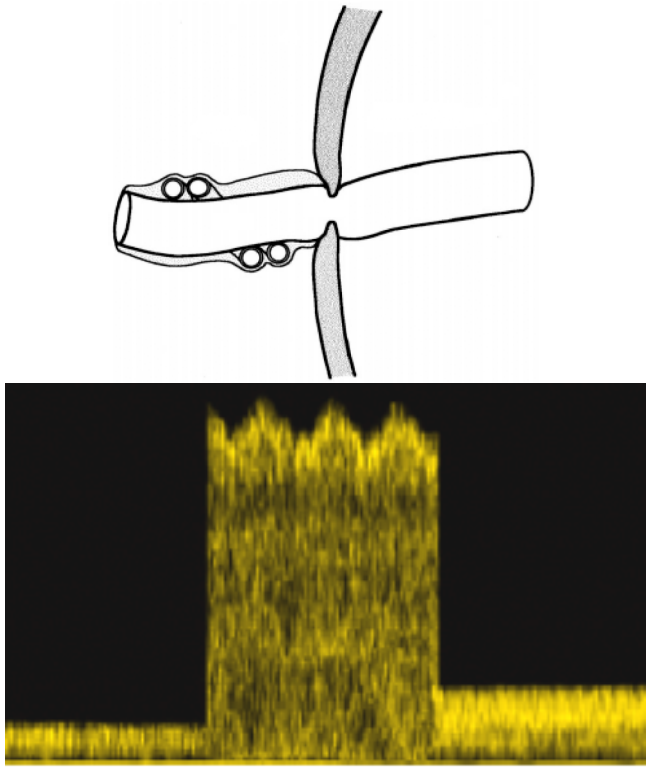
Umbilical vein constriction at the abdominal wall

An ultrasound study in low risk pregnancies



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Abbreviatons

2D–imaging	Two-dimensional ultrasound, gray scale ultrasound,
AD	Anno Domini, after Christ
BC	Before Christ
BW	Birthweight
BW/PW	Birthweight/placental weight ratio
CI	Confidence intervals
DV	Ductus venosus
ECMUS	European Committee for Medical Ultrasound Safety
EFSUMB	European Federation of Societies for Ultrasound in Medicine and Biology
EPOR	Erythropoietin receptor gene
ET	Endothelin
FDA	Food and Drug Administration (United States government agency)
fs	Sampling frequency in Doppler
IP	Index of pulsation of the pressure in the umbilical vein in a mathematical model
I_{spta}	Spatial Peak Temporal Average Intensity (mW/cm^2); commonly used measure of the acoustic energy that the tissues are exposed to
IVC	Inferior vena cava
kHz	Kilohertz
λ	Wavelength
LHV	Left hepatic vein
MHz	Megahertz
MI	Mechanical index; empirical factor correlated to the formation of bubbles in living tissue (cavitation)
mm Hg	Pressure expressed in terms of the weight of a column of mercury of unit cross section
MPa	Megapascal; million Newton per square metre (pressure)
MRG	Multi range gated
mW/cm^2	Milliwatt per square centimetre (energy disposal in the tissue)

NO	Nitric oxide
pCO_2	Partial pressure of carbon dioxide in arterial blood
pH	Quantitative measure of the acidity or basicity of blood
PI	Pulsatility index: (systolic velocity – diastolic velocity)/mean velocity
pO_2	Partial pressure of oxygen in arterial blood
PRF	Pulse repetition frequency in Doppler
PW	Placental weight at birth
PW	Pulsed wave Doppler
RC	Reflection coefficient
Re_d	Critical Reynolds number when a transition from laminar flow to turbulence occurs
Reynolds number	In fluid mechanics: a number that expresses the risk of laminar flow developing into turbulence; it depends on vessel dimension, density, velocity and viscosity of the fluid
SD	Standard deviation
UV	Umbilical vein
V_{max}	Maximum time averaged blood velocity in a vessel measured by pulsed Doppler technique
$V_{max.abd}$	Maximum time averaged blood velocity in the umbilical vein at the abdominal wall
$V_{max.cord}$	Maximum time averaged blood velocity in the umbilical vein in the cord
V_{mean}	Mean time averaged blood velocity in a vessel
Z	Impedance, resistance to pulsatile flow
Z_{DV}	Impedance in the ductus venosus
z–score	The distance in standard deviations between the observation and the mean: (observed value–mean)/SD
Z_{UV}	Impedance in the umbilical vein

List of original papers

1. Skulstad SM, Rasmussen S, Iversen OE, Kiserud T. The development of high venous velocity at the fetal umbilical ring during gestational weeks 11–19.
   Br J Obstet Gynaecol 2001; 108: 248–253.

2. Skulstad SM, Kiserud T, Rasmussen S. Degree of fetal umbilical venous constriction at the abdominal wall in a low–risk population at 20–40 weeks of gestation.
   Prenat Diagn 2002; 22: 1022–1027.

3. Skulstad SM, Kiserud T, Rasmussen, S. The effect of vascular constriction on umbilical venous pulsation.
   Ultrasound Obstet Gynecol 2004; 23: 126–130.

4. Skulstad SM, Rasmussen S, Seglem S, Svanaes RH, Aareskjold HM. The effect of umbilical venous constriction on placental development, cord length and perinatal outcome.
   Early Human Dev 2004; In press

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Svein Magne Skulstad

Summary

The umbilical vein is the only vessel supplying the fetus with blood supplying oxygen and nutrients from the placenta. Case reports indicate that the fetal end of the umbilical cord is susceptible to mechanical complications.

Hypothesis: We assume that umbilical ring constriction may affect the umbilical vein and have haemodynamic effects on fetal development and birth.

Aims: To describe the occurrence and degree of umbilical venous constriction in low risk–pregnancies. To establish reference ranges. To determine whether such a constriction has a haemodynamic effect, or any effect on fetal development or perinatal outcome.

Material and methods*:* 384 low–risk singleton pregnancies were included in the cross–sectional studies after written consent and ethical approval. 2D–imaging, colour Doppler and pulsed Doppler were used to measure diameters and blood velocity in the umbilical vein before, at or beyond the abdominal wall. All blood flow velocimetry was performed during fetal quiescence. Perinatal outcome was noted.

Results: From 13 weeks onwards, after the period of physiological umbilical herniation, umbilical venous constriction was noted in increasing numbers and severity until 19 weeks of gestation *(paper I)*. For the latter half of the pregnancy, during gestational weeks 20–40, the pattern remained constant; 41/191 (21%) had a venous constriction corresponding to a diameter reduction to the half, while the corresponding venous blood velocity increment was ≥300%, and 5% of the fetuses had velocities ≥107 cm/s, which is exceptionally high compared with other blood velocities in the body, whether arterial or venous *(paper II)*. The reproducibility study showed that the measurements of venous blood velocity in the cord and at the umbilical ring had SD of 0.58 and 1.83 cm/s respectively and that the diameter measurements both at the umbilical ring and at the cord had a mean SD of 0.07 mm *(paper II).*

The incidence of umbilical venous pulsation was higher at the umbilical ring in the abdominal wall, 242/279 (87%) than in the cord, 43/198 (22%) or intra–abdominally, 84/277 (30%). When pulsation was observed intra–abdominally, the pulsatility was not different from that at the umbilical ring. The lowest pulsatility was found in the cord vein, where the largest vein diameter was found *(paper III).*

Umbilical venous constriction had a significant negative correlation to the birthweight/placental weight ratio in male but not in female fetuses. Umbilical venous constriction was also associated with and increased length of the cord, but only in female fetuses. Constriction was also associated with Apgar score ≤7 at one minute after birth but not after five, and was not associated with emergency delivery *(paper IV).*

Conclusions: The umbilical ring seams to tighten in the following weeks after the period of physiological herniation, causing an increasing number and degree of umbilical vein constriction. After 19 weeks of gestation, the occurrence of umbilical vein constriction is constant until term. The degree of constriction can be considerable, and in 20% of the fetuses the umbilical vein diameter is ≤50% of that in the cord. We have established reference ranges. Within physiological ranges, i.e. in a low–risk population, such constrictions have a haemodynamic effect leading to increased incidence of pulsations and gender specific effects on fetal, cordal and placental growth. The significant effect on Apgar score at one minute warrants further studies of the effect of extreme constriction on perinatal morbidity.

1 Introduction

1.1 History

The umbilical cord is the fetal lifeline, which supplies the oxygen and nutrition needed, and transports the waste products from the fetus to the mother. In Japanese folklore the umbilical cord has been called the “The flower stalk of life”, and it has been surrounded by an aura of magic and the supernatural in different cultures all over the world. The legitimacy of the child was determined by the specific gravity of the umbilical cord, the child’s future virility by the size of its stump. Sterility or diminished fecundity was forestalled by tasting its blood or by eating its substance. The umbilical cord was worn as a talisman to protect its bearer from various sicknesses and misfortunes (Spivack 1946). In the Kingdom of Buganda (the largest of the four kingdoms in the western region of Uganda, East Africa), from the dawn of legend up to the middle of the nineteenth century, the jawbone and umbilical cord of the *kabaka* (king or clan leader) were preserved in a special shrine after his death, and became the means through which his successor could consult him in affairs of state (Welbourn 1964).

Since long, the existence and function of the cord has been related to fetal survival. In the Brahmanas, a constituent of the Vedas (ancient Indian literature, compiled around 3500 BC), this description is to find: "The *dhamanis* (ducts with thick walls equivalent to arteries) in the fetus take their rise from the umbilical cord, thus bringing nourishment from the mother. The embryo is held at the navel. It grows without taking food, that is, there is no effort made on the part of the embryo to take food and no food is specially served to it. The food in its final form is assimilated automatically and directly into the system of the embryo. The child is nourished of its own accord as it were. The mother is not conscious of the nourishment given to the young one below her heart" (Bhargava and Chakrabarti 1995).

In Hippocrates’ (460 – 377 BC) treatise on “The Nature of the Child” it was stated that the embryo is nourished by maternal blood, which flows to the fetus and coagulates, forming the embryonic flesh (Hippocrates *et al.* 1978). According to Hippocrates the first nutrient for the fetus is supplied through the umbilical cord. The umbilical cord is also considered the

means by which fetal respiration is carried on. Galen (131–200 AD) was convinced that the venous and arterial systems were each sealed and separated from each other. William Harvey, discoverer of the circulation of the blood including that of the fetus (Harvey 1653), wondered how Galen, having got so close to the answer, did not himself arrive at the concept of the circulation.

After Harvey, a vast literature exists on the anatomy of the fetal cardiovascular system, much of it in the German language. Most of it came into existence in the nineteenth century, reviewed by Barclay et al (Barclay *et al.* 1945). The focus of interest has shifted in accordance with the tools available for investigation. As early as in the 19th century, by various histological techniques, scientists showed that the umbilical vessels are different compared to vessels of the body (Hyrtl 1870), which was verified by other investigators in the twentieth century (Chacko and Reynolds 1954).

During the last 50 years ultrasound investigation has gradually become the most important tool in obstetrics to ascertain fetal conditions. The evidence to support the use of Doppler velocimetry in clinical management of pregnancies has been analysed more thoroughly and systematically than the evidence regarding other techniques used in modern obstetrics (Goffinet *et al.* 1997; Thornton 2001; Westergaard *et al.* 2001). Although the umbilical flow was determined with Doppler technique at an early stage (Gill and Kossoff 1979), the different sections of the umbilical vein have not been studied thoroughly. Focus has been on the arterial side of the circulation. However, during the past 10 to 15 years the assessment of the blood flow on the venous side has been shown to provide valuable information. Ductus venosus velocimetry was introduced (Kiserud *et al.* 1991) and proved to be of great value in the evaluation of fetal well–being (Baschat *et al.* 2001; Kiserud 2001a).

1.2 Developmental anatomy and physiology

1.2.1 Developmental anatomy

In classical terms, the human pregnancy can be divided into two distinct phases. The first, or early pregnancy period, corresponds approximately to the first 8 weeks after conception or 10 weeks after the last menstrual period. During this time the key events of embryogenesis, placentation, and organogenesis take place (Burton *et al.* 2001; Burton and Jaunaiux 2001). The remainder of pregnancy is characterised by fetal growth and maturation by the means of the umbilical circulation.

The embryo formed after four gestational weeks consists of two layers between the amniotic cavity and the primary yolk sac. The ectoderm is the first and top germ layer, from which the nervous system and epidermis develop, while the second germ layer (endoderm) gives rise to the epithelia of all gut–derived organs. The embryo proper consists of the two germ layers, and is spread within the umbilical ring.

The third germ layer, the mesoderm, is formed by the gastrulation process, which starts at the dorsocaudal half of the embryo in a groove–like structure, the primitive streak (Larsen 1997; Moore and Persaud 2003; Sadler and Langman 2004). It has been shown that during the early stages the entire ectoderm is capable of depositing cells into the mesodermal compartment (Smits-van Prooije *et al.* 1987; Hartwig *et al.* 1989). Gradually some areas will loose this quality, while the surface ectoderm placodes (Smits-van Prooije *et al.* 1985; Smits-van Prooije *et al.* 1988) like the umbilical ring (also called the body wall placode) continues to have this ability, contributing cells to the mesodermal compartment of the future ventral body wall (Hartwig *et al.* 1989; Hartwig *et al.* 1991). The deposition of ectodermal cells into the mesodermal compartment is made possible by apoptosis and phagocytosis. Rapid proliferation of the neurectoderm and the underlying mesoderm initiates the embryonal change in form from a disk to a cylinder (O'Rahilly and Müller 1987). These two cell layers increase quickly in size and grow beyond the yolk sac. The embryonic folding process is thought to occur due to a relative growth delay of the umbilical

ring based on the apoptotic cell death (Hartwig *et al.* 1991; Vermeij-Keers *et al.* 1996). The umbilical cord formation is completed by the attachment of the amnion to the connecting and yolk stalks in both embryonic and placental direction.

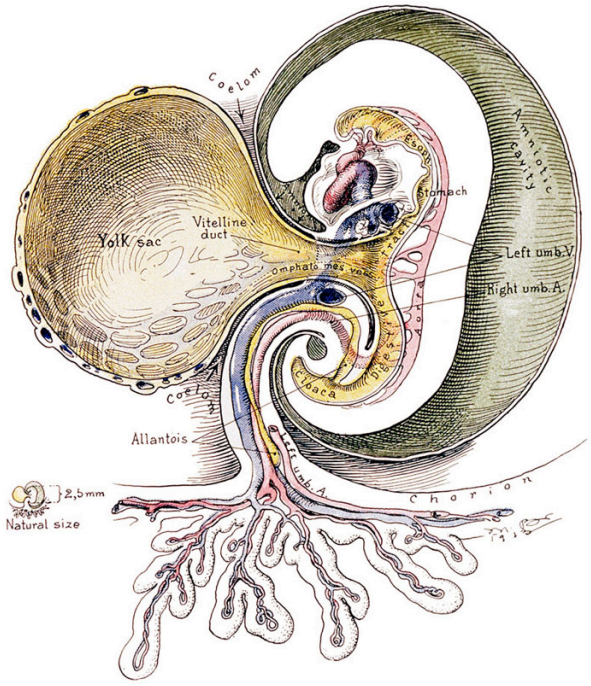

Figure 1. The formation of the umbilicus in an embryo of 2.5 mm. The amnion almost completely encircles the embryo, and in so doing has combined the vitelline duct with the body-stalk, containing the chorionic vessels and the allantois into a common cord. As the development advances the cord will become more compact, thinner, and longer. (From http://www.netembryo.org/broedel3.htm)

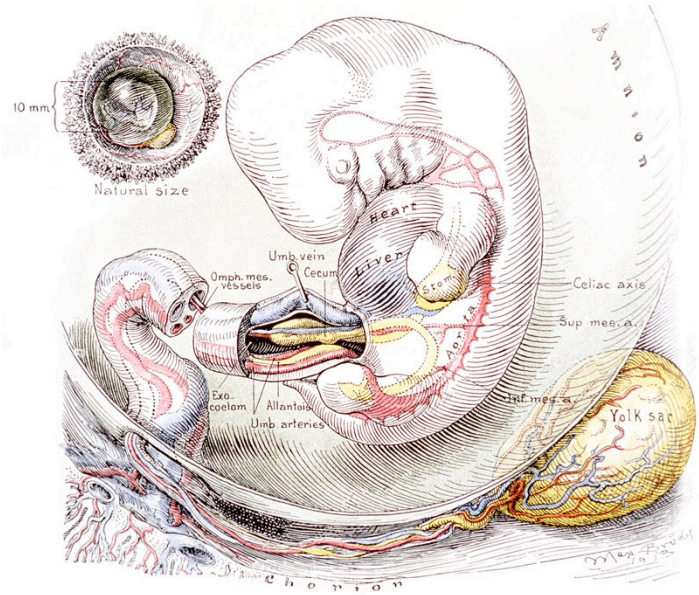

Figure 2. The umbilical region of a human embryo of 10 mm. The yolk-sac is now disposed far from the umbilical cord. The umbilical cord shows the first indication of a twist. The small intestine extends a considerable distance into the exocoelom of the cord. The omphalomesenteric vein passes on the left side of the intestinal loop; the artery, on the right side. (From http://www.netembryo.org/broedel8.htm)

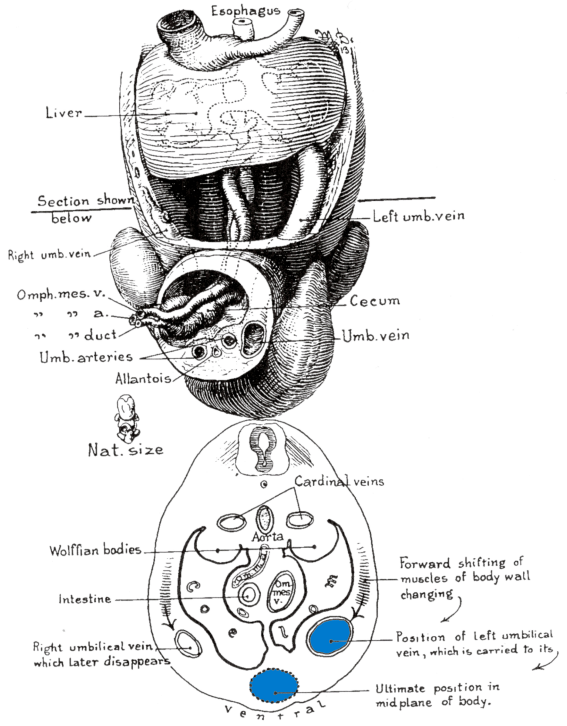

Figure 3. Another of Max Brödels drawings depicting the embryology of the umbilicus. The forward shifting of the body wall muscles changes the position of the left umbilical vein that is carried to its ultimate position in the midline of the body. (From Polin, R.A., Fox, W.W. (1998). Fetal and neonatal physiology. Philadelphia, Saunders, with permission)

The primitive venous system consists of three components, all of which are initially bilaterally symmetrical and converge on the right and left sinus horns of the sinus venosus: the cardinal system (drains the head, neck, body wall and limbs); the vitelline veins (drain initially the yolk sac); and the umbilical veins developing in the connecting stalk and carrying oxygenated blood from the placenta to the embryo. All three systems undergo extensive modifications during development. Regarding the umbilical vein system, the right umbilical vein disappears, while the left umbilical vein persists as the umbilical vein in the cord. It enters the abdomen to follow the inferior surface of the liver as the intra–abdominal portion of the umbilical vein that terminates as it branches into the liver parenchyma. At this point the portal sinus starts, and it ends at the junction with the right portal branch (Mavrides *et al.* 2001). The ductus venosus arises from this sinus (Mavrides *et al.* 2001; Kiserud *et al.* 2003). The main portal stem divides into a left and right branch, and all that is to the left of the main stem is the left portal venous system. For practical reasons the intra–abdominal umbilical vein can be regarded as connected to the ductus venosus. In this approach, the short section beyond the ductus venosus connected to the main portal stem is called the left portal branch (Kiserud 2001b; Kiserud *et al.* 2003).

The cord is covered by the amniotic epithelium, a cuboidal/squamous epithelium that is continuous with the squamous epithelium of the umbilicus and the cuboidal/columnar epithelium of the placental/membranous amniotic surface. This epithelium does not differ from the type covering the ventral body wall, but later on (during the3rd and 4th month) when a dermis is constituted over the embryonic body and over the proximal part of the cord, a keratinized, squamous epithelium is induced to cover both the body and the proximal part of the cord (Schramm 1962a). This transitional zone corresponds to the embryological umbilical ring. At term, this part of the cord extends 1.5 cm from the abdominal wall, providing cutaneous covering of the cord stump at birth (Schramm 1962b). The cord amnion is firmly adherent to the underlying connective tissue, Wharton´s jelly, which is derived from the extraembryonic mesoderm and is mucoid and compressible (by the distended umbilical vessels). Wharton´s jelly is nourished by diffusion, and like other avascular tissues it is composed of a ground substance rich in hyaluronic acid, collagen and chondroitin sulfate (mainly small chondroitin/dermatan sulphate proteoglycans), (Gogiel *et al.* 2003). There are sparse myofibroblasts (resembling vascular smooth muscle cells) embedded in collagen meshwork (Eyden *et al.* 1994; Nanaev *et al.* 1997), which are postulated to be part of the mechanism regulating cord turgor (Nanaev *et al.* 1997).

Since long it has been known that the architecture of the umbilical vessels is different compared to vessels of the body. The walls are much thicker, due to a rich supply of muscular elements arising from the single layer endothelium as interlacing spirally arranged fibers, passing from the lumen toward the outside of the muscle layer and forming a fine reticulum throughout (Chacko and Reynolds 1954). The vessels in the cord have certain morphologic characteristics. The vein, but not the arteries, has a well–developed lamina elastica interna, although the arteries have considerable elastic tissue within their media. The media in all the umbilical vessels consists of decussating helicoidal smooth muscle bundles that shorten to become nearly circular with contraction. The umbilical vessels are devoid of an adventitia and vasa vasorum. Wharton´s jelly, derived from extraembryonic mesoblast, consists of connective tissue and a small number of isolated muscle cells and forms the protective casing of the vessels and binds them into a single functional unit (Chacko and Reynolds 1954).

At present there is no consensus on the subject of autonomic innervation of the umbilical cord (Spivack 1943; Pearson and Sauter 1969; Pearson and Sauter 1970; Ellison 1971; Fujiyama *et al.* 1971; Reilly and Russell 1977; Fox and Khong 1990; Sexton *et al.* 1996), but the existence of nerve fibres in the paraumbilical segment is considered reasonably well established (Heifetz 1996).

1.2.2 Developmental physiology

The early fetus relies heavily on anaerobic pathways to support energy requirements, and the placental metabolism is essentially anaerobic for the first nine weeks (Beckman *et al.* 1996). After that time, the trophoblastic plugs are progressively dislocated, allowing maternal blood to progressively flow freely and continuously within the intervillous space. This process starts in the periphery of the placenta, and advances to the central area between 10 and 14 weeks of gestation (Jauniaux *et al.* 2003b). Simultaneously, the umbilico–placental unit is established, connecting the fetal heart with the capillary network of the villi (Jauniaux *et al.* 2000). Following this, there is a dramatic increase in pO_2 in the intervillous space from < 20 mm Hg (2–4%) at 10 weeks of gestation to > 50 mm Hg (10%) at 12 weeks (Jauniaux *et al.* 2000; Jauniaux *et al.* 2001). The fetal metanephros starts the production of urine, which is emptied into the amniotic cavity (Gulbis *et al.* 1996), and the exoceolomic cavity is gradually obliterated by the growing amniotic cavity (Jauniaux *et al.* 2003b).

Haematopoiesis starts in the wall of the yolk sac around 5–6 weeks of gestation, and the first haemoglobin synthesised is of the embryonic type (Peschle *et al.* 1985), which has a particularly high affinity for oxygen and is saturated at low pO_2 levels. Due to the low mean radius of the villous vascular system and high viscosity (all erythrocytes being nucleated), the resistance to flow in the early umbilico–placental circulation is high. This suggests that during the first two months of gestation the extraembryonic circulation is mainly vitelline, and that the chorioallantoic circulation is essentially limited to the chorionic plate (Jauniaux *et al.* 2003a).

