resistance and impaired utero-placental perfusion [57, 58]. Hematocrit is raised. The same applies to red cell aggregation, which especially affects low shear flow such as in the intervillous space of the placenta and therefore may reduce oxygen supply to the fetus. An increase in red cell aggregation causes a steep rise in blood viscosity. Hemodilution has been advocated as an effective treatment in pre-eclampsia to lower the increased blood viscosity [59-60]. This may not only expect to improve uteroplacental perfusion, but also lower maternal systemic blood pressure, one of the symptoms of pre-eclampsia [61]. Maternal administration of antihypertensive drugs in addition to plasma expanders has been advocated to further reduce systemic vascular resistance and blood pressure [62]. Dihydralazine is a drug frequently used in the treatment of pre-eclampsia. It has vasodilating properties, but crosses the placenta.

The aim of the present study was to establish the effects of maternal administration of plasma expanders followed by intravenous dihydralazine on maternal whole blood viscosity and hematocrit, uteroplacental and feto-placental downstream impedance and umbilical venous volume flow in 13 women with pre-eclampsia.

Method

Subjects

Umbilical venous volume flow, umbilical artery and uterine artery flow velocity waveforms were recorded in a consecutive series of 13 pre-eclamptic women with a singleton pregnancy. They were enrolled from the obstetric high care unit during the second half of 2002. Eight women were nulliparous and five were multiparous. Maternal age ranged between 25-39 years (median 30 yrs). The women were not known to have pre-existing hypertension, renal disease or heart disease. Pre-eclampsia was defined as the occurrence of a diastolic blood pressure >90 mmHg measured in sitting position and proteinuria ≥0.3 g/l. Five out of thirteen patients displayed HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low Platelet count) which was defined as the simultaneous occurrence of a platelet count of less than $100x10^9$/L, serum aspartate aminotransferase (ASAT) and serum alanine aminotransferase (ALAT) concentrations greater than 30 U/l and haptoglobin value less than 0.3 g/l [63]. Moreover, seven out of thirteen women used the antihypertensive drug methyldopa before admission. In each woman a blood test was performed including a complete blood count, urine analysis, serum creatinine, uric acid, liver enzymes, whole blood viscosity and platelet count before fluid infusion. Pregnancy duration was determined from the last menstrual period and confirmed by a fetal crown-

rump or by biparietal diameter before 20 weeks gestation. Fetal growth restriction existed in six out of thirteen fetuses according to a fetal upper-abdominal circumference below the 5th centile [11]. Gestational age varied between 27 and 33 weeks (median 30 wks). On admission, umbilical artery Pulsatility Index was situated above 95th centile of the normal reference chart [64] in four out of thirteen fetuses. Individual clinical data and laboratory findings of the 13 pre-eclamptic women are presented in Table 1.

Maternal hematocrit, whole blood viscosity as well as umbilical venous volume flow, umbilical artery and uterine artery pulsatility index were determined on four different occasions. The first measurement (measurement 1) served as baseline before any intravenous fluid treatment; the second measurement (measurement 2) was carried out one hour after starting maternal administration of pasteurised plasma as a volume expander. A total of 500 ml was administered during the first hour of treatment. The third measurement (measurement 3) was taken one hour after initiating maternal antihypertensive treatment by means of dihydralazine, which was administered intravenously one hour after starting plasma infusion, as a bolus of 10 mg during the first 60 minutes followed by 1 mg/hour or more depending on patients conditions. The fourth and last measurement was performed between 20 and 25 hours following the baseline measurement. During the first 24 hours of treatment, fluid intake and diuresis were recorded and blood pressure was either monitored continuously by means of an intra arterial catheter in the maternal radial artery using a Hewlett-Packard monitor system (n=8) or by means of an automatic blood pressure device (Dinamap™1846 SX Critikon) (n=6). Arterial blood pressure measurements were recorded and the mean of at least 3 values (between 3-9 values) was taken over a period of 15 minutes for further analysis, sonographic measurements were performed in the same time period as well as maternal blood samples. In 10 out of 13 women, after the third measurement had been performed, plasma administration was continued for another 24 hours with a total amount ranging from 250 to 1000 ml. Antihypertensive treatment was aimed at achieving a diastolic blood pressure of $\leq$ 90 mm Hg.

Whole blood viscosity determination

Blood sample (6 ml) was collected during the first 24 hours, simultaneously with the four Doppler ultrasound measurements for hematocrit determination and for whole blood viscosity. For pratical reason we did not have the opportunity to perform viscosity measurements in all patients. Data were available in a subset of 10 out of 13 women for hematocrit determination and in a subset of 8 out of 13 women for whole blood viscosity determination. The latter was determined using the Contraves LS 30 cone-plate viscometer

Table 1. Individual clinical data and laboratory findings of pre-eclamptic patients (n=13) on admission

Patient No	Age (year)	Parity*	Gestational age (weeks +days)	Blood pressure (mmHg)	Associated HELLP Syndrome	Uric acid (mmol/L)	Proteinuria (g/L)	Liver function tests**	Platelet count (10^9/L)	Haptoglobin (g/l)
1	29	N	29+3	180/110	-	0.27	3.4	N	225	0.72
2	32	N	30+5	170/110	-	0.43	22.0	I	182	-
3	28	M	29+3	165/105	-	0.27	3.1	N	179	<0.05
4	39	M	30+2	202/115	-	0.30	11.2	N	117	0.51
5	33	N	31+5	222/104	+	0.33	4.6	I	66	<0.05
6	29	M	33	180/120	-	0.81	2.7	N	197	1.53
7	27	N	27+3	180/120	+	0.43	8.8	I	89	0.05
8	31	N	29+6	150/95	+	0.30	0.6	I	61	<0.05
9	36	M	31	155/107	+	0.38	3.0	I	82	0.25
10	25	N	30+4	161/114	-	0.30	0.4	I	134	0.13
11	30	M	30+5	160/100	-	0.29	1.0	I	80	0.97
12	30	N	27+5	183/101	+	0.22	0.8	I	84	<0.05
13	25	N	27	163/105	-	0.34	49.4	I	151	0.28

*: N=Nulliparous M=multiparous
**: I=Increased; N= normal

(Zurich, Switzerland) allowing measurements at different shear rates (0.19; 0.87; 4.04; 18.74; 87 s^{-1}). The temperature was fixed at 37 C°. For statistical analysis we selected the blood viscosity at the lowest (0.19 s^{-1}), medium (4.04 s^{-1}) and highest (87s^{-1}) shear rate.

Umbilical venous volume flow

A Toshiba SHH 140A (Toshiba Corporation, Medical Systems Division, Tokyo, Japan) unit was used for all Doppler and B mode ultrasound recordings.

Umbilical venous cross-sectional area (mm^2) was expressed by the mean of three tracings of the inner edge of the vessel [38]. The software programme to obtain these tracings was developed in our department using Labview and Imaq Vision software (National Instruments Austin, Tx). Umbilical venous maximum flow velocity (mm/s) was measured using a combined two-dimensional real-time and colour-coded Doppler system with a transducer carrier frequency of 3.75 MHz, respectively. The system operates at output intensities of < 100 mW / cm^2 spatial peak temporal average in both imaging and Doppler mode. Sample volume length for all flow velocity waveforms ranged between 0.2-0.3 cm; the high-pass wall filter was set at 100 Hz. All ultrasound studies were carried-out with the woman in the semi-recumbent position. All ultrasound / Doppler recordings were performed by the same investigator (SB).

A more detailed description of both vessel area and umbilical venous flow velocity measuring techniques for calculation of volume flow have been presented in detail previously. In brief, umbilical venous (UV) volume flow was calculated according to the formula: UV volume flow (ml/min)= 0.06 × time-averaged velocity (mm/sec) × cross sectional vessel area (mm^2).

Arterial Doppler data acquisition

Uterine artery flow velocity waveforms were recorded in all patients from the ascending vasculature of the uterine artery bilaterally [65]. Flow velocity waveforms were defined as abnormal if there was persistent notching, or if the Resistance Index (RI) was >95th centile whether or not a notch was present [66]. Umbilical artery flow velocity waveforms from the free loop of the umbilical cord were recorded for calculation of the Pulsatility Index (PI). All Doppler recordings and two-dimensional real-time images were stored on SVHS videotape in PAL format using a Panasonic AG 7350 (Matsushita Elect. Ind. Co., Japan) for off-line analysis.

Statistical analysis

All calculations were performed with SPSS 10 software package (SPSS Inc., Chicago, IL). Data are reported as median values with ranges. We used nonparametric two-way analysis

of variance (Friedman) and the Wilcoxon matched pair signed rank test to assess differences between paired variables such as measurements for maternal systolic and diastolic blood pressure, hematocrit and whole blood viscosity as well as fetal and maternal Doppler ultrasound data. A p-value less than 0.05 was considered to be statistically significant.

Results

Table 2 demonstrates median values with ranges for maternal arterial systolic and diastolic blood pressure, maternal hematocrit and whole blood viscosity, as well as uterine and umbilical venous/arterial Doppler ultrasound data at each of the four measurements. Median maternal arterial systolic blood pressure only becomes significantly reduced after dihydralazine administration and 24 hours following the baseline measurement. Median maternal arterial diastolic pressure already displays a significant reduction after administration of the plasma expander. The same pattern exists for median maternal hematocrit, median maternal whole blood viscosity (apart from the low shear rate subset at 24 hours) and median umbilical artery pulsatility index. Median umbilical venous cross-sectional vessel area increases significantly (apart from the measurement at 24 hours). No significant changes were established for the median uterine artery resistance index, median time-averaged (TA) velocity and median umbilical venous volume flow during the entire study period.

After maternal dihydralazine administration, median arterial systolic and diastolic blood pressure and umbilical artery pulsatility index (PI) show a statistically significant decrease compared with the measurement following plasma expansion. At 24 hours, only median maternal arterial systolic and diastolic blood pressures display a significant further decrease.

Figure 1 shows the relation between maternal whole blood viscosity and shear rate at each of the four measurements. Note that median values for maternal whole blood viscosity after plasma expansion and after dihydralazine administration coincide.

Abnormal uterine artery flow velocity waveforms are present in eight out of thirteen women and persisted throughout the four measurements.

Median gestational age at delivery was 32 weeks (range 28–34) and the median lag time between ultrasound examinations and delivery was 7 days (range 2–30). Median umbilical cord pH at delivery was 7.23 (range 7.01–7.14) and median fetal birthweight was 1350 g. (range 635–2305). Three out of thirteen newborn had a birthweight below the 5th centile according to the Kloosterman Tables adjusted for maternal parity and fetal sex [12].

Table 2. Median and ranges values for maternal arterial systolic and diastolic blood pressure (n=13), hematocrit (n=10), maternal blood viscosity measurements (n=8) and fetal / maternal Doppler flow parameters (n=13).

	Measurements							
	1 (Baseline)		2 (After plasma expansion)		3 (After dihydralazine)		4 (After 24 hours)	
	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max
Maternal arterial systolic blood pressure (mmHg)	175	150 – 222	165	140 – 218	150*#	140 – 205	140 *Δ	117 – 170
Maternal arterial diastolic blood pressure (mmHg)	106	90– 120	101 *	77 – 125	90 *#	75 – 105	80*Δ	59 – 87
Maternal hematocrit (%)	35.0	31.0 – 44.5	32.5 *	28.0 – 42.5	33.5*	29.0 – 41.5	32.5 *	27.0 – 40.0
Maternal whole blood viscosity (mPa*s) at low shear rate (0.187 s^{-1})	17.8	8.42 – 30.0	16.0*	6.5 – 31.4	16.4 *	8.3 – 28.6	16.4	4.4 – 26.2
Maternal whole blood viscosity (mPa*s) at medium shear rate (4.04 s^{-1})	6.9	5.3 – 11.0	5.2 *	4.2 – 10.7	5.6 *	3.7 – 9.9	5.5 *	3.5 – 8.1
Maternal whole blood viscosity (mPa*s) at high shear rate (87 s^{-1})	4.5	3.42 – 5.18	3.6 *	3.1 – 5.3	3.8 *	3.8 – 4.0	3.7 *	2.9 – 4.2
Uterine artery RI	0.6 3	0.5 – 0.9	0.64	0.5 – 0.8	0.64	0.4 – 0.9	0.7	0.5 – 0.8
Umbilical Artery PI	1.51	0.9 – 2.2	1.32 *	0.9 – 1.96	1.21 *#	0.8 – 1.7	1.25 *	0.8 – 1.7
Umbilical venous cross-sectional area (mm^2)	33.6	22.2 – 43.0	37.7*	27.6 – 52.3	38.7 *	25.4 – 47.8	34.4	27.6 – 53.5
Umbilical venous TA velocity (mm/s)	55.1	44.2 – 81.4	58.1	43.2 – 70.2	56.5	35.7 – 69.5	56.3	35.6 – 77.9
Umbilical venous volume flow (ml/min)	109.2	58.8 – 164.6	121.4	77.9 – 215.6	127.3	54.3 – 189.5	116.1	64.5 – 202.4

$p \leq 0.05$ measurements 2, 3 and 4 compared with measurement 1; # $p \leq 0.05$ measurement 3 compared with measurement 2;
Δ $p \leq 0.05$ measurement 4 compared with measurement 3.

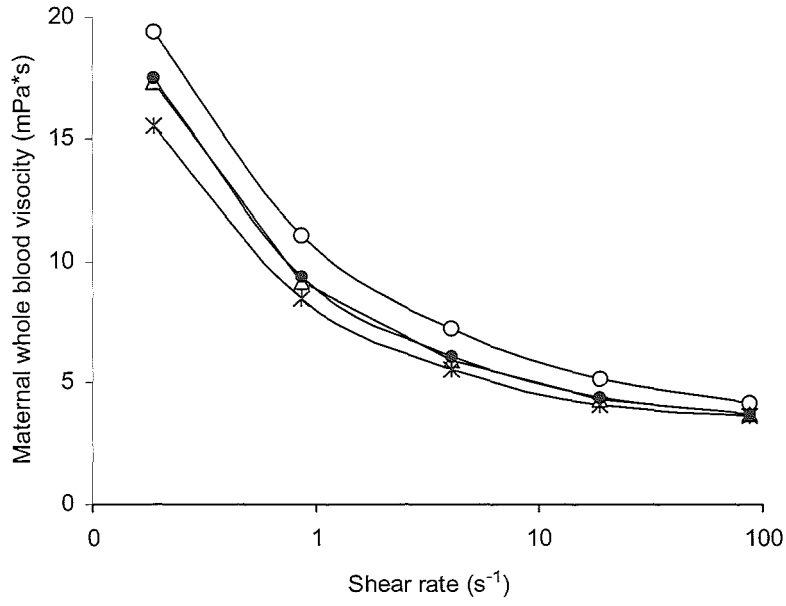

Figure 1 *shows the relation between maternal whole blood viscosity (mPa*s) and shear rate (s^{-1}) at each of the four measurements. Baseline measurement: -*-; Measurement after plasma expander -6-; Measurement after dihydralazine treatment -)-Measurement after 24 hours: -H-.*

Discussion

The objective of this study was to assess circulatory effects of hemodilution and antihypertensive treatment with the vasodilator dihydralazine in pre-eclampsia on both mother and fetus. In the pre-eclamptic syndrome an increased vascular resistance and a diminished circulating blood volume with impaired perfusion of various organs, including the utero-placental unit are important pathophysiologic features [67].

Generally, antihypertensive drugs are administered in the course of pre-eclampsia to avoid maternal vascular complications induced by elevation of maternal arterial blood pressure. Their administration may result in a marked reduction in blood pressure affecting both the maternal [67] and fetal circulations [68]. Therefore, it has been suggested that antihypertensive treatment, especially with a vasodilator, should be preceded by administration of a plasma expander as to normalise maternal circulatory volume [67-69].

Effects of maternal plasma expansion

Maternal site

In our study a small but significant fall in maternal arterial diastolic blood pressure following plasma expansion was established. This is in line with some studies [70-72], but not with others [67,73].

It is known that plasma expansion will give rise to an increase in maternal cardiac output as well as a reduction in peripheral vascular resistance. The latter is documented by the drop in maternal arterial diastolic blood pressure. In pre-eclampsia there is a considerable change in the rheological properties of maternal blood, including an increase of red cell aggregation, which causes a steep rise in whole blood viscosity. Plasma expansion will lead to hemodilution as expressed by a lower hematocrit. This will result in a drop in maternal whole blood viscosity. In our study, baseline measurements of maternal whole blood viscosity were above the normal range for normotensive pregnant women according to Buchan [74]. A significant reduction in maternal whole blood viscosity was observed after plasma expansion at virtually all shear rates. This suggests a relationship between reduction in viscosity and drop in blood pressure after volume expansion. A further explanation for the drop in maternal arterial diastolic blood pressure could be that volume expansion induces an activation or release of a substance at endothelial level such as nitric oxide and prostacyclin, with vasodilatation as a result [75]. Moreover, another pathway that seems to be involved in the possible beneficial effects of volume expansion, is the suppression of the renin-angiotensin system at the utero-placental level [76-77].

