Maternal diabetes mellitus and fetal venous liver flow – a longitudinal study

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Thesis for the degree of Philosophiae Doctor (PhD)
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Scientific environment

This project was planned and conducted by the Research group for Pregnancy, Fetal development and Birth at the Department of Clinical Science, K2, University of Bergen (UiB), Norway.

The clinical part of the research was performed with practical support from the Department of Fetal medicine and Ultrasound, and from the Department of Obstetrics, at Haukeland University Hospital, Helse Bergen, Norway.

Mark Hanson contributed by reviewing and editing Paper II. M. Hanson works at the Institute of Developmental Sciences, University of Southampton, UK.

Elisabeth Qvigstad contributed by reviewing and editing Paper III. She works at the Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Norway

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My years in clinical research have been very valuable to me, as a doctor and on a personal level. I have the deepest respect for the researchers who came before me and have laid the foundations for our current work. I am grateful to have taken part in the whole process; from planning and organizing, the meticulous collection of data, to analyzing and presenting results. I have learned about teamwork and collaboration, about self-discipline and the degree of accuracy and methodological rigor that scientific enquiry demands. This project has broadened my perspectives but also taught me the value of narrowing down my scope.

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Abbreviations

BMI	body mass index (kg/m^2)
CI	confidence interval
DIP	diabetes diagnosed in pregnancy expected to continue postnatally
DM	diabetes mellitus
DOHaD	Developmental Origins of Health and Disease
DV	ductus venosus
FGR	fetal growth restriction
GDM	gestational diabetes
GWG	gestational weight gain
HbA_{1C}	glycated hemoglobin (% or mmol/L)
IADPSG	International Association of Diabetes in Pregnancy Study Group
IOM	Institute Of Medicine
IVC	inferior vena cava
LGA	large for gestational age
LPV	left portal vein
OR	odds ratio
PGDM	pregestational diabetes mellitus
PI	pulsatility index
PV	portal vein (main stem)
Q	Blood flow (ml/min)
TAMXV	time-averaged maximum flow velocity
UV	umbilical vein
WHO	World Health Organization

Abstract

Background: Despite adequate glycemic control, the risks of perinatal complications and fetal macrosomia are increased in pregnancies with pregestational diabetes (PGDM). Maternal overweight, obesity and excess gestational weight gain add significantly to the risk of large for gestational age offspring in PGDM pregnancies. Umbilical perfusion of the fetal liver has a key role in regulating fetal growth. We hypothesized that PGDM alters umbilical venous distribution and fetal liver blood flow depending on maternal anthropometry and glycemic control.

Aims: The aims were to study a population with PGDM to 1) Compare the longitudinal development of the venous liver flow with a low-risk population 2) Assess the relation between maternal HbA_{1C} and fetal venous liver flow 3) Explore the influence of maternal body mass index (BMI) and weekly gestational weight gain (GWG) on the venous liver flow 4) Test if fetal flow was related to birthweight differently in PGDM compared with the reference population.

Materials and methods: In a prospective longitudinal observational study, 49 women with PGDM underwent monthly ultrasound examinations in gestational weeks 20 – 36. The time average maximum blood velocity was measured by Doppler in the umbilical vein (UV), ductus venosus (DV), left portal vein (LPV) and portal vein (PV). The inner vessel diameter was measured in UV, DV and PV, and the blood flow was calculated. Flow was normalized for estimated fetal weight.

Mean and percentile curves were modelled by multilevel regression and compared with reference curves from a low-risk population (n=160). In addition, differences between mean fetal flow *z* -scores in the PGDM and low-risk populations were tested by independent sample *t*-test. HbA_{1C} was measured in the first trimester and the relation to fetal venous flow was assessed by multilevel regression.

Pre-pregnancy BMI and weekly GWG were calculated from self-reported pre-pregnancy weight and maternal height, and the last maternal weight that was measured before delivery. ANOVA was used to test fetal flow differences between the BMI and GWG categories, and to test differences between birthweight in fetal flow categories. The impact of BMI and weekly GWG on the fetal flow variables was investigated by log-likelihood statistics.