1.2.3 Umbilical cord growth

The exact mechanisms in control of cord growth are unknown, but growth has by some authors been linked to the incidence of fetal activity and the availability of intrauterine space (stretch hypothesis) (Miller *et al.* 1981; Moessinger *et al.* 1982). Like all other endothelial cells in the vasculature, human umbilical vein endothelial cells are exposed to three types of mechanical forces: 1) fluid shear stress, due to blood flow; 2) wall stretch, due to compliance of the blood vessel wall tissue; and 3) fluid pressure, due to containment of blood within the lumen of the vasculature. Shear stress modulates human umbilical vein endothelical cell function through mechanosensors, which activate intracellular signalling pathways, leading to the transcription of specific genes (Illi *et al.* 2003). Several of the up–regulated genes are directly involved with structural and contractile properties of the cellular cytoskeleton (Garcia-Cardena *et al.* 2001). In this way, shear stress acts as a strong modulator of endothelial gene expression and cell growth. The response of human umbilical vein endothelical cell gene expression is found to be a function of the magnitude of shear stress (Chien *et al.* 1998). Sustained mechanical strain (wall stretch) has been found to trigger cell proliferation and vascular remodelling in vitro (Stula *et al.* 2000). In other words, the flowing of blood in the cord vessel and the effects created on the vessel wall is perhaps the single most important factor regarding vessel and cord growth.

Blood flow from the placenta to the fetus depends on human umbilical vein vascular tone. The contribution of human umbilical vein vascular tone to the regulation of umbilical blood flow was earlier regarded as negligible because the human umbilical vein was thought to operate at maximal vasodilatation already at resting conditions (Goodwin 1968; Paulick *et al.* 1991). More recent studies have shown that this assumption is not true. Human umbilical vein vascular tone has been shown to increase with rising local pO_2 and to decrease with declining pO_2 (Mildenberger *et al.* 1999), just like blood vessels of the systemic vasculature (Smith and Vane 1966; Siegel *et al.* 1991). In one study, vascular tone of the umbilical vein showed a decrease to 88% of resting tension at PO_2 values below the normal intrauterine PO_2 value, indicating that the human umbilical vein has a

vasodilator reserve at hypoxic conditions, and that this vasodilatation was endothelium dependent (Mildenberger *et al.* 1999). In fetal sheep, hypoxaemia causes reduced heart rate, reduced maximum and weighted mean blood velocity, and augmented pulsation in the umbilical vein (Kiserud *et al.* 2001). Vascular tone in the umbilical vessels is prone to regulation by a vast number of vasoactive agents (LeDonne and McGowan 1967; Dyer 1970; Winters and Dyer 1970; Altura *et al.* 1972; Park *et al.* 1972; Adamson *et al.* 1989; Haugen and Stray-Pedersen 1991). The endothelium of the umbilical vessels also has the capability of production of vasoactive substances, e.g., nitric oxide (Dimmeler *et al.* 1999), prostanoids (Haugen *et al.* 1990) and different cholinergic substances (Loesch 2002). Besides prostanoids, endothelial nitric oxide (NO) and endothelin (ET) are the most important locally released factors regulating vascular tone in the umbilical vein. There is a continuous basal release of NO from the vascular endothelium. Increased release of NO contributes to endothelium–dependent vasodilatation in response to various stimuli (Moncada *et al.* 1991). The hypoxic vasodilatation of the human umbilical vein has been found to be under the control of NO, ET being less important (Mildenberger *et al.* 2003).

Upon constriction, the venous vessel wall thickens about tenfold, the arterial wall even more, and the artery shortens by 20 % (Chacko and Reynolds 1954). This may be part of the explanation why there is no bleeding from the infant after incision of the cord without ligation (Rachmanow 1914). In 1914 Rachmanow published a study of 10.000 deliveries were the cord was cut when cord pulsation no longer could be felt (12–18 minutes after birth), without doing ligation of the fetal stump. In just 2.4 % of all these infants, a ligation had to be performed due to bleeding (Rachmanow 1914). Yao et al. found that the placenta was emptied within a few minutes after birth; at birth the blood distribution between infant and placenta was 67% and 33%, after 1 minute 80% and 20% and after 3 minutes 87% and 13% (Yao *et al.* 1969).

1.3 Some aspects of the fetal circulation

1.3.1 Cardiac function, output and blood pressure

Much of the present knowledge of fetal cardiac output and distribution is derived from studies of fetal sheep. Measurements of fetal cardiac output and its distribution have been done by employing radionucleotide–labeled microspheres, or by the application of electromagnetic flow transducers around the ascending aorta and the pulmonary trunk. During the last half of pregnancy, the combined ventricular output has been found to be 450–500 ml/min *x* kg of fetal weight (Rudolph and Heymann 1967; Rudolph and Heymann 1970; Anderson *et al.* 1981). During the past twenty years, Doppler measurement of the combined cardiac output has been possible on the human fetus, yielding quite similar results. The right ventricle ejects 1.2 times more blood than the left ventricle (Maulik and Nanda 1985; Kenny *et al.* 1986; Allan *et al.* 1987; De Smedt *et al.* 1987). In fetal sheep, about 90 % of the right ventricular output bypasses the pulmonary circulation via the ductus arteriosus to reach the descending aorta, and only 30 % of the left ventricular output passes the aortic arch to reach the descending aorta (Rudolph 1985). The umbilical–placental blood flow (measured by Doppler ultrasound technique) is approximately 120 ml/(min *x* kg fetal weight) in humans (Griffin *et al.* 1983; Erskine and Ritchie 1985), representing about 30 % of human fetal cardiac output, and 50–60 % of the flow in the thoracic descending aorta.

1.3.2 Umbilical venous blood flow

The normalised umbilical flow in human fetuses under physiological conditions is found to be 115 mL min^{-1} kg^{-1} at 20 weeks, decreasing to 64 mL min^{-1} kg^{-1} at term (Kiserud *et al.* 2000b). Others have found a stable weight–dependent umbilical vein blood flow through the last half of gestation (from 123 ml/min/kg to 109 ml/min/kg) (Gill *et al.* 1984; Sutton *et al.* 1990; Bellotti *et al.* 2000). High frequency transducers, memory buffers for selecting the optimal image, and the method of repeat measurements seem to make measurement of blood flow in fetal veins reliable (Kiserud and Rasmussen 1998; Kiserud *et al.* 1999). The

results obtained in sheep fetuses is generally higher, 100–250 min^{-1} kg^{-1}, and vary with techniques and experimental set–up, being lowest near term (Dawes 1968; Jensen *et al.* 1991). A different developmental physiology including higher growth velocity, higher temperature and lower hemoglobin concentration, may account for the higher flow rates in sheep (Kiserud 2000).

Studies on fetal lambs have shown that oxygenated blood returning from the placenta via umbilical veins flows through the DV and left hepatic vein (LHV), and is mainly directed towards foramen ovale and the left atrium (Edelstone and Rudolph 1979). Both animal experiments (Barclay *et al.* 1942; Behrman *et al.* 1970; Edelstone and Rudolph 1979) and a few experiments in the human fetus (Lind and Wegelius 1949; Rudolph *et al.* 1971), have demonstrated that there is a preferential streaming of umbilical blood through the foramen. Ultrasound studies under physiological conditions have demonstrated that this mechanism also operates in humans (Kiserud *et al.* 1991; Kiserud *et al.* 1992; Kiserud *et al.* 2000b). By means of acceleration of the blood flow through the ductus venosus (the velocity reaches 60–85 cm s^{-1} during the second half of pregnancy) and of direction, this blood is predominantly injected into the foramen ovale orifice, distending its valve and forcing blood into the left atrium (Kiserud 2000). Another difference between the human fetus and animal fetuses is the degree of shunting of umbilical blood through DV. In animal experiments it is found to be 40–50% of the combined cardiac output, in the human fetus under physiologic conditions it is reported to be less: 30–40% at 20 weeks and decreasing to 20% at 32 weeks, and remaining low for the rest of pregnancy (Bellotti *et al.* 2000; Kiserud *et al.* 2000b). The estimated decrease in weight–indexed DV volume blood flow was from 60 ml/min/kg to 17 ml/min/kg (Bellotti *et al.* 2000). This suggests that the fetal liver, receiving 80 % of the umbilical blood, has a high demand for oxygenated blood in late pregnancy (Kiserud *et al.* 2000b). In agreement with this observation, Rudolph et al. (Rudolph *et al.* 1991) demonstrated that experimental obstruction of DV in fetal sheep at term did not change oxygen delivery to the vital organs. The right hepatic vein and IVC carry the lowest oxygen saturated blood mainly from the fetal lower body to the right atrium and across the tricuspid valve to the right ventricle (Rudolph 1985). The distribution of the venous return is designed to optimise adequate oxygen supply to the organs vital for fetal survival: the brain, the heart and the adrenal glands. It has been estimated in studies on fetal lamb that during

hypoxemia or reduced umbilical flow, the blood shunted across DV may increase up to as much as 70% of the umbilical blood flow (Behrman *et al.* 1970; Edelstone and Rudolph 1979). Active dilatation of DV and increased shunting have also been observed in human fetuses (Rudolph *et al.* 1971; Bellotti *et al.* 1998; Tchirikov *et al.* 1998; Bellotti *et al.* 2004), but the degree of shunting is found to be considerable less than in sheep. The distribution between the flow to the liver and the ductus venosus is a delicate equilibrium that is easily altered by small changes in the umbilical venous pressure or hematocrit (Kiserud *et al.* 1997). In recent years, it has been shown that even in the growth retarded fetus, which shunts more of the venous blood to the DV, blood flow to the liver is maintained by increasing flow through the hepatic artery (Kilavuz and Vetter 1999). These data may indicate that the fetal liver has a higher circulatory importance during intrauterine development than has previously been suggested (Brezinka 2001).

The blood flow velocity in the umbilical vein both in the cord and in the intra–abdominal section is low and steady. According to fluid dynamic principles, such flows are laminar with a parabolic profile of the velocity distribution across the vessel (Hatle and Angelsen 1985). The mean velocity is half of the maximum velocity found in the centre of the vessel ($V_{mean} = 0.5V_{max}$). In the DV, the blood flow is accelerated, and the velocity profile becomes partially blunted (Pennati *et al.* 1997; Kiserud *et al.* 1998; Pennati *et al.* 1998). The mean velocity will be higher in relation to the maximum velocity ($V_{mean} = 0.7V_{max}$). By more powerful acceleration (e.g. in the outlets of the heart) the velocity profile will be blunted (Hatle and Angelsen 1985). In the DV, the velocity profile may be skewed to one side due to the inlet geometry, variation of vessel axis and curvatures, and may vary according to the tapering shape of the vessel and the pulsation imposed from the atrium (Pennati *et al.* 1997).

1.3.3 Umbilical venous blood flow in fetal disease

Both in animals (Fan *et al.* 1980) and in humans (Rosenkrantz and Oh 1982; Moise *et al.* 1990) there is a hyperdynamic circulation (Rightmire *et al.* 1986), with an increased cardiac output and a decline in blood viscosity in the fetus exposed to anemia, which tends to

preserve tissue oxygenation. Fetal anemia in humans has been shown to be associated with both umbilical vein dilatation (Jouppila and Kirkinen 1984b) and a high blood flow velocity (Kirkinen *et al.* 1981; Kirkinen *et al.* 1983; Jouppila and Kirkinen 1984b; Oepkes *et al.* 1994; Hecher *et al.* 1995b; Iskaros *et al.* 1998; Dukler *et al.* 2003). High blood velocities can also be observed on the arterial side of the circulation, and Doppler velocimetry of the peak velocities in the middle cerebral artery has turned out to be the most sensitive method for non–invasive diagnosis and follow up of this condition, (Mari *et al.* 2000; Detti *et al.* 2001; Dukler *et al.* 2003), the reason being that the brain circulation responds quickly to hypoxemia (Mari *et al.* 2000).

A variety of animal models have been created for the study of fetal growth restriction. In the pregnant sheep, repetitive embolization of the uteroplacental circulation has resulted in fetuses with characteristics similar to those of the growth restricted human fetus: low birth weight, low ponderal index, high brain weight/body weight ratio (Clapp *et al.* 1980; Clapp *et al.* 1982). A rapid, progressive, and persistent decrease in umbilical flow has been found to occur in growth–retarded group fetal lambs after microsphere embolization of the uteroplacental circulation. In contrast, a progressive increase in umbilical blood flow and decrease in umbilical vascular resistance were noted in controls (Clapp *et al.* 1980). All controls showed a significant increase in oxygen and glucose consumption during the remainder of pregnancy. The uptake curve for the growth retarded fetuses remained flat despite a significant increase in the venoarterial differences, clearly indicating that blood flow is the rate–limiting factor for oxygen and glucose consumption (Clapp *et al.* 1981). These studies suggest a strong relation between alterations in the uterine blood flow and that of the umbilical circulation, preceding the onset of fetal growth retardation. It is therefore likely that most cases of human growth retardation are haemodynamically mediated at some point, making Doppler technology an ideal diagnostic tool. For the last 20 years, human fetal growth restriction has been shown to be associated with changes in umbilical venous flow measured by Doppler. (Gill *et al.* 1984; Jouppila and Kirkinen 1984a; Laurin *et al.* 1987; Kiserud *et al.* 1994; Boito *et al.* 2002). Umbilical vein volumetric blood flow in fetuses with abnormal umbilical artery blood velocity waveforms was significantly lower than in control fetuses (63–98 ml/min/kg versus 117–124 ml/min/kg) at any gestational age between 25 and 38 weeks (Ferrazzi *et al.* 2000). Growth–restricted fetuses

with abnormal blood velocity waveforms in their IVC had significantly increased atrial natriuretic peptide levels, indicating increased systemic venous pressure in these fetuses (Capponi *et al.* 1997).

1.3.4 Umbilical vein pulsation

The atrial contraction wave gives rise to pressure waves transmitted into the central venous system (Reed *et al.* 1990; Reed *et al.* 1997). The duration of time from peak pressures in the inferior vena cava to decreases in velocity in the venous system (ductus venosus, intra–abdominal umbilical vein, and the umbilical vein in the cord) have been found to increase with the distance from the atrium (Schroder *et al.* 2003). The wave form of precordial venous flow velocity, expressed either by the absolute velocities of the different phases of the cardiac cycle or by a ratio, has become a widely accepted way of assessing fetal cardiac performance (Kanzaki and Chiba 1990; Reed *et al.* 1990; Kiserud *et al.* 1991; DeVore and Horenstein 1993; Hecher *et al.* 1994; Hecher *et al.* 1995a). Ductus venosus velocimetry in particular serves as a predictor for the early recognition of fetal chromosomal aberrations (Borrell *et al.* 1998; Matias *et al.* 1998a; Matias *et al.* 1998b; Antolin *et al.* 2001; Bilardo *et al.* 2001) and congenital heart malformations (Kiserud *et al.* 1993; Matias *et al.* 1999; Matias *et al.* 2000; Antolin *et al.* 2001; Bilardo *et al.* 2001). An increase in afterload results in augmented atrial contraction and a correspondingly increased pulsation of the venous blood flow velocity (Reuss *et al.* 1983). This effect is mediated by an increased adrenergic drive (Hasaart and de Haan 1986), and is also observed during hypoxemia in fetal sheep (Gudmundsson *et al.* 1999; Kiserud *et al.* 2001). Kiserud (Kiserud 1999b) found a 5 % rate of absent or reversed flow during atrial contraction in the ductus venosus in normal fetuses at 8–15 weeks gestation. Later, Germer et al. made a similar observation while screening for fetal chromosomopathies or congenital heart disease (Germer *et al.* 2002). This is in accordance with the occurrence of pulsation in the umbilical vein during early pregnancy (Rizzo *et al.* 1992) and with the concept of a generally increased propagation of pulse waves down the venous system at this stage of pregnancy. Other determinants for venous pulsations are the vessel diameter, the stiffness of the vessel wall (Hellevik *et al.* 1998; Hellevik *et al.* 2000) and the intravascular pressure.

Wave reflection at the ductus venosus inlet is regarded as the most important reason why the venous pulse transmission across the ductus venosus to the umbilical vein is so poor (Kiserud 1999a; Hellevik *et al.* 2000). The venous pressure wave from the heart will be partially reflected and partially transmitted when there is a change in impedance (Z) along the transmission line. The degree of reflection depends on the Reflection Coefficient (R_C), expressed as

$$R_C = \frac{\text{Reflected wave}}{\text{Incident wave}} = \frac{Z_{UV} - Z_{DV}}{Z_{UV} + Z_{DV}}$$

where Z_{UV} and Z_{DV} represent the impedance of the umbilical vein and the ductus venosus, respectively. If the impedance is the same in both sections, $R_C = 0$, there will be no reflection but full transmission. The most important determinant of impedance Z is the cross section of the vessel. Normally, the diameter of the umbilical vein is four times larger than that of the ductus venosus (Kiserud 1999a), which results in low transmission of waves. A similar relation exists for the IVC–ductus venosus junction, which is shown to be affected by fetal position. Fetal bending results in squeezing of the DV outlet (small diameter compared to IVC diameter), and the wave transmission is stopped, giving a non–pulsatile DV velocity tracing (Kiserud 2000).

In contrast to the pulsatile flow in precordial veins, the blood flow in the umbilical vein (UV) is usually steady. However, UV pulsation is a normal phenomenon in fetuses of 13 weeks or lower gestational age (Nakai *et al.* 1992; Rizzo *et al.* 1992; Van Splunder *et al.* 1996a). Part of the reason for these pulsations is thought to be the low compliance due to the small dimensions of the UV during the first part of the pregnancy (Hellevik *et al.* 2000). Both the stiffness parameter (the mechanical properties), and the size (the UV cross–sectional area), influenced the index of pulsation (IP) of the pressure in the UV in a mathematical model (Hellevik *et al.* 2000). UV pulsations have also been found late in the pregnancy in fetuses with no heart anomalies and with no sign of increased preload (Van Splunder *et al.* 1996b; Nakai *et al.* 1997b; Nakai *et al.* 1997a). In these cases the umbilical cord was either hypercoiled or compressed, and the umbilical venous pulsation occurred in these segments of the cord only, most probably due to a reduced UV compliance in these regions. In

general, the large dimension of the vein makes it function like a reservoir which requires a high amount of pulse energy to produce visible changes in blood velocity (Hellevik *et al.* 1998; Kiserud *et al.* 2003). However, increased stiffness of the wall (e. g. increased muscular tone), increased intravascular pressure (e. g. congestion), and small diameter (e. g. early pregnancy) all promote the transformation of pressure wave into kinetic energy and visible velocity waves. The cross–section of the vessel is usually the most decisive factor (Kiserud *et al.* 2003). If this converted energy results in a velocity increase (as seen in the left portal vein during atrial contraction) the velocity wave and the pressure wave have the same direction. In contrast, when they have opposite directions (as seen in the ductus venosus during atrial contraction), a decrease in flow will be noticed (Kiserud *et al.* 2003).

1.3.5 Umbilical vein pulsations in fetal disease

Lingman et al showed that fetuses with signs of distress had umbilical venous flow pulsation (Lingman *et al.* 1986). In the years to follow it was shown that pulsation occurred more commonly in growth–restricted fetuses (Nakai *et al.* 1992; Kiserud *et al.* 1994; Gramellini *et al.* 2001) and fetuses with cardiac malformations (Kiserud *et al.* 1993). The phenomenon was also identified as a poor prognostic factor in the fetus with nonimmune hydrops (Gudmundsson *et al.* 1991). It is now integrated as a regular part of the fetal haemodynamic evaluation (Huhta 2001).

An increased intravascular and transmural pressure in the venous system reduces compliance, increases the speed of the wave, and promotes the transport of pulsation further to the periphery. The distance that these pressure waves are transmitted is determined by the central venous pressure, the venous compliance and the force of atrial contraction. The abnormal end–diastolic umbilical venous pulsation in the cord has been shown to be an ominous sign of the severely compromised fetus with congestive heart failure (Gudmundsson *et al.* 1996). A further development would be double pulsation, especially if extending to the cord. This sign has been connected to poor prognosis in high–risk pregnancy (Hofstaetter *et al.* 2001). Even triphasic patterns have been described, in association with tricuspid regurgitation and increased end–diastolic ventricular pressure

due to myocardial dysfunction as a late consequence of long–lasting placental compromise. (Baschat and Gembruch 1996).

Umbilical venous pulsations in ventricular systole are sometimes seen in oligohydramniotic fetuses and fetuses with an arteriovenous fistula. The systolic pulsation is thus transmitted directly to the local venous flow signal (Nakai *et al.* 1997b). In severe placental insufficiency, pulsations in the umbilical vein, increased reverse flow component in the fetal IVC and hepatic veins, and decreased or reversed flow component in DV during the atrial contraction have been observed (Hecher *et al.* 1995a). It has been demonstrated that growth restricted fetuses with pathological umbilical venous pulsations have significantly lower pH and pO_2 values and higher pCO_2 values than those without pulsations (Rizzo *et al.* 1995).

1.4 Umbilical cord complications

Nuchal cord entanglement is reported in approximately 20 –30 % of all deliveries (Dippel 1964; Spellacy *et al.* 1966; Lamberti *et al.* 1973; Sornes 1995; Osak *et al.* 1997; Lackman *et al.* 2001). A clear association between fetal cord length, fetal gender and risk of nuchal cord have been established, male fetuses with long umbilical cords bearing the highest risk (Horwitz *et al.* 1964; Rhoades *et al.* 1999). In a group of patients with nuchal cord entanglement, spontaneous fetal movements caused transient complete cessation of the umbilical venous flow (Ramon y Cajal 2002). A similar effect was observed when applying external pressure on the maternal abdominal wall for short periods of time (1–2 seconds) (Ramon y Cajal 2002). Nuchal cord entanglement has also been associated with increased risks of fetal distress (Dhar *et al.* 1995; Jauniaux *et al.* 1995; Larson *et al.* 1995; Rhoades *et al.* 1999), operative delivery (Dhar *et al.* 1995; Jauniaux *et al.* 1995; Larson *et al.* 1995), one–minute Apgar score <7 (Jauniaux *et al.* 1995), and five–minute Apgar score <7 (Rhoades *et al.* 1999). The condition has also been associated with increased risk of need for neonatal resuscitation and admission to the neonatal intensive care unit (Jauniaux *et al.* 1995). Multiple coils of umbilical cord around the fetal neck at delivery have been found to

be frequent (15 % of all pregnancies) and to interfere more intensely with the condition of the neonate at birth than did a single nuchal cord loop. In this subgroup, the incidence of low arterial and venous pH, low Apgar score at 1 minute, of meconium stained amniotic fluid and obstetric or neonatal interventions, was more common than in the group with a single loop around the neck only (Jauniaux *et al.* 1995). In addition to these short–term effects, more lasting consequences have been described, such as a lower weight/placental weight ratio (BW/PW) in pregnancies with a nuchal cord entanglement. It has been suggested that restriction of the transfer of nutrient to the fetus and thus growth, or a pooling of blood in the placenta, may be responsible for this shift in weight development (Osak *et al.* 1997). In a large Canadian study (more than 27.000 infants included), infants with birth weights ≤ 10^{th} percentile were more likely to have a nuchal cord noted at the time of delivery, whereas in infants with birth weights ≥ 90^{th} percentile this was less likely (Lackman *et al.* 2001).

Cord knots are uncommon (0.3 to 2.1 % of all births), but because the condition is associated with increased perinatal mortality (Blickstein *et al.* 1987; Joura *et al.* 1998) perhaps due to arrest of the umbilical venous blood flow, it is worth mentioning. Knots are associated with long umbilical cords, polyhydramnios, small fetus and monoamniotic twin pregnancy (Blickstein *et al.* 1987). The sonographic diagnosis of the condition is difficult (Sepulveda *et al.* 1995), and just a few cases have been reported (Jeanty 1989; Collins 1991b; Collins *et al.* 1993; Gembruch and Baschat 1996). There is one report of a stenotic effect in the umbilical vein du to the presence of a cord knot. A clear post–stenotic acceleration with blood flow velocity of 100 cm/s was demonstrated, showing a pulsatile pattern, compared to the low velocity (15 cm/s) non–pulsatile flow pre–stenotically (Gembruch and Baschat 1996). The blood flow velocities were found to normalise to pre–stenotic levels approximately 2 cm distal to the stenosis, and no other region of the umbilical vein had pulsatile blood flow velocities.