We could not observe any change in the uterine artery resistance index after hemodilution. This is in agreement with another report [78] and could be explained by the assumption that the utero-placental circulation is a low impedance vascular system and therefore the degree of pulsatility does not reflect downstream impedance [79-80].

Fetal site

In pre-eclampsia, an association between increased impedance in the fetal circulation and abnormal fetal blood rheology with or without fetal growth restriction has been described by several authors [81-82]. For example, Buchan [83] reported a higher median whole blood viscosity, hematocrit and fibrogen concentration in cord blood of pregnancies complicated by pre-eclampsia as compared with controls. In our study umbilical artery PI decreased following maternal plasma expansion reflecting reduced downstream impedance at feto-placental level. This is in agreement with a previous report [84] in which a reappearance of end-diastolic flow velocity in the umbilical artery was established after hemodilution.

Hemodilution was associated with a significant increase in umbilical venous cross-sectional vessel area, resulting in a non-significant increase of 11% in umbilical venous volume flow. Regulation of blood volume between the fetus and the placenta is controlled by the different pressure gradients between placental and the umbilical blood flow [85]. In an animal experimental study [86], it was reported that hypervolemic hemodilution with albumin leads to slight fetal hemodilution, which can be identified by a further decrease in fetal colloidosmotic pressure. The elevated hydrostatic pressure in the intervillous space, induced by plasma volume expansion, may be the mechanism underlying the net flow of water into the fetal circulation. Our data are at

variance with a previous study [87] in which a significant increase in umbilical venous volume flow was observed after maternal hemodilution with 500 ml of dextran.

Effects of Dihydralazine treatment

Maternal site

Both maternal arterial systolic and diastolic blood pressures are significantly reduced following administration of dihydralazine. However, no significant changes were observed in uterine artery resistance index, which may be due to its state of maximum dilatation [88-90] nor in maternal hematocrit and maternal whole blood viscosity.

Fetal site

Dihydralazine crosses the placenta barrier into the fetal circulation [91], which may explain the further reduction in feto-placental downstream impedance expressed by the umbilical artery pulsatility index. This does not exclude, however, a possible prolonged plasma expander effect. Dihydralazine administration is not associated with a further increase in umbilical venous cross-sectional vessel area. Although Jouppila et al [92] reported no change in the intervillous blood flow, they found an elevated umbilical venous volume flow following dihydralazine administration. This is at variance with our data, which demonstrated an unaltered umbilical venous perfusion.

Our results underline that during pre-eclampsia, maternal administration of plasma expansion, followed by antihypertensive treatment by means of dihydralazine, lead to a significant reduction in maternal diastolic blood pressure, maternal hematocrit and whole blood viscosity. Maternal plasma expansion is associated with a significant increase in umbilical venous cross-sectional vessel area and a non-significant rise of 11% in umbilical venous volume flow. Maternal dihydralazine administration does not result in any change in umbilical venous cross-sectional vessel area. However umbilical artery pulsatility index, as a measure of feto-placental downstream impedance, decreases significantly after both plasma and dihydralazine treatment. Our study does not provide evidence whether or not the use of plasma volume expansion, in the treatment of pre-eclampsia, is beneficial to the mother or the fetus. On the other hand, it can be concluded that combined maternal administrations of plasma expander and dihydralazine infusion has no deleterious effect on the feto-placental circulation as expressed by umbilical artery pulsatility index and umbilical venous volume flow measurements.

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Chapter 5

Fetal liver volume assessment

Introductory remarks

Three-dimensional ultrasonography allows assessment of the shape and volume of fetal organs. This subchapter describes reproducibility and normal values for fetal liver volume in normally developing fetuses using three-dimensional ultrasound scanning. Moreover, our investigation is directed at pregnancies complicated by fetal growth restriction. Measurement of fetal upper abdominal circumference is considered the mainstay of sonographic determination of fetal growth and estimation of fetal weight. Considering that fetal liver comprises most of the abdomen, we investigated which role fetal liver volume measurement plays in the early detection of fetal growth restriction relative to head and upper abdominal circumference.

This is presented in subchapter 5.1 Insulin dependent diabetes during pregnancy is considered to be responsible for fetal growth acceleration. In post mortem specimen, weight gain of fetal organs has been demonstrated particularly in some structures and tissues such as liver and fatty tissue. Using 3-dimensional ultrasound we measured liver volume in fetuses of diabetic mothers and compared these data with normally developing fetuses as well as their possible correlation with maternal glycemic metabolism. In addition, we tested whether in maternal insulin-dependent diabetes mellitus umbilical venous volume flow and feto-placental arterial down stream impedance are altered (subchapter 5.2).

5.1 Three-dimensional assessment of liver volume, head circumference and abdominal circumference in healthy and growth restricted fetuses.

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Abstract

Purpose: To establish reproducibility and normal values for fetal liver volume and its significance in identifying fetal growth restriction relative to head (HC) and upper abdominal circumference (AC) according to a cross-sectional study design.

Material and methods: Using a Voluson 530-D, a total of 135 pregnancies were enrolled. The coefficient of variation (CV) for liver volume scans (t_0, t_{20} min) and liver area tracings (t_0: 2x; t_{20}: 2x) was determined (n:20; 23-36 wks). Normal charts for liver volume, HC, and AC were constructed (n:85; 20-36 wks) and related to data from growth restricted fetuses (birthweight< P5) (n:24; 22-36 wks).

Results: A total of 109 fetal liver measurements was obtained. CV was 2.9% for volume scans and 1.6% for area tracings. In 85 uncomplicated cases, mean fetal liver volume (P50) ranges between 9.7 ± 4.4 (SD) ml at 20 weeks and 96.4 ± 8.2 (SD) ml at 36 weeks of gestation. In 24 cases of fetal growth restriction, liver volume, HC and AC expressed as percentage of the normal P50 was 45%, 90% and 82%, respectively. Mean difference in liver size between fetal growth restriction and normal fetal development as expressed by the Z-score (-4.32 ±1.4SD), was significantly different ($p<0.05$) from that for HC: -3.04 ± 1.3 (SD), but not for AC: -4.7 ± 1.2 (SD).

Conclusion: Acceptable reproducibility exists for liver volume determinations. In fetal growth restriction, reduction is more pronounced for liver volume than HC or AC; liver volume is a better discriminator than HC, but not AC.

Introduction

Measurement of fetal upper abdominal circumference is considered the mainstay of sonographic determination of fetal growth and estimation of fetal weight [1,2] The fetal liver comprises most of the abdomen measured by the abdominal circumference. Measurement of fetal liver volume to identify fetal growth restriction is of interest since both human and rat have severely depleted hepatic glycogen stores associated with growth restriction [3]. Due to fetal malnutrition, reduction in fetal liver weight is more pronounced than reduction in brain weight due to the brain sparing effect, reflecting redistribution of fetal blood flow during chronic fetal hypoxaemia [4]. Measurement of fetal liver volume may thus contribute to the early detection of fetal growth restriction. Another study dealing with measurements of fetal liver length in fetal growth restriction suggests that also other intra-abdominal organs, reduced amounts of fat or elevation of the diaphragm due to poor lung growth are contributing factors to a reduced fetal upper abdominal circumference [5].

Recently, a more direct approach to measurement of fetal liver volume using three-dimensional ultrasound was reported [6], including preliminary data on normal fetal liver volume from our own centre [7]. To the best of our knowledge, no data seem to be available on 3D sonographic measurement of fetal liver volume in fetal growth restriction. Therefore, the purpose of our study was to establish reproducibility and normal values for fetal liver volume and its significance in identifying fetal growth restriction relative to head and upper abdominal circumference according to a cross-sectional study design.

Material and Methods

During the period 1997-2000 a total of 135 women with a singleton pregnancy from our outpatient unit consented to participate in the study following approval granted by the institutional Ethics Review Board, this includes 34 normal singleton pregnancy from a previous study [7]. Technically acceptable sonographic liver recordings were obtained in 85 out of 104 uncomplicated pregnancies and in 24 out of 31 pregnancies associated with fetal growth restriction, resulting in a success rate of 82% and 77%, respectively. A sonographic liver recording was qualified as acceptable if the entire contour of the liver could be manually outlined. Recording failures were mainly due to maternal obesity, fetal position or excessive fetal movements. A liver volume calculation took approximately 15 minutes. Each woman was evaluated in the study only once. Uncomplicated pregnancy constituted the delivery of a healthy infant with a birthweight between the 5th and 95th centile for weight of gestation, according to the Kloosterman tables [8]. Fetal growth restriction was represented by fetal upper abdominal circumference below the 5th centile [9] and a birthweight below the 5th centile for weight of gestation was confirmed after delivery according to the Kloosterman tables [8]. Pregnancy duration

was determined from the last menstrual period and confirmed by ultrasound before 20 weeks of gestation. It varied between 20 and 36 weeks (median: 30 wks) in uncomplicated pregnancies and between 22 and 36 weeks (median: 29 wks) in the subset of fetal growth restriction. Maternal age in all 109 pregnancies ranged between 19 and 42 years (median: 29 yrs). The 5 MHz annular array transducer (VSW 3-5) of the Voluson 530 ultrasound machine (Kretz technik AG, Zipf, Austria) was used for volume scanning. Two examiners (S.B. and J.A.M.L.) obtained the data, each of which also performed the liver volume measurements.

Organ volumes were determined by slicing through collected images and recording a truncated pyramidal volume. Depth as well as longitudinal and transverse dimensions of this volume are adjustable, with an initial area of 6.6 cm $\times$ 3.3 cm, up to a maximum area of 28 cm $\times$ 22 cm at a depth of 20 cm. This provides a maximum scanned volume of 5.9 l. The maximum resolution in the longitudinal direction is 512 ultrasound lines for each of the maximum of 500 slices in the transverse (sweep) direction.

In the present study, the opening angle of each plane was 70 degrees and the angle of the volume sample was limited to a maximum of 60 degrees. The depth of the volume was restricted to 12-16 cm, with a resolution of 210 slices per volume. Following each scan, the volume is presented in three perpendicular planes on the monitor representing a frontal, sagittal and transverse cross-section of the fetus. Data are stored on hard-disc (Io. Mega Jaz Drive) for off-line analysis. In a first step towards fetal liver volume determination, a frontal cross-section of the liver anterior to the stomach is visualised. In a simultaneously demonstrated sagittal cross-section, the outline of the liver is traced manually in approximately 10 sagittal sections between the most lateral left and right points of the diaphragm in the frontal plane. The liver is measured from its upper limit at the diaphragm to its distal rim as the lower limit. The system automatically keeps track of the distances between the sections and calculated the total volume after each area measurement was completed. In each instance, fetal liver volume recording was followed by measurement of fetal head and upper abdominal circumference and femur length. Fetal weight was estimated from these biometric parameters using the Hadlock formula [10].

Reproducibility

The first 20 uncomplicated singleton pregnancies examined by SB were enrolled into the reproducibility study. Pregnancy duration varied between 23 and 36 weeks of gestation. Liver volume recordings were obtained on t=0 min and t=20 min, for determining the intra-observer variability as expressed by the coefficient of variation (CV). To establish the CV for fetal liver volume tracings, these were carried-out twice for each liver volume recording (t_0: 2$\times$ and t_{20}: 2$\times$). Analysis of variance was used to calculate the variance and the standard deviation (SD) within patients split up into recordings and tracings. The CV was calculated by dividing the SD by the overall mean of the measurements.

Statistics

The gestational age related reference centiles p5, p50 and p95 were constructed using the method described by Altman (11). According to this procedure, we modelled the relationship between liver volume, head circumference, upper abdominal circumference and gestational age by means of a cubic polynomial. Either the linear or quadratic term was left out because of too low a tolerance (SPSS Version 8.0 for Windows). The age specific standard deviation was obtained from linear regression of the absolute residuals on gestational age. Polynomial linear regression was used to calculate the relationship between liver volume and estimated fetal weight. Data from liver volume, head circumference (HC), and upper abdominal circumference (AC) for fetal growth restriction, were expressed as a ratio of the normal p50 values. Abdominal circumference and head circumference ratios were raised to the power of three to estimate the abdominal and head volume reduction in growth restricted fetuses. These values were compared with the three dimensional liver volume data during fetal growth restriction. Mean difference in liver volume between fetal growth restriction (FGR) and normal fetal development was also determined by the Z score = liver volume$_{(FGR)}$ – P50$_{(normal)}$ / SD$_{(normal)}$. In the group of fetal growth restriction the percentage of normal P50 values and Z-scores of the liver volume were compared with AC and HC by means of the paired sample t-test. The level of statistical significance was set at 0.05.

Results

The number of confirmed normally developed infants was 85 and the number of growth restricted infants was 24. CV for liver volume recordings and liver volume tracings was 2.9% and 1.6%, respectively, resulting in a total CV of 3.3% within fetuses. The number of uncomplicated pregnancies per gestational week varied between 3 and 12. In uncomplicated pregnancy, mean fetal liver volume was 9.7 ±4.4 (SD) ml at 20 weeks and 96.4 ± 8.2 (SD) ml at 36 weeks of gestation (Fig. 1). At the same time, mean fetal head circumference (Fig. 2) measured 17.5 ± 0.7 (SD) cm and 31.7 ± 0.9 (SD) cm and mean fetal upper-abdominal circumference (Fig. 3) 14.8 ± 0.8 (SD) cm and 31.4 ± 1.0 (SD) cm at 20 and 36 weeks, respectively. A significant linear relation was established between fetal liver volume and estimated fetal weight (Fig. 4).

In fetal growth restriction, mean gestational age at delivery was 31 ± 4.6 (SD) weeks, mean fetal birth weight was 1140 ± 636 (SD) g and mean lag time between liver volume measurement and delivery was 2.5 ± 1.4 (SD) weeks. Fetal liver volume, fetal head circumference and upper-abdominal circumference were situated below the 5th centile of the reference chart in most or in all cases (Fig. 1-3). Mean difference in liver volume between fetal growth restriction and normal fetal development as expressed by percentage of the normal p50 values was 45 ± 13.0 (SD) %, which is significantly different ($p<0.001$) from head circumference:

90 ± 4.2 (SD) % and fetal upper abdominal circumference: 82 ± 4.1 (SD) %. Within the subset of growth restricted fetuses, mean liver volume is also significantly smaller ($p<0.001$) than the estimated head volume (75 ± 11 (SD) %) and estimated abdominal volume (56 ± 8.4 (SD) %), respectively. Mean difference in liver volume between fetal growth restriction and normal fetal development as expressed by the Z-score, was –4.32 ± 1.4 (SD), which is significantly different ($p<0.05$) from the Z-score for head circumference: -3.04 ± 1.3 (SD), but not for fetal upper abdominal circumference: -4.7 ± 1.2 (SD).