Results: Compared with the reference, UV flow, LPV velocity, umbilical venous liver flow and total venous liver flow were larger, and the DV flow was smaller in PGDM pregnancies. In the PGDM population birthweights were high and when

normalized for estimated fetal weight the UV and total venous liver flow were smaller than the reference values. The most prominent deviations from the reference curves were seen after 30 weeks of gestation and near term.

DV shunting and PV fraction of total venous liver flow were negatively, and LPV velocity positively related to first trimester HbA_{1C}.

There was a graded positive association between UV flow, umbilical venous liver flow, total venous liver flow, LPV velocity and birthweight, and this effect was more pronounced in PGDM pregnancies than in the low-risk reference population. BMI and GWG modified venous liver flow to a larger extent in PGDM pregnancies than in the reference population. Overweight women with PGDM had the highest umbilical venous liver flow, total venous liver flow and LPV velocity, while PV fraction was lower. Those with excessive GWG had the largest UV flow, umbilical venous liver flow and LPV velocity, and lower PV fraction, compared with the other GWG categories.

Conclusion: This study provides new insight to the fetal development and the physiological mechanisms contributing to increased risks in PGDM pregnancies. UV flow to the liver was prioritized at the expense of DV shunting. Reduced DV shunting could increase neonatal risks by inhibiting fetal compensatory responses to hypoxia near term and during labour.

Increased distribution of UV blood to the liver contributed to larger birthweight in PGDM pregnancies, and maternal glycemic control influences the distribution of fetal liver flow. After 30 gestational weeks however, the blunted development of the umbilical venous liver flow caused an increasing mismatch between fetal growth and venous blood supply in the third trimester. The modification of fetal flow and birthweight by BMI and GWG was larger in PGDM pregnancies than in the reference population.

Our study supports the concept that fetal liver perfusion is an important regulator of fetal growth. We found this mechanism to be augmented in PGDM pregnancies.

What was already known	What this study adds
Paper I In low-risk pregnancies, umbilical venous (UV) and normalized UV flow increase during the second half of pregnancy with blunting near term. In fetal growth restriction (FGR) the UV flow is reduced depending on the degree of circulatory compromise. UV flow is higher in macrosomic fetuses of healthy women, also when normalized for fetal weight. In low-risk pregnancies, 20-30% of the UV flow is shunted through the ductus venosus (DV). Non-diabetic macrosomic fetuses shunt less UV blood through the DV in late pregnancy. In FGR a higher proportion of the UV flow is shunted through the DV.	In pregnancies with pregestational diabetes mellitus (PGDM) UV flow was larger than in low-risk pregnancies, but reduced when normalized for fetal weight. In pregnancies with PGDM, the DV flow, normalized DV flow and DV shunt fraction were lower, and this reduction was more pronounced near term. There was a negative relation between the degree of DV shunting, and maternal glycemic control in the first trimester (HbA_{1C}). The difference in estimated flow between the reference and pregnancies with PGDM was caused by larger UV size, and lower DV flow velocity.
Paper II The flow velocity in the left portal vein, portal vein flow and contribution to the total venous liver supply increases towards term in low-risk pregnancies. In macrosomic fetuses of healthy mothers, liver flow volumes are higher than the reference, but similar when the flow is normalised for fetal weight. In fetal growth restriction the total venous liver flow is reduced, also relative to fetal weight. Fetal liver volume is positively related to HbA_{1C} in pregnancies with diabetes.	In PGDM pregnancies, the fetal venous liver flow was larger than the reference before 30 gestational weeks. After this time, when normalized for fetal weight, total venous liver flow was smaller. The left portal vein velocity as a measure of UV flow to the right liver lobe was positively related to maternal glycemic control in the first trimester, while the portal fraction of the total venous liver flow was negatively related to first trimester HbA_{1C}, in PGDM pregnancies.
Paper III In low-risk pregnancies, low maternal weight gain is associated with a preferential supply of UV blood to the left liver lobe. In PGDM maternal weight and weight gain is related to degree of overgrowth and macrosomia, even when the maternal glycemic control is good.	The association between UV flow distribution and birthweight was more pronounced in PGDM pregnancies than in the low-risk population. In pregnancies with PGDM, body mass index and gestational weight gain modified fetal venous liver flow. Maternal overweight and excessive weight gain was associated with higher umbilical and total venous liver flows. Women with PGDM combined with overweight or excessive weight gain gave birth to neonates with the highest birthweights.