Significant reductions in the size of the umbilical cord are referred to as stricture, torsion and coarctation. Stricture of the umbilical vein is repeatedly reported in umbilical cord abnormalities and intrauterine demise in early gestation. These abnormalities are not uncommon, and are frequently found at the abdominal wall of the macerated fetuses with

long, heavily spiralled cords (Javert and Barton 1952; Benirschke 1994). A significant number of fetal deaths in early gestation is associated with umbilical cord abnormalities. The most common anomaly found was constriction at the fetal end of the cord, eventually associated with hypercoiling, accounting for 11% of the miscarriages in one study (Singh *et al.* 2003). Strictures are said to be less common in later pregnancy (Benirschke 1994), but in the second half of pregnancy umbilical cord complications have been found to be associated with stillbirth in 9 % of cases, in a prospective autopsy study (Petersson *et al.* 2002). Numerous case reports support the hypothesis that twisting, stricture or externally imposed constriction may cause complications and fetal demise (Weber 1963; Gilbert and Zugibe 1974; Tavares Fortuna and Lourdes Pratas 1978; Virgilio and Spangler 1978; Robertson *et al.* 1981; Ahrentsen and Andersen 1984; Ghosh *et al.* 1984; Labarrere *et al.* 1985; Glanfield and Watson 1986; Kiley *et al.* 1986; Herman *et al.* 1991; Benirschke 1994; Hallak *et al.* 1994; Sun *et al.* 1995; Bakotic *et al.* 2000). All of these reports have some features in common: (1) The phenomenon is most often confined to the fetal end of the cord, close to the fetal abdomen, and rarely at the placental end (Weber 1963; Virgilio and Spangler 1978; Glanfield and Watson 1986) or in multiple sites along the cord (Ghosh *et al.* 1984; Sun *et al.* 1995). (2) Absence of Wharton's jelly, stenosis, or obliteration of cord vessels at the narrow segment and intravascular cord thrombosis are the major pathological features. (3) Fetal death associated with cord constriction characteristically occurs in the middle trimester.

The absence of Wharton's jelly and replacement of the stroma by dense collagenous tissue is thought to be the primary defect (Robertson *et al.* 1981; Sun *et al.* 1995). King likened the normal cord to a thick rubber band, with the constricted part behaving more like a cotton string, easily twisting when committed to torsion (King 1926). Interestingly, the constriction most often occurs at the (embryological) umbilical ring, which in fetal life is the transitional zone, where the amniotic epithelial covering of the distal cord meets the malpighian keratinized epithelium continuos with the covering of the fetal abdominal wall. The single layer amniotic epithelium rests directly on Wharton's jelly, while the multilayer, keratinized epithelium rests on the dermis (Schramm 1962b). Histological sections indicate a gradually diminishing amount of Wharton's jelly towards this transitional zone (Schramm 1962b;

Schramm 1962a), which makes this portion of the cord more vulnerable to mechanical forces (Benirschke and Kaufmann 2000).

The twist or coiling of the umbilical blood vessels is established by the ninth week of gestation in about 95% of all pregnancies (Lacro *et al.* 1987). The cause of umbilical vascular coiling is unknown, but there are several hypotheses: fetal movement, different umbilical vascular growth rates, fetal haemodynamic forces and umbilical vascular wall mechanics (Strong *et al.* 1993). Vascular coiling is believed to confer turgor to the umbilical unit, producing a cord that is strong but flexible and able to resist external forces that might compromise umbilical vascular flow (Lacro *et al.* 1987; Strong *et al.* 1993). Gestational diabetes mellitus is associated with both reduced and increased coiling (Ezimokhai *et al.* 2000; Ezimokhai *et al.* 2001), while FGR is associated with both increased coiling of the umbilical cord (hypercoiling) (Nakai *et al.* 1997b; Machin *et al.* 2000) and absence of coiling in combination with a lean cord with reduced amount of Wharton's jelly (Goodlin 1987; Raio *et al.* 1999; Di Naro *et al.* 2001). An association of noncoiled umbilical cords with known causes of reduced fetal activity, such as multiple pregnancy, oligohydramnios, and chromosomal abnormalities, has been demonstrated (Strong *et al.* 1993; Strong *et al.* 1994). An in vitro study failed to show any difference in umbilical venous flow between coiled and noncoiled cords when external compression, twisting and longitudinal stretching were applied to the cord segments (Dado *et al.* 1997), while another study established a significant inverse relationship between vascular coiling and susceptibility to cord venous occlusion when traction was applied to a cord encirclement (Georgiou *et al.* 2001).

Varix in the umbilical cord is believed to be associated with a poor outcome and is mostly an autopsy finding (Ghosh *et al.* 1984; Heifetz 1988; Schrocksnadel *et al.* 1991; White and Kofinas 1994). In the intra–abdominal portion of the umbilical vein, varix is a rare finding, representing 4% of the malformations of the umbilical cord (Konstantinova 1977). It can be detected prenatally on ultrasonography and appears as an oval, elongated or fusiform dilatation of the umbilical vein within the fetal abdomen, usually close to the anterior abdominal wall (Estroff and Benacerraf 1992; Mahony *et al.* 1992; Zalel *et al.* 2000; Rahemtullah *et al.* 2001). No criteria are defined for the size of the varix, but the diameter has been compared to the diameter of the nondilated portion of the intrahepatic umbilical

vein. In one study the distension of the umbilical vein varix was about 2 times this diameter (Rahemtullah *et al.* 2001). The vascular nature of the umbilical vein varix can be confirmed by Doppler ultrasonography, thereby excluding nonvascular causes of a cystic mass in this region. Some authors have reported normal outcomes only (Estroff and Benacerraf 1992; White and Kofinas 1994) while others have found an increased risk of fetal anomalies and poor pregnancy outcome (Fuster *et al.* 1985; Mahony *et al.* 1992; Sepulveda *et al.* 1998). In a recent review of 44 cases, a varix was found to be associated with a fetal death in 10 , aneuploidy in five, and hydrops in four of them (Zalel *et al.* 2000). There appears to be two different groups of fetuses with this finding: one of presumptive normal fetuses, showing no problems in the perinatal period, and another displaying malformations and problems in the perinatal period. Interestingly, in the former group, some fetuses also showed symptoms of heart strain. In one study a fetus diagnosed with a varix at 19 weeks, cardiomegaly was observed from weeks 25–32. The condition resolved spontaneously, and after birth the cardiac function was normal and at two years follow–up there was no sign of sequelae (Estroff and Benacerraf 1992). Another report noticed pericardial effusion in a fetus with an umbilical vein varix, which also disappeared at birth (Rahemtullah *et al.* 2001).

The cause of the umbilical vein varix is unknown. One of the hypotheses is that the dilatation is due to an intrinsic weakness in the wall of the dilated portion of the extrahepatic portion of the umbilical vein (Mahony *et al.* 1992), but autopsy studies have failed to confirm this (Konstantinova 1977). On the arterial side of the circulation poststenotic dilatation is a well–known phenomenon. It appears as a dilation of the vessel wall 1–3 cm distal to the area of a partial stenosis (Roach 1963; Roach and Harvey 1964). It commonly exist in the normal human, as it is reported that 72% of normal common femoral arteries exhibit some degree of dilation where the vessels emerge from under the inguinal ligament (Lord *et al.* 1979). Usually, poststenotic dilation occurs distal to coarctation of the aorta, abdominal aorta, or pulmonary arteries (Vito *et al.* 1975), but the phenomenon is also demonstrated to exist on the venous side of the circulation (Sugimoto *et al.* 2002). The exact flow disturbance that causes poststenotic dilation is uncertain. However, biomechanical forces (abnormal shear stresses and vortices/turbulence) causing the vessel wall to vibrate are thought to produce alterations in wall elastin and possibly in vascular smooth muscle tone to produce the distension (Dobrin 1991; Stehbens 1999). Descriptions of colour Doppler

examinations showing vortex formations in the varix (Rizzo and Arduini 1992; Zalel *et al.* 2000) strengthen this assumption. As for turbulence to occur a "Reynolds number" of 2000 must be reached. Reynolds number is a dimensionless parameter of flow that is directly related to the density of the fluid, the diameter of the vessel and the velocity of flow, and is inversely related to the viscosity of the fluid. It is an indicator of when the inertial stresses in a flow becomes sufficiently large compared with the viscous stresses to cause a breakdown of the laminar flow, first to a transitional state (with vortex formations) and then to a turbulent regime. The critical Reynolds number gives the condition below which the flow remains unaffected by disturbances, i.e. remains laminar. Transition is caused by instabilities in the flow which, when the Reynolds number is sufficiently high, leads to turbulence. In steady flow, if U is the mean flow velocity through the tube, d is the diameter of the tube, v is the viscosity, it is found that flow breakdown occurs at a value of Re_d ($=Ud/v$) of at least 2000 (Wood 1999; Schlichting *et al.* 2000).

Umbilical cord thrombosis is a rare event, with an incidence of only 1/1300 deliveries among prospectively examined placentas (Heifetz 1988). In retrospective studies the condition is linked to late gestational fetal distress or death. This was not so in a prospective study (6 fetuses of 7738 examined with cord thrombosis) were all born alive with no sign of fetal distress (Heifetz 1988). Male fetuses are affected more often than female fetuses (Heifetz 1988). The umbilical vein is affected by thromboses more often than the arteries (85% of cases, 23% of cases in combination with one or both arteries) (Heifetz 1988). Interestingly, cases of thrombosis in intra–abdominal umbilical vein varix have been reported (Leinzinger 1969; Schrocksnadel *et al.* 1991; Mahony *et al.* 1992; Allen *et al.* 1998; Viora *et al.* 2002), suggesting that umbilical vein varices could be associated with increased risk of thrombosis.

1.5 The ultrasound examination

1.5.1 Physics

The transabdominal transducer

Sweeping the ultrasound beam transversely over the examination field will create two–dimensional images of the organs. Movement of the beam can be performed by mechanical rotation of the transducer, called mechanical scanning, or by various kinds of electronic arrays. Transducers for diagnostic medical ultrasound for the transabdominal approach are usually calibrated in the range 2 – 10 MHz. In obstetrical ultrasound the most common frequencies are in the range 3 – 5 MHz, but frequencies up to 7.5 MHz are occasionally used. When using the transabdominal approach, we encounter several well–known physical problems: the abdominal wall with its different layers consisting of muscles, tendon and fat creates acoustic noise, like reverberations and phase front aberrations, in the ultrasound image. Another problem in early pregnancy is the distance between the transducer and the embryo lying deep in the pelvis, which requires the use of low ultrasound frequencies, resulting in images with low resolution (coarse–grained).

Mechanical sector scanners use electric motors to rotate or oscillate the active transducer elements for sweeping the ultrasound beam to scan the tissue plane (Ebina *et al.* 1967; Griffith and Henry 1974; Feigenbaum 1981). The transducer elements are encased in a fluid–filled enclosure with a flexible membrane that provides an acoustic coupling with the skin. A rotating transducer head is most common, since it offers a wide scan angle and provides constant sweeps with greater uniformity of line distribution in the scan field. This scanhead is also less prone to vibrations. For duplex function, most mechanical systems use the same transducer elements for imaging and Doppler scanning. The Doppler sample volume is placed at the desired target by use of the cursor line representing the subsequent Doppler beam path. To obtain a satisfactory Doppler scanning, the transducer must be

kept in position for several seconds, during which the real time image is frozen. The advantages with the mechanical sector system is the improved image resolution and Doppler sensitivity due to the greater dimensions of the transducer crystals and acoustic focusing (Maulik 1996; Angelsen 2000). The main disadvantage is the discontinuity between these two functions. For the studies integrated in this thesis, annular array mechanical sector scanners with options for 2D colour flow mapping and pulse wave (PW) Doppler were used.

In most modern transducers, the ultrasound beam is formed with electronic arrays. The bars of elements are mounted on a backing with fill–mass between the elements, either on a central plate in concentric rings (*annular array transducer*), or in a linear array (*linear array transducer)*.

All designs of the *annular array transducer* are made to sweep the ultrasound beam through a pie–shaped wedge or sector with an opening angle ranging from 30 to 100 degrees. Due to the wide elements, high frequencies can be applied. The sector image is created by rapid to and fro movements of the annular array. This system has the advantage of symmetric focusing, improving the spatial resolution also in the elevation plane, resulting in thin ultrasound slices of high quality. Their high manoeuvrability and their ability to visualise large areas at greater depths through small acoustic windows surpass the limited view of superficial structures by sector scanners. In the phased–array type of transducers (elements may be arranged in annular or linear arrays) all the elements of an array are used for each interrogation pulse, and variable time delays are introduced between the various elements, both on transmission and reception, to ensure that effective launching and reception of an acoustic wave occur predominantly in one defined direction.

The *linear array transducers* have one–dimensional arrays where the ultrasonic beam are focused in the scan plane (the azimuth plane) only by controlled of the excitation of the elements. The distance between two elements in the array is called “pitch”. In the plane perpendicular to the scan plane (elevation plane), the beam has a fixed focal distance with a relatively thick slice. An impedance matching layer is usually a single layer $\lambda/4$ thick, placed in front of the elements. The function of this layer is to shorten the emitted pulse (by

restraining the “ring–down” of the emitted pulse). Imaging very small structures may be affected by the poor resolution in the elevation plane. The scanning process involves steering of the beam by appropriately varying the individual time delays (Somer 1968). To achieve this, the distance between the centres of the single elements (pitch), must be less than $\lambda/2$ (Angelsen 2000). This type of transducer has a small aperture and produces a sector scan. Due to the low pitch of this transducer ($<\lambda/2$), it cannot be manufactured to emit very high frequencies.

The steering of the ultrasound beam has been a challenge, especially in linear array transducers. During recent years, there have been considerable achievements in computer–technology, allowing sophisticated, high–speed, computer–controlled pulsing of the individual elements circuitry. The "steered–beam, phased–array" system requires a unique total element pulse sequence for each scan line (typically 128), since each line has its own unique angle with respect to the transducer face in the sector format. Electronic focusing on both transmitting and receiving (similar to annular array designs) provides a longer focal zone with a narrower beam width than conventional single element designs. Similar to linear array designs, focusing in the direction at right angles to the scan plane determines the slice thickness and is accomplished by use of acoustic lenses. Since the beam path is electronically controlled, the direction (vector) of each A–line can be selected at random. This unique advantage over mechanical designs allows the system to perform simultaneous B–mode imaging and M–mode or Doppler functions.

Resolution of the ultrasound image

Two important factors are crucial for the quality of the ultrasound images:

1. Spatial resolution
2. Contrast resolution

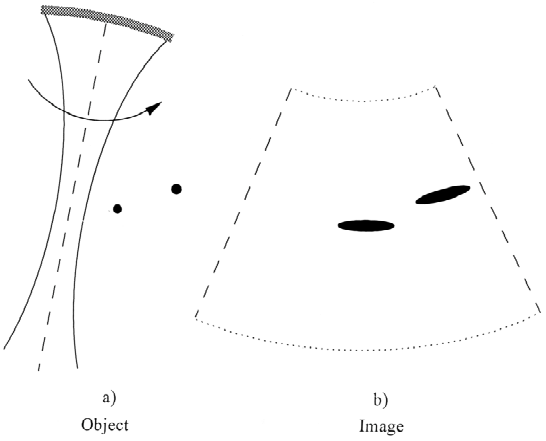

Figure 4. The smeared out images in b), from the point targets in a), defining the spatial resolution. (From B. A. J. Angelsen: "Ultrasound Imaging", Emantec, Trondheim, 2000, http://www.ultrasoundbook.com).

1. Spatial resolution has three components: Axial–, lateral and transversal resolution. Axial resolution is influenced mainly by the ultrasound frequency used and very little by the design of the transducer. In contrast, the spatial resolution in lateral and transverse planes is dependent on both the design of the probe, the method used for its focusing and the processing of the signal pathway from probe to output as image. In fact, spatial resolution is determined by the smallest distance between two points or surfaces at which two identifiable signals can be distinguished (Angelsen 2000).
2. The contrast resolution describes the ability to detect small variations in the intensity of the back–scattered signal form targets located close to each other. Signal generated noise is the main problem, caused by sidelobes and multiple reflections of the ultrasound pulse (reverberations). Electronic noise generated in the receiver will also limit the contrast resolution (Angelsen 2000). The contrast resolution is sometimes referred to as the dynamic range of the image, and often presented in decibel (dB). Due to the nature of the problem, it is not possible to increase the signal to noise ratio by increasing the transmitted power.

Doppler investigations

Christian Doppler (1803–1853) was the first to describe the frequency shift that occurs when sound or light is emitted from a moving source relative to the observer. The change

in relative motion between the observer and the object is known as the Doppler shift. The Doppler shift (Fd) can be calculated with the following formula: $Fd = 2vf\cos\alpha/c$, where v is the speed of the moving target, f is the frequency of the emitted pulse (i.e. the frequency of the transducer), α is the angle between the direction of emitted sound wave and the direction of the moving target, and c is the average speed of sound within the tissue) (Angelsen 2000). Doppler shift is dependent on the speed of blood flow, the angle between the transducer and the blood vessel, and the operating frequency of the Doppler transducer (Angelsen 2000). The Doppler angle in these equations is measured from the B–mode image of the vessel. The $\cos\alpha$ in the denominator of the last equation requires that the Doppler angle be less than about 70° (the less the better), otherwise a small error in α will produce a large error in the measured velocity. The acoustic velocity in blood is often assumed to be 1.54×10^5 cm/s. The Doppler shift of the moving blood is monitored continuously to form the Doppler signal. Because the transmit frequency is about 2 to 4 MHz, the Doppler shift of moving blood is within the audible range, e.g., ~2 kHz, and can thus be heard through a pair of stereo loudspeakers. The forward Doppler signal is made audible through one loudspeaker and the reverse Doppler signal is made audible through the other loudspeaker. The resulting sound is distinct and provides feedback to the skilled operator, allowing the appropriate placement of the Doppler sample volume.

There are three main techniques for making Doppler ultrasound velocity measurements of blood flow; *continuous wave Doppler, pulsed Doppler, and colour Doppler.*

Continuous wave Doppler

Continuous wave Doppler will be mentioned briefly, as it was not in use in this work. In these systems, there are two different transducers, one emitter and one receiver operating continuously. The transmitted and reflected beams begin to overlap a short distance from the surface of the probe, and the overlap extends until the beams attenuate (Angelsen 2000). When several vessels are focused within the sensitive volume, the Doppler signals are superimposed and detected simultaneously (Gill 1987). This explains why investigation

of certain locations is not possible. On the other hand, continuous Doppler ultrasonography is not dependent on the depth of the location and speed of the blood flow.

Pulsed wave Doppler

Pulsed wave Doppler (PW) ultrasonography is a system with range resolution, allowing selection of the location where the Doppler signals are obtained. This is made possible by ultrasound waves emitted in pulses whereby only waves from certain areas return before the next pulse is transmitted. In order to analyse reflected waves during a certain time period after pulse transmission, a sample volume can be set located in a predetermined range (Gill 1987). The axial length of the sample volume is determined by the time period during which the gate is open. Changing frequencies of waves reflected from a moving target limits the use of pulsed Doppler. Each Doppler signal is sampled once for every pulse transmission, and the sampling frequency is hence equal to the pulse repetition frequency, PRF for the instrument used. The sampling frequency is $f_s = 1/T_s$, where T is the elapsed time between transmitted pulses. To avoid frequency aliasing with the PW Doppler, the Doppler shift must be less than half the sampling frequency (PRF), $f_D < f_s/2$. This is the requirement of the Shannon sampling theorem for error free reconstruction of a sampled signal, and is referred to as the Nyquist limit of the frequency (Angelsen 2000). If this limit is exceeded, aliasing occurs, produced by a frequency shift that is wrong both in magnitude and direction. Thus, the higher blood velocitites to be recorded, the higher pulse repetition frequency is needed for a correct reproduction. However, the high pulse repetition restricts the range where recording is possible. The velocity of the blood flow and the depth of the object are the most important limitations of pulsed Doppler ultrasonography.

Colour Doppler

In multi range gated (MRG) pulsed Doppler instruments the backscattered signal from a transmitted pulse is range gated for a multitude of depths. The MRG unit sweeps the beam across the vessel and samples the return signal at the multiple depth ranges along a pulsed ultrasound beam and estimates the Doppler shift in frequency at each depth (Maulik 1996;

Angelsen 2000). The Doppler shift in each sample volume is proportional to the radial component of the blood velocity in the cell. In this manner the MRG unit generates a profile of the radial component of the blood velocity along each beam direction. These profiles generate a colour–coded pattern, and the colour Doppler image is created. Flow towards the transducer is in most systems visually demonstrated as red and flow away from the transducer as blue, while non–moving targets remain grey. The saturation of the colour is related to the velocity of the flow. The limitations of colour flow imaging are similar to pulsed Doppler ultrasonography. Generally speaking, the scan methods used to generate 2D images equals the method used to generate a flow image, but there are two major differences. One is that the spatial changes of the blood velocities occur less rapidly than the spatial variation in the tissue structures. Thus the colour flow image is hampered by less spatial resolution than the tissue (2D) images. 2D images are based on 512 samples along each ultrasound line compared to 64–128 samples for a colour flow image. Secondly, the frame rate for colour flow imaging is much slower, due to the transmittance of several pulses (4–16) in each beam direction (Angelsen 2000).

1.5.2 Safety

Mechanical index is an empirical factor that is correlated to the formation of bubbles in living tissue (cavitation). It is defined as

$$\mathbf{MI} = \mathbf{P}_{\mathbf{neg}}/\sqrt{\mathbf{f}}$$

where $\mathbf{P}_{\mathbf{neg}}$ is the negative amplitude of the pressure in Mpascal (pressure generated in the tissue by the ultrasound wave) and **f** is frequency of the transmitted signal in MHz. The physical argument for dividing with $\sqrt{\mathbf{f}}$ is that a bubble requires time to develop.

Table I. Magnitudes of frequency and intensity dependent variable MI (Mechanical index, given as $P/\sqrt{f}$). The I_{spta} for the transducers used in our study were considerable lower (50 mW/cm^2), and will give a lower MI than listed in this table.

P *negative amplitude of the pressure in Mpascal (pressure generated in the tissue by the ultrasound wave)*

f *frequency of the transmitted signal in MHz.*

ω *Fourier transform variable for time*

k *real component of wave number*

(From B. A. J. Angelsen: "Ultrasound Imaging", Emantec, Trondheim, 2000, http://www.ultrasoundbook.com/.)

f	MHz	1	1.5	2	3.5	5	7	10	20	30	60
ω	10^6 s^{-1}	6	9	13	21	31	42	63	126	188	377
k	10^3 m^{-1}	4.0	6	8.1	14	20	28	40	81	121	242
$P/\sqrt{f}$ 0.1W/cm^2	10^{-2}	6	4.9	4.2	3.2	2.7	2.3	1.9	1.34	1.1	0.77
$P/\sqrt{f}$ 1W/cm^2	10^{-2}	19	15.5	13.4	10.2	8.5	7.2	6	4.3	3.5	2.5
$P/\sqrt{f}$ 10W/cm^2	10^{-1}	5.9	4.8	4.2	3.15	2.7	2.3	1.9	1.3	1.1	0.8
$P/\sqrt{f}$ 50W/cm^2	10^{-1}	13.1	10.7	9.3	7	5.9	5	4.1	2.9	2.4	1.7
$P/\sqrt{f}$ 100W/cm^2	10^{-1}	18.5	15.1	13	9.9	8.3	7	5.9	4.1	3.4	2.4

Mechanical index indicates cavitation potential in the tissues, and thus the frequency of ultrasonographic pulses at any time. Increased pulse frequency results in proportionally lower mechanical index values. Capillary bleeding has been observed in the lung after exposure of neonatal, young and adult mice (Child *et al.* 1990; O'Brien and Zachary 1996; Dalecki *et al.* 1997b), swine (O'Brien and Zachary 1996; Dalecki *et al.* 1997a) adult rats , rabbits (O'Brien and Zachary 1996) and monkeys (Tarantal and Canfield 1994) to diagnostically relevant, pulsed ultrasound. Thresholds for capillary bleeding in adult mice and neonatal and young swine are of the order of 1 MPa at 2 MHz, which is within the range of output values of commercially available diagnostic ultrasound systems. This particular effect results from a cavitation–related mechanism of interaction that seems to depend on the presence of a tissue/gas interface. It is therefore unlikely to be implicated in fetal tissue. However, unexplained nonthermal lesions have been observed near tissue/bone boundaries when exposed to pulsed ultrasound at unusually low pulse

repetition frequency (Dalecki *et al.* 1997c). Research data are quite limited on nonthermal effects on embryonic development. The measured cavitation pressure amplitude threshold has been found to depend almost linearly on frequency, with a slope of about 5.3 MPa MHz^{-1} (Hynynen 1991). Focused ultrasound systems with low emitting frequencies, could expose the tissue for energies above the critical point for cavitation (Hynynen 1991). This is particularly important for the ultrasound modalities that provide the highest energy deposit in tissue, low pulse repetition Doppler with a focused beam being the most dangerous. Theoretical predictions in a computer model indicate that tissue rheology and, in particular, elastic properties should be considered in investigations of ultrasound cavitation bioeffects (Allen and Roy 2000). It is known that cavitation potential can be of major concern when intensities exceed 3300 mW/cm^2. Fetal Doppler studies usually use emitted levels below 100 mW/cm^2, and in our studies the setting was ≤ 50 mW/cm^2.