Figure 1. *Liver volume (ml) relative to gestational age (wks). The figure shows that all liver volumes of the growth-restricted fetuses are situated below the P5 reference level. Note that these liver volume data are even more reduced relative to the P5 reference level than the abdominal circumference data (figure 3). However since for the liver volume, the corresponding Standard Deviation is larger than that for head and upper-abdominal circumference, the standardized distance (Zscore) is not significantly different. Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. Closed circles (•) represent fetal growth restriction. GA = gestational age. P50: cubic fit =* $0.0012 \times GA^3 + 0.0443 \times GA^2 - 18.268$, *P5-P95=* $P50 \pm 1.64 (- 0.2408 \times GA - 0.4560)$

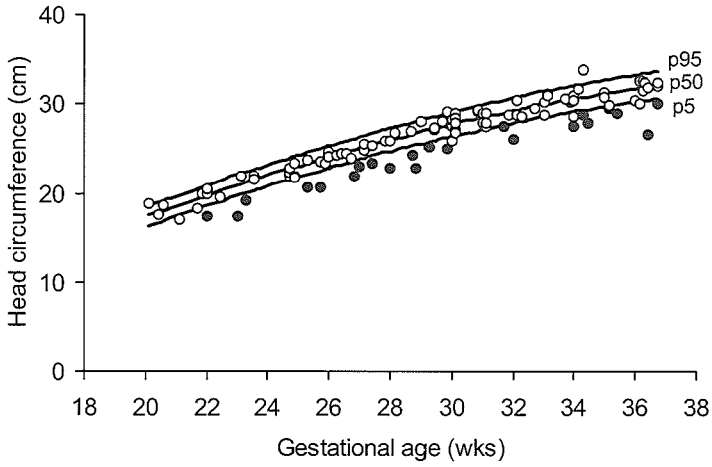

Figure 2. *Head circumference (cm) relative to gestational age (wks). This figure shows that the head circumference data of the growth restricted fetuses are less reduced to the P5 reference level than liver volume and upper-abdominal circumference. Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. Closed circles (•) represent fetal growth restriction. GA = gestational age. P50: cubic fit = -0.000274 × GA^3 +1.564502 × GA – 11.801200, P5-P95 = P50 ± 1.64 (0.016072 × GA + 0.343292)*

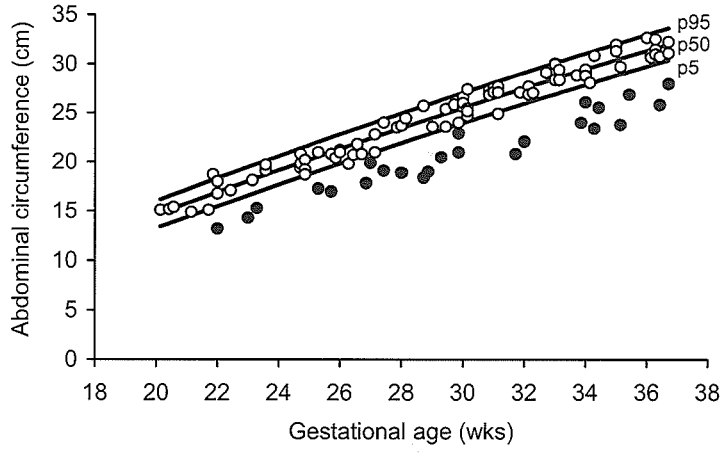

Figure 3. *Upper abdominal circumference (cm) relative to gestational age (wks). This figure should be interpreted as a reference figure to be compared with figure 1 and figure 2. Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. Closed circles (•) represent fetal growth restriction. GA = gestational age.*

P50: cubic fit = -0.000079 × GA^3 + 1.2381 GA – 9.493536, P5-P95= P50 ± 1.64 (0.009144 × GA + 0.665685)

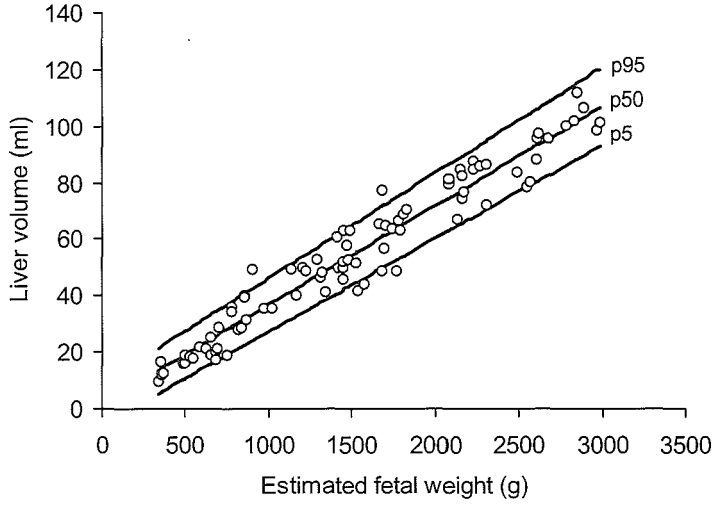

Figure 4. *Liver volume (ml) relative to estimated fetal weight (g). The regression line demonstrates that the liver volume is proportional to estimated fetal weight during the second half of pregnancy. Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. EFW = estimated fetal weight. P50: linear fit = 35.190623 × EFW + 1.560381, P5-P95= P50 ± 1.64 (1.300713 × EFW + 4.447085)*

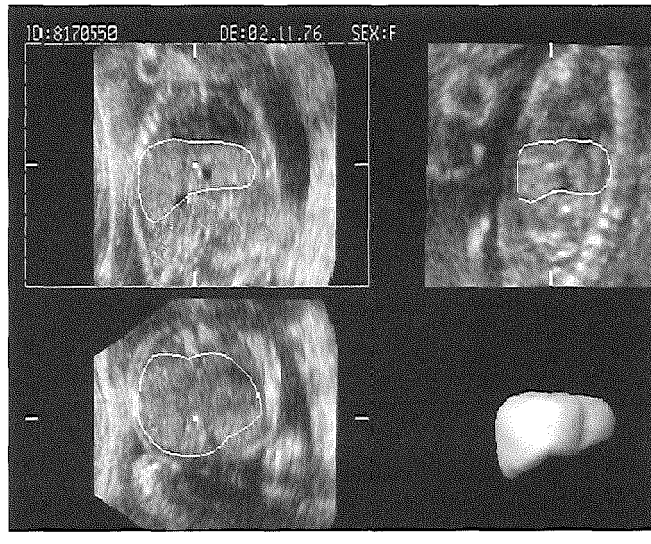

Figure 5. *A picture representing how liver volume calculations were performed and the final three-dimensional image of the fetal liver.*

Discussion

The present study focussed on 3D sonographic measurements of fetal liver volume in normal and abnormal fetal development during the second half of pregnancy. The success rate in acquiring technically acceptable fetal liver volume measurements was as high as 82% in uncomplicated pregnancy and somewhat lower (77%) in fetal growth restriction. Low image quality occurred in the presence of maternal obesity or when the fetal back was in the anterior position. In this case, fetal bones, particularly vertebra and ribs, cause shadows on some parts of the fetal abdomen, limiting the definition of some slices inside the volume box. Reduced amniotic fluid constituted a reason for not obtaining a technically acceptable liver volume measurement in the growth-restricted fetus.

Selection of the second half of pregnancy in the present study was not determined by limitation of 3D measurements of liver volume before 20 weeks of gestation [6], but by the fact that fetal growth restriction usually does not set in until 22-24 weeks.

An acceptable reproducibility of liver volume measurements is essential before embarking on a clinical study. We demonstrated that the reproducibility of fetal liver volume recordings and tracings is good with a total coefficient of variation of less than 4%. We did not study the accuracy of fetal liver volume measurements using 3D ultrasound, but many investigations have been undertaken in to the past to confirm the accuracy of 3D sonographic volume measurements [12-14].

In uncomplicated pregnancy, fetal liver volume demonstrates a 10-fold increase with advancing gestational age and increasing fetal weight. The regression line, shown in figure 4, demonstrates that the liver volume is proportional to estimated fetal weight during the second half of pregnancy.

Currently, a sonographic measurement of fetal head and upper-abdominal circumference is the most widely used method of establishing impaired fetal growth [15,16]. The detection of fetal growth restriction by means of head circumference measurements may be limited due to fetal brain sparing in the presence of chronic fetal hypoxaemia. The majority of studies has shown that the most effective method of detecting fetal growth restriction is the upper abdominal circumference. However, this measurement is not all that satisfactory in that the positive predicted value for detecting fetal growth restriction may be as low as 21% [17].

Therefore accurate assessment of liver volume may contribute to the early detection of growth restricted fetuses since liver weight in these fetuses is reduced due to reduction in hepatic glycogen stores [3].

Fetal liver volume has been estimated indirectly through measurement of liver length, circumference and area using 2 – dimensional ultrasound [18,19] and more recently using 3D ultrasound [6,7]. A linear relationship has been described between 2D fetal liver length and fetal

upper abdominal circumference in uncomplicated pregnancies [18,19]. 3D ultrasonographic measurements of the normal liver volume demonstrated a close linear relation between liver volume and estimated fetal weight [6,7]. In an earlier study, Baker et al [17], were able to show that fetal liver volume measurements by means of MRI allowed accurate detection of fetuses, which were subsequently found to be growth restricted. However, MRI scanning is associated with reduced patient acceptability and high costs.

The present study shows that fetal growth restriction is associated with reduced liver volume in every instance. Moreover, when expressed as percentage of the normal P50, the decrease in liver volume is more pronounced than the reduction in head circumference or upper abdominal circumference. When looking at the mean difference in liver volume between normal and reduced fetal growth, as expressed by the Z-score, we only found a significant difference when compared with head circumference, confirming the brain sparing effect during abnormal fetal development.

It can be concluded that 3D sonographic measurements of fetal liver volume show a good reproducibility. Normal fetal liver volume is 10-fold larger at the end of gestation compared with the beginning of the second half of pregnancy. Fetal size is affected in fetal growth restriction, but fetal liver volume measurement is not a better discriminator than measurement of the upper abdominal circumference.

5.2 Assessment of fetal liver volume and umbilical venous volume flow in pregnancies complicated by insulin-dependent diabetes mellitus.

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Abstract

Objectives To determine fetal liver volume and its relation with umbilical venous volume flow and maternal glycosylated haemoglobin (Hb1Ac) in pregnancies complicated by diabetes mellitus type I (IDDM).

Design a cross sectional study;

Setting Obstetric out-patient clinic, Erasmus MC, Rotterdam

Population Data from fetuses of diabetic women (n=32;18–36 wks) were compared with data from normal controls (n=32) matched for gestational age.

Methods Umbilical venous (UV) cross-sectional area (mm^2) and time-averaged velocity (mm/s Doppler) were determined for calculation of volume flow (ml/min) and flow/kg fetus (ml/min/kg). Fetal liver volume (LV) measurements were obtained using a Voluson 530-D. Main outcome measures Fetal liver volume is 20% higher whilst UV volume flow is not essentially different in IDDM compared with normal pregnancy.

Results A statistically significant difference between fetuses of diabetic women and normal controls was found for LV (45.9 ± 34.0ml vs. 38.3 ± 28.7ml), abdominal circumference (22.2 ± 6.6cm vs. 21.3 ± 5.6cm), estimated fetal weight (1162 ± 898g vs. 1049 ± 765g) and feto-placental weight ratio (0.22 vs. 0.19). UV volume flow (ml/min) and umbilical artery PI were not essentially different between the two study groups, but UV volume flow/kg fetus was lower ($p<0.05$) in the diabetes group (94.3 ± 26.1 ml/min/kg) compared with normal controls (109.5 ± 28.0 ml/min/kg). A positive correlation existed between fetal LV and maternal HB1Ac ($p=0.002$).

Conclusions Measurement of fetal liver volume by 3D ultrasound may play a role in identifying fetal growth acceleration in diabetic pregnancies. Fetal LV increase appears to be positively related to maternal HB1Ac levels reflecting degree of maternal glycemic control. UV volume flow and feto-placental downstream impedance are not different between diabetic and normal pregnancies.

Introduction

Ultrasonography is a useful tool for the diagnosis and surveillance of fetal conditions in the management of diabetic pregnancy [20]. Previous findings indirectly confirm the greater sensitivity of the abdominal circumference in the detection of macrosomia, and indicate the usefulness of the latter parameter in predicting the large for gestational age fetus [21-24].

Pedersen and colleagues popularised the hypothesis that macrosomia is due to the increased transfer of glucose from the diabetic mother to the fetus and placenta resulting in fetal hyperglycaemia and hyperinsulinaemia [25]. Susa and colleagues found significant enlargement of the fetus and placenta in rhesus monkey fetuses that had received exogenous insulin [26]. In infants of diabetic mothers macrosomia seems to be more apparent in some fetal structures and tissues than in others. Examples are the liver and subcutaneous fat [27].

More recently, the introduction of 3-dimensional ultrasound has opened the possibility of evaluating fetal organ volumes as has been demonstrated by our centre for fetal liver volume [7,28].

Fetal growth is influenced both by maternal concentration of various nutrients and the total amount of substrate crossing the placenta [29,30]. The transfer of essential substrates to the fetus is not only determined by maternal placental but also by feto-placental blood flow. Therefore feto-placental blood flow is an essential component in our understanding of fetal growth disturbances in IDDM pregnancies. Doppler ultrasound has been employed in identifying fetal compromise in diabetic women.

Olofsson recorded raised umbilical venous blood flow patterns in fetuses of insulin dependent mothers in the early third trimester of pregnancy [31]. Recently, also we have been collecting umbilical venous volume data in both normal and underdeveloped infants [32].

We hypothesised that IDDM may be associated with fetal liver enlargement depending on the degree of maternal glycaemic control. Increased fetal liver size is a result of fetal hyperglycaemia and hyperinsulinemia rather than raised placental perfusion.

The following questions were addressed: is fetal liver volume increased in maternal insulin-dependent diabetes mellitus when compared with normal pregnancy, and if so, does a relation exists between fetal liver volume and maternal glycosylated haemoglobin concentrations reflecting maternal glycaemic control; is maternal insulin-dependent diabetes mellitus associated with altered feto-placental arterial down stream impedance and umbilical venous volume flow compared with normal pregnancy.

Methods

Subjects

During the period 2000-2002, a consecutive series of 36 women with pre-gestational insulin-dependent diabetes mellitus (IDDM) and a singleton pregnancy and 36 women with a normal

singleton pregnancy serving as a matched controls consented to participate according to a cross-sectional study design. All women originated from the antenatal department of the Erasmus MC. The study was approved by the Regional Ethics Review Board. In every case pregnancy duration was determined from the last certain menstrual period and confirmed by ultrasound measurement of fetal crown rump length (10-12 weeks) or fetal biparietal diameter (13-20 weeks). Within the IDDM subset, no fetal liver volume measurement was possible in four women resulting in 32 IDDM women for further analysis. Consequently, four women with similar pregnancy duration from the normal control subset were removed from the study. Failure to obtain a fetal liver volume measurement was always due to maternal obesity. Maternal age ranged between 19-39 years (median 32 yrs) in IDDM (n=32) and between 19-42 years (median 31 yrs) in normal controls (n=32). Gestational age in both subsets varied between 18 and 36 weeks (median 26 wks).

Within the IDDM subset, according to the White classification [33], 18 women belonged to group B, four women to group C_1, three women to group C_2, two women D_5, three women to group R and two women to group F/R. The medical records of each women were reviewed and information regarding medical disease, glycemic control, diabetic complications and pregnancy outcome were collected. Hb1Ac (glycosylated haemoglobin) level in all 36 diabetic patients was available within three weeks before fetal liver volume measurement were performed. Hb1Ac (glycosylated haemoglobin) showed a mean value of 6.7% (range 4.5-12.5%) in these patients. In our centre, the normal reference value for glycosylated haemoglobin ranges between 4.0 – 6.0%. Within the subset of normal controls, pregnancy was uneventful resulting in the delivery of a healthy infant without anomalies and a birth-weight between 10-90th centile for weight of gestation according to the Kloosterman tables [8] corrected for maternal parity and fetal sex. Apart for iron tablets, no other medication was given. Relevant basic data from both the IDDM and normal control subsets are presented in Table 1.

Data analysis

Liver volume determination:

The 5 MHz annular array transducer (VSW 3-5) of the Voluson 530 ultrasound machine (Kretz technik AG, Zipf, Austria) was used for volume scanning of the fetal liver. Organ volumes were determined by slicing through collected images and recording a truncated pyramidal volume. Depth as well as longitudinal and transverse dimensions of this volume are adjustable, with an initial area of 6.6 cm $\times$ 3.3 cm, up to a maximum area of 28 cm $\times$ 22cm at a depth of 20 cm. This provides a maximum scanned volume of 5.9 l. The maximumresolution in the longitudinal direction is 512 ultrasound lines for each of the maximum of 500slices in the transverse (sweep) direction.In the present study, the opening angle of each plane was 70 degrees and the angle of the volume sample was limited to a maximum of 60 degrees. The depth of the volume was

restricted to 12-16 cm, with a resolution of 210 slices per volume. Following each scan, the volume was presented in three perpendicular planes on the monitor representing a frontal, sagittal and transverse cross-section of the fetus. Data were stored on hard-disc (Io. Mega Jaz Drive) for off-line analysis. In a first step towards fetal liver volume determination, a frontal cross-section of the liver anterior to the stomach was visualised. In a simultaneously demonstrated sagittal cross-section, the outline of the liver was traced manually in approximately 10 sagittal sections between the most lateral left and right points of the diaphragm in the frontal plane. The liver was measured from its upper limit at the diaphragm to its distal rim as the lower limit. The system automatically keeps track of the distances between the sections and calculates the total volume after each area measurement was completed.