List of Publications

Paper I: Lund A, Ebbing C, Rasmussen S, Kiserud T, Kessler J. Maternal diabetes alters the development of ductus venosus shunting in the fetus. Acta obstetricia et gynecologica Scandinavica. 2018 (1)

Paper II: Lund A, Ebbing C, Rasmussen S, Kiserud T, Hanson M, Kessler J. Altered development of fetal liver perfusion in pregnancies with pregestaional diabetes. PLOS ONE. 2019 (2)

Paper III: Lund A, Ebbing C, Rasmussen S, Qvigstad E, Kiserud T, Kessler J. Maternal body mass and gestational weight gain are associated with augmented fetal liver blood flow and birthweight in pregnancies with pregestational diabetes. Manuscript

1. Introduction

1.1 Diabetes in pregnancy

1.1.1 **History**

Descriptions of a condition causing "too great emptying of urine" appeared in Egypt around 1500 B.C and "sweet urine" was noted in India, but it was the Greek physician Aretaeus (probably 1st century A.D) who first defined diabetes (Fig.1) (3). Until the discovery of insulin in 1921, by the Banting, Best and MacLeod collaboration, treatment was primitive and life expectancy short for people with diabetes mellitus (4, 5). Consequently, pregnancies in women with diabetes were very rare, and according to the 1920 edition of Williams *Textbook of Obstetrics*, the prognosis was "ominous for mother and child". Indeed, women with diabetes were "giving birth astride of a grave" (6).

Figure 1 ***Portrait of Aretaeus*** (Courtesy of the Bibliothèque Nationale de France), who authored *On the Causes, Symptoms and Cure of Acute and Chronic Diseases* (7).

1.1.2 Definitions and epidemiology

The International Association of Diabetes in Pregnancy Study Group (IADPSG) and the World Health Organization (WHO) have reclassified hyperglycemia in pregnancy into three groups; diabetes diagnosed in pregnancy expected to continue postnatally (DIP), gestational diabetes (GDM) (8, 9) and pregestational diabetes (PGDM) diagnosed before pregnancy; type 1 or type 2 diabetes mellitus. Other forms of diabetes will not be discussed in this thesis.

In 2016 WHO estimated the global prevalence of diabetes to be 8.5%, an almost fourfold increase since 1980, reflecting the rise in risk factors such as overweight and obesity (10). These factors also affect women of fertile age, but mainly because of variations in the diagnostic criteria for GDM and ethnic differences, reported prevalence of diabetes in pregnancy range widely, from 2 – 25% (11, 12). According to the Medical Birth Registry of Norway, 5.8% of women had diabetes in pregnancy in 2016; 3.7/1000 type 1 diabetes mellitus (DM), 1.9/1000 type 2 DM and 51.5/1000 GDM (13). In 2017 the IADPSG recommendations for GDM were incorporated into Norwegian guidelines, and the prevalence of GDM is expected to reach 8 – 10% (14).

Fortunately, the prognosis in PGDM pregnancies has improved greatly over the last century (5). Still, the advances in medical therapy and routines for close clinical follow-up have not resulted in outcomes for women with PGDM approximating those of the background population (15). The mothers have increased risks of preeclampsia and operative delivery (16), and in a Dutch nationwide study the combined perinatal morbidity in type 1 DM pregnancies was 80% (17). Complications such as congenital anomalies, premature delivery, macrosomia, neonatal intensive care admission, neonatal hypoglycemia and perinatal death are more frequent in PGDM pregnancies (16-20).