Homeothermic animals, including humans, can experience body temperature elevations induced by febrile infections, heavy exercise and hot environments. In each species of animal, the critical threshold temperature is an elevation of approximately 2 to 2.5° C above its normal resting temperature known to cause a syndrome of embryonic resorption, abortion, and malformations in experimental animals (Edwards 1986; Edwards 1993; Edwards *et al.* 1995). Heat is effective as a teratogen only when a susceptible stage of development and a threshold temperature elevation coincide (Edwards *et al.* 1995). Thermal index is an indicator for tissue heating by diagnostic ultrasound, set as an estimate of the tissue temperature rise in ºC, which might be possible in “reasonable worst case conditions". It is divided into soft tissue, bone and cranial thermal indices. Areas most likely to absorb ultrasonic energy are those directly associated with bone and at muscle/bone interfaces (Lehmann *et al.* 1967; Drewniak *et al.* 1989; Carstensen *et al.* 1990). Proliferating embryonic and fetal neural tissue is especially susceptible to thermal injury (Edwards 1986; Edwards 1993; Edwards *et al.* 1995). Ultrasonographic equipment has to display the emitted energy in settings in which thermal and/or mechanical indices might be over or equal to 0.4 (ECMUS 1999a; ECMUS 1999b). Sample graphical displays showing duration of each ultrasound mode, thermal and mechanical index levels and overall elapsed time of scan and modes, have been developed to keep ultrasound exposure as low as possible (Deane and Lees 2000). Diagnostic exposure that produces a

maximum in situ temperature rise of no more than 1.5ºC above normal physiologic level (37ºC) may be used clinically without reservation on thermal grounds (Ziskin and Barnett 2001). Fetal temperature elevation above 41ºC for five minutes or more is considered hazardous (Barnett *et al.* 1997). The risk of inducing thermal effects is greater in the second and third trimesters, when fetal bone is intercepted by the ultrasound beam and significant temperature increase may occur in the fetal brain (Ziskin and Barnett 2001).

In animal ultrasonographic studies during organogenesis and later pregnancy, no long–term ramifications, abortions, gross malformations, or stillbirths have been observed in the exposed animals (Tarantal and Hendrickx 1989b; Tarantal and Hendrickx 1989a; Tarantal *et al.* 1993; Tarantal *et al.* 1995). Only in the most recent of these studies were doses of exposure (I_{spta} 645 to 714 mW/cm^2) presently allowed to be used in obstetrical ultrasound examinations according to the latest US FDA regulations applied (NCRP 2002). Follow–up studies after human fetal ultrasonographic exposure has been summarised in a Cochrane review (Neilson 2004) which concludes that there is no statistically significant difference in the proportion of low birth weight children (<2.5 kg) between ultrasound–screened children and controls (odds ratio 0.96; 95% confidence interval: 0.82—1.12). There seems to be no association between the risk of childhood malignancy and in utero ultrasound exposure (Salvesen 2002). In a safety tutorial (ECMUS) for the European ultrasound organisation (EFSUMB), Salvesen concluded that there seems to be no association between ultrasound exposure in early fetal life and growth or impaired vision or hearing during childhood (Salvesen 2002). In one of the first follow–up studies to be published, a statistically significant association between two routine ultrasound examinations at 18 and 32 weeks of pregnancy and subsequent non–right–handedness among 8– and 9–year–old children was found (Kieler *et al.* 1993; Salvesen *et al.* 1993). Subsequently, this association was restricted to boys only (Salvesen *et al.* 1993; Kieler *et al.* 1998). However, a metaanalysis from Cochrane did not demonstrate any statistically significant differences between screened children and controls with regards to non–right–handedness, left–handedness or ambidexterity (Neilson 2004). When considering the results from the different studies available today on long term effect, we should remember that most participants in these studies have been exposed to just one or two examinations performed with low acoustic output ultrasound equipment (B–mode before 1990).

Modern sophisticated ultrasound equipment operates at source pressures significantly higher than those in use 15 years ago (Henderson *et al.* 1995; Duck and Henderson 1998; Duck 1999). Advances in transducer technology have resulted in the use of increased acoustic frequencies, focal depths and focal gains, with very different acoustic beam character that was in use 20 years ago (Duck 1999). There is ample evidence that the maximum acoustic output of the equipment in use today is capable of producing biological effects (Barnett 1998). A UK survey has noticed output values far above the recommended values for current equipment in use; for pulsed Doppler mode ≈ 9,000 mW/cm^2 and B–mode ≈ 990 mW/cm^2 (Henderson *et al.* 1995). The median I_{spta} intensity value for pulsed Doppler clinical equipment used in Britain is given as 1180 mW/cm^2. The absence of a regulated source quantity in the FDA regulations has been criticised, and it has been argued that the calculated saturation values for different measures of emitted acoustic intensity imply that conditions exist for which regulatory limits set by the FDA can be exceeded (Duck 1999). At present, we are unaware of the possible long time effects. To obey the ALARA principle (as low as reasonably achievable risk (Kossoff 1997)) that frequently repeated ultrasonographic exposures should be of a clinical benefit is therefore mandatory.

2 Hypothesis, aims and objectives

2.1 Hypothesis

The umbilical ring at the abdominal wall may represent a constrictive force with individually different haemodynamic effects on the umbilical vein flow and possible adverse effects on the pregnancy.

2.2 Aims and objectives

1. To determine the occurrence and degree of umbilical vein constriction in low risk population after the completion of the period of physiologic herniation (weeks 13–19) (*paper I*), and during the second half of pregnancy (weeks 20–40) (*paper II*).

2. To determine haemodynamic effects of an umbilical vein constriction as expressed in
   I) occurrence of venous pulsation (*paper III*) and
   II) effect on placental weight and birthweight, cord length, emergency operative delivery and Apgar score (*paper IV*).

3 Subjects and methods

3.1 Selection of subjects

Healthy women with singleton pregnancies were recruited from the low risk antenatal clinic for the cross–sectional studies at the Fetal Medicine Unit, Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway, during the years 1997 and 1998. The regional committee for medical research ethics had acknowledged the protocol,

and they all gave written informed consent. Smoking, diabetes, hypertension, or any general chronic disease excluded participation, and so did previous hypertensive complication of pregnancy, growth restriction and abruption of the placenta. Gestational age was assessed by last menstrual period and confirmed or corrected by ultrasound measurement of the fetal biparietal diameter. Malformations and chromosomal aberrations identified prior to recruitment excluded participation. Chromosomal aberration or malformation discovered during the course of the project and after birth were not reasons for withdrawal. Mode of delivery was noted, as well as urgent delivery by ventouse, forceps or caesarean section due to fetal distress. After birth, Apgar score, gender, birthweight, placental weight and umbilical cord length were noted, and a paediatrician examined the newborn. Chart 1 gives an overview of how participants contributed to different sections of the study.

Sample size was determined based on previous studies using similar examination techniques (Kiserud *et al.* 1992). At least 10 – 15 observations per gestational week were considered appropriate to construct the reference ranges, and a success rate (ability to perform) of 80 % was common for these types of measurements. A total of 384 women were included in the studies.

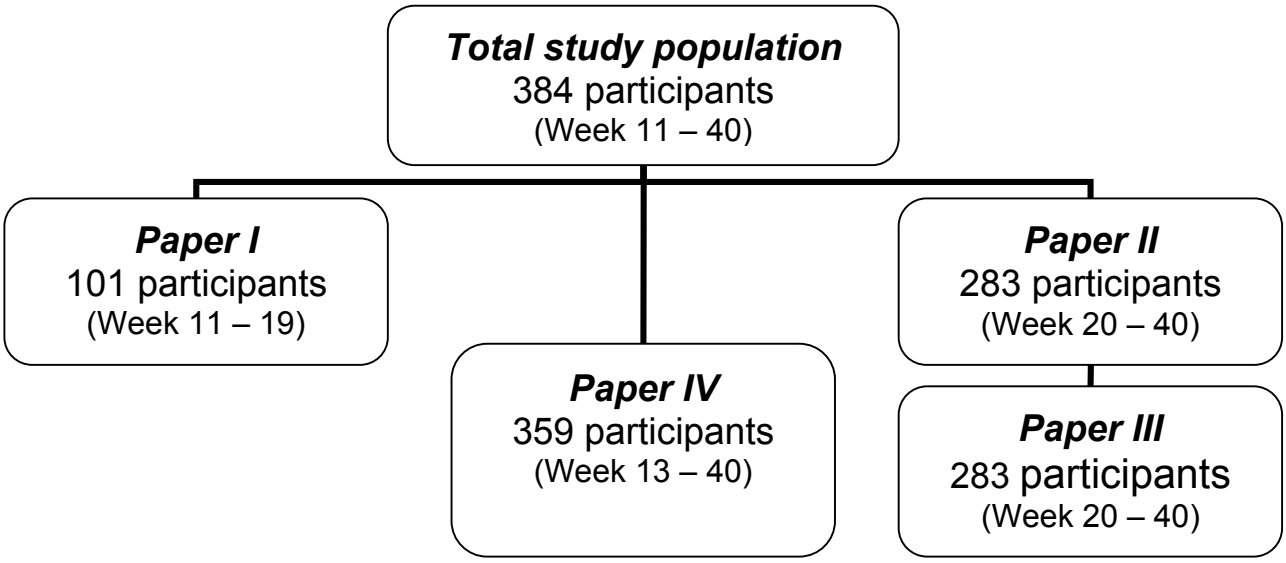

Chart 1. Total study population and publications.

3.2 Methods

3.2.1 Ultrasound equipment

The participants were examined transabdominally once at gestational age 11–40 weeks during a 45 minutes ultrasound session. We used a Vingmed CFM 800 ultrasound scanner (GE Vingmed Sound, Horten, Norway) with one of three multifrequency mechanical sector transducers (centre frequency 3.25, 5 or 7.5 MHz) carrying colour Doppler and pulsed Doppler facilities (2.5 or 4 MHz). The high pass filter was not used. Each Doppler recording took 2–12s. All the ultrasonographic data were transferred digitally to a Macintosh computer for later analyses, which were done with dedicated software (EchoDisp, Vingmed Sound, Horten, Norway).

3.2.2 2D–imaging

One set of measurements of the umbilical vein was taken at the fetal end of the umbilical cord. The inner diameter of the vein was measured in a perpendicular insonation, with the scan plane along the axis of the vein. Alternatively, for the earliest observations (until gestational week 15) the measurement was done as a transection to obtain a circular cross section. The second set of measurements was done at the inlet through the abdominal wall (the umbilical ring), and a third at the straight intraabdominal portion of the vein. The diameter was determined as an average of ≥ 5 repeated measurements (Kiserud and Rasmussen 1998).

3.2.3 Colour Doppler

In general, colour Doppler was added to identify the insonation with the lowest angle for the pulsed Doppler measurements. Colour Doppler was particularly useful during insonation at

the abdominal wall entrance, but was also of help for identifying the vessels of the cord and the intraabdominal portion of the umbilical vein.

3.2.4 Doppler velocimetry

The blood velocity was recorded in the umbilical cord at the same site as the diameter measurements were done, but in a new insonation along the long axis of the vein and with an expanded sample volume, in order to include the entire cross–section of the vessel. Since the insonation was kept strictly along the axis of the vessel, no correction of angle was needed. Assuming that the highest measured velocity represented the lowest angle of insonation, the measurement was repeated 3 to 5 times, and the time–averaged maximum velocity was calculated as an average of these recordings and included in the statistics.

Similarly, a second set of measurements of the umbilical vein was taken at the inlet through the abdominal wall (the umbilical ring) assuming that the cross–section of the umbilical vein was circular. The velocity measurements were taken as close to perpendicular to the abdominal wall as possible and, as with the previous set of measurements, the highest time–averaged velocity recordings (average of 3–5 measurements) was included in the statistics for each fetus. A third set of measurements was done in the intraabdominal portion of the umbilical vein, following the same guidelines.

The velocity increment at the level of the umbilical ring (papers I, II and IV) was calculated as the difference between the velocity at the umbilical ring ($V_{max.abd}$) and that found in the cord ($V_{max.cord}$) presented as a percentage of the velocity in the cord:

$$100\%(V_{max.abd} - V_{max.cord})/V_{max.cord}$$

Umbilical venous pulsation (paper III) was defined as a velocity variation synchronised with the fetal heart rate. The assessment was done both visually (the examiner noting whether pulsation was present or not) and by the temporal maximum velocity tracing of the Doppler shift. The degree of pulsation was calculated as the difference (ΔV) between the maximum

velocity and minimum velocity during the pulsation, calculated in centimetres per second (cm/s) or as the pulsatility index (PI) (ΔV/time–averaged velocity). The average of three or more pulses was entered into the statistics for the three sites of the umbilical vein.

3.2.5 Data quality assurance

The measurements were taken during fetal quiescence, as changes in intrathoracic pressure during breathing movements have a profound influence on flow velocity waveforms recorded in fetal veins (Marsal *et al.* 1984; Chiba *et al.* 1985; Trudinger 1987; Huisman *et al.* 1993). The digital transfer of data from the ultrasound machine to an online computer ensured that data were kept in original form for analysis. Data backup on magnetoptic (MO) disks and tape–streamer prevented loss of data.

3.2.6 Statistical analysis

In general, ln–transformation was performed if needed to achieve normal distribution, and polynomial or fractional polynomial regression models were fitted to the data in order to construct mean curves for blood velocities, diameters and their changes according to gestational age. To construct the 10th and 90th centile curves, the method of scaled absolute residuals was applied (Altman and Chitty 1994; Royston and Wright 1998). The 10^{th} centile was obtained from: mean – 1.28SD, and the 90^{th} centile from: mean + 1.28SD (papers I and II).

Analysis of variance for dependent observations was used to assess the means of differences with 95% confidence intervals (papers I – III). Intraobserver variation of the diameter and velocity measurements was studied for the participants using the repeated measurements included in the study (paper II). One–way analysis of variance was used to calculate the within subject mean variance and mean SD, which reflects the intra–observer variation (Bland and Altman 1996). The mean SD was calculated as $\sqrt{}$(mean square). In paper III, the Chi–square test was employed to assess differences between observations.

Regression analysis was used to determine the effect of constriction (and reduced compliance) on the magnitude of pulsation.

In paper IV, standard deviation scores (z–scores) were calculated for the $V_{max,abd}$ and for the percentage velocity increase, based on our previously estimated results (Skulstad *et al.* 2001; Skulstad *et al.* 2002). Similarly, the z–scores were calculated for BW/PW ratio and the umbilical cord length. Z–score for an observation was calculated based on the distance in standard deviations between the observation and the mean: (observed value–mean)/SD. To assess the distribution of operative delivery due to fetal distress and low Apgar score at one minute, the study population was stratified into tertiles according to percent velocity increase and to time–averaged maximum venous blood velocity at the umbilical ring. Differences were assessed by linear and logistic regression analyses. In general, statistical significance was considered to be achieved when $p < 0.05$. The SPSS statistical packages (SPSS 10.0 for Macintosh/ SPSS 10.0–12.0 for Windows, SPSS inc., Chicago, Illinois, USA) and Sigmaplot statistical packages (SigmaPlot 6.0 for Windows, SPSS inc., Chicago, Illinois, USA) were used for the analyses.

4 Results

In **paper I,** we investigated the umbilical vein blood flow velocity and diameter at the umbilical ring and in the umbilical cord in 101 fetuses age 11–19 weeks of gestation. High venous velocity at the umbilical ring was increasingly common with progressing gestation, while the venous velocity in the cord remained low. Before 13 weeks of gestation there was hardly any difference between the velocity recordings at the two sites, but after 13 weeks the difference was more pronounced and there were an increasing number of fetuses with a substantial velocity increment (i.e. >50%) at the umbilical ring. After week 16, 14% had a velocity increase of 300% or more, corresponding to a reduction in diameter to the half or less.

In **paper II**, the umbilical vein blood flow velocity and diameter at the umbilical ring and in the cord in 283 fetuses at 20–40 weeks of gestation were investigated with the same examination technique. We found that the time–averaged maximum venous blood velocity in the cord was a generally low (mean 13–19 cm/s), while the corresponding velocity at the umbilical ring at the abdominal wall was substantially higher (mean 34–41 cm/s), signifying a certain degree of umbilical vein constriction. Since the pattern was the same in the whole 20 to 40 weeks' span, the results were combined and percentiles were calculated for the entire period.

The acceleration of blood at the umbilical ring was calculated, and we found that 78 % had a velocity increase of ≥50%, and that 21% had ≥300%, which corresponds to a diameter reduction of ≥50%. The velocity increment showed substantial variation, and a few fetuses exceeded 900%. The reproducibility study showed that the diameter measurements both at the umbilical ring and at the cord had a mean SD of 0.07 mm. The variation of venous blood velocity measurements at the cord and at the umbilical ring had SDS of 0.58 and 1.83 cm/s.

In **paper III**, occurrence and magnitude of umbilical venous pulsation at the abdominal inlet was investigated in the same study group as in paper II, and the results were compared with corresponding observations in the cord and in the intraabdominal section of the vein. We found that visible pulsation in the umbilical vein was more common and more pronounced at the abdominal inlet where the umbilical ring tended to exert a constrictive impact on the vein, thus reducing its compliance. The incidence of pulsation at this site was remarkably high: 87% compared to 30% intraabdominally and 22% in the cord. When pulsation was recorded simultaneously at the three sites, the pulsatility was at its lowest in the cord where the diameter of the vein was largest (and compliance highest), illustrating the role of compliance as a local determinant for pulsation. In general, a smooth and prolonged pulse pattern was seen in the recordings, indicating an arterial origin. The other alternative, a short and distinct deflection indicating the atrial contraction wave, was rarely observed.

In 359 women of the study population (**paper IV**), with gestational ages 13 to 40 weeks, we found that the umbilical vein constriction did not represent any significant disadvantage at birth, apart from an increased incidence of Apgar score ≤7 at one minute after birth, but one fetus with a high degree of constriction later developed cerebral palsy. Using z–scores statistics we showed that constriction was linked to longer umbilical cords (females only) and relatively larger placentas (males only).

5 Discussion

5.1 Methodological considerations

5.1.1 Subjects studied

Interventions and outcome seemed slightly more favourable in the study group than in the general population (data from The Medical Birth Registry of Norway, year 1998), as evidenced by caesarean sections (6.8% versus 13.6%), perinatal deaths (0 versus 0.4%), birthweights (3724g versus 3525g). However, the recruited group was selected from a low–risk population that is expected to have less pregnancy and birth complications than the general population. On the other hand, it is worth noting that we never excluded participants because of complications once they were included.

5.1.2 Reproducibility of measurements

Ultrasound measurements

Diameter measurements of fetal vessels are liable to errors (Gill *et al.* 1981; Eik-Nes *et al.* 1984). Random error is particular prominent in small vessels, such as the ductus venosus and the umbilical vein in early pregnancy. A method of controlling such random error is to repeat the measurement.

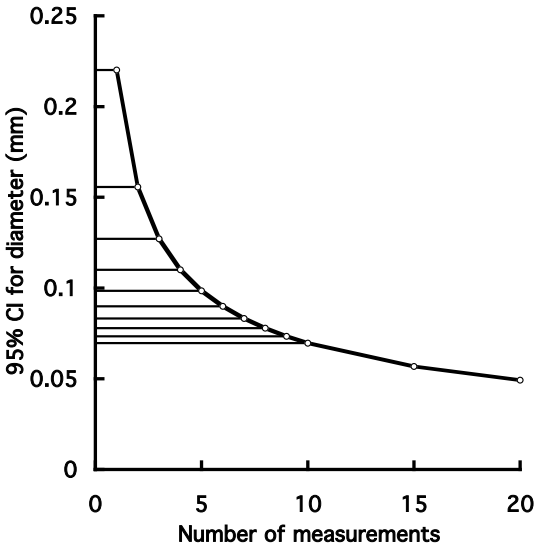

Figure 5. Upper 95% confidence limit for the intraobserver error of in vitro ultrasound assessment of diameters of 0.6–6 mm based on one measurement or an average of repeated measurements (2–20). (From Kiserud et al. (1999). "Validation of diameter measurements by ultrasound: intraobserver and interobserver variations assessed in vitro and in fetal sheep." Ultrasound Obstet Gynecol 13: 52-7, with permission.

To minimise measurement error, all measurements were done five times and averaged (Kiserud and Rasmussen 1998). It is shown that this simple method lowers the error to the half (Kiserud *et al.* 1999). The within subject mean variance and mean SD, which reflects the intraobserver variation, were examined for measurements (diameter and velocity) during gestational weeks 20 – 40 (Paper II). The diameter measured both at the umbilical ring and at the cord had a mean SD of 0.07 mm, which are comparable with previous results (Kiserud and Rasmussen 1998; Kiserud *et al.* 1999). This low value reflects a standardisation of the measurement procedure followed, but may also be due to use of the memory buffer in the ultrasound machine, creating many frames within a short period of time. The use of high–resolution transducers is also part of the explanation. The axial resolution was ≤ 0.7 mm for 5 MHz and ≤ 0.6 mm for 7.5 MHz for the range of 5–80 mm on the phantom (according to the company). The high reproducibility does not reflect the problems of getting valid diameter measurements of the vessel in the umbilical ring. Due to the narrow passage both the two arteries and the ring structure will compress the umbilical vein, and the resulting pressure may not exert evenly distributed impact round the circumference.

The intraobserver variation for the velocity measurements of venous blood velocity measurements at the cord and at the umbilical ring had SDs of 0.58 and 1.83 cm/s respectively. Regarding the Doppler velocimetry, focus was on achieving satisfactory measurements in the umbilical vein at the abdominal wall inlet. Efforts were made to obtain pulsed Doppler measurements in parallel with the vessel to avoid any angle correction. Colour Doppler was particularly useful in this process. Large sample volumes were used to cover the vessel area entirely, to include all velocity components. If the sample volume is inadequate to cover the vessel entirely, the result will be uneven insonation. Partial insonation of a vessel in the periphery of the lumen will underestimate the blood flow velocity (in particular the mean), while a similar insonation in the centre will overestimate it (Evans *et al.* 1989). On the other hand, if the large sample volume includes low–velocity signals from structures in the neighbourhood (such as the umbilical vein in the cord or intraabdominally) the mean blood flow velocity (V_{mean}) will be underestimated and cannot be properly quantified.

In DV it has been shown that a more correct and robust method is to derive V_{mean} from the easily identifiable V_{max} (Kiserud *et al.* 1998). The velocity profile (ratio V_{mean}/ V_{max}) in DV has been found to be partially blunted (ratio=0.69) on the basis of measurements, which confirms the results of a mathematical computational model (ratio=0.68) (Pennati *et al.* 1998). The blood flow in the umbilical vein has until now been assumed as being steady with low velocity, and with a parabolic distribution of blood flow velocities in the cross section of the vessel. In the present study, we have shown that quite a few fetuses have high blood flow velocities in the umbilical vein at the umbilical ring. It was outside the scope of the investigation to study the velocity distribution at this particular point of the circulation, but according to investigations of the velocity profile in ductus venosus (DV) with its narrow inlet and high blood flow velocities, we expect a more blunted profile at the abdominal wall (Kiserud *et al.* 1998). Pennati and co–workers evaluated the velocity distribution in the two investigated cross–sectional areas of the DV (inlet and outlet) of computational model simulations and the velocity shape coefficients (i.e. the ratios between the maximal and mean spatial velocities) were calculated as a function of vessel geometry. A more flat velocity profile (blunt) was found at the inlet than at the outlet, where the profile was more parabolic.

Since the section of the umbilical vein in the abdominal wall is short and the exact points for diameter assessment are difficult to define, we relied on the velocity recording to determine constriction. The simple assumption is that the amount of blood in the extraabdominal umbilical vein (V_{cord} π $(D_{cord}/2)^2$ is the same as the volume that passes at the umbilical ring ($V_{abd.wall}$ π $(D_{abd.wal}/2)^2$. Thus $V_{cord} / V_{abd.wall} = (D_{abd.wall} / D_{cord})^2$. Small differences in the diameter result in larger differences in velocity. If the diameter $D_{abd.wall}$ is half of that in the cord, the velocity will be four times higher at the abdominal wall compared to that in the cord. This makes the velocity measurement more sensitive than the diameter measurement. However, it could be argued that we have recorded only maximum velocities. The weighted mean velocity determines the volume flow. In the extraabdominal portion of the umbilical vein, this would be only half of the maximum flow, since flow is parabolic. For the velocity at the abdominal wall, this relation is different, due to acceleration. The mean velocity at this point would be between 0.5 and 1 of the maximum velocity. It follows that we have systematically underestimated the degree of constriction of the umbilical vein.