Table 1. Clinical data of pregnant women with diabetes mellitus type I (n=32) and normal pregnant controls (n=32)

	IDDM (n = 32)	Controls (n = 32)
Maternal age (years)	31 (19-39)	31 (19-42)
Parity (n)		
0-para	12	10
>= 1-para	20	22
Racial origin		
Caucasian	22	25
African	7	5
Asiatic	3	2
Smokers	4	0
Alcohol	0	0
Gestational age at measurement (wks)	25.7 (18-36)	25.6 (19-36)
Gestational age at delivery (wks)	37.43 (28-41)*	38.8 (36-42)
Lag time between measurements and delivery (wks)	11.7 (2-20)	12.2 (2-21)
Spontaneous delivery	13	26
Assisted delivery	4	2
Caesarean section	15	4
Pre-eclampsia	5	0
Intra-uterine death	1	0
Fetal birthweight (g)	3377 (1250-4830)	3252 (2410-4120)
Birth weight >90th centile (n)	15	1
Birth weight <10th centile (n)	4	0
Placental weight (g)	699 (400-1050)	621 (405-815)
Feto-placental weight ratio	0.22 (0.16-0.36) *	0.19 (0.14-0.25)
Complications new-born:		
Hypoglycemia	6	0
Dismaturity	3	0
Fetal anomalies	2^{+}	0
Infection	1	0

Data are presented as mean (range). IDDM: insulin dependent diabetes mellitus.
**: P-value ≤ 0.05 compared to normal controls. +: Anal atresia; thoracic hypoplasia associated with cardiac atrial and ventricular septum defects.*

Umbilical Doppler blood flow:

A Toshiba SHH 140A (Toshiba Corporation, Medical Systems Division, Tokyo, Japan) unit was used for all Doppler and B mode ultrasound recordings.

Umbilical venous cross-sectional area (mm^2) was expressed by the mean of three tracings of the inner edge of the vessel [32]. The software programme to obtain these tracings was developed in our Department using Labview and Imaq Vision software (National Instruments Austin, Tx). Umbilical venous maximum flow velocity (mm/s) was measured using a combined two-dimensional real-time and colour-coded Doppler system with a transducer carrier frequency of 5 MHz. The angle of insonation was less than 15 degrees. The system operates at output intensities of < 100 mW/cm^2 spatial peak temporal average in both imaging and Doppler mode. Sample volume length for all flow velocity waveforms ranged between 0.2-0.3 cm; the high-pass wall filter was set at 100 Hz. All ultrasound studies were carried-out with the women being in a semirecumbent position.

A more detailed description of both vessel area and umbilical venous flow velocity measuring techniques for calculation of volume flow have been presented in detail previously [30]. In brief, umbilical venous (UV) volume flow was calculated according to the formula: UV volume flow (ml/min) = 0.06 × time-averaged velocity (mm/s) × cross sectional vessel area (mm^2).

In each instance, Doppler recordings were followed by measurement of fetal biparietal diameter (BPD, cm) head (HC, cm) and upper abdominal circumference (AC, cm) and femur length (FL, cm). Fetal weight was estimated from these parameters according to Hadlock' formula [10]. This was followed by calculation of umbilical venous volume flow per kilogram fetal weight. Doppler recordings of the umbilical artery flow velocity waveforms for calculation of the Pulsatility Index were obtained from the free loop of the umbilical cord. All liver volume and flow recordings were performed by one examiner (SB).

Statistical analysis

All calculations were performed with SPSS 10 software package (SPSS Inc., Chicago, IL). Data are reported as mean and standard deviation. The paired t-test was used to compare differences between the diabetic and normal control group. To compare liver volume between the two subsets, multiple linear regression was used, after a logarithmic transformation. Also multiple linear regression was applied to relate fetal liver volume to gestational age, glycosylated haemoglobin and maternal insulin dosage. A p-value less than 0.05 was considered to be statistically significant.

Results

Table 1 shows that mean fetal birthweight and placental weight were not essentially different between the two subsets. However, a statistically significantly higher mean feto-placental weight ratio was established in diabetic pregnancy.

Table 2 demonstrates mean ± S.D. values for fetal biometry, fetal liver volume and umbilical venous/arterial Doppler data in insulin dependent diabetes mellitus (IDDM) and normal controls. A statistically higher mean value was found in the IDDM subset for fetal abdominal circumference (cm), fetal liver volume (ml) and ultrasonically estimated fetal weight (g.) and a statistically lower mean value for umbilical venous volume flow per kg fetal weight (ml/min/kg). No statistically significant difference existed for umbilical PI between both subsets.

The log linear regression line for fetal liver volume relative to gestational age (GA) in the normal group was as follows: Ln Liver volume (ml) =0.14 (SE = 0.007) * GA - 0.306 (SE = 0.178). The corresponding regression line for the IDDM subset was:

Ln Liver volume (ml) =0.138 (SE=0.007) * GA – 0.0351 (SE=0.189) (Figure 1).

The slopes of these regression lines were not essentially different. Assuming a common slope, a multiple linear regression model was fitted, resulting in an estimated gestational age corrected difference of 0.18 (SE = 0.05, p=0.001). This corresponds to a 20% higher liver volume (95% CI: 8%-32%) at each week of gestation for fetuses in the diabetic group.

In the IDDM subset, ninety-three percent of the variance in fetal liver volume could be explained by gestational age. When adding the glycosylated hemoglobin (HbA1c) data to the log linear multiple regression model, this percentage would increase significantly ($p<0.002$) to 95 percent, resulting in the following regression formula:

Ln Liver volume (ml) = 0.135(SE =0.006)*GA +0.076 (SE = 0.021)*HBA1c–0.481(SE = 0.20) This means that liver volume is increased by 8.1% for each unit increase in HbA1c (95% CI = 3.5-13.0%) and by 14% (95% CI = 13.0-15.8%) per week of gestational age. Addition of maternal insulin dosage did not further explain the variance in fetal liver volume according to the multiple regression model.

Since the gestational age related variance in umbilical venous (UV) volume flow could not be improved by logaritmic transformation, linear regression lines were determined for both the IDDM and normal control subsets relative to gestational age resulting in the following equations:

UV flow (ml/min) IDDM = 12.03 (SE = 1.01) * GA – 213.6 (SE = 27.00) and

UV flow (ml/min) normal controls = 11.21 (SE = 0.79) * GA – 185.6 (SE = 21.2).

Figure 2 shows that the regression lines for both subsets are not essentially different. In the IDDM subset, addition of glycosylated hemoglobin or maternal insulin dosage did not significantly explain the variance in umbilical venous volume flow according to the multiple regression model.

Table 2. Mean and standard deviation for growth and flow parameters obtained from women with diabetes type I (n=32) and normal pregnant controls (n=32)

	IDDM (n=32)		Controls (n=32)		
	Mean	SD	Mean	SD	p-value
Biparietal diameter (cm)	6.6	1.5	6.5	1.3	0.8
Head circumference (cm)	23.5	5.1	23.4	4.8	0.4
Abdominal circumference (cm)	22.2	6.5	21.3	5.6	0.01*
Femur length (cm)	4.8	1.3	4.7	1.3	0.2
Estimated fetal weight (g)	1161	897	1049	764	0.006*
Liver volume (ml)	45.9	34.1	38.2	28.8	0.001*
Umbilical venous area (mm^2)	26.6	15.6	28.0	14.3	0.2
Umbilical venous velocity (mm/s)	60.1	12.3	60.2	11.9	0.9
Flow (ml/min)	100.5	69.3	106.2	63.4	0.3
Flow/kg (ml/min/kg)	94.2	26.1	109.4	27.9	0.03*
P I Umbilical Artery	1.1	0.2	1.2	0.3	0.7

*: statistically significant according to the paired t-test.

Discussion

The present study provides data on fetal liver volume and umbilical venous volume flow and its components in fetuses of diabetic mothers matched with normal controls. Our findings indicate that 3-dimensional ultrasound may identify disparate growth rates of fetal structures in pregnancy complicated by maternal insulin dependent diabetes. Data from the current study demonstrate that mean liver volume in the diabetes group was 20% higher compared with the normal control group. Our data are in line with another report [34] in which 2-dimensional ultrasound was used to determine liver size. It is assumed that production of insulin by the fetal pancreas is increased in fetuses of diabetic mothers and that this is the major growth factor in diabetic macrosomia [25]. Fetal structures grow at a different rate on the basis of their responsiveness to insulin [25,35] for instance, muscle and liver tissue are highly sensitive to fetal insulin whereas the latter has no direct role in brain metabolism. Naeye [36] found in post mortem specimen liver size in fetuses of diabetic mothers to be increased by approximately 80% when compared with normal controls. This difference was due to both cellular hyperplasia and hypertrophy. Moreover, the enlarged fetal liver in the diabetic group contained more than three times as much hematopoietic tissue as the fetal liver in controls. This abnormal growth is mostly attributable to fetal hyperinsulinemia [37].

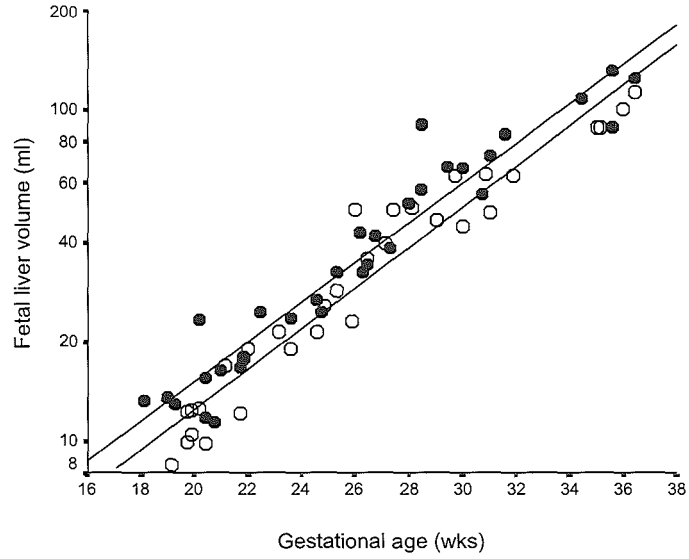

Figure 1. *Fetal liver volume (ml) relative to gestational age (wks). Open circles (o) represent individual normal values. Closed circles (•) represent fetuses of diabetic women. Solid line (—): regression lines. The regression lines demonstrate a log-linear relationship.*

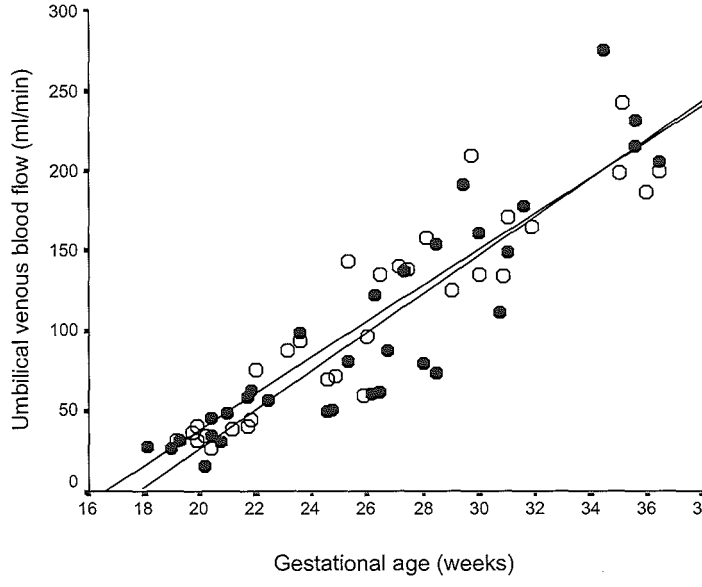

Figure 2. *Umbilical venous volume flow (ml/min) relative to gestational age. Open circles (o) represent individual normal values. Closed circles (•) represent fetuses of diabetic women. Solid line (—): regression lines. The slopes of the two regression lines are not statistically different from each other.*

Similar results were found in the rhesus monkey [26] Indeed, various animal studies were undertaken to demonstrate a direct relationship between maternal carbohydrate metabolism, fetal insulin levels and fetal growth in the presence of diabetes [38,39]. For instance, Susa and Schwartz [26] reported fetal hyperglycaemia and fetal hyperinsulinemia under chronic infusion of insulin into the fetus of a normal pregnant rhesus monkey, resulting in selective fetal organomegaly, macrosomia, intrauterine death and placental hyperplasia. Considering that insulin promotes and controls the utilisation of metabolic fuels by the fetal tissue, maternal homeostasis and therefore fetal insulin may play a pivotal role in the control of fetal development.

Glycosylated hemoglobin is representative of long-term glucose control in pregnant and non-pregnant diabetic patients, and it is directly correlated with mean plasma sugar values [40]. Widness et al [41] found a significant elevation of glycosylated haemoglobin in insulin-dependent pregnant diabetic patients. In our data we observed a positive correlation between levels of maternal glycosylated haemoglobin and fetal liver volume. The intrinsic limitation of this observation is determined by the fact that only few patients displayed higher HB1Ac levels as a result of inadequate insulin treatment. To find the true relation between fetal liver volume and glycosylated haemoglobin it would be necessary to examine an untreated population.

It is believed that exogenous insulin administered to a pregnant woman with IDDM does not cross the human placenta, therefore it has not been considered an etiologic factor in diabetic macrosomia and fetopathy [42]. However Menon and co-authors [43] reported that insulin does cross the placenta as an insulin-antibody complex and that the amount of transfer directly correlated with the amount of anti-insulin antibody in the mother. Moreover, high concentrations of animal insulin in cord blood were significantly associated with the development of fetal macrosomia, suggesting that the transferred insulin expressed biologic activity [43]. No correlation between maternal insulin dosage and fetal liver volume could be demonstrated in the present study.

In fetuses of diabetic women both umbilical venous time-averaged flow velocity and cross-sectional area were not essentially different between the diabetic and normal control group, resulting in similar umbilical venous volume flow data. This is at variance with an earlier paper [31] in which an increase in umbilical venous volume flow in diabetic women was established. This discrepancy may be determined by the mixture of type I and type II diabetes in the latter study, as opposed to only type I diabetes in the present report. The reduced umbilical venous volume flow per kg fetal weight in our diabetic group was determined by the raised estimated fetal weight values.

Umbilical artery pulsatility index (PI) is believed to reflect placental vascular resistance [44]. When comparing umbilical artery PI between the diabetic and normal control group, there were no differences. Therefore umbilical artery PI does not seem to be influenced by long term blood glucose regulation which is in agreement with previous reports [45,46]. Our observation, that in

pregnancies complicated by maternal diabetes mellitus the feto-placental circulation is essentially unchanged, can be explained from animal studies in which it was demonstrated that the increase in fetal glucose uptake is not determined by elevated umbilical venous volume flow but by raised glucose transfer across the placenta [47, 48]. Considering that in fetuses of diabetic mothers, growth acceleration is more pronounced in organ tissues such as fat, muscle and liver on the basis of their responsiveness to fetal hyperinsulinemia [49], this phenomenon appears not to be associated with a rise in venous volume flow.

The placental features most observed during maternal diabetes are excessive weight and immaturity of chorionic villi, both relative to gestational age [50]. Moreover, fetal macrosomia defined as a birth weight at term in excess of 4,000 g, is associated with increased placental size. Like in the fetus, increased placental size can be attributed to cell hypertrophy and hyperplasia [50]. In the present study, mean birthweight and placental weight were not essentially different between the diabetes and control subset. However, a large-for-date infant (birtweight >90 centile) was delivered in 15/32 (46.8%) diabetic pregnancies versus only 1/32 (3.12%) normal controls. Moreover, feto-placental weight ratio was significantly increased in the diabetic subset.

It can be concluded that measurement of fetal liver volume by 3D ultrasound may play a role in identifying fetal growth acceleration in diabetic pregnancies. Fetal liver volume increase appears to be positively related to maternal HB1Ac levels reflecting degree of maternal glycemic control. Umbilical venous volume flow and feto-placental downstream impedance are not different between diabetic and normal pregnancies.

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Chapter 6

Umbilical venous inflow and fetal brain/liver size

Introductory remarks

From post-mortem studies it has been established that fetal growth restriction is associated with a raised brain/liver ratio. During fetal hypoxaemia reduction in fetal brain weight is less pronounced than fetal liver weight and this phenomenon is caused by fetal circulatory centralisation and fetal brain sparing, resulting in asymmetrical growth restriction. Using three-dimensional ultrasound scanning we describe normal values for fetal brain/liver volume ratio in normally developing fetuses compared with pregnancies complicated by fetal growth restriction. Since asymmetrical growth restriction is associated with fetal circulatory adaptation to utero-placental insufficiency, we investigated the role of fetal brain/liver volume ratio in the early detection of fetal growth restriction relative to umbilical venous blood flow and downstream impedance. Assessment of combined umbilical venous inflow and brain/liver volume ratio should provide a more complete picture of circulatory redistribution and organ adaptation in growth restriction.

6.1 Fetal brain/liver volume ratio and umbilical volume flow parameters relative to normal and abnormal human development.

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Abstract

Objectives To estimate fetal brain volume from head circumference and published post-mortem literature data; to determine normal values for fetal brain volume (BV) / liver volume (LV) ratio relative to gestational age; to establish the relation between BV/LV ratio and fetal circulatory parameters during normal and restricted fetal growth (SGA) in a cross-sectional study design.