1.2 Maternal and fetal physiology

1.2.1 The normal development

During the embryonic period, the 3rd to 8th week of gestation, organs develop from three germ layers (organogenesis). Diffusion is no longer sufficient for nutrition, and the complex formation of the cardiovascular system initiates.

The fetal venous system

Three major pairs of veins can be distinguished from gestational week 5; the vitelline, umbilical and cardinal veins. The vitelline veins drain the yolk sac and form the hepatic sinusoids, and from this anastomotic network the portal vein (PV) develops. The umbilical veins drain the chorion and connect to the hepatic sinusoids, the right vein disappears, and the left umbilical vein (UV) then becomes the dominant vessel for blood flow from the placenta. The cardinal veins develop to become the venous drainage of the embryo, including the formation of the proximal inferior vena cava (IVC) (21). With increasing UV flow the ductus venosus (DV) forms, and by the 8th gestational week DV is a well-defined shunt between the UV and the IVC (Fig. 2) (22).

The fetal liver

From endodermal epithelium at the distal end of the foregut, the liver bud forms in gestational week 3. Epithelial liver cords, the vitelline veins and the umbilical veins develop to become hepatic sinusoids, and the afferent and efferent venous network of the fetal liver is formed through complex vessel growth and asymmetric degeneration of vessels (23). The afferent veins include the UV, PV and DV, and the efferent system is constituted of the hepatic veins (24).

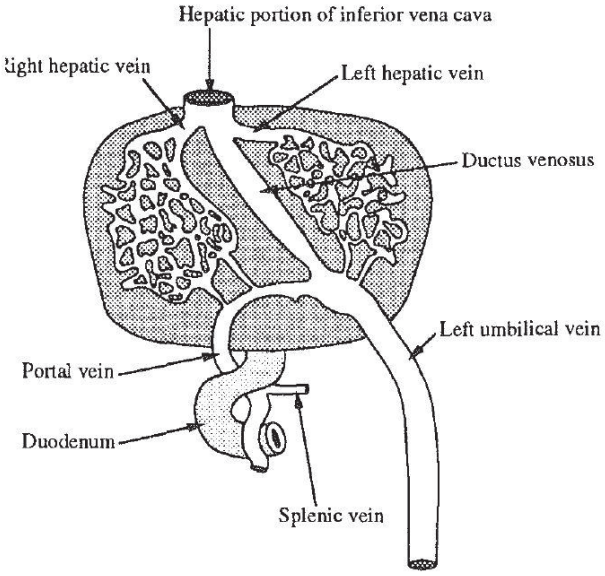

Figure 2 ***After the 8th gestational week the ductus venosus forms*** a shunt between the umbilical vein and inferior vena cava. The vitelline veins form the superior mesenteric, splenic and portal veins. The left umbilical vein has become the dominant vein for flow from the placenta. Reprinted with permission from T. Kiserud; The ductus venosus in the human fetus (Univ. of Trondheim, 1994)

Fetal blood

Hematopoiesis occurs in the liver during fetal life (25). Fetal erythrocytes are larger than adult erythrocytes, with a shorter life span, and the fetal hemoglobin concentration reaches 18 g/dL near term (26). The feto-placental blood volume is approximately 125 mL/kg fetal weight or 10 – 12% of the body weight at term (27). The combined cardiac output per kilo fetal weight, approximately 400 ml/min/kg, is constant during pregnancy (28-30). There is a high capacity for diffusion between the fetal compartments making blood volume compensation in response to events like hypoxia possible (31).