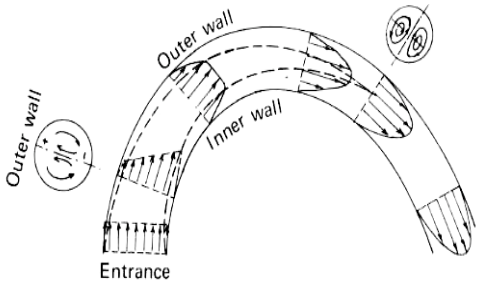

Figure 6. The blood flow velocity profile in a vessel loop. In this example, the profile is blunted at the entrance, but is gradually converted to a skewed, parabolic profile when passing the loop. Additionally, the flow is spiralling.
(From Fung, Y. C. (1984). Biodynamics : circulation. New York, Springer, with permission)

Another point would be a possible skewness of the velocity profile due to the curvatures. The profile would then change to be more acute, shifted to the wall with an increase in maximal compared to mean velocity. This would then overestimate the degree of constriction. However, based on the results of the ductus venosus modelling, we expect this effect to be less prominent than the effect of blood acceleration (Pennati *et al.* 1998). We therefore believe that our measurements underestimate the degree of constriction rather than overestimate it.

Weighing of the infant and the placenta

In general, an electronic balance was used for the weighing of the infant and the placenta. This type of balance is considered accurate and easy to operate. The weight of the newborn child was controlled by two persons (the nursing assistant and the midwife), and was registered immediately. This routine should minimise registration errors. As the weighing of placenta is concerned, this was also accomplished by the use of an electronic balance, but even though the procedure was uniform, some aspects need to be mentioned. Placentas were weighed with membranes and umbilical cord attached. No attempt was made to remove placental blood prior to weighing. This method has been used by others (Osak *et al.* 1997; Lackman *et al.* 2001). The dissimilarities in residual placental blood volume after clamping of the cord on the basis of different time passing from birth to clamping could introduce an error in calculation of the birthweight/placentalweight (BW/PW) relationship.

Placental transfusion has been found to be completed in 3 minutes (Yao *et al.* 1968). When clamping the umbilical cord less than 5 seconds after birth, the infant–placental blood volume distribution has been found to be 67% and 33%, after 1 minute the distribution was 80% – 20%, and after 3 minutes 87% – 13% (Yao *et al.* 1969). The placental recidual blood volume (PRBV) was estimated to 127 ml if the cord was clamped five seconds after birth, declining to 48 ml if the cord was clamped three minutes after birth. Theoretically, clamping–time could interfere with the placentalweight/birthweight relationship in percent (% PW/BW), but it was found to be approximately 17% (mean), bearing no relation to the time passed from birth to clamping (Yao *et al.* 1969). If the data in paper IV is precented in this way, the % PW/BW is 18% (mean, range 11% – 36%). In our study, for most newborns, the cord was clamped when pulsations were no longer felt. The problem with placental residual blood volume should therefore be negligible. Others describe the removal of cord and even membranes, and emptying of placental blood preceding weighing (Molteni *et al.* 1978), which makes a direct comparison with their results impossible.

5.2 Discussion of results

The umbilical ring constriction is established during early 2nd trimester (paper I). At mid–trimester, about one fifth of the fetuses showed a velocity increase of ≥ 300%, corresponding to a diameter reduction to the half or less (paper II). However, we do not know the duration of this constriction. The study was not designed to answer this question. We showed that a long cord and an umbilical ring constriction both represent increased resistance to flow, and shift the placental weight compared to birthweight (reduced BW/PW ratio)(paper IV). This is in line with previous reports on nuchal cord entanglement, which seems to give long term effects, causing low birthweight (Lackman *et al.* 2001) and shift in the BW/PW ratio (Osak *et al.* 1997), probably due to reduced transmission of nutrients to the fetus or pooling of fetal blood in the placenta (Osak *et al.* 1997).

Another possible mechanism would be a direct haemodynamic effect on placental growth, which depends on intravascular pressure to develop its villi (Karimu and Burton 1994). We assume that the effect on placental weight and length of the cord requires that the causal factor is prolonged for days and weeks.

The umbilical vein at the abdominal inlet could also be subject to humoral or neural regulation, or a combination of both. The ductus venosus seems to be under a tonic adrenergic control (Ehinger *et al.* 1968; Coceani *et al.* 1984; Kiserud *et al.* 2000a; Tchirikov *et al.* 2003), by distending under the influence of nitric oxide (Kiserud *et al.* 2000a) and prostaglandins (Adeagbo *et al.* 1982; Adeagbo *et al.* 1985; Coceani and Olley 1988; Adeagbo *et al.* 2004). The umbilical vein in the cord is also subject to regulation. In the rat, two separate sets (vitelline and allantoic) of umbilical vessels originate from the umbilicus, which do not anastomose as they pass to the yolk sac and placenta. Only the vitelline vessels seem to be innervated (Ellison 1971; Anthonioz and Maillet 1972a; Anthonioz *et al.* 1973; Jutee *et al.* 1977). A similar pattern has been described in the guinea pig (Anthonioz and Maillet 1972b). A phylogenetic explanation for this is suggested, as control over the blood supply of the yolk sac may have existed in primitive forms before the evolution of the allantoic placenta (Ellison 1971). In humans, the umbilical vein and arteries also develop

from the allantoic part of the cord, which makes a direct nervous influence on the vessels less probable. Some authors have been unable to identify nervous tissue in the human cord (Spivack 1943; Reilly and Russell 1977; Fox and Khong 1990). However, most investigators have been able to demonstrate the existence of unmyelinated nerve fibers and nerve endings in the proximal part of the cord; close to the umbilical ring, by the use of different stains and light microscopy (Mabuchi 1924; ten Berge 1963; Ehinger *et al.* 1968; Pearson and Sauter 1968; Pearson and Sauter 1970; Ellison 1971; Fujiyama *et al.* 1971; Bettzieche 1978; Baljet and Drukker 1982), some have been able to demonstrate nerves more distally in the cord (Mabuchi 1924; Fox and Jacobson 1969), and others even in the placenta (Coujard *et al.* 1952; ten Berge 1963; Jacobson and Chapler 1967). Structures resembling myelinated nerves have been identified by electron microscopy within the smooth muscle cells of the umbilical arteries (Nadkarni 1970; Matsubara and Tamada 1988) and in periarterial plexus formation (Kawano and Mori 1989). None of the investigators using electron microscopy was able to demonstrate vasomotor nerve terminals. Nerve fibers positive for calcitonin gene–related peptide (CGRP), a vasodilator peptide, neuropeptide Y (NPY), a vasoconstrictor peptide, and tyrosine hydroxylase (TH), a key enzyme for the synthesis of the neurotransmitter of adrenergic nerves, have been demonstrated on the fetal side of the umbilical cord by immunohistochemistry (Sato 1998). The immunopositive nerve fibers were observed in the smooth muscle of the media of the umbilical artery and in the margins of Wharton's jelly. They were not observed around the umbilical vein (Sato 1998). Others have found nerve terminals immediately external to the umbilical vein and in the cord matrix between the vein and the arteries, but no direct contact with the vessels was found (Ellison 1971). This finding led the author to conclude that the nerves were probably sensory. He proposed that the terminals around the vein sense venous distension, and that the freely ending terminals in Wharton's jelly detect stretch or compression (Ellison 1971). Intra–abdominally, continuity between the nerve plexus along the umbilical arteries and the plexus along the internal iliac arteries and the umbilical vein has been established. Interestingly, the nerves in the deep layer of the sheath of the musculus rectus abdominis has been found to be in contact with the plexus of the umbilical vein (Baljet and Drukker 1982). This finding may imply that the nerve plexuses along the umbilical vessels both intra– and extra–abdominally together with output from mechanoreceptors in the rectus sheath (Gray *et al.* 1974; Tomilova 1975) give sensory

output transmitted via the sacral plexus and the phrenic and celicac ganglions (Pearson and Sauter 1969; Pearson and Sauter 1970). From these ganglions, nerve fibers run with the vagal trunks, and may eventually interact with the phrenic nerve by way of the medulla and the respiratory center, creating a reflex arch which could induce contractions of the diaphragm causing fetal hiccups or fetal respiratory movements. Compression or stretch of the umbilical cord (Collins 1991a) or umbilical ring variation, could be the stimulus causing this reaction. The total afferent output could then alert the central nervous system of impending dangerous situations, which could then be averted through reflex mechanisms.

Hypoxemia in sheep have been found to increase resistance in the umbilical veins more than twofold, without affecting resistance in the umbilical arteries or placenta (Paulick *et al.* 1990). This effect is probably mediated by norepinephrine and epinephrine which have been demonstrated to increase the vascular resistance of the umbilical veins in a dose–dependent manner in the ovine fetus (Paulick *et al.* 1991). The increased resistance in the umbilical veins may improve maternal–fetal blood gas exchange by increasing the fetal surface area in the placenta. On the contrary, hypoxemia has been shown to dilate DV (Kiserud *et al.* 2000a), an effect also found to be mediated by catecholamines (Paulick *et al.* 1991; Tchirikov *et al.* 2003) and nitric oxide (Kiserud *et al.* 2000a).

The wide variation in the umbilical vein constriction in healthy fetuses could indicate that it is somehow related to normal fetal physiology. Several studies have demonstrated the existence of a fluid transport across the placenta (Power and Longo 1974; Kaufmann *et al.* 1982; Schroder 1982; Schroder *et al.* 1982). The fluid transport in the materno–fetal direction has been described as pinocytosis (King and Enders 1971). A transtrophoblastic channel system between the maternal and the fetal trophoblastic surfaces in the human placenta has also been demonstrated (Kertschanska *et al.* 1994; Kertschanska *et al.* 1997). These membrane–lined channels are distended by small increases in fetal venous hydrostatic pressure (Kertschanska *et al.* 1997). When the venous hydrostatic pressure is increased, an edematous fluid accumulation in the stroma of the trophoblasts occurs, likely to be the result of water filtration out of the fetal vascular system. Small increases in venous pressure (>4 mm Hg) have been found more effective than a large increase in arterial pressure (> 80 cm Hg) (Kertschanska *et al.* 1997). It is suggested that the

transtrophoblastic channels act as pressure dependent valves, which enable the fetus to eliminate excess water. The umbilical vein narrowing at the umbilical ring may be involved in the regulation of this valve system. Provided that the umbilical venous vessel wall is under autonomous nerve control, the fetus could quickly adapt to changes in circulatory loads.

The different effect of umbilical vein constriction on placental development for the male and the female conceptus may correspond to other known differences in gender related to the cardiovascular system. A stress response has been shown to induce an increase in whole blood viscosity (WBV) and hematocrit in men, but not in women, probably mediated by an increase in plasma noradrenaline (Ross *et al.* 2001). Males have a higher hematocrit (Hct) than females at all ages. Sequence–based polymorphism for the erythropoietin receptor gene (EPOR) has been established, and this may be partly responsible for the gender–based variation in Hct level (Zeng *et al.* 2001). According to Hagen–Poiseuilles' law, a higher blood viscosity would result in a higher resistance, demanding higher perfusion pressure in the placentas of the male fetuses. The net result could be heavier placentas for this sex, but information on Hct is not available in the present study (Paper IV).

6 Conclusions

The present study has shown that some degree of venous constriction at the fetal umbilical ring is quite common during the second and third trimester. The constriction is increasingly present during gestational weeks 13 – 19, i.e., after the end of the period of physiological umbilical herniation. Reference ranges for low–risk pregnancies were established for the second half of pregnancy by help of colour Doppler and pulsed Doppler techniques. Interestingly, as many as 1 in 5 of the fetuses had a vein constriction to half of the vein diameter.

Constriction had haemodynamic effects, shown by the locally increased incidence of venous pulsations, elongation of the cord and a shift towards higher placental weight compared to birthweight. These effects had gender specific differences.

In spite of the unexpectedly common and pronounced umbilical venous constriction, this had no major effect on perinatal outcome, possibly signifying that the fetal circulation is normally a robust system, able to cope with such challenges. However, the significant effect on Apgar score at one minute after birth may be an indication that extreme constriction may represent some kind of risk. The results warrant further studies designed to address such issues.

7 Perspectives

We have studied a phenomenon that until now has hardly been addressed in the literature. We have established a method to examine the umbilical venous constriction and provided reference ranges as a basis for further studies in the field. Our results also point out possible directions for such studies.

Studies are needed to clarify whether the constriction is stable or varies with time. Both short times variation (hours, days) and variation in the long run (weeks, months) could be explored. Secondly, the blood flow at the umbilical ring could be studied during fetal movements, such as respiratory movements. To further elucidate the role of the constriction in fetal physiology regarding umbilical cord properties, placenta and the fetal circulation, both cross–sectional and longitudinal studies are useful. Key words could be fetal fluid balance and fetal heart effects.

Last but not least, the role of the constriction in perinatal outcomes demands further studies.

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9 Research papers I – IV

Paper I

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The development of high venous velocity at the fetal umbilical ring during gestational weeks 11–19

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Objective To determine the occurrence of high venous velocities at the umbilical ring in the normal early second trimester, based on the assumption that a narrow umbilical ring may cause obstruction and increased venous blood velocity at the abdominal wall.

Design Cross-sectional study.

Setting Hospital antenatal clinic.

Population One hundred and one low risk singleton pregnancies specifically recruited for the study.

Methods Ultrasound was used at 11–19 weeks to determine the diameter and velocity in the umbilical vein at the fetal end of the cord and at the inlet through the abdominal wall.

Outcome measures 10th, 50th and 90th centiles were estimated for the time-averaged maximum velocity in the cord and at the abdominal inlet. The increase of velocity as the blood entered the abdominal wall was calculated in percent of the velocity in the cord.

Results During weeks 11-12 there was hardly any difference between blood velocity in the umbilical vein at the umbilical ring and that in the cord. From week 13 onwards it was increasingly common to find blood acceleration at the umbilical ring of 50–500%. Velocity increment >50% was found in 0/12 fetuses (0%) at 11–12 weeks, 5/20 (25%) at 13–14 weeks, and in 21/28 (75%) at 17–19 weeks.

Conclusions Blood velocity is higher in the umbilical vein at the abdominal wall than the cord, particularly after 13 weeks of gestation. If acceleration of blood velocity at the umbilical ring is a sign of a narrow inlet, it seems that a progressive tightening occurs during the second trimester.

INTRODUCTION

Adequate umbilical venous flow is essential for fetal development. Combining ultrasound imaging and Doppler technique, Gill[1] measured this flow in the human fetus *in utero* for the first time in 1979. He found that the average umbilical flow increased from 100 mL minute ^{1} at 26 weeks of gestation to 300 mL minute^{-1} at term and the corresponding normalised flow was 145 and below 100 mL minute^{-1} kg^{-1}, respectively[2]. These results have proved to be reasonably reproducible even with the refined equipment of today[3]. In the growth restricted fetus the blood flow (and normalised blood flow) tends to be lower than in normally grown fetuses[2,4,5]. The generally low pressure and low velocity system of the umbilical vein is susceptible to external forces. Fetal movements and respiratory activity influence umbilical venous flow[6]. For a long time a more lasting constriction (or obliteration) as a possible cause of death has been discussed[7]and numerous case reports support the hypothesis that twisting, stricture or externally imposed constriction can cause complications and fetal demise[8–11]. Compared with the velocities recorded in the large fetal arteries, the umbilical venous blood velocity is low. However, in a recent study of fetuses in the second half of pregnancy a generally higher velocity was found at the entrance through the abdominal wall, the umbilical ring, and in quite a few cases the velocity was considerably higher suggesting a natural venous constriction[12]. Whether fetuses with such a narrow umbilical ring are at increased risk of complications during pregnancy is not known. Nor is it known when this type of stricture is formed. The formation of the umbilical ring is probably completed at 12 weeks of gestation, when the period of the physiological umbilical herniation ends[13,14]. We hypothesise that this process tightens the umbilical ring, and in extreme cases may cause a venous stricture.

Based on the assumption that a constriction of the umbilical vein causes a corresponding acceleration in blood velocity, we aimed at determining the tightening of the umbilical ring by measuring the increase of velocity as the umbilical venous blood enters the abdominal wall as well as the corresponding diameter at 11–19 weeks of gestation.

METHODS

A total of 101 healthy women with singleton pregnancies were recruited from the low risk antenatal clinic for

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the cross-sectional study. They all gave written informed consent and the procedure followed had been accepted by the regional ethics committee. Smoking, diabetes, hypertension, or any general chronic disease excluded participation, and so did previous hypertensive complication of pregnancy, growth restriction and abruption of the placenta. Gestational age was assessed by last menstrual period and confirmed or corrected by ultrasound measurement of the embryonic crown–rump length or fetal biparietal diameter. Serious malformations and known chromosomal aberrations were excluded prior to recruitment. Chromosomal aberration or malformation discovered during pregnancy and after birth were not reasons for withdrawal. After birth, Apgar score, gender, and birthweight were noted and the newborn was examined by a paediatrician.

The participants were examined transabdominally once at gestational age 11–19 weeks during a 45 minutes ultrasound session using a Vingmed CFM 800 ultrasound scanner (GE Vingmed Sound, Horten, Norway) with one of two multifrequency mechanical sector transducers (centre frequency 5 or 7.5 MHz) carrying colour Doppler and pulsed Doppler facilities (4 MHz). The spatial peak temporal average intensity was set at 50 mW/cm^2 for the pulsed Doppler, and was less for the colour Doppler mode. Each Doppler recording took 2-12s.

One set of measurements of the umbilical vein was taken at the fetal end of the umbilical cord (Fig. 1). The inner diameter of the vein was measured in a perpendicular insonation with the scan plane along the axis of the vein. Alternatively, the measurement was done as a transection to obtain a circular cross-section. The diameter was determined as an average of $\geq$5 repeat measurements[15]. The blood velocity was recorded at the same site but in a new insonation along the long axis of the vessel and with an expanded sample volume, in order to include the entire cross-section of the vessel. Colour Doppler was used to find the insonation with the lowest angle. The measurements were taken during fetal quiescence. All measurements were done by one operator (S.M.S). The velocity measurement was repeated 3-5 times, and the time-averaged maximum velocity determined. Assuming that the highest measured velocity represents the lowest angle of insonation, this velocity recording was included in the statistics.

Similarly, a second set of measurements of the umbilical vein was taken at the inlet through the abdominal wall (the umbilical ring, Fig. 1) assuming that the cross-section of the umbilical vein is circular at this site. The velocity measurements were taken as close to perpendicular to the abdominal wall as possible and, as with the previous set of measurements, the highest time-averaged velocity recording was included in the statistics for each fetus.

The velocity increment at the level of the umbilical ring was calculated as the difference between the velocity at the umbilical ring ($_{Vmax,abd}$) and that found in the cord ($_{Vmax,cord}$) presented as a percentage of the velocity in the cord:

$$100\%(V_{max,abd} - V_{max,cord})/V_{max,cord}$$

ln-transformation was performed if needed to achieve normal distribution, and polynomial or fractional polynomial regression models were fitted to the data in order to construct mean curves for blood velocities, diameters and their changes according to gestational age. To construct the 10th and 90th centile curves, the method of scaled absolute residuals was applied[16,17]. The 10th centile was obtained from: mean – 1.28SD, and the 90th centile from: mean + 1.28SD. Analysis of variance for dependent observations was used to assess the means of differences with 95% confidence intervals.

RESULTS

There were no withdrawals among the 101 participants. Average gestational age at birth was 39 weeks 5 days (median 40 weeks 4 days, range 32 weeks 4 days to 42 weeks 3 days), and the average birthweight was 3510 g (range 1630-4600 g). Five babies were delivered by caesarean section. There were no perinatal deaths, one newborn had Apgar score 7 after one minute, and none had Apgar score $\leq$7 after five minute. One baby had an occult spina bifida and intraspinal lipoma, one had bilateral clubfoot, and another had hypospadia.

The time-averaged maximum venous blood velocity in the cord was recorded in 83 fetuses (Fig. 2 a) and showed a generally low velocity (7-15 cm/s) during 11–19 weeks of gestation (Fig. 3). The venous velocity at the umbilical

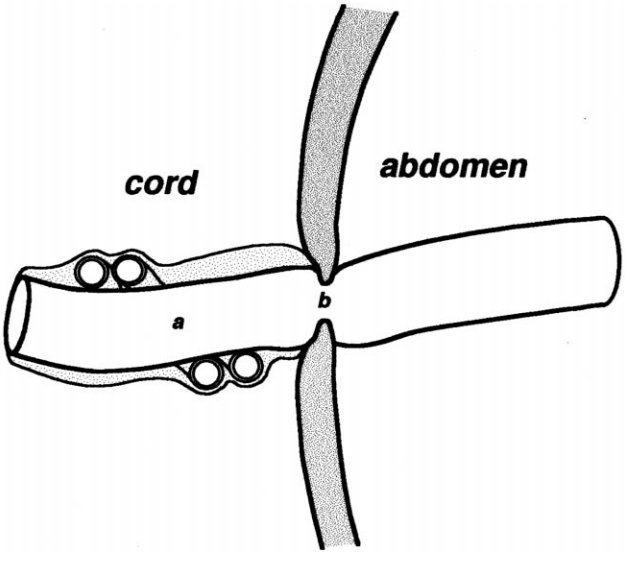

Fig. 1. The umbilical vein enters the fetal abdomen through the umbilical ring. To assess the degree of constriction at this point, the inner diameter and blood velocity were determined in the cord outside the abdominal wall (a) and in the umbilical ring (b).

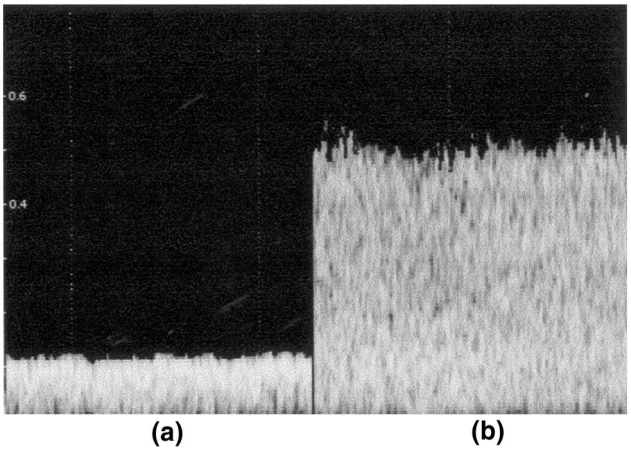

Fig. 2. Doppler recording in a fetus of 17 weeks gestation showing a typical low blood velocity in the vein of the umbilical cord (a) and a high velocity at the umbilical ring in the abdominal wall (b). The velocity increment is attributed to the tightening of the umbilical ring during the second trimester. Duration of each recording: 1 s; velocity scale: m/s.

ring in the abdominal wall was recorded in all 101 participants and showed a marked increase with gestational age (Fig. 2b and 4). Before 13 weeks of gestation there was hardly any difference between the venous flow velocity in the cord and that at the umbilical ring (Table 1). However, after 13 weeks the difference was more pronounced and there were an increasing number of fetuses with a substantial velocity increment (i.e. $\geq$50%) at the umbilical ring (Table 1 and Fig. 5).

The mean inner diameter of the vein of the umbilical cord increased from 1 to 3.5 mm during gestational week 11–19 (Fig. 6). The corresponding mean diameter of the vein at the umbilical ring seamed to grow less during the same period, from 1 to 2.5 mm (Fig. 7). Correspondingly, the mean difference between the diameters at the two sections of the umbilical vein increased during the period observed, but with wide ranges (Table 1). Unfavourable position, fetal movements, maternal obesity, and reduced time for examination were the reasons for a reduced number of observations in some of the participants.

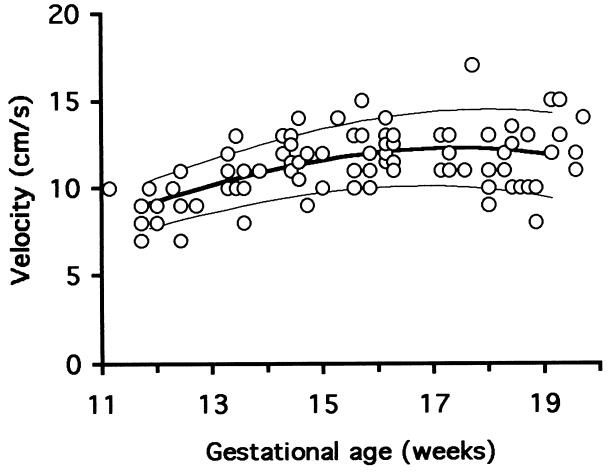

Fig. 3. Time-averaged maximum velocity of the venous flow in the umbilical cord in 83 fetuses presented with the 10th, 50th and 90th percentile. The equation for the 50th percentile is: $y = -0.01 + 0.0013x^2 - 0.00005x^3$. SD $-0.003 + 0.001x$.