Patients and Method A total of 47 uncomplicated pregnancies (AGA) and 23 pregnancies resulting in the delivery of a growth-restricted fetus (SGA) was included in the study. At enrolment gestational age ranged between 20 and 36 weeks in both groups. Umbilical venous cross-sectional area (mm^2) and time-averaged velocity (mm/s) for calculation of volume flow (ml/min) as well as velocity waveforms from the umbilical artery, middle cerebral artery and ductus venosus were recorded using a Toshiba SSA 140A machine. Fetal liver volume measurements were obtained using a Combison 530 D Ultrasound machine. Fetal brain volume was estimated from fetal head volume following comparison with post-mortem literature data on fetal brain weight.

Results A significant correlation exists between prenatally estimated fetal head volume and post-mortem fetal brain volume. Fetal brain volume is approximately half of fetal head volume. Normal fetal BV/LV ratio demonstrates a significant gestational age-related reduction (R= -0.54; $p<0.001$). Normal mean fetal BV/LV ratio (3.4 ± 0.7 (SD)) is significantly different ($p<0.001$) from mean fetal BV/LV ratio in the SGA group (n=23) (5.9 ± 1.9 (SD)). A significant difference exists for mean UV blood (ml/min/kg) between AGA (104.7 ± 26.9 (SD)) and SGA fetuses (59.6 ± 22.7 (SD)). In the SGA fetus, these is a significant inverse relationship ($p<0.001$) between fetal weight-related umbilical venous volume flow and fetal BV/LV ratio. In a subset of 16 SGA and 16 AGA fetuses matched for gestational age, a significant difference exists for umbilical artery PI (2.30 ± 1.52 (SD) vs.0.99 ± 0.19 (SD)), fetal middle cerebral artery PI (1.3 ± 0.4 (SD) vs. 2.1 ± 0.3(SD)) and late diastolic flow velocity (cm/s) in the fetal ductus venosus ((6.9 ± 14.2 (SD) vs. 23.9 ± 8.8

(SD)), but not for peak systolic, early diastolic and time-averaged velocity in the latter vessel.
Conclusion Sonographic estimates of fetal brain volume can be obtained. Fetal brain/liver volume ratio is a predictor of fetal outcome in the growth-restricted fetus. An inverse relation exists in SGA fetuses between brain/liver volume and fetal weight-related umbilical venous blood flow.

Introduction

From post-mortem studies it has been established that fetal growth restriction is associated with a raised brain/liver weight ratio [1]. The ratio of brain weight to liver weight is normally about 3:1. In the presence of fetal growth restriction, this ratio may be increased to 5:1 or more during the last 12 weeks of pregnancy [2-6]. Looking at the components of this ratio, reduction in fetal brain weight is less pronounced than fetal liver weight due to the so-called brain sparing effect, reflecting circulatory redistribution during fetal hypoxaemia [7-9]. This explanation is supported by animal models showing that fetal hypoxaemia results in redistribution of cardiac output with maintenance of flow to the brain, heart, and adrenals whereas flow is decreased to muscle, viscera, and bones [10-13]. This sequence of events can theoretically result in *asymmetrical growth restriction* with an abnormal relative increase in brain size when compared with the small liver.

Neither liver weight nor brain weight can be determined during fetal life. However, fetal liver volume can be reliably measured using 3D dimensional ultrasound [14].

It has been shown recently in two overlapping patient cohorts that fetal liver volume [15] and umbilical venous volume flow are reduced in the growth-restricted fetus [16]. We hypothesise that in the growth restricted fetus, reduced umbilical venous volume flow is related to a raised fetal brain/liver volume ratio. The aim of this study were to: (i) estimate fetal brain volume as part of fetal brain/liver volume ratio calculations; (ii) determine normal values for fetal brain/ liver volume ratio relative to gestational age; (iii) establish the relation between the fetal brain/liver volume ratio and fetal circulatory parameters, i.e. umbilical venous volume flow and downstream impedance in the umbilical artery, fetal middle cerebral artery and ductus venosus during normal and restricted fetal growth.

Material and Methods

During the period 1 January - 1 November 2000, simultaneous recordings of fetal biometry, liver volume and umbilical venous volume flow were obtained in 70 singleton pregnancies from our out patient unit. All women consented to participate in the study following approval granted by the Regional Ethics Review Board. Each woman was included in the study only once. Scanning was carried-out by a single investigator to avoid variation in scanning technique. Pregnancy was uneventful as expressed by an appropriate for gestational age fetus (AGA) (upper abdominal

circumference above the 5th centile) [17] in 47 women resulting in the delivery of a healthy term infant with a birth weight between the 5th and 95th centile according to the Kloosterman tables [6]. A small for gestational age fetus (SGA) defined by an upper-abdominal circumference below the 5th centile [17] and a birth weight below the 5th centile for weight of gestation according to the Kloosterman tables [18] was established in 23 women. At enrolment, pregnancy duration was determined from the last reliable menstrual period or, in case of uncertainty, adjusted by ultrasound before 20 weeks of gestation. Data on gestational age at sonographic examination and at birth as well as ultrasonically estimated fetal weight and birthweight for AGA and SGA fetuses are presented in Table 1. Maternal age in both subsets ranged between 18 and 42 years (median: 29 years).

In fetal growth restriction, mean gestational age at delivery was 32 ± 4.3 (SD) weeks, mean fetal birth weight was 1204 ± 611 (SD) g. Mean lag time between fetal sonographic measurements and delivery was 2.2 ± 1.7 (SD) weeks. There were six cases of intra-uterine death and two cases of post partum death (≤ 14 days) resulting in a survival rate of 15 out of 23 (65.2 %) infants. The time interval between the sonographic examination and subsequent perinatal death (n=4) was at least 14 days, rendering a reliable comparison between sonographic estimation of brain volume and true brain volume impossible.

In 16 out of 23 SGA fetuses, data were also available on the pulsatility index (PI) from the umbilical artery and middle cerebral artery and on the different components of the flow velocity waveform of the ductus venosus. These data were compared with data from 16 normally developing fetuses matched for gestational age which were selected from the 47 normal pregnancies in which the above Doppler velocimetry data were obtained in every instance. The matching difference was at most one gestational week. A combined two-dimensional real-time and colour-coded Doppler system (Toshiba SSA 140 A Toshiba Corp. Medical Systems Division, Tokyo, Japan) with a transducer carrier frequency of 5 MHz and 3.75 MHz, respectively was used. The system operates at output intensities of < 100 mW/cm² spatial peak temporal average in both imaging and Doppler mode. Umbilical artery flow velocity waveforms were recorded from a straight section of the free-floating loop of the umbilical cord. The middle cerebral artery can be visualised as a major branch of the circle of Willis running anterolaterally towards the edge of the orbit [19]. The fetal ductus venosus is localised in the liver and originated from the umbilical sinus. The sample volume was placed immediately above the umbilical sinus, visualised in a transverse cross-sectional view [20]. The degree of pulsatility in the umbilical artery and middle cerebral artery was expressed by the pulsatility index (PI). Waveforms analysis in the ductus venosus consisted of measurement of peak velocity (cm/s) during both the systolic and early diastolic phase of the cardiac cycle and calculation of the time-averaged velocity (cm/s). Moreover, the late diastolic flow velocity component coinciding with atrial contraction was determined. Three consecutive

flow velocity waveforms with the highest velocity and of similar appearance were used to calculate the different parameters in each vessel. Cross-sectional venous vessel area (mm^2) of the free floating loop of the umbilical cord was expressed by the mean of three tracings of the inner edge of the vessel [16]. The software program to obtain these tracings was developed in our Department using Labview and Imaq Vision software (National Instruments Austin, Tx). Umbilical venous maximum flow velocity (mm/s) was measured using the Toshiba SSA 140 A equipment. Volume flow determination from flow velocity and vessel diameter has been described by others [21-23]. In our study we used direct tracing of the vessel area. Our methodology to achieve umbilical venous volume flow has been reported in detail earlier in this journal [16]. In brief, umbilical venous (UV) volume flow was calculated according to the formula: UV volume flow (ml/min) = 0.06 × time-averaged maximum velocity (mm/s) × cross sectional vessel area (mm^2). Fetal biometry including biparietal diameter (BPD, cm), head (HC, cm) and upper-abdominal circumference (AC, cm) and femur length (FL, cm) was performed following each Doppler flow velocity and vessel area determination. Fetal weight was estimated according to Hadlock's formula [24]. This was followed by calculation of umbilical venous volume flow per kilogram fetal weight [16]. The 5 MHz annular array transducer (VSW 3-5) of the Voluson 530 ultrasound machine (Kretz technik AG, Zipf, Austria) was used for volume scanning of the fetal liver. The method used by us for the measurement of fetal liver volume has been described earlier [14-15]. In brief, a frontal cross-section of the liver anterior to the stomach is visualised. In a simultaneously demonstrated sagittal cross-section, the outline of the liver is traced manually in approximately 10 sagittal sections between the most lateral left and right points of the diaphragm in the frontal plane. The liver is measured from its upper limit at the diaphragm to its distal rim as the lower limit. The system automatically keeps track of the distances between the sections and calculates the total volume after each area measurement is completed [14-15].

To obtain the fetal brain / liver volume ratio, next to liver volume, brain volume had to be determined. Assuming the fetal head to approach the shape of a sphere, head volume (HV) was estimated according to the formula $HV(ml)=1/6\ \pi \times d^3$, in which d (diameter) was determined from HC/π . The prenatal fetal head volume data were compared with post-mortem data on fetal brain weight from literature [25] matched for gestational age. To this purpose post-mortem brain weight was transferred to brain volume (BV) according to the formula: BV (ml)= brain weight / specific gravity of the brain (1.04) [26]. To obtain an estimation factor for brain volume, the regression line through the origin of prenatal head volume versus post mortem brain volume was calculated. Combined with fetal liver volume, brain / liver volume ratio was determined.

Statistics

Linear regression was used to calculate the relationship between brain/liver volume ratio and gestational age as well as umbilical venous volume flow per kilogram fetal weight. Using the

method described by Altman [27] we modelled the relationship between brain/liver volume ratio and gestational age by means of a linear polynomial. According to this procedure the gestational age related reference centiles p5, p50 and p95 were constructed. Differences in umbilical artery Doppler PI, middle cerebral artery PI and the peak systolic, peak early diastolic and time-averaged velocity in the ductus venosus between the AGA and SGA fetuses were compared by means of the paired sample t-test. The level of statistical significance was set at 0.05. Calculations were performed with SPSS software Version 10.0 for Windows (SPSS Inc., Chicago, IL).

Results

Linear regression demonstrates a statistically significant positive correlation (adjusted Rsquare = 0.96, $p< 0.001$) between prenatally estimated fetal head volume and post-mortem fetal brain volume. Since prenatally estimated head volume is approximately twice (2.016) post-mortem brain volume, the former volume was divided by two to obtain an estimate of prenatal fetal brain volume. Ultrasonically estimated and post-mortem fetal brain volume relative to gestational age is shown in Figure 1. The vast majority of the ultrasonically estimated data are within ± 1 SD of the post-mortem data. Normal fetal brain/liver volume ratio demonstrates a significant reduction with advancing gestational age ($R= -0.54$, $p<0.001$) (Figure 2). Fetal brain/liver volume ratio was situated above the p95 in 18/23 (78%) SGA fetuses. Note that eight out of nine SGA fetuses below 29 weeks of gestational age and displaying a brain/liver volume ratio above p95 did not survive (Figure 2). In the AGA fetus there is no correlation between brain/liver volume ratio and umbilical venous volume flow per kg fetal weight (Figure 3). However, in the SGA fetus, there is a statistically significant negative correlation ($R = -0.55$, $p<0.001$) between these two variables. A statistically significant difference ($P<0.001$) exists for umbilical venous blood flow per kilogram fetal weight between AGA (mean 104.7 ml/min/kg ± 26.9 (SD)) and SGA fetus (mean 59.6 ml/min/kg ± 22.7 (SD)). Table 2 presents the actual data for fetal brain/liver volume ratio and umbilical venous volume flow per kg fetal weight relative to gestational age (wks) from the 23 SGA fetuses.

Within the subset of 16 SGA fetuses and 16 AGA fetuses matched for gestational age, there was a statistically significant difference between umbilical artery PI (2.3 ± 1.5 (SD) vs. 1.0 ± 0.2 (SD)), fetal middle cerebral artery PI (1.3 ± 0.4 (SD) vs. 2.1 ± 0.3 (SD)) and late diastolic flow velocity (cm/s) in the fetal ductus venosus (6.9 ± 14.2 (SD) vs. 23.9 ± 8.8 (SD)). No statistically significant difference was established for the peak systolic, peak early diastolic and time-averaged velocity in the ductus venosus.

Table 1.

	Aga group			Sga group		
	p25	median	p75	p25	median	p75
Ga (wks) at scan	24	30	32	27	29	34
Estimated fetal weight (g)	698	1452	1792	622	901	1441
Ga (wks) at birth	38	39	40	29	33	40
Birthweight	3075	3280	3725	600	1070	1830

Table 2.

Case No.	Gestational age (wks)	Fetal BV/LV Ratio	Umbilical Venous volume flow/kg fetus (ml/min/kg)
1	22	6.9	25.2
2	23	5.8	93.6
3	25	6.2	66.3
4	25	6.5	46.3
5	26	7.2	32.4
6	27	4.9	93.8
7	27	3.8	107.5
8	28	9.1	28.6
9	28	9.9	61.6
10	28	8.1	53.4
11	29	5.8	47.1
12	29	4.1	91.0
13	29	7.9	56.6
14	31	8.1	26.4
15	32	6.0	42.5
16	33	4.8	76.2
17	34	4.7	67.8
18	34	5.7	43.7
19	34	4.5	52.5
20	35	5.0	63.5
21	35	3.6	77.2
22	36	3.5	55.6
23	36	3.1	62.2

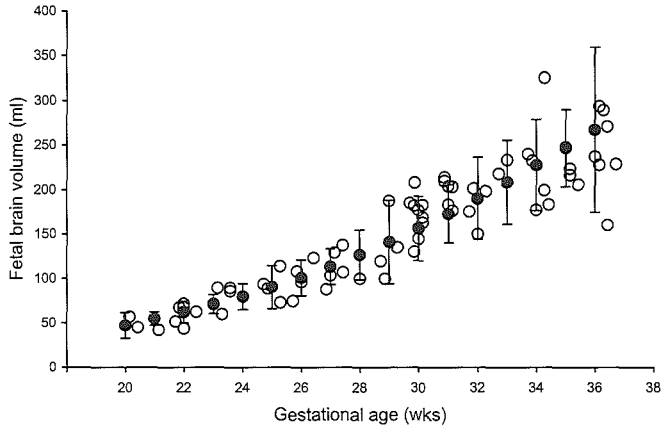

Figure 1. *Fetal brain volume (ml) relative to gestational age (wks). Open circles (o) represent ultrasonically estimated brain volume (ml). Closed circles (•) represent post-mortem brain volume (ml). Error bars represent ± 1 standard deviation from post mortem data compiled by Cj Sung abd DB Singer with 1975-1984 data from Women and Infant's Hospital, Providence, RI 1988* [25].

Discussion

This study provides estimates in -utero of fetal brain volume and its relation with liver volume and umbilical venous volume flow measurements. It is feasible to obtain head volume and subsequently brain volume from routine biometric measurements. We determined fetal brain volume by converting brain weight in to brain volume using specific gravity [26] and found an estimate for brain volume when comparing sonographic derived head circumference data with post-mortem fetal brain volume specimen from literature [4,25]. In normal fetuses, estimated brain volume to liver volume ratio depicts a significant decrease with advancing gestational age (Figure 2). These findings are in agreement with previous reports in which MRI was used [28,29] and could be due to the fact that the brain grows faster than the liver, with a less rapid increase in brain weight during the last six weeks of gestation [28-30]. We also considered estimated brain/liver volume ratio in impaired fetal growth. Because placental insufficiency may result in diminished glucose transfer and hepatic storage [10-12], and considering that the liver comprises the bulk of the upper abdomen, fetal abdominal circumference, which reflects liver size, would be reduced. Regarding the post mortem data, in the growth restricted fetus the necropsy diagnosis of dysmaturity depends on the recognition of characteristic abnormalities of organ weight and composition [2]. Various organs are reduced in size but the liver is particularly small, while in contrast the brain remains near normal in size for gestational age [1]. The ratio of brain weight to

liver weight in neonatal post-mortem

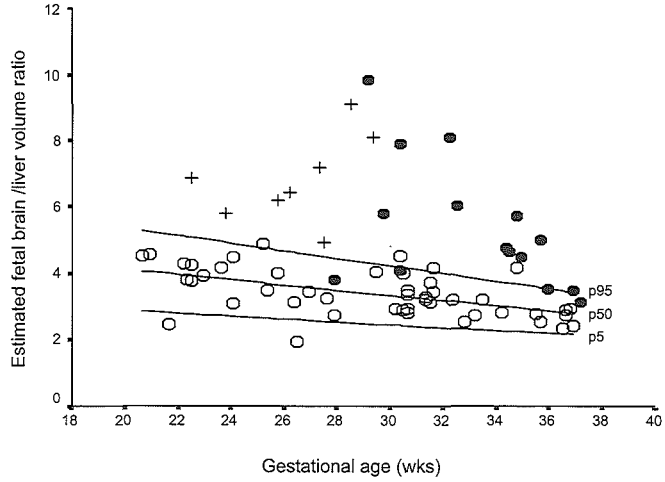

Figure 2. *Estimated brain/liver volume ratio relative to gestational age (wks). Open circles (o) represent individual normal values, solid line (—): P5, P50, and P95 reference lines. Closed circles (•) represent SGA resulting in a live-born infant, crosses (+) represent SGA resulting in intra-uterine or post-partum death. P50: linear = 5.639 – 0.078 × GA; P5-P95: P50 ± 1.64 (0.937 – 0.017 × (GA))*

normally is 3:1, and may be doubled in SGA infants up to 6-7:1 [1,3]. A similar approach was adopted in the present study in which brain / liver volume ratio rather than head to upper abdominal circumference ratio was determined to ensure a more reliable reflection of change in organ size associated with fetal growth restriction. Our fetal data are close to the fetal neonatal post-mortem data: a mean brain /liver volume ratio in the AGA fetus of 3.4:1, and a mean brain /liver volume ratio in the SGA fetuses of 5.9:1.

All fetuses except one with a gestational age below 29 weeks and an estimated fetal brain liver volume ratio above p95 did not survive (Figure 2). This strongly indicates that brain liver volume ratio can predict fetal outcome for fetuses younger than 29 weeks of gestation, whereas all fetuses older than 29 week with estimated brain liver volume ratio above p95 survived. We speculate that asymmetry of the fetal brain/liver volume ratio indicates pronounced growth impairment secondary to utero-placental insufficiency.

In normal pregnancy there is no relationship between fetal brain/liver volume ratio and fetal weight-related umbilical venous inflow, an inverse relationship was established in the SGA fetus. Markedly raised ratio values (≥ 6) were observed only at umbilical venous volumes below 60 ml/min/kg. The asymmetry itself appears to represent a fetal adaption to the uteroplacental insufficiency, and suggests preferential redistribution of fetal cardiac output favouring brain

development [7]. Moreover, it is proposed that there is preferential shunting of oxygen and nutrients

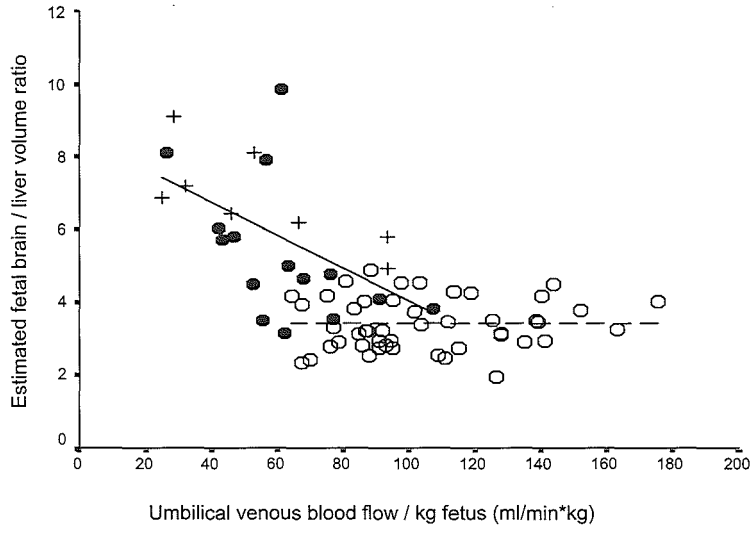

Figure 3. *Estimated brain/liver volume ratio relative to umbilical venous volume flow/ kg estimated fetal weight (ml/min/kg). Open circles (o) represent individual normal values, shaded line (---) represents mean of normal estimated brain/liver volume ratio reference line. Closed circles (•) represent SGA resulting in a live-born infant, crosses (+) represent SGA resulting in intra-uterine or post-partum death. Solid line (—) represents linear regression line (8.578-0.045 × (GA)).*

directly through the forame ovale in to the left atrium to spare the main organs such as the heart and brain [31,32]. The cross-sectional design of the present study does not allow information on the time relationship between impairment of umbilical venous inflow and increase in fetal brain/liver volume ratio.

Traditional Doppler parameters such as umbilical artery PI, middle cerebral artery PI and absolute flow velocities in the ductus venosus were compared in a subset of 16 SGA and AGA fetuses matched for gestational age. Umbilical artery PI, and middle cerebral artery PI as well as late diastolic flow velocities in the ductus venosus were significantly different between AGA and SGA fetuses. Umbilical artery PI is an important parameter for downstream impedance and indirectly for fetal-placental perfusion. Increased umbilical artery PI as a result of raised down-stream impedance is associated with fetal hypoxaemia [9]. In the literature a preferential blood flow pattern in the ductus venosus in the presence of growth restriction in both animals [10-13] and in human [31] has been reported. In the latter study both flow velocity and vessel area were measured, indicating maintenance of normal volume flow through the ductus venosus in the SGA

fetus. In our study no significant differences were observed in the ductus venosus peak systolic velocity and time averaged flow velocity when comparing AGA and SGA fetuses in spite of reduced umbilical venous volume flow values in the latter. This phenomenon could reflect the same pattern reported by others [31-33] and suggests that in the SGA fetus the presence of reduced umbilical venous volume flow is associated with a larger portion of this volume flow being shunted through the ductus venosus to maintain adequate oxygen supply to the fetus. Whereas a reduced middle cerebral artery PI reflects brain sparing [34], reduced late diastolic velocities in the ductus venosus indicate impaired atrial contraction due to the raised arterial afterload in the SGA fetus [31, 33].

It can be concluded, that sonographic estimates of fetal brain volume can be obtained. Fetal brain/liver volume ratio is a predictor of fetal outcome in the growth restricted fetus. An inverse relation exists in SGA fetus between fetal brain/liver volume and fetal weight-related umbilical venous blood flow.

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Chapter 7

Conclusions and future developments

7.1 Conclusions

The studies presented in this thesis were designed to establish the feasibility of 3D ultrasonography in the assessment of liver volume in the presence of normal fetal development and fetal pathology associated with utero-placental insufficiency and maternal diabetes. Moreover the nature of fetal venous inflow as expressed by umbilical venous volume flow was determined under these circumstances.

Doppler studies sofar have focussed on the description of arterial, cardiac and venous flow velocity waveforms. Volume flow determinations were previously hampered by the inaccuracy of vessel size measurements. With the introduction of Labview and Imaq software used for measurement of dorsal aortic size in the chick embryo model, it became possible to obtain reliable data on the umbilical venous cross-sectional vessel area in the human fetus.

Umbilical venous volume flow demonstrated no differences at the fetal, placental or free loop site of the umbilical cord. A seven-fold increase between 20-36 weeks in umbilical venous volume flow was established under physiological circumstances, which was mainly determined by an increase in cross-sectional vessel size. There was a significant reduction in fetal weight-related umbilical venous volume flow.

In normal fetal development at term we demonstrated fetal behavioural related changes in umbilical venous cross-sectional area. During fetal behavioural state 2F (active sleep) a significant increase in umbilical venous cross-sectional area was detected on the basis of high venous vessel wall compliance. The increase in vessel size may be determined by a rise in mean venous pressure. Moreover a significant rise in fetal heart rate was established reflecting a possibly elevated fetal cardiac output during fetal behavioural state 2F. This was further supported in part by a mild increase in umbilical venous volume flow.

Fetal growth restriction was associated with a significant reduction in umbilical venous volume flow, which again was mainly determined by a reduction in cross-sectional vessel size. In fetal growth restriction, umbilical artery Pulsatility Index reflecting feto-placental down stream impedance was significantly raised when fetal weight-related umbilical venous volume flow was situated below the lower limit (5^{th} centile) of the normal range compared with normal values.

The umbilical venous circulation is responsible for oxygen delivery to the fetal tissues. Fetal hypoxaemia will result in fetal growth restriction as clinically expressed by a raised fetal head-to-abdominal ratio. The slow-down in upper abdominal circumference is mainly determined by a reduced fetal liver size. As we were able to establish reproducible fetal liver volume measurements, it became possible to determine fetal liver volume under normal and abnormal fetal conditions. Normal fetal liver volume increases ten-fold during the second half of pregnancy, whereas in fetal growth restriction fetal liver size is reduced. It was demonstrated that the

reduction is more pronounced for liver volume than for the traditional fetal head-and upper-abdominal circumference. However, fetal liver volume measurement is not a better discriminator for growth restriction than the measurement of the fetal upper-abdominal circumference.

During pre-eclampsia maternal and fetal hemodynamics changes were observed after maternal administration of plasma expander followed by antihypertensive treatment by means of dihydralazine. After maternal plasma expansion, the results constituted a significant reduction in maternal diastolic blood pressure, maternal hematocrit and whole blood viscosity associated with a significant increase in umbilical venous cross-sectional vessel area and a non-significant rise of 11% in umbilical venous volume flow. Maternal dihydralazine administration did not result in any change in umbilical venous cross-sectional vessel area. However, umbilical artery pulsatility index, as a measure of feto-placental downstream impedance, decreased significantly after both plasma and dihydralazine treatment.

Another pathological condition, which was studied, was maternal insulin dependent diabetes mellitus (IDDM). IDDM is associated with fetal macrosomia. From animal experimental work it is known that the fetal liver is enlarged in IDDM. We were able to establish a 20% higher fetal liver volume in IDDM compared with normal controls during the second half of pregnancy. Increase in fetal liver volume in IDDM was found to be positively related to maternal glycosylated haemoglobin reflecting degree of maternal glycemic control.

Since we were able to measure fetal liver volume using 3D ultrasound, we became interested in data on fetal brain volume, thus allowing calculation of the brain/liver volume ratio. In the present study presented in this thesis, fetal brain volume was estimated from 2D sonographic measurements of fetal head circumferences, assuming the fetal head to approach the shape of a sphere. It was shown that prenatally estimated head volume is approximately twice post mortem brain volume. The former volume was, therefore, divided by two to obtain an estimate of prenatal brain volume.

Being able to calculate fetal brain / liver volume ratio as a means of establishing fetal growth restriction, and indirectly indicating fetal hypoxaemia, it became of interest of how this ratio related to umbilical venous volume flow responsible for oxygen transfer to the fetus. An inverse relation was found in the growth-restricted fetus between fetal brain / liver volume ratio and fetal weight-related umbilical venous blood flow. Raised fetal brain / liver volume ratios were first found at reduced fetal weight-related umbilical venous volume flows of 70-80 ml/min/Kg.

Also in maternal insulin dependent diabetes mellitus (IDDM), we were interested in the relation between fetal liver size as a marker of fetal growth and umbilical venous volume flow. Enlarged fetal liver size in IDDM was not associated with an increase in placental perfusions. This suggests that in pregnancies complicated by maternal diabetes mellitus, raised fetal glucose

uptake is not determined by elevated umbilical venous volume flow but by raised glucose transfer across the placenta.

7.2 Limitations and future direction

The research presented in this thesis was designed to establish the feasibility of 3D ultrasonography in the assessment of fetal liver volume and combined colour-Doppler velocity and vessel size measurements in determining umbilical venous volume flow. These techniques were studied separately and in combination, the latter in relation to normal and abnormal fetal development, notably fetal growth retardation, maternal insulin dependent diabetes and pre-eclampsia.

Despite the accuracy and the advantages in the representation of organ volume, the actual 3-D ultrasonography technique has some limitations, which currently constrain a widespread and routinely used in the clinical setting. The acquisition of the data requires 3-6 second per rotation, depending on maternal respiratory movements and fetal body movements. The patient should not move during the procedure in order to avoid artefacts. Processing and reconstruction are still off-line procedures and require a special 3-D workstation. The time needed to process and reconstruct 3D images is considered one of the major limitations of the technique. However, recently new software versions have appeared in which the time requested for processing has diminished from 20 minutes to 5-10 minutes. Also the final creation of 3D images, so called *volume rendered images,* has become faster. The effective time for displaying such image has been reduced from 5 to less than 1 minute. Drawbacks present in 2D ultrasonography will persist and sometimes be enhanced in the 3D reconstruction. Resolution decreases during transfer of the original ultrasound data from 2D equipment to the 3D-acquisition system as a result of the assembling and segmentation process. Furthermore it is important to include all the images of the organ of interest in the conical data set otherwise it is not possible to perform the analysis of the data volume. The awareness of these limitations is important for the correct interpretation of the images in order to avoid diagnostic mistakes. Probably our conclusion that fetal liver volume measurement is not a better discriminator for fetal growth restriction than the measurement of abdominal circumference is due to the intrinsic limitations of the 3D technique mentioned above. We believe that the improvement of technology will further reduce the variability of repeated fetal liver volume measurements.

Also, umbilical venous volume flow determinations are still carried-out on an off-line basis and are, therefore, as yet not suitable for day-to-day clinical settings. However, with further improvement and more efficient collection of 3D fetal liver and brain volume data, a longitudinal study should be set up to establish more precisely at what reduced umbilical venous volume flow range, fetal head / liver volume ratio starts to increase.

Chapter 8

Summary

Chapter 1

The objectives of the present thesis are focused on the calculation of umbilical venous volume flow from cross-sectional area and flow velocity measurements with emphasis on the reproducibility of component measurements. We hypothesise that changes in fetal venous inflow are directly related to fetal growth and fetal well being. Additionally, we emphasise the key role of fetal liver size to detect impaired fetal growth. Therefore, reproducibility and normal values for fetal liver volume as obtained by 3-dimensional ultrasound were established and the significance of fetal liver volume assessment in identifying fetal growth restriction was evaluated relative to head and upper abdominal circumference. Moreover, the reliability of 2-dimensional and 3-dimensional sonographic fetal brain volume estimates as part of fetal brain/liver volume ratio calculations was addressed. Normal values for fetal brain/ liver volume ratio relative to gestational age were established and the relation between fetal brain/liver volume ratio and umbilical venous volume flow was evaluated during normal and abnormal fetal development with emphasis on fetal growth restriction. Umbilical venous flow was studied in relation to fetal behavioural states in late normal pregnancies and to abnormal conditions such as fetal growth restriction, maternal insulin dependent diabetes mellitus and maternal pre-eclampsia.

Chapter 2

A literature survey is presented on umbilical venous volume flow both in animal and in human as well as on fetal liver size during normal and abnormal fetal development.

Chapter 3

The first part of the chapter deals with methodological aspects of sonographic measurement of umbilical venous volume flow. The second part of the chapter describes three-dimensional determination of organ volume, in particular the fetal liver.

Chapter 4

This chapter focuses on umbilical venous flow velocity waveform analysis, umbilical venous cross-sectional vessel size and subsequent volume flow calculation in the human fetus. The first part of the chapter (sub-chapter 4.1) defines umbilical venous volume flow in the normal and growth restricted fetus with emphasis on the reproducibility of component measurements. Measurements of umbilical venous vessel area and time-averaged velocity resulted in acceptable reproducibility of volume flow calculations. A gestational age related increase exists for vessel area, time-averaged flow velocity, and umbilical venous volume flow, which shows a 7-fold increase at 20-36 weeks. In the normally developing fetus a reduction in volume flow per

kilogram fetal weight was observed. In growth restricted fetuses, volume flow is significantly reduced. When calculated per kg/fetus umbilical venous volume flow is reduced in 63.6% of cases. In growth restricted fetuses, umbilical artery Pulsatility Index is significantly different between the subsets of normal and reduced volume flow/kg fetus.

Subchapter 4.2 presents data on umbilical venous volume flow relative to fetal behavioural states 1F (quiet sleep) and 2F (active sleep) in normal late third trimester pregnancies. Doppler ultrasound studies have demonstrated that changes in fetal behavioural state are associated with specific alterations in fetal hemodynamics. To determine the presence of state dependency of venous inflow at the level of the umbilical vein, volume flow in this vessel was determined during behavioural state 1F and 2F in 20 normally developing fetuses at 35-40 weeks of gestation. We hypothesise that during state 2F (active sleep) there is an increase of venous inflow into the left heart, which is based on preferential blood flow through the feto-placental circulation. A significant increase in umbilical venous cross-sectional area exists during fetal behavioural state 2F. We suggest that a rise in mean venous pressure may be the underlying mechanism, considering the high venous vessel wall compliance. The significant rise in fetal heart rate during behavioural state 2 F seems to reflect an elevated fetal cardiac output. This is further supported in part by a mild increase in umbilical venous volume flow.