DISCUSSION

In the present study we have shown that the umbilical venous velocity increases notably at the umbilical ring in the abdominal wall during the early second trimester and in quite a few fetuses the velocity is 200% to 500% higher than in the cord. The period of physiologic umbilical herniation starts at 7 weeks of gestation and ends before 12 weeks. Interestingly, the increase in umbilical venous velocity at the umbilical ring coincides with the end of the physiologic umbilical herniation and suggests that the umbilical ring is progressively tightened during the following weeks leading to a relative narrowing of the vein.

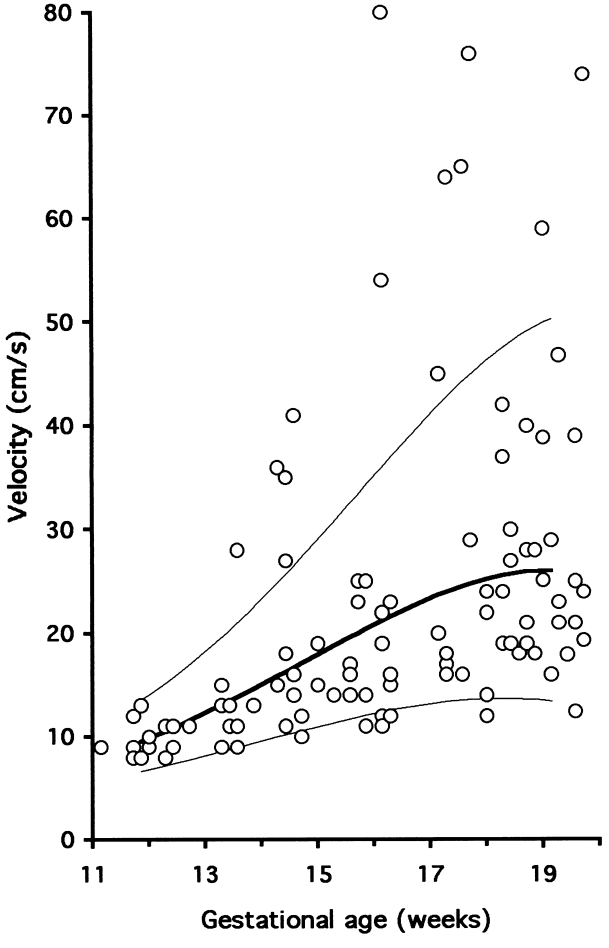

Fig. 4. Time-averaged maximum velocity of the venous flow at the umbilical ring in the abdominal wall in 101 fetuses presented with the 10th, 50th and 90th percentile. The equation for the 50th percentile is: $y = -4.43 + 0.03x^2 - 0.00088x^3$. ln-transformation was performed. SD $-0.115 + 0.033x$.

Table 1. The normal tightening of the umbilical ring in 101 fetuses expressed as the difference in diameter or blood velocity between the umbilical vein in the cord and in the abdominal wall, presented with mean and 95% CI. Fetuses with a tightening of the umbilical ring associated with >50% or >300% increase of the venous blood velocity, compared to the vein in the cord, are presented as a fraction of the total sample at the corresponding gestational age.

Gestational age (weeks)	Diameter difference (mm)		Velocity increment (cm/s)		Cases with increased velocity	
	Mean	[95% CI]	Mean	[95% CI]	> 50%	> 300%
11–12	-0.2	[-0.3;-0.1]	2.3	[0.7;3.9]	0/12	0/12
13–14	-0.2	[-0.3;-0.1]	6.6	[2.3;10.9]	5/20	0/20
15–16	-0.3	[-0.6;-0.1]	8.7	[1.4;15.9]	9/21	2/21
17–19	-0.7	[-0.8;-0.5]	21.9	[16.8;27]	21/28	4/28

The same tendency towards a relative physiologic stricture at the abdominal wall is found in the measurements of the diameters of the vein. However, the variation of the diameter measurements at the umbilical ring is great. This may be due to biological variation also expressed in the velocity measurements. Another important source of variation is the error of measurement in small vessels. The diameter measurement in small vessels (0.5-2.5 mm) has an upper 95% confidence limit of 0.15 mm when the diameter is determined as the average of five measurements[15]. We are not sure that our assumption of a circular cross-section is valid for this section of the vein. Furthermore, the section of the umbilical vein that passes through the umbilical ring is short and in a plane less favourable for measurements and, accordingly, less reproducible. Since a reduction of the radius of the vessel results in an increase in blood velocity by the power of two, the velocity is a more sensitive and reliable indicator of a stricture than the diameter measurement.

The blood velocity found in the vein of the umbilical cord agrees with that mentioned in previous reports[18]. However, the blood velocity pattern at the umbilical ring demonstrates a progressively increasing difference from the velocity in the cord but with considerable individual variation as pregnancy proceeds through the second trimester (Fig. 5 and Table 1). We can only speculate what implication an extreme tightening of the umbilical ring might have. The increase of blood velocity by 300% corresponds to a reduction of the diameter to the half and might represent an increased resistance to venous return to the fetus. After 16 weeks of gestation

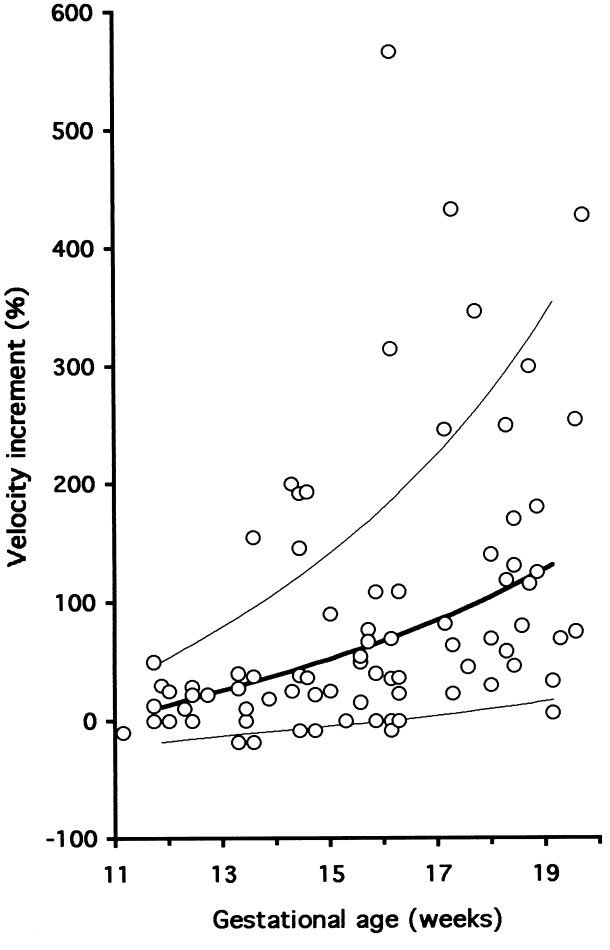

Fig. 5. The increase in velocity of the venous flow at the umbilical ring in 81 fetuses calculated as percent of the velocity in the cord and presented with the 10th, 50th and 90th percentile. The equation for the 50th percentile is: $y = 5.01 - 61.59x^{-2} + 0.000086x^{3}$. ln-transformation was performed. SD – 0.24 + 0.04x.

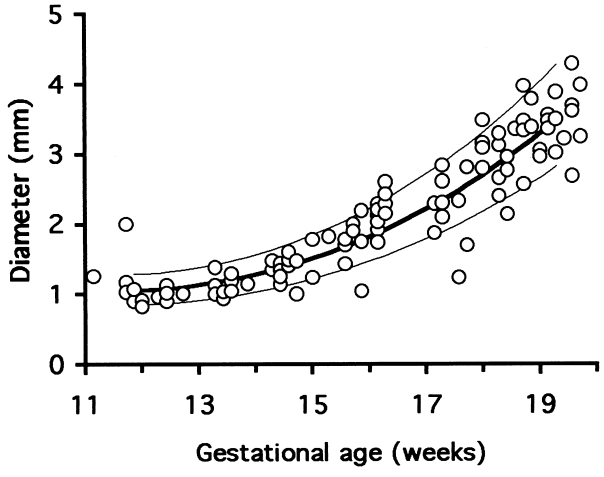

Fig. 6. The inner diameter of the vein in the umbilical cord in 97 fetuses presented with the 10th, 50th and 90th percentile. The equation for the 50th percentile is: $y = 5.68 + 1106.92x^{-2} - 187.33x^{-1}$. ln transformation was performed. SD 0.16 + 0.00028x.

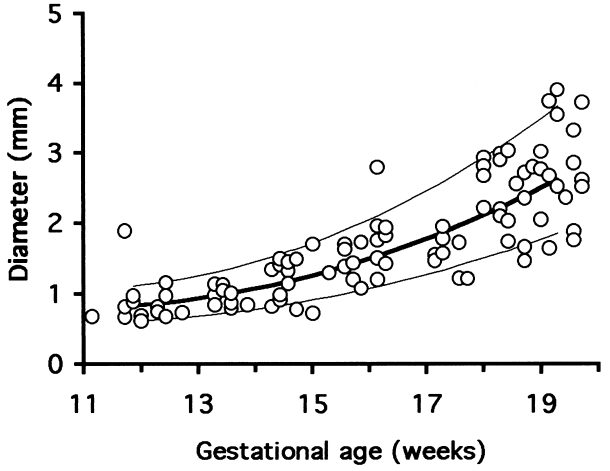

Fig. 7. The inner diameter of the vein at the umbilical ring in the abdominal wall in 94 fetuses presented with the 10^{th}, 50^{th} and 90^{th} percentile. The equation for the 50^{th} percentile is: $y = 3.9 + 783.6x^{-2} - 141.9x^{-1}$. ln transformation was performed. SD 0.18 + 0.0044x.

≥10% of the fetuses may have this type of tightening (Table 1).

It has been suggested that flow and resistance to flow in the placental vascular bed, and the development of villi, depend on maternal intervillous pressure and the fetal intravascular pressure (or rather the transmural pressure)[19–21]. An increased resistance in the umbilical vein could be a cause of increased transmural pressure and thus influence villi proliferation and flow. The present research continues in order to address these questions.

Apart from demonstrating the normal process of tightening of the umbilical ring that follows the period of physiologic umbilical herniation, is there any clinical implication? Through the years, quite a number of reports of perinatal complications and death associated with stricture or obliteration of the umbilical vein suggest that this portion of the fetal circulation is vulnerable. The present study shows that in a normal population of fetuses quite extensive squeezing of the umbilical vein at the abdominal wall is rather common (Fig. 5). However, the present study was not designed to examine the association between the tightening of the umbilical ring and complications during pregnancy and the perinatal period. We may speculate that fetuses with extreme tightening of the umbilical ring carry a higher risk of complications being more susceptible to twisting or squeezing, particularly in late pregnancy. A second implication could be that a segment of increased resistance to flow at the umbilical ring might lead to slower response when increased placental flow is demanded. Obviously, the next step in this research would be to examine whether extreme tightening of the umbilical ring influences the development of pregnancy and the perinatal outcome.

In short, the present study has demonstrated the normal progressive tightening of the umbilical ring during the second trimester, leading to a pronounced increase of umbilicial venous velocity, and in quite a few fetuses, a velocity increment of 200% to 500%. Several hypotheses can be derived from the results, such as: does extreme tightening that squeezes the umbilical vein influence placental or fetal development, or represent any perinatal risk?

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Paper II

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Degree of fetal umbilical venous constriction at the abdominal wall in a low-risk population at 20–40 weeks of gestation

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Objectives To determine the degree of constriction of the umbilical vein at the abdominal wall in the second half of pregnancy.

Methods A total of 283 low-risk singleton pregnancies were recruited for a cross-sectional study, and examined once at 20–40 weeks of gestation. Two sets of ultrasound measurements of the umbilical vein were taken: one at the fetal end of the umbilical cord and another at the inlet through the abdominal wall, the umbilical ring. The diameter was determined as an average of ≥5 repeat measurements. The blood velocity was recorded at the same site.

Results The time-averaged maximum venous blood velocity in the cord was low (mean 13–19 cm/s during 20–40 weeks of gestation), and the corresponding mean diameter 3.6–8.2 mm. In contrast, the mean of the venous blood velocity at the umbilical ring was 34–41 cm/s and the diameter was 2.8–5.9 mm during the same period. Of 191 pairs of observations, 41 (21%) had a velocity increment of ≥300 %, which corresponds to a diameter reduction to half or more at the umbilical ring.

Conclusion Constriction of the umbilical ring is a common phenomenon in the second half of pregnancy.

KEY WORDS: fetus; circulation; ultrasound; Doppler; blood flow; umbilical vein

INTRODUCTION

Fetal development depends on the umbilical venous return, the average being 115 ml/min/kg at 20 weeks of gestation and 64 ml/min/kg at 40 weeks (Kiserud *et al*., 2000). The low blood flow velocity and pressure, 4–10 mmHg (Ville *et al*., 1994), makes the umbilical vein particularly susceptible to external mechanical forces. Twisting, stricture, or externally imposed constriction are believed to cause complications and death *in utero* (Browne, 1925; Weber, 1963; Virgilio and Spangler, 1978; Ghosh *et al*., 1984; Sun *et al*., 1995). We have previously shown that after the period of physiological herniation is completed at 12 weeks of gestation, the umbilical ring is increasingly tightened (Skulstad *et al*., 2001). During gestational weeks 13–19, an increasing number of fetuses had established a venous constriction, reflected by the corresponding acceleration in venous blood velocity. That study showed that at 17–19 weeks 14% of the fetuses had a velocity increment ≥300%, which corresponds to ≥50% reduction of the diameter. Whether this pattern is maintained during the second half of pregnancy is not known. We hypothesise that venous constriction of the abdominal inlet is a common phenomenon during the second half of pregnancy.

The aim of the present study was to determine the degree of constriction of the umbilical vein at the abdominal wall of the fetus in the second half of pregnancy.

METHODS

A total of 283 singleton pregnancies were recruited from the low-risk antenatal clinic for a cross-sectional study. They all gave written informed consent according to a protocol approved by the Regional Committee for Medical Research Ethics. Smoking, diabetes, hypertension or any general chronic disease excluded participation, as did previous hypertensive complication of pregnancy, growth restriction or abruption of the placenta. Gestational age was assessed by ultrasound measurement of the biparietal diameter at 17–20 weeks of gestation. Chromosomal aberrations and serious malformations detected at this stage were not included. However, chromosomal aberrations or malformations discovered at a later stage or after birth were not reasons for withdrawal. After birth, Apgar score, gender, and birth weight were noted and a paediatrician examined the newborn.

The participants were examined once at 20–40 weeks of gestation during a 45-min session using a Vingmed CFM 800 ultrasound scanner (GE Vingmed Sound, Horten, Norway) with one of two multifrequency mechanical sector transducers (centre frequency 3.25 or 5.0 MHz) carrying colour and pulsed Doppler facilities (2.5 or 4.0 MHz). The spatial peak temporal average intensity was set at 50 mW/cm^2 for the pulsed Doppler and was less for the colour Doppler mode. Each Doppler recording took 2–12 s.

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Two sets of measurements of the umbilical vein were taken: one at the fetal end of the umbilical cord, and the other at the inlet through the abdominal wall, the umbilical ring (Figure 1). The measurements were taken during fetal quiescence. In the cord, the diameter of the vein was measured in a perpendicular insonation with the scan plane along the long axis of the vessel. Alternatively, the measurement was done as a transection to obtain a circular cross-section, and in both cases, the inner–inner distance determined. The diameter was determined as an average of ≥ 5 repeat measurements (Kiserud and Rasmussen, 1998; Kiserud *et al*., 1999). The blood velocity was recorded at the same site but in a new insonation along the long axis of the vein and with an expanded sample volume in order to include the entire cross-section of the vessel. Colour Doppler was used to ensure an insonation orthograde with the blood flow. Since the insonation was kept strictly along the axis of the vessel, no correction of angle was needed. The velocity measurement was repeated three to five times. The time-averaged maximum velocity was calculated as an average of these recordings and included in the statistics.

A corresponding set of measurements was taken at the abdominal wall (Figure 1). The velocity measurements were taken perpendicular to the abdominal wall. Colour Doppler was particularly useful for optimising the insonation. Assuming that the highest velocity represented the lowest angle of insonation to the blood flow direction, the Doppler measurement was repeated three to five times in this position, and the averaged value included in the statistics.

The velocity increment at the level of the umbilical ring was calculated as the difference between the time-averaged blood velocity at the umbilical ring ($V_{max,abd}$) and that found in the cord ($V_{max,cord}$) presented as a percentage of the velocity in the cord: $100\%\ (V_{max,abd} - V_{max,cord})/V_{max,cord.}$ Ln-transformation was performed, if needed, to achieve normal distribution. Polynomial or fractional polynomial regression models were fitted to the data in order to construct mean curves for blood velocities, diameters and their changes with gestational age. The method of scaled absolute residuals was used to construct the 10th and 90th centile curves (Royston and Wright, 1998). The 10th percentile was obtained from mean−1.28 SD, and the 90th percentile from mean +1.28 SD.

Intraobserver variation of the diameter and velocity measurements was studied for the participants using the repeated measurements included in the study. One-way analysis of variance was used to calculate the within-subject mean variance and mean SD, which reflects the intraobserver variation (Bland and Altman, 1996). The mean SD was calculated as $\sqrt{}$(mean square). The SPSS statistical package was used for all the analyses.

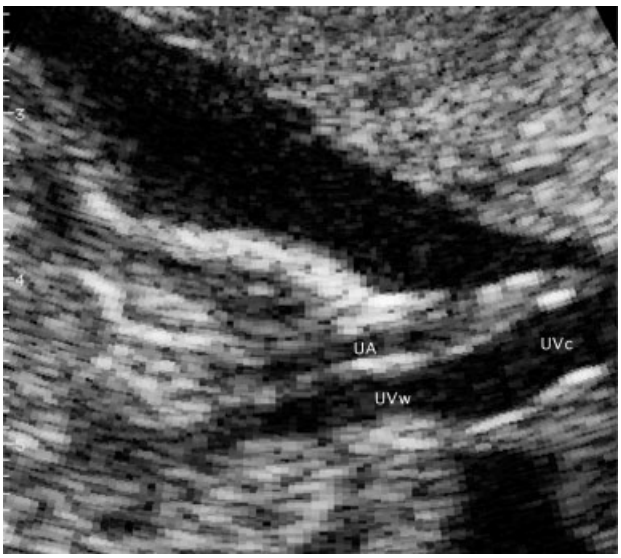

Figure 1—Ultrasound scan of the junction of the cord at the fetal abdominal wall at 21 weeks of gestation showing the umbilical vein in the cord (UVc) and the constricted portion of the vein at the abdominal wall (UVw). UA, Umbilical artery

RESULTS

There was no withdrawal among the 283 participants. The median gestational age at birth was 40 weeks 2 days (range: 28 weeks 0 days–42 weeks 5 days), and the median birth weight was 3720 g (range: 1220–5060 g). Twenty babies were delivered by caesarean section. There were no perinatal deaths, but 21 babies had an Apgar score of ≤ 7 at 1 min after birth. One baby had atrial septal defect, secundum type, another baby had stenosis of the pulmonary valve (infundibular type), and one baby had a muscular ventricular septal defect. Four babies were born with congenital hip dysplasia.

The time-averaged maximum venous blood velocity in the cord was recorded in 195 fetuses and showed a generally low velocity (mean 13–19 cm/s) during 20–40 weeks of gestation (Figure 2A). The corresponding velocity at the umbilical ring in the abdominal wall was recorded in 279 participants and was found to be substantially higher (34–41 cm/s) (Figures 2B and 3). Since the velocity did not change significantly with gestation [r = 0.01, 95% CI (−0.001; 0.2)], the results were combined and percentiles were calculated for the entire second half of pregnancy

Table 1—Percentiles for the time-averaged maximum blood velocity in the umbilical vein at the abdominal wall (umbilical ring) based on observations in 279 low-risk pregnancies

	Percentile														
	10	20	30	40	50	55	60	65	70	75	80	85	90	95	97.5
Velocity (cm/s)	21	24	27	30	33	35	38	41	44	50	60	74	84	107	129

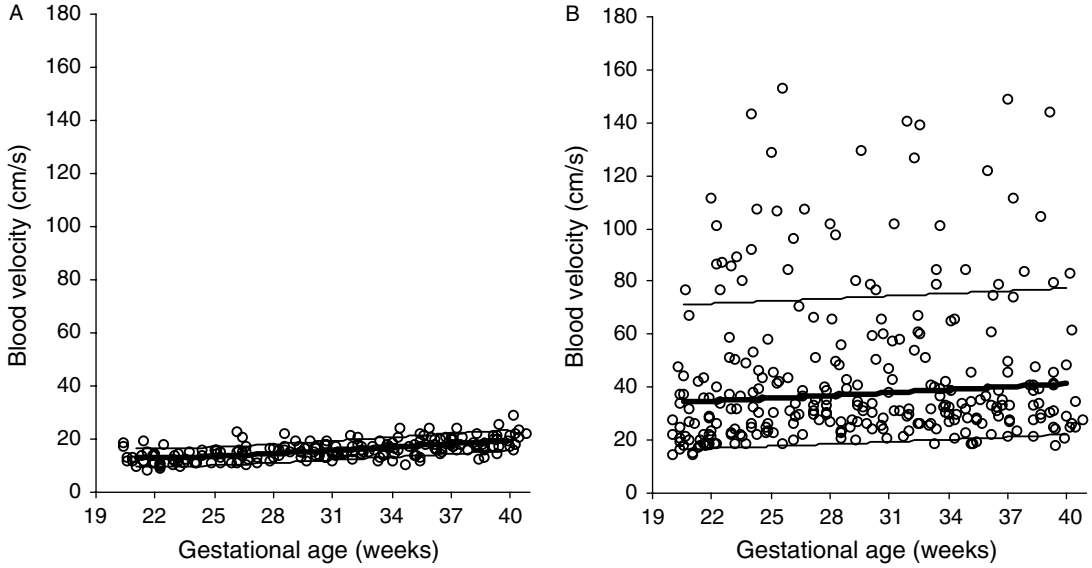

Figure 2—The 10th, 50th and 90th percentiles of the time-averaged maximum blood velocity in the umbilical vein of the cord in 195 low-risk pregnancies (A). The equation for the 50th percentile is: $y = 68.3 + 8611.5x^{-2} - 342.7x^{-0.5}$. $SD = 2.2 + 0.02x$. The corresponding velocity at the abdominal wall was higher (B). The equation for the 50th percentile ($n = 279$) was: $y = 3.34 + 0.01x$. Ln-transformation was performed. $SD = 0.65 - 0.004x$

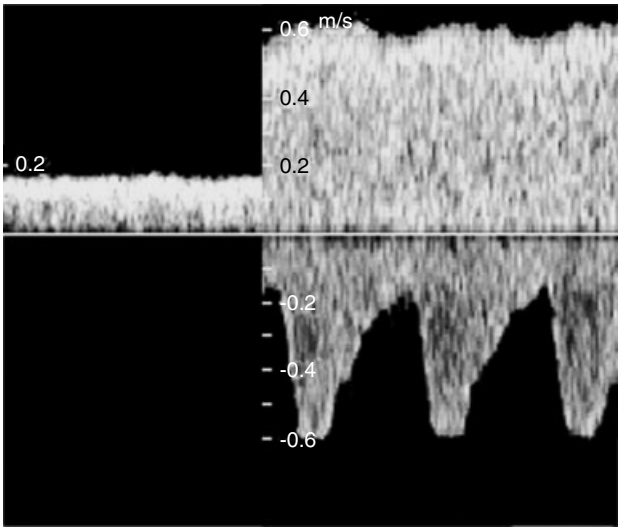

Figure 3—Doppler recording of the umbilical venous blood velocity at the fetal end of the cord (left panel) in a fetus at 32 weeks of gestation. The velocity is low compared to the recording at the abdominal inlet (right panel), which shows the simultaneously recorded blood velocity in the umbilical artery

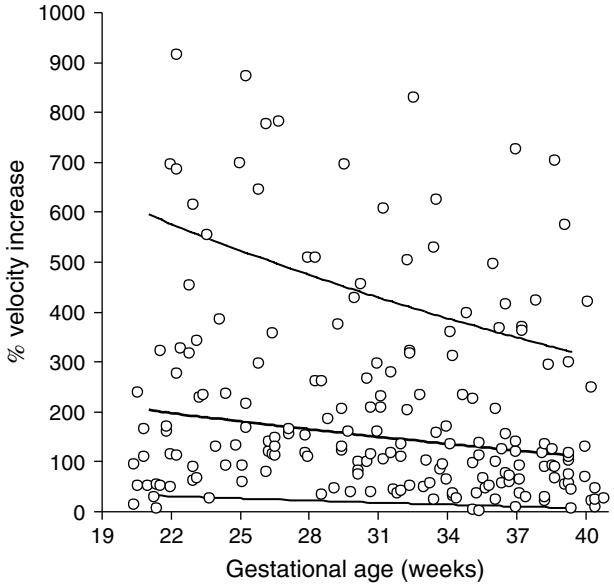

Figure 4—Umbilical venous velocity acceleration at the abdominal wall calculated as the percentage increment of the blood velocity in the umbilical cord and presented with the 10th, 50th and 90th percentiles ($n = 191$). The equation for the 50th percentile is: $y = 6.12 - 0.02x$. Ln-transformation was performed. $SD = 0.778 - 0.006x$

(Table 1). The acceleration of blood at the umbilical ring was calculated based on 191 pairs of observations (Figure 4). We found that 78 % (149/191) had a velocity increase of $\geq$50%, and that 21% (41/191) had $\geq$300%, which corresponds to a diameter reduction of $\geq$50%. The velocity increment showed substantial variation and values exceeding 900% were noted (Figure 4). There was a small tendency towards reduced relative velocity increment with increasing gestational age [$r = -0.02$, 95% CI (-0.032; -0.005)], mainly due to the small increase of venous blood velocity in the cord with gestational age (Figure 2A), which formed the 100% for calculating the increment.