The aim of subchapter 4.3 is the evaluation of the effects of maternal administration of plasma expanders followed by dihydralazine infusion on maternal whole blood viscosity and hematocrit, utero-placental and feto-placental downstream impedance and umbilical venous volume flow in 13 women with pre-eclampsia. Hemodilution has been advocated as an effective treatment in pre-eclampsia to lower the increased blood viscosity. Maternal administration of vasodilating drugs such as dihydralazine associated with plasma expanders has been indicated to further reduce systemic vascular resistance and blood pressure. We observed a significant reduction in maternal diastolic blood pressure, maternal hematocrit and whole blood viscosity after maternal plasma expansion followed by dihydralazine administration. Maternal plasma expansion is associated with a significant increase in umbilical venous cross-sectional area and a non-significant rise of 11% in umbilical volume flow. Maternal dihydralazine administration does not result in any change in umbilical venous cross-sectional area. However, we observed a significant decrease in umbilical artery pulsatility index after both plasma expansion and dihydralazine treatment.

Chapter 5

In this chapter, a direct approach to measure fetal liver volume using three-dimensional ultrasound is described. We evaluated the diagnostic relevance of 3D-Ultrasonography in fetal liver volume determination in normal pregnancies, in fetal growth restriction and maternal insulin

dependent diabetes mellitus. We established reproducibility and normal values for fetal liver volume and its significance in identifying fetal growth restriction relative to head and upper abdominal circumference according to a cross-sectional study design. Currently, an ultrasound measurement of fetal head and upper-abdominal circumference is the most widely used method of establishing impaired fetal growth. The fetal liver comprises most of the abdomen measured by the abdominal circumference. We hypothesised that due to fetal malnutrition, reduction in fetal liver weight is more pronounced than reduction in brain weight due to the brain sparing effect, reflecting redistribution of fetal blood flow during chronic fetal hypoxaemia. We demonstrated that in uncomplicated pregnancy, fetal liver volume depicts a 10-fold increase with advancing gestational age and increasing fetal weight. Liver volume is proportional to estimated fetal weight during the second half of pregnancy. Acceptable reproducibility was found for liver volume determinations. The present study shows that fetal growth restriction is associated with reduced liver volume. Fetal growth restriction affects fetal liver size, but fetal liver volume measurement is not a better discriminator for growth restriction than is upper abdominal circumference mesurement.

Subchapter 5.2 presents data on fetal liver volume and its relation to umbilical venous volume flow and maternal glycosylated haemoglobin (Hb1Ac) in pregnancies complicated by maternal diabetes mellitus type I (IDDM). Measurement of fetal liver volume by 3D ultrasound may play a role in identifying fetal growth acceleration in diabetic pregnancies. In infants of diabetic mothers (IDDM) macrosomia seems to be more apparent in some fetal structures and in particular the liver and subcutaneous fat. We hypothesised that in IDDM women fetal macrosomia may be associated with fetal liver enlargement depending on the degree of maternal glycaemic control. Fetal liver size increase appears to be positively related to maternal HB1Ac levels reflecting degree of maternal glycaemic control. We observed that umbilical venous volume flow and feto-placental downstream impedance are not different between diabetic and normal pregnancies. We suggest that an increased fetal liver size is a result of fetal hyperglycaemia and hyperinsulinemia rather than raised placental perfusion. Therefore, fetal growth seems to be influenced by both maternal concentration of various nutrients and the total amount of substrate crossing the placenta and not by an increase in umbilical venous volume inflow.

Chapter 6

In this Chapter data are presented of a study designed to determine brain/liver volume ratio in the second half of pregnancy and its relation to umbilical venous volume flow in normally developing and growth restricted fetuses. Fetal brain volume was estimated from sonographically determined fetal head circumference and subsequently compared with post-mortem data on fetal brain weight from literature. To this purpose, conversion of fetal brain weight into brain

volume was carried-out. A good correlation was found and normal values for fetal brain volume (BV) /liver volume (LV) ratio were calculated and related to gestational age. Moreover, the relation was established between BV/LV ratio and fetal circulatory parameters, i.e. umbilical venous volume flow and downstream impedance in the umbilical artery, fetal middle cerebral artery and ductus venosus during normal and restricted fetal growth (SGA) according to a cross-sectional study design. In agreement with data from literature, we found in normal fetal development a ratio of brain volume to liver volume of about 3:1. In the presence of fetal growth restriction, this ratio may increase to 5:1 or more during the last 12 weeks of pregnancy. Looking at the components of this ratio, reduction in fetal brain volume is less pronounced than reduction in fetal liver volume due to the so-called brain sparing effect. We proposed that in the growth restricted fetus, reduced umbilical venous volume flow is related to a raised fetal brain/liver volume ratio. In the growth-restricted fetus, an inverse relation in SGA fetuses was observed between fetal brain/liver volume and fetal weight-related umbilical venous blood flow.

Samenvatting

Hoofdstuk 1

De doelstellingen van dit proefschrift concentreren zich op de berekening van het bloeddoorstromingsvolume van de vena umbilicalis, verkregen via oppervlakte en bloedstroom snelheidsmetingen van de vena umbilicalis De nadruk werd gelegd op de reproduceerbaarheid van de verschillende metingen. Onze hypothese is dat veranderingen in de foetale veneuze instroom direct gerelateerd zijn aan de foetale groei en gezondheid. Daarbij benadrukken wij de sleutelrol van de foetale leveromvang als mogelijkheid om groeivertraging op te sporen. Normaalwaarden voor foetaal levervolume zijn verkregen met behulp van 3-dimensionale echoscopie. Tevens werd de reproduceerbaarheid van deze methode getoetst en werd het foetale levervolume als maat voor foetale groeivertraging geëvalueerd ten opzichte van de hoofd- en buikomtrek. Ook de betrouwbaarheid van 2- en 3-dimensionale echoscopische foetale hersenvolume schattingen als onderdeel van de hersen/lever volume ratio komt aan de orde. Normaalwaarden voor de foetale hersen/lever volume ratio in relatie tot de zwangerschapsduur werden vastgesteld. Tevens werd de relatie tussen foetaal hersen/lever volume ratio en bloeddoorstromingsvolume van de vena umbilicalis gedurende normale en abnormale foetale ontwikkeling met de nadruk op foetale groeivertraging vastgesteld.
Veneuze navelstrengdoorbloeding werd bestudeerd in relatie met foetale gedragstoestanden in gevorderde normale zwangerschappen en in relatie met abnormale situaties zoals, foetale groeivertraging, maternale insuline afhankelijke diabetes mellitus en pre-eclampsie.

Hoofdstuk 2

Een literatuuroverzicht wordt gegeven over bloeddoorstromingsvolume van de vena umbilicalis gemeten in dier en mens en ook een overzicht van foetale levergrootte tijdens normale en abnormale foetale ontwikkeling.

Hoofdstuk 3

Het eerste deel van dit hoofdstuk beschrijft de methodologische aspecten van echoscopische metingen van het bloeddoorstromingsvolume van de vena umbilicalis. Het tweede deel beschrijft de 3-dimensionale bepaling van orgaanvolume, in het bijzonder de foetale lever.

Hoofdstuk 4

Dit hoofdstuk concentreert zich op de analyse van de bloeddoorstromingsprofielen van de vena umbilicalis, de afmeting van bloedvatoppervlakte en vervolgens de bloeddoorstromingsvolume berekening in de humane foetus. Het eerste deel van dit hoofdstuk (subhoofdstuk 4.1)

definieert de veneuze navelstrengdoorbloeding in de normale en groeivertraagde foetus met de nadruk op de reproduceerbaarheid van deze meting. Metingen van de oppervlakte en de gemiddelde bloedstroomsnelheid van de vena umbilicalis demonstreerden een acceptabele reproduceerbaarheid van bloeddoorstromingsvolume berekeningen.

Een zwangerschapsafhankelijke toename is aangetoond voor bloedvatoppervlakte, gemiddelde bloedstroomsnelheid en bloeddoorstromingsvolume van de vena umbilicalis, welke een zevenvoudige toename liet zien tussen 20 en 36 weken. In de normaal ontwikkelende foetus werd een afname van het bloeddoorstromingsvolume per kilogram foetaal gewicht waargenomen. In de groeivertraagde foetus was het bloeddoorstromingsvolume significant verlaagd en in 63,6% was er ook sprake van een afname van het bloeddoorstromingsvolume per kilogram foetaal gewicht. In de groeivertraagde foetus is de pulsatiliteitsindex van de navelstrengarterie significant verschillend tussen groepen met een normaal of een verlaagd bloeddoorstromingsvolume per kilogram foetaal gewicht.

Subhoofdstuk 4.2 behandelt de gegevens van bloeddoorstromingsvolume van de vena umbilicalis ten opzichte van de foetale gedragstoestanden 1F (rustige slaap) en 2F (actieve slaap) in het late derde trimester van de normale zwangerschap. Doppler echoscopisch onderzoek heeft aangetoond dat veranderingen in de foetale gedragstoestand geassocieerd zijn met specifieke aanpassingen in de foetale hemodynamiek. De aanwezigheid van een gedragstoestand afhankelijk veneuze instroom ter hoogte van de vena umbilicalis gedurende gedragstoestand 1F en 2F in 20 normaal ontwikkelende foetus van 35-40 weken zwangerschapsduur is bestudeerd. Wij veronderstellen dat er tijdens de 2F (actieve slaap) gedragstoestand een toename is van de veneuze instroom in het linkerhart, vanwege de preferentie van de bloedstroom naar foetus en placenta. Een significante toename van de oppervlakte van de vena umbilicalis werd aangetoond tijdens de foetale gedragstoestand 2F. Wij suggereren dat een toename van de gemiddelde veneuze bloeddruk misschien een onderliggend mechanisme is met het oog op de hoge veneuze vaatwand compliantie. De significante toename van hartslagfrequentie tijdens gedragstoestand 2F lijkt een gevolg van een verhoogd foetaal hartminuutvolume. Dit wordt gedeeltelijk onderbouwd door een lichte stijging van het bloeddoorstromingsvolume van de vena umbilicalis.

Het doel van subhoofdstuk 4.3 is het beschrijven van de effecten van het toedienen van plasmavolumevergroters gevolgd door dihydralazine op de maternale bloedviscositeit en hematocriet, uteroplacentaire en foeto-placentaire vaatweerstand en het bloeddoorstromingsvolume van de vena umbilicalis bij 13 pre-eclamptische zwangere vrouwen. Hemodilutie wordt gezien als de aanbevolen behandeling voor verlaging van de volbloed viscositeit bij pre-eclampsie. Maternale toediening van vasodilaterende geneesmiddelen zoals dihydralazine samen met plasmavolumevergroters wordt gebruikt om de systemische

vaatweerstand en bloeddruk verder te verlagen. Wij constateerden een significante verlaging van maternale diastolische bloeddruk, hematocriet en volbloed viscositeit na maternale plasmavolumevergroting en dihydralazine toediening. Maternale plasmavolumevergroting is geassocieerd met een significante toename van de bloedvatoppervlakte en een niet-significante stijging van 11% van het bloeddoorstromingsvolume van de vena umbilicalis. Maternale dihydralazine toediening leidt niet tot een verandering in bloedvatoppervlakte van de vena umbilicalis. Echter wij zagen een significante afname van de pulsatiliteitsindex van de navelstrengarterie na gecombineerde behandeling met plasmavolumevergroting en dihydralazine.

Hoofdstuk 5

In dit hoofdstuk wordt een directe benadering beschreven om de foetale lever te meten met behulp van 3-dimensionale echoscopie. Wij evalueerden de diagnostische relevantie van 3-dimensionale echoscopie in normale zwangerschappen, bij foetale groeivertraging en bij maternale insuline afhankelijke diabetes mellitus. Wij stelden de reproduceerbaarheid en normaalwaarden voor foetaal levervolume vast. In een cross-sectioneel onderzoek werd de waarde van deze methode bij het identificeren van foetale groeivertraging in relatie tot hoofd- en buikomtrek bepaald. Momenteel wordt de echoscopische meting van de foetale hoofd- en buikomtrek het meest gebruikt voor het bepalen van verstoorde foetale groei. De foetale lever omvat het grootste gedeelte van het abdomen, bepaald aan de hand van de buikomtrek. Wij veronderstellen dat bij foetale ondervoeding de afname in het levergewicht meer uitgesproken is dan de afname van het hersengewicht. Dit is een gevolg van het 'brain-sparing effect' wat leidt tot redistributie van bloedstroom tijdens foetale hypoxie. Wij hebben aangetoond dat in de ongecompliceerde zwangerschap het foetale levervolume een tienvoudige stijging laat zien met het vorderen van de zwangerschapsduur en met toenemend foetaal gewicht. Het levervolume stijgt proportioneel met het geschat foetaal gewicht in de tweede helft van de zwangerschap. Voor levervolume determinanten werd een acceptabele reproduceerbaarheid gevonden. Deze studie laat zien dat foetale groeivertraging geassocieerd is met een verlaagd levervolume. Foetale groeivertraging beïnvloedt de levergrootte, maar de meting van het foetale levervolume is geen betere maat voor groeiachterstand dan de buikomtrek.

Subhoofdstuk 5.2 beschrijft de gegevens over levervolume en de relatie met het bloeddoorstromingsvolume van de vena umbilicalis en maternaal geglycoliseerd haemoglobine (Hb1Ac) in zwangerschappen gecompliceerd met type I diabetes mellitus (IDDM). Metingen van het foetale levervolume met behulp van 3-dimensionale echoscopie zouden een rol kunnen spelen bij vaststelling van foetale groeiversnelling bij diabetische zwangerschappen. Bij kinderen van diabetische moeders (IDDM) lijkt marcosomie meer aanwezig te zijn in bepaalde foetale

structuren en in het bijzonder in de lever en in het subcutane vet. Wij veronderstellen dat bij diabetische vrouwen met een macrosome foetus, foetale leververgroting geassocieerd kan zijn met de mate van maternale bloedglucose instelling. Er lijkt een positieve relatie te bestaan tussen de foetale leververgroting en het maternaal HB1Ac wat een afspiegeling is van de maternale bloedglucose instelling. Wij hebben vastgesteld dat het bloeddoorstromingsvolume van de vena umbilicalis en de foetale-placentaire weerstand niet verschillend zijn in diabetische en normale zwangerschappen. Wij stellen voor dat een foetale leververgroting eerder een gevolg is van foetale hyperglycemie en hyperinsulinemie dan van een verhoogde placentaire perfusie. Foetale groei lijkt meer beïnvloed te worden door de maternale concentraties van verschillende nutriënten en de totale hoeveelheden substraat welke de placenta passeren en niet door een toename van veneuze bloed instroom via de navelstreng.

Hoofdstuk 6

In dit hoofdstuk wordt een studie gepresenteerd over de bepaling van de hersen/lever volume ratio in de tweede helft van de zwangerschap en de relatie van deze ratio met het bloeddoorstromingsvolume van de vena umbilicalis in de normale en groeivertraagde foetus. Het foetaal hersenvolume werd geschat met behulp van de echoscopisch verkregen foetale hoofdomtrek en het werd vervolgens vergeleken met post-mortum literatuur gegevens over foetaal hersengewicht. Teneinde deze vergelijking te maken werd het foetaal hersengewicht omgezet in hersenvolume. Er werd een goede correlatie gevonden tussen het geschatte hersenvolume en de post-mortum gegevens. Normaalwaarden voor de foetale hersen/lever volume ratio werden berekend en gerelateerd aan de zwangerschapsduur. Ook werd in een cross-sectioneel onderzoek de relatie vastgesteld tussen deze ratio en foetale circulatie parameters, te weten het bloeddoorstromingsvolume van de vena umbilicalis en de vaatweerstand in de navelstrengarterie, in de arteria cerebri media en in de ductus venosus bij de normale en groeivertraagde foetus. In overeenstemming met literatuurgegevens, vonden wij tijdens normale foetale ontwikkeling een hersen/lever volume ratio van ongeveer 3:1. In de groeivertraagde foetus kan deze ratio oplopen tot 5:1 of meer in de laatste 12 weken van de zwangerschap. Ten gevolge van het zogenaamde 'brain-sparing effect' is de afname in het foetale hersenvolume minder uitgesproken dan de afname in het levervolume. Wij veronderstellen dat in de groeivertraagde foetus, het verlaagde bloeddoorstromingsvolume van de vena umbilicalis gerelateerd is aan een toegenomen foetale hersen/lever volume ratio. In de groeivertraagde foetus werd een omgekeerde relatie gevonden tussen een toegenomen foetale hersen/lever volume ratio en het van het foetale gewicht afhankelijke bloeddoorstromingsvolume in de vena umbilicalis.