The mean inner diameter of the vein in the cord was 3.6–8.2 mm during gestational weeks 20–40 while the corresponding diameter at the umbilical ring was less at 2.8–5.9 mm (Figure 5).

The reproducibility study showed that the diameter measurements both at the umbilical ring and at the cord had a mean SD of 0.07 mm (Table 2). The variation

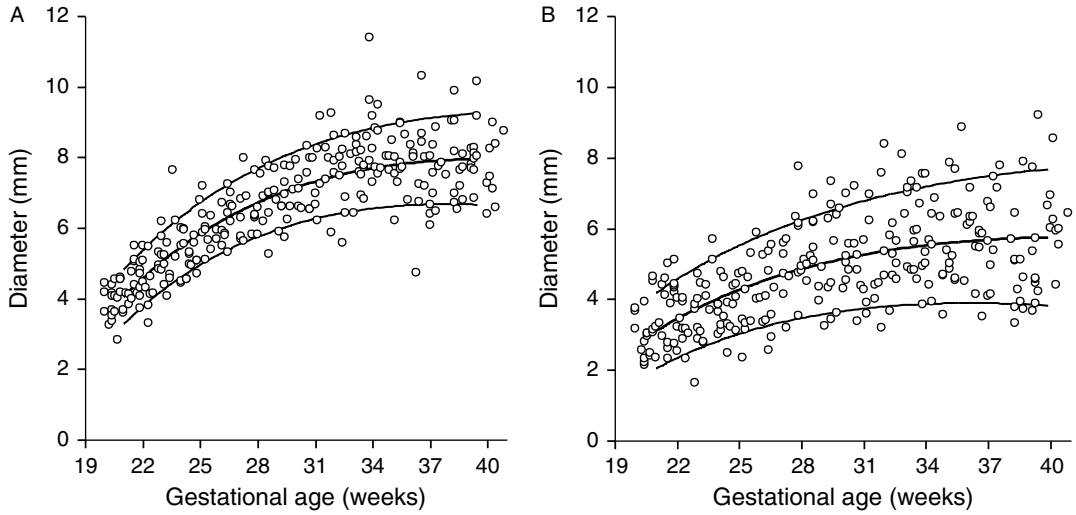

Figure 5—The 10th, 50th and 90th percentiles of the inner diameter of the umbilical vein in the cord of 273 fetuses (A). The equation for the 50th percentile is: $y = 49.2 - 173.6x^{-0.5} - 0.34x$. $SD = 0.14 + 0.02x$. The corresponding venous diameter at the abdominal wall was determined in 276 fetuses (B). The equation for the 50th percentile is: $y = 30.96 - 108x^{-0.5} - 0.2x$. $SD = 0.09 + 0.036x$

Table 2—Intraobserver variation for diameter measurements (mm). One-way analysis of variance for repeated measurements of the umbilical vein **in the cord** in 273 participants and at the umbilical ring in 275 participants

	Degrees of freedom	Sum of squares	Mean square	Mean SD	F ratio	*p*
In the cord						
Between subjects	272	3424.179	12.5889		2302.7155	<0.0001
Within subjects (intraobserver)	1079	5.899	0.0055	0.0739		
Total	1351	3430.078				
At the umbilical ring						
Between subjects	274	2849.568	10.3999		1853.1137	<0.0001
Within subjects (intraobserver)	1077	6.0442	0.0056	0.0749		
Total	1351	2855.612				

Table 3—Intraobserver variation for Doppler flow velocity measurements (cm/s). One-way analysis of variance for repeated measurements in the umbilical vein in the cord in 195 participants and **at the umbilical ring** in 279 participants

	Degrees of freedom	Sum of squares	Mean square	Mean SD	F ratio	*p*
In the cord						
Between subjects	194	9958.3137	51.3315		153.7631	<0.0001
Within subjects (intraobserver)	598	199.6333	0.3338	0.5777		
Total	792	10157.947				
At the umbilical ring						
Between subjects	278	1059671.35	3811.7675		1142.9098	<0.0001
Within subjects (intraobserver)	1004	3348.4833	3.3351	1.8262		
Total	1282	1063019.83				

of venous blood velocity measurements at the cord and at the umbilical ring had SDs of 0.58 and 1.83 cm/s (Table 3).

Fetal movements, unfavourable position, maternal obesity, and reduced time for observation were the reasons for a reduced number of observations in some of the participants. The velocity and diameter measurements in the umbilical ring had the highest priority, sometimes leaving less time for measurements of the cord, particularly during the initial phase of the study.

DISCUSSION

In the present study we have shown that the umbilical ring is tight during the second half of pregnancy and commonly influences the umbilical venous diameter and blood velocity. As many as 78% of the fetuses in the present study had ≥50% increase in velocity at the umbilical ring, and 21% had ≥300%, which corresponds to a constriction of the vein to half of the diameter. Since the population examined was drawn from the

low-risk antenatal clinic, we assume that the results represent the physiological ranges of venous constriction at the inlet to the abdomen and that these ranges are wide.

The period of physiological umbilical herniation ends at 12 weeks of gestation. We have previously shown that in the following weeks, the umbilical ring is progressively tightened causing a venous constriction at the abdominal wall and a corresponding increase of venous blood velocity, sometimes exceeding an acceleration of 500% (Skulstad *et al*., 2001). The present study shows that the venous velocity at the umbilical ring remains unchanged during the second half of pregnancy (Figure 2B) and that the calculated percentiles can by used for the entire second half of pregnancy (Table 1).

A previous small study of 11 fetuses (gestational age 24–34 weeks), showed that the venous velocity was higher immediately inside than outside the abdominal wall (Kilavuz and Vetter, 1998), which supports the same assumption: that there is a physiological venous constriction at the abdominal ring during the second half of pregnancy. However, based on the Bernoulli theorem, we believe measurement of blood velocity at the umbilical ring itself (used in the present study) reflects the reduction of diameter more precisely than the technique used by Kilavuz and Vetter, sampling inside the abdomen where the vein is wider and the velocity rapidly reduces.

The present results of blood velocity and diameter in the umbilical vein in the cord are in agreement with a previous report (Barbera *et al*., 1999). The mean of the velocities at 20–40 weeks was 12.8–19.2 cm/s in the present study, whereas Barbera and co-workers found 15.7–21.7 cm/s. Correspondingly, the mean inner diameter of the vein in the cord was 3.6–8.2 mm during gestational weeks 20–40 in the present study, compared to 4.1–8.7 mm in their study.

In the present study we have based our conclusions on velocity measurement rather than on diameter assessments. The reasons for this are two-fold. First, velocities increase by the power of two compared with the diameter changes, which makes Doppler a sensitive measurement. Second, the measurement of vessel diameters carry a high risk of error (Gill *et al*., 1981; Eik-Nes *et al*., 1984). Diameter measurements in small vessels (0.3–2.3 mm) have been shown to have an intraobserver variation with SD 0.17 mm, and for vessels of diameter 2.0–8.0 mm SD 0.23 mm (Kiserud and Rasmussen, 1998; Kiserud *et al*., 1999). In order to control for this kind of random error we used repeat measurements as suggested in previous studies (Kiserud and Rasmussen, 1998; Kiserud *et al*., 1999). We achieved SD 0.07 mm both for the umbilical vein in the cord and at the umbilical ring when using $\geq$5 repeat measurements to establish the diameter (Table 2), which are comparable with the previous results.

We are not sure that our assumption of a circular cross-section is valid for the section of the vein situated in the umbilical ring. This cross-section may be influenced both by the degree of compression from the umbilical ring and the umbilical arteries, which may not exert evenly distributed impact round the circumference.

It has been suggested that intravascular pressure may be a determinant for the development of the villi (Karimu and Burton, 1994). Whether an increase in resistance due to an umbilical venous constriction can have any impact on the umbilical circulation is open to speculation, leaving room for the hypothesis that an extreme degree of umbilical venous constriction constitutes a risk factor for perinatal complications (Skulstad *et al*., 2001).

In the present study we have shown that the umbilical vein constriction is a common finding that does not change during the second half of pregnancy. We have established reference ranges for this phenomenon. Whether extreme degrees of constriction affect placental circulation or are associated with any type of pregnancy complication are hypotheses still to be tested.

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Paper III

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The effect of vascular constriction on umbilical venous pulsation

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KEYWORDS: blood flow; circulation; compliance; Doppler; fetus; hemodynamics; pulsation; ultrasound

ABSTRACT

Objectives *Umbilical venous pulsation is an important sign of hemodynamic compromise, but is also found under normal physiological conditions. Mathematical modeling suggests that vascular compliance is a determinant for pulsation, and we tested this by studying velocity pulsation at three sites on the umbilical vein.*

Methods *In a cross-sectional study of 279 low-risk pregnancies (20–40 weeks' gestational age) blood flow velocity in the umbilical vein was determined before, within and after the umbilical ring in the fetal abdominal wall, and the incidence and magnitude of pulsation (the difference between the maximum and minimum velocity during a pulse, and pulsatility index) were noted. Based on the fact that the vessel cross-sectional area is an important determinant of compliance, we measured the diameter and time-averaged maximum velocity to reflect variation in diameter and compliance at the three sites.*

Results *The incidence of umbilical venous pulsation was higher at the umbilical ring in the abdominal wall (242/279, 87%, 95% CI 82–90) than in the cord (43/198, 22%, 95% CI 16–27) or intra-abdominally (84/277, 30%, 95% CI 25–36) ($P < 0.001$). When pulsation was observed intra-abdominally, the pulsatility was not different from that at the umbilical ring ($P = 0.16$). However, the lowest pulsatility was found in the cord vein ($P < 0.0001$), where the largest vein diameter was found.*

Conclusion *The high incidence of venous pulsation at the umbilical ring where diameter and compliance are low supports the suggestion that local compliance is an important factor influencing pulsation in fetal veins.*

INTRODUCTION

In 1986, Lingman *et al.*[1] suggested pulsation in the umbilical vein as a clinical sign of pending fetal asphyxia, and in the years that followed it was shown that pulsation occurred more commonly in growth-restricted fetuses[2,3] and fetuses with cardiac malformations[4]. The sign proved to be an indicator of poor prognosis in hydropic fetuses[5] and was later integrated as a regular part of the fetal hemodynamic evaluation[6].

The rationale for this is that an augmented atrial contraction wave, which reflects increased afterload and adrenergic drive[7,8], is transmitted along the transmission line formed by the inferior vena cava and ductus venosus into the umbilical vein[9,10]. As the pulse wave travels along the venous transmission line, it is modified according to the local variation in vascular impedance[11–13], with reflection mechanisms playing a major role[14,15]. The wave reflection is particularly effective at the junction between the ductus venosus and umbilical vein due to the substantial step in impedance; the diameter ratio is 4 (95% CI 2–6)[15]. Accordingly, during normal late pregnancy, the umbilical vein receives a small proportion of the wave energy, which is usually not sufficient to cause visible velocity pulsation. However, the fact that pulsation occurs in normal pregnancies but varies with gestational age[16,17] and site of recording[18] indicates that determinants other than the magnitude of the emitted wave are in play, and have to be controlled in order to make the pulsation sign a meaningful clinical tool.

Another local determinant is vascular compliance. In a computer model it was shown that a stronger pulse was needed to induce velocity pulsation in a compliant compared to a less compliant umbilical vein[13]. Compliance in the umbilical vein is determined by vascular wall stiffness[19], transmural pressure and vessel cross-sectional area. We have previously shown that after the period of physiological umbilical herniation

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is completed, the umbilical ring is tightened, imposing a physiological constriction of the umbilical vein in quite a few fetuses[20,21]. This tightening and constricting process at the umbilical ring also represents a reduction in compliance.

In the present study we hypothesize that the constricted section of the umbilical vein at the abdominal entrance (the umbilical ring) is associated with more velocity pulsation compared to the more compliant neighboring sections. Based on this assumption we determined the occurrence and magnitude of umbilical venous pulsation at the abdominal inlet and compared the results with corresponding observations in the cord or intra-abdominal section of the vein in a clinically relevant setting.

METHODS

A group of 283 women with low-risk pregnancies had been recruited for a detailed study of the umbilical circulation after written informed consent was obtained according to a protocol acknowledged by the Regional Committee for Medical Research Ethics. The participants were examined once at 20–40 weeks of gestation during a 45-min session using a Vingmed CFM 800 ultrasound scanner (GE Vingmed Sound, Horten, Norway) with one of two multifrequency mechanical sector transducers (center frequency 3.25 or 5.0 MHz) carrying color and pulsed Doppler facilities (2.5 or 4.0 MHz). The spatial peak temporal average intensity was set at 50 mW/cm^2 for the pulsed Doppler and was less for the color Doppler.

The blood velocity was recorded in three portions of the umbilical vein: one in a free loop at the fetal end of the umbilical cord, another at the inlet through the abdominal wall (the umbilical ring), and a third at the straight intra-abdominal portion of the vein (Figure 1). In the cord and in the intra-abdominal part, the insonation was along the long axis of the vein and with an expanded sample volume. Color Doppler was used to ensure that the insonation was orthograde with the blood flow. Umbilical venous pulsation was defined as a velocity variation synchronized with the fetal heart rate. The assessment was done both visually (noting whether pulsation was present or not) and by the temporal maximum velocity tracing of the Doppler shift. The degree of pulsation was calculated as the difference (ΔV) between the maximum velocity and minimum velocity during the pulsation calculated in centimeters per second (cm/s) or as the pulsatility index (PI) (ΔV/time-averaged velocity). The average of three or more pulses was entered into the statistics for the three sites of the umbilical vein.

An expression of compliance was sought in two ways. First, assuming that the variation in diameter along the vein reflects a corresponding variation in compliance we determined the diameter as an average of three or more measurements at each of the three sites. Second, based on the fact that blood velocity has to be increased correspondingly to the reduction in diameter in order to maintain volume flow, $\pi(D_1/2)^2V_1 = \pi(D_2/2)^2V_2$ (D, diameter; V, velocity), we used the measurement of time-averaged maximum velocity (TAMXV) and the percentage velocity increment (umbilical venous velocity in the cord being 100%) as indicators of venous constriction (and low compliance).

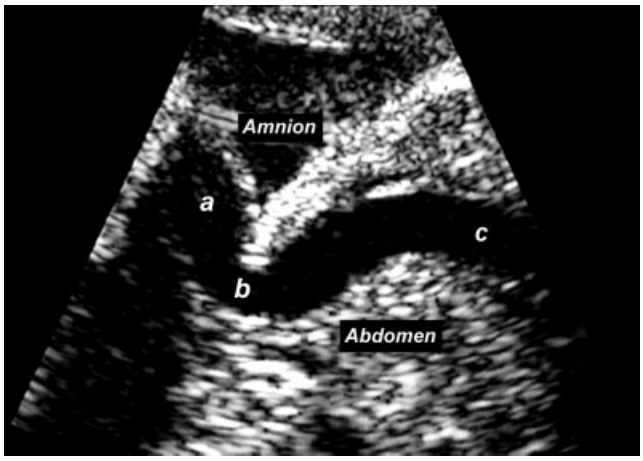

Figure 1 Ultrasound image of the umbilical cord insertion at the fetal abdomen at 26 weeks of gestation demonstrating a modest constriction of the vein at the umbilical ring. Measurements were taken in the free loop of the cord (a), at the abdominal wall (b) and at the straight intra-abdominal portion of the vein (c).

Analysis of variance was used to calculate mean and 95% CI values, and the Chi-square test was employed to assess differences between observations. Regression analysis was used to determine the effect of constriction (and reduced compliance) on the magnitude of pulsation. $P = 0.05$ was chosen as the significance level.

RESULTS

A total of 279/283 participants had acceptable umbilical venous velocity recordings. The average gestational age at examination was 29.6 (range, 20–41) weeks. The median gestational age at birth was $40 + 2$ (range, $28 + 0$ to $42 + 5$) weeks and the median birth weight was 3720 (range, 1220–5060) g. Twenty babies were delivered by Cesarean section. There were no perinatal deaths.

We found visible pulsation in the umbilical vein at the abdominal inlet (umbilical ring) in 242/279 participants (87%, 95% CI 82–90), which was more common than in the cord (43/198, 22%, 95% CI 16–27) and in the intra-abdominal section of the vein (84/277, 30%, 95% CI 25–36) ($P < 0.001$) (Figure 2).

The velocity variation, ΔV, during the venous pulse was more pronounced at the abdominal wall (mean 7.3 cm/s, 95% CI 7.0–7.6) than in the cord (mean 2.6 cm/s, 95% CI 2.2–3.0) and the intra-abdominal segment (mean 4.1 cm/s, 95% CI 3.8–4.4) ($P < 0.001$) (Figures 3 and 4). When comparing the PI of the pulsation identified in the intra-abdominal umbilical vein with that at the umbilical ring no difference was found ($P = 0.16$). Conversely, when comparing umbilical cord with the umbilical ring there was a reduction in PI of 0.03 ($P < 0.0001$, 95% CI 0.04–0.02). However, for the majority of cases of

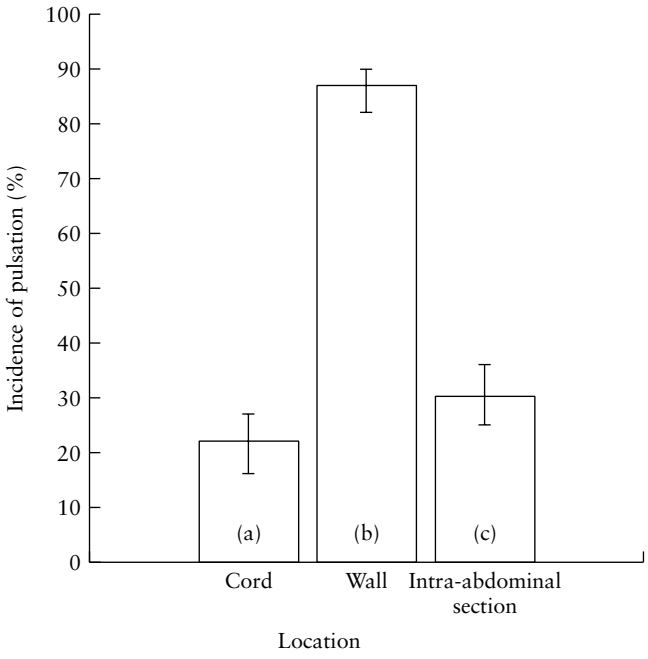

Figure 2 The percentage of velocity recordings with visible pulsations presented with 95% CI for (a) the extra-abdominal portion of the umbilical vein ($n = 198$), (b) the abdominal wall ($n = 279$) and (c) the intra-abdominal section of the vein ($n = 277$).

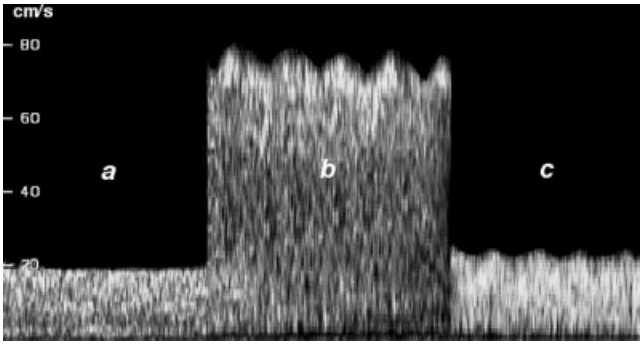

Figure 3 Umbilical venous blood velocity recording (a) in the cord, (b) at the abdominal inlet and (c) in the intra-abdominal section at 26 weeks of gestation. On average, there is no difference in the pulsatility index between sites (b) and (c). Compared to (c), the pulsation at (b) is scaled up by the increased velocity. The lack of pulsation at (a) is attributed to a higher compliance in the cordal vein than in the two other sections.

pulsation at the umbilical ring there was no corresponding pulsation in the intra-abdominal or extra-abdominal vein (Figure 2).

The umbilical vein diameter at the abdominal wall was significantly smaller than in the cord (mean difference 1.8 mm, 95% CI 1.5–2.0), but the difference between the vein diameter at the abdominal wall and in the intra-abdominal section was not significant (mean difference 0.1 mm, 95% CI −3.1 to 1.8). The diameter of the umbilical vein in the cord was significantly larger than in the intra-abdominal section (mean difference 1.7 mm, 95% CI 1.5–2.0) ($P < 0.01$) (Figure 1). The TAMXV at the abdominal wall was significantly higher than in the cord (mean difference 27.8 cm/s, 95% CI 24.6–31.1) and

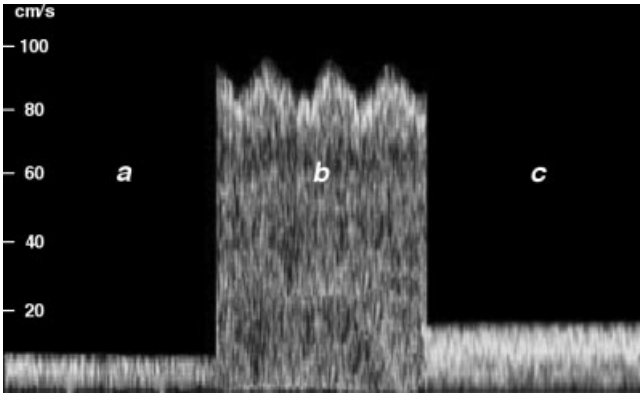

Figure 4 Doppler recording showing pulsation in the umbilical vein at the abdominal wall (b), but no pulsation in the cord (a) or in the intra-abdominal section (c), at 25 weeks of gestation, a common pattern observed during the second half of pregnancy.

in the intra-abdominal section (mean difference 22.7 cm/s, 95% CI 19.7–25.6), indicating a constriction and reduced compliance at the abdominal inlet ($P < 0.001$).

DISCUSSION

In the present study we have shown that visible pulsation in the umbilical vein is more common and more pronounced at the abdominal inlet where the umbilical ring tends to exert a constrictive impact on the vein thus reducing its compliance. The incidence of pulsation at this site was remarkably high: 87% compared to 22% and 30% in the neighboring sections (Figure 2). Interestingly, when pulsation was recorded simultaneously at the three sites, the pulsatility was at its lowest in the cord where the diameter of the vein was largest (and compliance lowest), illustrating the role of compliance as a local determinant for pulsation[13].

The high velocity at the constricting umbilical ring scales up the pulsation and makes it more visible (Figure 3). However, in the majority of cases the pulsation at the umbilical ring is not accompanied with pulsation in the neighboring sections of the vein (Figures 2 and 4), signifying that mechanisms other than scaling must be involved, compliance being one likely candidate.

What is the origin of the venous pulsation at the abdominal wall? One obvious candidate is the atrial contraction wave. This wave follows the transmission line inferior vena cava–ductus venosus–umbilical vein to reach the abdominal wall. However, the pulse energy is reduced at the junction between the ductus venosus and umbilical vein due to reflections and may not suffice to induce velocity changes in the reservoir-like intra-abdominal umbilical vein[22], but may do so when the pulse reaches the umbilical ring where the compliance is lower. For some of the current observations this may be the case. The sudden increase in impedance at the umbilical ring would also cause reflections and reduce the energy transmitted into the cordal section of the vein. The result, reduced pulsation, is actually what we observed in the cord.

Another candidate for inducing pulsation in the vein is the neighboring umbilical artery at the abdominal inlet. The umbilical ring tightens around the three vessels thus reducing the compliance at this point and facilitating the transmission and induction of a pulse in the vein in as many as 87% of the fetuses. Extra-abdominally, in the cord, the diameter and compliance of the umbilical vein are higher and the incidence of pulsation correspondingly low (i.e. 22%). The smooth and prolonged pulse pattern commonly seen in the recordings (Figures 3 and 4) suggests an arterial origin rather than the atrial contraction wave, which tends to be a short and distinct deflection.

The low compliance is probably an important reason why umbilical venous pulsation is common in early pregnancy[16,17] when vessel dimensions are minute. Conversely, the high compliance of the sizeable intra-abdominal umbilical vein in late pregnancy makes pulsation a rare event. The commonly recorded pulsation in the portal system during the second half of a normal pregnancy[18,23] may have a similar reason: the transverse portal sinus (left portal branch) has a considerably smaller dimension compared to the umbilical vein[24].

During abnormal cardiac physiology[25,26] an augmented atrial contraction is transmitted along the inferior vena cava and ductus venosus, which act as a transmission line to reach the intra-abdominal umbilical vein[9,15]. During hypoxia the ductus venosus distends[27,28] and reduces the reflections that normally take away most of the pulse energy before it reaches the umbilical vein[15]. A congestion in the umbilical vein (commonly seen in such situations[29]) stretches the wall, or a twisting of the cord[30] causes a reduced compliance, which enhances the induction of velocity pulsation. An increased tone in the vessel wall due to adrenergic stimulation acts in the same direction. Thus compliance[13], together with velocity direction[24] and differences in impedance at venous junctions[14,15], are important issues when interpreting pulsations in a clinical setting.

Although we have found an increased blood velocity in the section of the umbilical vein passing through the abdominal wall, and shown that the diameter at this point is less than in the cord, the diameter was not found to be significantly smaller than in the intra-abdominal portion of the vein. We have previously reported that the diameter measurement at the abdominal wall is less reliable than at other sections of the vein, and have recommended the use of velocity measurements to indicate constriction. We are not sure that the assumption of a circular cross-section is valid for the abdominal wall section of the vein[20], or whether the constriction is situated slightly more into the cord in some cases and therefore not included in the diameter measurement at the umbilical ring. Reports of umbilical cord stricture close to the abdominal insertion in some stillborns support the latter scenario[31]. In the latter case the velocity measurement would be the more reliable one.

A point could be made that it is the weighted mean velocity that actually reflects the constriction, not the maximum velocity tracing. Compared to weighted mean velocity the maximum velocity tracing, which is used in the present study, is an overestimation but a more practical and robust method. Since a constriction causes an acceleration of the blood, the spatial velocity profile changes from a parabolic to a blunted pattern in much the same way as in the ductus venosus[32,33]. The consequence for the present study would be that the velocity measurement overestimates less the stricture than the two other sections so that the diameter difference actually is underestimated. Another possible source of error would be the impact of vessel curvature causing a skewed spatial velocity profile with an increased maximum velocity. This has been shown in mathematical models of the ductus venosus[32], but the effect does not reach the extreme velocity changes described at the umbilical ring. We therefore believe that the measurements we have obtained represent real constrictions of the umbilical vein. However, to assess the degree of modification imposed by curvatures of the vein, a mathematical model would be helpful.

In short, based on our observations in the umbilical vein at sections with various diameters (and compliance) we have demonstrated that compliance is a determinant for velocity pulsation and should be taken into account when interpreting Doppler recordings in a clinical setting.

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Paper IV

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The effect of umbilical venous constriction on placental development, cord length and perinatal outcome

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KEYWORDS
Fetus;
Umbilical vein;
Placenta;
Doppler;
Growth;
Gender

Abstract

Background: Umbilical vein constriction at the fetal abdominal inlet is a common finding after week 13, when the period of umbilical herniation is brought to an end.
Aims: To test the hypothesis that a constricting umbilical ring within physiological ranges affects fetal hemodynamics by either pooling blood in the placenta or restricting nutrient transfer to the fetus and thus shift the birthweight/placental weight (BW/PW) ratio. A constriction could also cause pressure changes and elongation of the cord and possibly be a disadvantage during labour.
Study design: Cross-sectional.
Subjects: 359 Low-risk singleton pregnancies at 13—40 weeks of gestation.
Outcome measures: Standard deviation score (z-score) and regression analysis were used to determine the effect of umbilical vein constriction (expressed by increased blood velocity) on birthweight/placental weight ratio (BW/PW), cord length, Apgar score and emergency delivery due to fetal distress.
Results: Umbilical venous constriction had a mild but significant effect on BW/PW in male (p=0.018) but not in female fetuses. Increased constriction was also associated

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with increased length of the cord but only in female fetuses (p=0.019). Cord length was positively related to birthweight and placental weight, but an increased length of the cord was also associated with decreasing BW/PW ratio for the male fetuses only (p=0.044). Increasing degree of venous constriction was associated with Apgar score ≤7 at 1 (p=0.009) but not at 5 min after birth and was not associated with emergency delivery.
Conclusion: Physiological umbilical venous constriction exerts a mild but significant gender-specific hemodynamic impact on intrauterine development.

1. Introduction

The umbilical vein with its compliant walls [1,2] and low blood pressure (4—10 mm Hg) [3] is particularly susceptible to external mechanical forces. Twisting, stricture or externally imposed constriction may cause complications and death in utero [4—18]. In a group of patients with nuchal cord entanglement, spontaneous fetal movements caused transient complete cessation of the umbilical venous flow [19]. A similar effect was observed when applying external pressure on the maternal abdominal wall for short periods of time (1—2 s) [19]. Nuchal cord entanglement has also been associated with increased risks of fetal distress [20,21], operative delivery [21] and 5-min Apgar score <7 [20]. In addition to these short-term effects, more lasting consequences have been described. The fact that the birthweight/placental weight ratio (BW/PW) is lower in pregnancies with a nuchal cord entanglement probably reflects such a lasting mechanical impact. It has been suggested that restriction of the transfer of nutrient to the fetus and thus growth or a pooling of blood in the placenta may be responsible for this shift in weight development [22].

We have previously shown that after the period of physiological umbilical herniation has been completed at 12 weeks of gestation, the umbilical ring at the abdominal wall is increasingly tightened, causing a constriction of the umbilical vein in quite a few fetuses [23]. In low-risk pregnancies, 20% of the fetuses have a constriction at 20 weeks of gestation corresponding to a diameter reduction of the vein to the half [24]. This pattern is found during the entire second half of pregnancy. Based on these findings, we hypothesise that an umbilical venous constriction represents a hemodynamic factor that could either pool blood in the placenta or restrict nutrient transfer to the fetus and thus shift the BW/PW ratio. Similarly, a constriction could cause pressure changes and prolongation of the cord and possibly be a hemodynamic disadvantage during labour.

Thus, the aim of the present explorative study was to determine the effect of venous constriction at the umbilical ring on the BW/PW ratio in a low-risk population, whether such a constriction had any impact on cord length and whether it was associated with a more frequent acute operative delivery and low Apgar score at birth.

2. Methods

A total of 370 women with a singleton pregnancy were recruited from the low-risk antenatal clinic for a larger evaluation prospective study of fetal venous hemodynamics. Previously, results on umbilical velocity distribution, degree of constriction at the umbilical ring and the effect of vascular constriction on venous pulsation have been published [23—25]. In the present paper, we address the effect of venous constriction on perinatal outcome, length of cord and birthweight/placental weight. The participants all gave written informed consent according to a protocol approved by the Regional Committee for Medical Research Ethics. Smoking, diabetes, hypertension or any general chronic disease excluded participation, as did a history of previous hypertensive complication of pregnancy, growth restriction or abruption of the placenta. Inasmuch as prematurity in itself is associated with less favourable perinatal outcomes [26], we chose to exclude the group of 11 participants born before 37 weeks of gestation. The final number of pregnancies left for the study was then 359. Gestational age was routinely assessed by ultrasound measurement of the crown-rump length in early pregnancy or fetal biparietal diameter before 20 weeks of gestation. Chromosomal aberrations and malformations were not included. However, chromosomal aberrations or malformations discovered during pregnancy or after birth were not reasons for withdrawal. Mode of delivery was noted, as well as urgent delivery by ventouse, forceps or caesarian section due to fetal distress. After birth, Apgar

score, gender, birth weight, placenta weight and umbilical cord length were noted, and a pediatrician examined the newborn.

The participants were examined once at 13—40 weeks of gestation during a 45-min session, using a Vingmed CFM 800 ultrasound scanner (GE Vingmed Sound, Horten, Norway) with one of two multifrequency mechanical sector transducers (centre frequency 3.25 or 5.0 Mhz) carrying colour and pulsed Doppler facilities (2.5 or 4.0 Mhz). The spatial peak temporal average intensity was set at 50 mW/cm^2 for the pulsed Doppler and was less for the colour Doppler mode. Each Doppler recording took 2—12 s.

We previously introduced the measurement of the high blood velocity or the increase in velocity as a better marker for vascular constriction at the umbilical ring than the direct diameter measurement [23]. Thus, in the present study, we either used the time-averaged maximum venous blood velocity at the umbilical ring ($V_{max,abd}$) (Fig. 1) or the percentage increase of velocity when compared to the time averaged maximum velocity recorded in the cord ($V_{max,cord}$): 100% ($V_{max,abd}-V_{max,cord}$)/$V_{max,cord}$ [23].

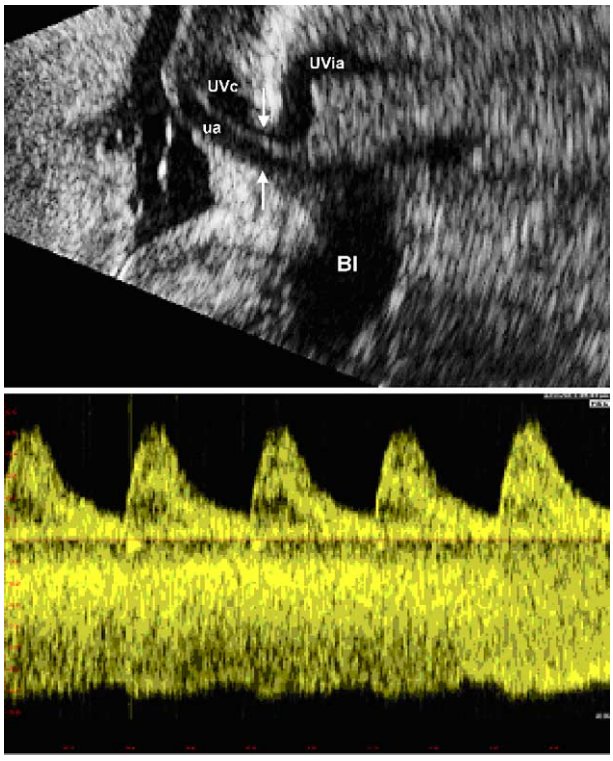

Figure 1 Upper panel—sagittal ultrasound scan of the junction of the cord at the fetal abdominal wall at 28 weeks of gestation showing the umbilical artery (ua) and vein (uvc) in the cord and the intra-abdominal portion of the vein (uvia). The vessels pass through the constricting ring at the abdominal wall (arrows). Bl—urinary bladder. Lower panel—doppler recording perpendicular to the abdominal wall showing ua velocity wave above the zero-line and the increased uv velocity (70 cm/s) corresponding to the venous constriction below the zero-line.

Immediately after delivery, nursing personnel weighed the infant using an electronic weight scale. Then the weight of the placenta was taken using the same weight scale. Membranes and umbilical cord was included without any attempt to remove placental blood before weighing.

Mean and percentiles were constructed, as previously proposed by Royston and Wright [27]. Polynomial regression analysis was performed to identify the regression curves that best fitted the mean and the standard deviation (S.D.) as a function of gestational age [25]. Ln tranformations were performed when appropriate. Standard deviation scores (*z*-scores) were calculated for the $V_{max,abd}$ and for the percentage velocity increase based on our previously estimated means [23, 24]. Similarly, the *z*-scores were calculated for BW/PW ratio and the umbilical cord length. *z*-Score for an observation was calculated based on the distance in standard deviations between the observation and the mean: (observed value−mean)/S.D. To describe the risk of operative delivery due to fetal distress and low Apgar at 1 min, the study population was stratified into tertiles according to percent velocity increase and to time-averaged maximum venous blood velocity at the umbilical ring. Differences were assessed by linear and logistic regression analyses. Statistical significance was considered achieved when $p<0.05$. The SPSS and Sigmaplot statistical packages were used for the analyses.

3. Results

There was no withdrawal among the 359 participants. Maternal age was at average of 29 years (range 18—41) and 36% were nullipara. The median gestational age at birth was 40 weeks, 2 days and the median birth weight 3710 g (Table 1). Twenty-five babies were delivered by caesarian section, eight of them due to fetal distress. Nineteen babies were delivered instrumentally, seven of them because of fetal distress. There were no perinatal deaths, but one neonate had signs of hypoxic brain injury with periventricular leukomalacia and later developed cerebral palsy.

Details of the umbilical venous velocity are published previously[23,24] and were, in the present study, used to establish individual *z*-scores.

Table 1 Birth weight (N=359), placental weight (N=340) and cord length (N=335) in the study group

	Mean*	Range
Gestational age at birth (weeks)	40.28	37.00–42.71
Females (N=168)	40.17	37.00–42.57
Males (N=191)	40.37	37.14–42.71
Birth weight (BW)[g]	3724	2460–5060
Females (N=168)	3668	2460–5060
Males (N=191)	3775	2580–5020
Placental weight (PW)[g]	666	350–1200
Females (N=152)	663	350–1200
Males (N=180)	669	350–1200
BW/PW ratio	5.60	2.77–8.75
Females (N=152)	5.54	3.50–8.75
Males (N=180)	5.66	2.77–8.74
Cord length (cm)	63.4	35–120
Females (N=153)	61.9	35–120
Males (N=182)	64.7	39–105

* Geometric mean.

In 354/359 fetuses, there was a recording of $V_{max,abd}$ to express degree of venous constriction, and in 255/359, there was a complete set of $V_{max,abd}$ and $V_{max,cord}$ to express the constriction by the percent velocity increase.

Of 23 babies with Apgar score ≤7 at 1 min after birth, three had ≤7 at 5 min. Apgar score ≤7 at 1 min after birth was related to venous constriction expressed both by the percentage velocity increase (p=0.009) and by $V_{max,abd}$ (p=0.022; Table 2). Apgar score at 5 min after birth was not related to venous constriction (N=349, slope=0.520, 95% CI [0.379; 7.457], p=0.494). The risk for acute operative delivery due to fetal distress was not linked to umbilical vein constriction (Table 3). One fetus with a high degree of venous constriction (318% velocity increase) later developed periventricular leukomalacia and cerebral palsy, as mentioned above. Mean placental weight was 666 g (range 350–1200 g). The mean BW/PW was 5.60 (range 2.77–8.75; Table 1). Based on z-score statistics, an effect of venous constriction (expressed as $V_{max,abd}$) on BW/PW was shown for male fetuses (N=178, slope=−0.179, 95% CI [−0.352; −0.007], p=0.041) but not for female (N=149, slope=0.074, 95% CI [−0.118; 0.172], p=0.715). The same effect was seen when the constriction was expressed as percent increase of blood velocity, i.e., reduced BW/PW in male fetuses (p=0.018) but not in female (p=0.441; Fig. 2). Adjustment for cord length had no effect.

The mean cord length was 63 cm (range 35–120 cm; Table 1). Cord length was positively related to birth weight (N=333, slope=53.591, 95% CI [4.290; 102.892], p=0.033) and placental weight (N=316, slope=23.190, 95% CI [6.837; 39.543], p=0.006). Venous constriction expressed by $V_{max,abd}$ was associated with an increase in cord length for the female fetuses (p=0.019) but not for the male fetuses (p=0.811) (Fig. 3). Cord length in itself was negatively related to the BW/PW ratio for male fetuses (N=171, slope=−0.166, 95% CI [−0.327; −0.004], p=0.044) but not for female (N=143, slope=−0.106, 95% CI [−0.258; 0.047], p=0.172).

4. Discussion

Umbilical vein constriction has been reported as a cause of fetal demise. However, we have recently shown that some degree of constriction of the umbilical vein is a normal phenomenon after 12 weeks in low-risk pregnancies. As many as 20% of the fetuses have a reduction of the umbilical vein diameter to the half or more at the inlet through the abdominal wall. In view of the present study of 359 pregnancies, such a constriction does not represent any significant disadvantage at birth, apart from leading to an increased number of

Table 2 Odds ratio for Apgar ≤7 at 1 min for different degrees of umbilical vein constriction (UV)

Degree of UV constriction	Apgar≤7	Apgar≥8	OR	95% CI		p
	N	N		Lower	Upper	
(a) Percentage increase in blood velocity (z-score, tertiles)						
1st tertile (low)	2	83	1			
2nd tertile (intermediate)	4	78	2.127	0.379	11.939	0.391
3rd tertile (high)	11	73	6.250	1.342	29.120	0.020
All tertiles	17	234	2.608	1.267	5.370	0.009
N=251						
(b) The time averaged maximum velocity (z-score, tertiles)						
First tertile (low)	4	112	1			
Second tertile (intermediate)	6	111	1.513	0.416	5.510	0.530
Third tertile (high)	13	104	3.500	1.106	11.075	0.033
All tertiles	23	327	1.944	1.100	3.435	0.022
N=350						

Table 3 Odds ratio for acute operative delivery for different degrees of umbilical vein constriction (UV)

Degree of UV constriction	Operative delivery Yes	Operative delivery No	OR	95% CI		p
	N	*N*		Lower	Upper	
(a) Percentage increase in blood velocity (z-score, tertiles)						
First tertile (low)	3	82	1			
Second tertile (intermediate)	6	79	2.076	0.502	8.587	0.313
Third tertile (high)	4	81	1.350	0.293	6.222	0.700
All tertiles	13	242	1.130	0.569	2.242	0.728
N=255						
(b) The time averaged maximum velocity (z-score, tertiles)						
First tertile (low)	3	115	1			
Second tertile (intermediate)	8	110	2.787	0.721	10.775	0.137
Third tertile (high)	4	114	1.345	0.294	6.142	0.702
All tertiles	15	339	1.110	0.588	2.095	0.747
N=354						

neonates with Apgar score ≤7 at 1 min after birth. This probably represents some hemodynamic effect of the constriction and is in line with our other findings; umbilical vein constriction was linked to longer umbilical cords and relatively larger placentas. Interestingly, these effects seemed to be gender-specific.

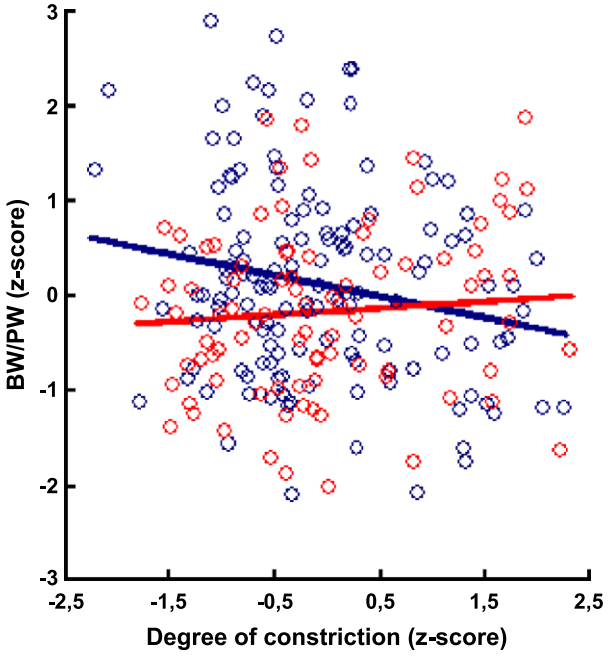

Figure 2 Effect of umbilical venous constriction (expressed by percentage increase in blood velocity) on the birth to placental weight ratio (BW/PW) for male fetuses (blue; *N*=137) and female fetuses (red; *N*=100) shown as *z*-scores (*z*-score=[observed value-mean]/S.D.). Male: $y=0.111-0.224x$, slope=−0.224, 95% CI (−0.409; −0.039), $p=0.018$. Female: $y=-0.159+0.070x$, slope=0.070, 95% CI (−0.110; 0.251), $p=0.441$. Adjustment for cord length had no effect. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Our data confirm the results of previous studies showing that cord length is positively related to placental size and birthweight [28–30]. Additionally, we showed that a long cord and an umbilical ring constriction, both representing increased

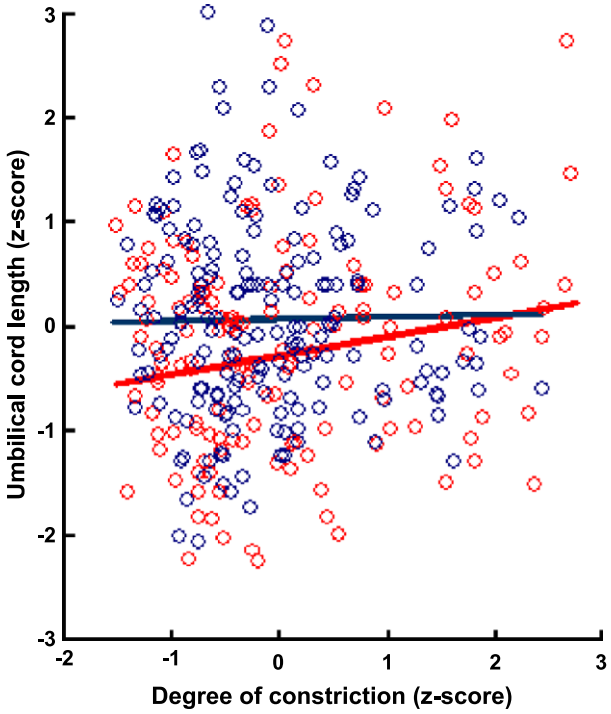

Figure 3 Effect of umbilical venous constriction (expressed by the maximum blood velocity) on the length of the umbilical cord for male fetuses (blue; *N*=180) and female fetuses (red; *N*=150) shown as *z*-scores (*z*-score=[observed value−mean]/S.D.). Male: $y=0.085-0.020x$, slope=0.085, 95% CI (−0.142; 0.181), $p=0.811$. Female: $y=0.183x-0.117$, slope=0.183, 95% CI (0.030; 0.336), $p=0.019$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

resistance to flow, shift the placental weight compared to birthweight (reduced BW/PW ratio). This is in line with previous reports on nuchal cord entanglement, which seems capable of long-term effects causing low birthweight [31] and shift in the BW/PW ratio [22], probably due to reduced transmission of nutrients to the fetus or pooling of fetal blood in the placenta [22]. Another possible mechanism would be a direct hemodynamic effect on placental growth, which depends on intravascular pressure to develop its villi [32].

Interestingly, the hemodynamic effect of the umbilical constriction was gender-specific (elongation of the cord in female and relative increase in placental weight in male fetuses). A recent study has shown gender-specific differences in the ductus venosus blood flow velocity at the gestational age of 10–14 weeks [33], which suggests that female and male fetuses develop differently their vascular system even in early pregnancy. Thus, gender-specific endocrine responses well known from postnatal life [34,35] seem to have an early fetal start [36].

We do not know from existing studies for how long time such an umbilical ring constriction may stay during pregnancy [23,24,37]. Twenty percent of the fetuses at any time during the second half of pregnancy have constriction to the half of the diameter [24], which suggests a rather long-standing or frequently repeated phenomenon. We believe that the effect on placental weight and length of the cord require that the effect is prolonged for days and weeks.

Nerves have been identified in the umbilical cord at the fetal end [37–39], and some of the nerve endings reach the media of the vein and could be motor in origin [39]. It is not known whether such nerves have any regulatory function, and it remains as speculation whether the umbilical ring exerts any regulation of umbilical blood flow in the fetus.

The study showed no association between venous constriction and emergency delivery due to fetal distress or Apgar score $\leq$7 at 5 min after birth. The study was exploratory in design and had insufficient power to answer these questions. However, the significant effect of physiological venous constriction on Apgar score at 1 min after birth (Table 2), all outcome OR>1 in Table 3 (admittedly not significant), the one fetus with high degree of constriction who later developed cerebral palsy and the hemodynamic effects on cord length and BW/PW ratio do indicate that further studies designed to assess the effect of extreme degrees of constriction on perinatal outcome are warranted. Considering brain damage during fetal life development to be multifactorial, we speculate that extreme, permanent or transient, umbilical ring constriction could be one of several factors contributing to increased risk.

In short, the present exploratory study has shown that a constricted umbilical vein at the abdominal inlet has a gender-specific effect on the development of the placenta and the cord and a possible hemodynamic disadvantage at birth. The results warrant further studies to assess whether the hemodynamic effect of the umbilical ring constriction is a risk factor during pregnancy and birth.

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