Sommario della tesi

Capitolo 1

Gli obiettivi di questa tesi sono incentrati sul calcolo del volume di flusso della vena ombelicale dedotto dalla sezione della stessa in sezione trasversa e dal calcolo della sua velocità. In particolare sono stati valutati: la riproducibilità delle misurazioni delle diverse componenti, il volume di flusso venoso nello sviluppo fetale normale e patologico e, in quest'ultimo, la correlazione con la velocimetria dell' arteria ombelicale. Abbiamo ipotizzato che le modificazioni nel flusso venoso in entrata siano direttamente correlate con il benessere e la crescita fetali; inoltre abbiamo messo in evidenza il ruolo chiave della misura del volume epatico fetale nella diagnosi del ritardo di crescita. Riproducibiltà e valori normali per il volume epatico fetale sono stati ottenuti grazie all'utilizzo dell'ecografia tri-dimensionale ed è stata valutata, inoltre, la significatività della misura del volume epatico fetale nell'identificare il feto affetto da ritardo di crescita relativamente alla circonferenza cefalica ed addominale. E' stata valutata l'affidabilità delle stime ultrasonografiche del volume cerebrale fetale ottenuto per mezzo, sia dell'ecografia bidimensionale sia dell' ecografia tridimensionale come parte del calcolo del rapporto dei volumi fetali cervello/fegato. Sono stati definiti i valori normali per il rapporto volumetrico cervello/fegato in rapporto all'età gestazionale ed è stata stabilita la correlazione tra rapporto volumetrico cervello/fegato e velocimetria fetale arteriosa e venosa nel corso dello sviluppo fetale normale ed anormale soprattutto nel caso di ritardo di crescita intra-uterino, nel corso di pre-eclampsia e nel diabete mellito materno insulino dipendente.

Capitolo 2

Viene presentata una revisione della letteratura relativa al volume di flusso venoso ombelicale fetale sia nell' animale che nell' uomo durante il 2° e 3° trimestre di gestazione; viene proposto inoltre, un riassunto delle opinioni presenti in letteratura sulla valutazione ecografica del fegato fetale, sia nel corso dello sviluppo normale che nel ritardo di crescita.

Capitolo 3

Questo capitolo tratta degli aspetti metodologici dell'acquisizione del volume di flusso per mezzo degli ultrasuoni. La seconda parte di questo capitolo descrive l'acquisizione tridimensionale del volume degli organi fetali ed in particolare del volume epatico.

Capitolo 4

In questo capitolo vengono analizzate le onde della velocità di flusso della vena ombelicale, la misura del vaso venoso in sezione trasversa ed il successivo calcolo del volume di flusso nel feto umano. La prima parte del capitolo (paragrafo 4.1) definisce il volume di flusso della vena ombelicale nel feto normale e nel feto affetto da ritardo di crescita, con particolare attenzione alla riproducibilità delle misurazioni delle variabili velocità ed area. Le misurazioni dell'area del vaso venoso e del tempo medio di velocità ottenute, documentano l'acquisizione di una riproducibilità accettabile nel calcolo del volume di flusso. E' stato evidenziato un aumento, correlato all'età gestazionale, dell'area del vaso, del flusso di velocità media e del volume di flusso della vena ombelicale; quest'ultimo evidenzia un aumento pari a 7 volte durante la seconda metà della gravidanza. Nel feto appropriato per epoca gestazionale (AGA) è stato osservata una riduzione del volume di flusso corretto per peso fetale. Nei feti affetti da ritardo di crescita, il volume di flusso è significativamente ridotto. Se corretto per il peso presunto del feto, il volume di flusso della vena ombelicale è diminuito nel 63.6% dei casi. Nei feti piccoli per epoca gestazionale (SGA), l'indice di pulsatilità dell'arteria ombelicale è significativamente diverso tra il sottogruppo con normali valori di volume di flusso corretto per kg di peso fetale, rispetto al sottogruppo con valori ridotti di volume di flusso per kg fetale.

Il paragrafo 4.2 presenta i dati sul volume di flusso della vena ombelicale relativamente agli stati di comportamento fetale 1F (sonno passivo) e 2F (sonno attivo) nella gravidanza fisiologica al termine del terzo trimestre. Studi ultrasonori Doppler hanno dimostrato che cambiamenti del comportamento fetale si associano a specifiche modificazioni delle emodinamiche fetali. Per determinare se esiste un rapporto causale fra flusso venoso a livello della vena ombelicale e diversi stati comportamentali fetali, è stato determinato il volume di flusso ombelicale durante gli stadi comportamentali fetali 1F e 2F in 20 feti tra la 35^ e la 40^ settimana di gestazione con appropriato sviluppo per epoca gestazionale. Abbiamo ipotizzato che durante lo stato 2F (sonno attivo) si verifica un aumento dell'afflusso venoso nel cuore sinistro, il quale si basa su un flusso preferenziale attraverso la circolazione feto-placentare. E' stato documentato un aumento significativo dell'area della vena ombelicale in sezione trasversale durante la fase comportamentale 2F. Considerando l'alta *compliance* della parete del vaso venoso, riteniamo che il meccanismo che giustifica questo riscontro possa essere un aumento della pressione venosa media. L'aumento significativo della frequenza cardiaca fetale durante la fase comportamentale 2 F sembra riflettere una gittata cardiaca fetale elevata. Questa osservazione è sostenuta anche dal concomitante moderato aumento del volume di flusso della vena ombelicale.

Scopo del paragrafo 4.3 è stata la valutazione degli effetti della somministrazione alla madre di *plasma expanders* seguiti dall'infusione di *diidralazina*, sulla viscosità totale del sangue e

sull'ematocrito materni, sull'impedenza al flusso utero-placentare e feto-placentare e sul volume di flusso della vena ombelicale in 13 donne affette da pre-eclampsia. L'emodiluizione ha rappresentato un efficace trattamento nel corso di pre-eclampsia per abbassare l'aumentata viscosità sanguigna materna. La somministrazione di farmaci vasodilatanti come la diidralazina associata a plasma expanders è stata indicata come uno dei trattamenti elettivi per ridurre le resistenze vascolari sistemiche e la pressione sanguigna nel corso di pre-eclampsia. Nel nostro studio abbiamo osservato una riduzione significativa della pressione arteriosa diastolica, dell'ematocrito e della viscosità sanguigna totale della paziente che riteniamo siano il risultato della somministrazione di plasma expanders e di diidralazina. L'emodiluizione materna è associata ad un significativo aumento dell'area della vena ombelicale ed a un aumento non-significativo dell'11 % del volume del flusso venoso. La somministrazione di diidralazina sembra non modificare l'area della vena ombelicale. Tuttavia, abbiamo osservato una diminuzione significativa dell'indice di pulsatilità dell'arteria ombelicale sia dopo la somministrazione di plasma expander che dopo il trattamento con diidralazina.

Capitolo 5

Questo capitolo descrive un approccio diretto della misura del volume epatico fetale per mezzo dell'ecografia tridimensionale. E' stata valutata la rilevanza diagnostica del 3D nella determinazione del volume epatico fetale nelle gravidanze normali, nel ritardo di crescita e nel diabete mellito materno insulino-dipendente. Sono stati determinati riproducibiltà e valori normali per il volume epatico fetale e la sua significatività nell'identificare l'iposviluppo fetale relativamente alla circonferenza fetale cefalica ed addominale. Attualmente, la misurazione ultrasonografica della circonferenza cefalica ed addominale e' il metodo ampiamente piu' in uso nello stabilire un difetto di crescita fetale. Il fegato fetale comprende la maggior parte dell'addome misurato dalla circonferenza addominale. Noi abbiamo ipotizzato che in seguito alla malnutrizione fetale, la diminuzione del peso del fegato fetale è più pronunciato di quella del peso cerebrale grazie all'effetto *brain sparing*, riflettendo una ridistribuzione del flusso di sangue fetale durante stati di ipossiemia fetale cronica. Abbiamo dimostrato che nelle gravidanze fisiologiche, il volume epatico fetale va incontro ad un aumento pari a 10 volte con l'avanzare della gravidanza e con l'aumentare del peso fetale. Il volume epatico è proporzionale al peso presunto fetale durante la seconda metà della gravidanza. E' stata raggiunta una riproducibilità accettabile per le determinazioni del volume epatico fetale. Il presente studio dimostra che il ritardo di crescita fetale è associato ad un diminuito volume epatico. Il ritardo di crescita intra-uterino influisce negativamente sull dimensioni del fegato fetale, ma la misurazione del volume epatico fetale non e' un parametro piu' discriminante per la diagnosi di ritardo di crescita di quanto lo sia la misurazione della circonferenza addominale.

Il paragrafo 5.2 presenta dati sul volume epatico fetale e la relazione che intercorre tra il volume di flusso della vena ombelicale e l'emoglobina glicosilata materna (Hb1Ac) nelle gravidanze complicate da diabete mellito tipo 1 (IDDM). Abbiamo ipotizzato che la misurazione del volume epatico fetale per mezzo dell'ecografia tridimensionale possa avere un ruolo nell'identificare un'accelerazione della crescita fetale nelle gravidanze diabetiche. Nei neonati di madri diabetiche (IDDM) la macrosomia sembra essere più evidente in alcune strutture fetali, in particolare il fegato e il grasso sottocutaneo. Abbiamo ipotizzato che nelle donne IDDM la macrosomia fetale sia associata ad un accrescimento epatico, che risente dei differenti livelli di controllo glicemico materno. L'aumento delle dimensioni del fegato fetale sembra correlarsi positivamente con i livelli di emoglobina glicosilata riflettendo gradi diversi di controllo glicemico materno. Abbiamo osservato che il volume di flusso della vena ombelicale e l'impedenza feto-placentare non differiscono tra la gravidanza normale e quella diabetica. I nostri dati suggeriscono che l'aumento delle dimensioni del fegato fetale sia il risultato di uno stato di iperglicemia ed iper-insulinemia fetale più che il risultato di un aumento della perfusione placentare. La crescita fetale quindi, sembra essere influenzata dalle concentrazioni di vari nutrienti e dalla quantità totale di substrati che attraversano la barriera placentare ma non da un aumento del volume di afflusso venoso ombelicale.

Capitolo 6

In questo capitolo vengono presentati i risultati di uno studio con cui ci siamo prefissi l'obiettivo di determinare il rapporto cervello/fegato nella seconda metà della gravidanza e la relazione che intercorre tra quest'ultimo parametro e il volume di flusso della vena ombelicale nei feti con normale sviluppo e nei feti affetti da ritardo di crescita. Il volume del cervello fetale è stato stimato in base al valore della circonferenza cefalica fetale determinata sonograficamente e successivamente è stato confrontato con dati autoptici ottenuti dalla letteratura. Per questo fine, è stata fatta una conversione del peso del cervello fetale in volume cerebrale. È stata riscontrata una buona correlazione tra questi due parametri e sono stati calcolati i valori normali per il rapporto volumetrico cervello/fegato fetali in rapporto all'età gestazionale. E' stata stabilita, inoltre, la relazione tra rapporto volumetrico cervello/fegato e parametri circolatori fetali come il volume di flusso della vena ombelicale e l'impedenza al flusso, rispettivamente nell'arteria ombelicale, nell'arteria cerebrale media e nel dotto venoso nel corso dello sviluppo fetale normale e patologico. In accordo con i dati della letteratura, abbiamo riscontrato nello sviluppo fetale normale un rapporto volumetrico cervello/fegato di circa 3:1. Nel caso di ritardo di crescita fetale, abbiamo osservato un aumento del rapporto fino a 5 a 1 o più, durante le ultime 12 settimane di gravidanza. Valutando i due indici di tale rapporto, la riduzione nel volume cerebrale fetale è meno pronunciata della riduzione del volume epatico fetale a causa del così detto effetto

"brain sparing". Noi suggeriamo che nel feto affetto da ritardo di crescita, un ridotto volume di flusso della vena ombelicale si associ ad un aumento del rapporto volumetrico cervello/fegato. Nel feto affetto da ritardo di crescita, è stato constatato che esiste una proporzione inversa tra rapporto volumetrico cervello/fegato fetale e il volume di flusso della vena ombelicale corretto per il peso fetale.

List of Publications

Boito SM, Struijk PC, Ursem NT, Stijnen T, Wladimiroff JW. Umbilical venous volume flow in the normally developing and growth- restricted human fetus. Ultrasound Obstet Gynecol 2002;19:344-349.

Boito SM, Laudy JA, Struijk PC, Stijnen T, Wladimiroff JW. Three-dimensional US Assessment of Hepatic Volume, Head Circumference, and Abdominal Circumference in Healthy and Growth-restricted Fetuses. Radiology 2002;223:661-665.

Boito SM, Struijk PC, Ursem NT, Wladimiroff JW. Fetal brain/liver volume ratio and umbilical volume flow parameters relative to normal and abnormal human development. Ultrasound Obstet Gynecol.2003;21:256-61.

Boito SM, Struijk PC, Stijnen T, Ursem NT, Wladimiroff JW. Assessment of fetal liver volume and umbilical venous volume flow in pregnancies complicated by insulin-dependent diabetes mellitus. Accepted for publication in BJOG.

Boito SM, Struijk PC, Ursem NT, Stijnen T, Wladimiroff JW. Umbilical venous volume flow and fetal behavioural states in the normally developing fetus. Accepted for publication in Ultrasound Obstet Gynecol.

Boito SM, Struijk PC, Pop GAM, Visser W, Steegers EAP, Wladimiroff JW. The impact of maternal volume plasma expansion and antihypertensive treatment with intravenous dihydralazine on fetal and maternal hemodynamics during pre-eclampsia. A clinical, echo-Doppler and viscometric study. Submitted

Roelfsema NM, Hop WCJ, Boito SM, Wladimiroff JW. Three-dimensional sonographic assessment of normal fetal brain volume during the second half of pregnancy. Accepeted for publication in Am J Ob Gyn.

ABSTRACTS:
Boito SM, Struijk PC, Ursem NT, Stijnen T, Wladimiroff JW. Umbilical venous volume flow: reproducibility and developmental data. Ultrasound Obstet Gynaecol 2000;16:47.

Boito SM, Laudy JA, Struijk PC, Stijnen T, Wladimiroff JW. Fetal liver volume in normal and reduced fetal growth. Ultrasound Obstet Gynaecol 2000;16:71.

Boito SM, Struijk PC, Stijnen T, Wladimiroff JW. Umbilical venous volume flow: reproducibility and developmental data. Presented at the congress "The woman and the child before, during and after pregnancy" 2001, Roma, Italy.

Boito SM, Laudy JA, Struijk PC, Stijnen T, Wladimiroff JW. Three-dimensional US Assessment of fetal liver volume in normal and reduced fetal growth. Presented at the congress "The woman and the child before, during and after pregnancy" 2001, Roma, Italy.

Boito SM, Laudy JA, Struijk PC, Stijnen T, Wladimiroff JW. Three-dimensional US Assessment of Hepatic Volume, Head Circumference, and Abdominal Circumference in Healthy and Growth-restricted Fetuses. Presented at Ducth Ultrasound Working Group 2002, Academic Hospital Utrecht, The Netherlands.

Boito SM, Struijk PC, Ursem NT, Fedele L, Wladimiroff JW. Fetal brain/liver volume ratio and umbilical volume flow parameters relative to normal and abnormal human development. Presented at 17th European Congress of Obstetric and Gynaecology 2002, Prague, Czech Republic.

Roelfsema NM, Boito SM, Hop WCJ, Wladimiroff JW. Three-dimensional sonographic assessment of normal fetal brain volume during the second half of pregnancy. Ultrasound Obstet Gynaecol 2002;20:3.

Boito SM, Ursem NT, Stijnen T, Fedele L, Wladimiroff JW. Umbilical venous volume flow in the normally developing term fetus demonstrates no relationship with fetal behavioral states. Ultrasound Obstet Gynaecol 2002;20:33.

Boito SM, Struijk PC, Stijnen T, Fedele L, Wladimiroff JW. Three-dimensional assessment of fetal liver volume and umbilical venous volume flow in pregnancies complicated by insulin-dependent diabetes mellitusUltrasound Obstet Gynaecol 2002;20:6.

Curriculum vitae

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List of abbreviations

UV	umbilical venous
PI	pulsatility index
RI	resistance index
BPD	biparietal diameter
HC	head circumference
AC	abdominal circumference
FL	femur length
EFW	estimated fetal weight
AGA	appropriate for gestational age
SGA	small for gestational age
FHR	fetal heart rate
1F	quite sleep in fetal behavioural state
2F	active sleep in fetal behavioural state
ASAT	serum aspartate aminotransferase
ALAT	serum alanine aminotransferase
Ht	hematocrit
WBV	whole blood viscosity
DH	dihydralazine
PVE	plasma volume expansion
LV	liver volume
IDDM	insulin dependent diabetes mellitus
HB1Ac	glycosylated haemoglobin
BV	brain volume
HV	head volume
BV/LV	brain/liver volume ratio

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Jacob Isaacksz van Ruisdael,
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