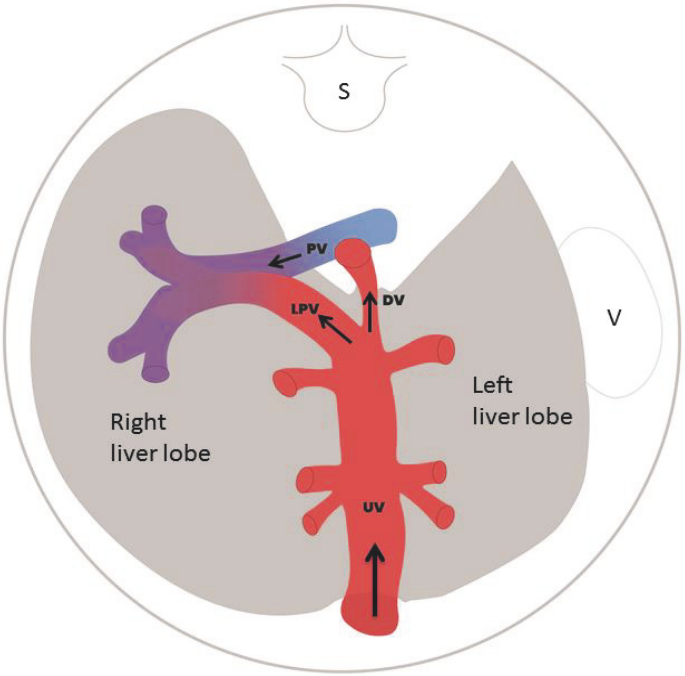

Figure 3 ***The venous supply to the fetal liver.*** A cross section of the fetal abdomen, with black arrows indicating physiological blood flow directions in the fetal liver (grey). The left liver lobe is supplied entirely by well-oxygenated umbilical venous (UV) blood (red), then UV blood is shunted through the ductus venosus (DV). Typically, UV blood blends in with deoxygenated portal blood (PV) (blue) to feed the right liver lobe; UV, umbilical vein; DV, ductus venosus; LPV, Left portal vein; PV, portal vein; S, spine; V, stomach

Venous anatomy and oxygenation

Nutrient- and oxygen-rich blood from the placenta enters the fetus through the UV. Most of the UV blood is distributed to the fetal liver, first via UV branches to the left liver lobe (Fig. 3). A fraction of the UV blood is directed to the heart through the DV. The rest of the UV blood is distributed to the right liver lobe through the left portal vein (LPV) where it blends blood form the portal vein (PV) (Fig. 3 and 4) (32).

The intraabdominal part of the UV, between the abdominal wall and the DV, is a relatively large fetal vein; the UV diameter grows from 2.5 to 6 mm during the last half of pregnancy (32). The DV remains a slender, trumpet shaped structure, with diameter 1mm, rarely up to 2 mm, and length 15 mm in the third trimester (32, 33).

Mavrides *et al.* demonstrated no sphincter at the DV inlet, but described an elastin-rich shelf structure and a narrow inlet, facilitating accelerated blood velocity through the DV (34). Also, a single layer of longitudinally arranged smooth muscle cells along the entire length of the DV were present. This allows active regulation of the vessel diameter, in response to stimuli (see below; Regulation of venous flow) (35).

The LPV is the short venous section, about 1 cm, between the DV and the right portal vein (Fig. 3). UV blood is normally directed to the right liver lobe through the LPV, but under certain conditions the LPV flow can reverse. The LPV is therefore described as a watershed area; between the umbilical venous and the portal venous circulation (36). Blood with low oxygen saturation (30%) from the spleen, stomach, pancreas and intestine is collected in the PV and transported to the right liver lobe (37). The right liver lobe thus receives a mixture of blood with high and lower oxygen content, through the LPV and PV (Fig. 3) (38).

Distinct pathways in the circulation enable the fetus to prioritize supply of blood to vital organs: Highly oxygenated blood from the UV is distributed through the "via sinistra"; the DV, foramen ovale, left atrium, left ventricle and ascending aorta. The anatomical and functional qualities of the DV, the IVC and the atrial septum, create a preferential flow of well-oxygenated blood to the left atrium (39, 40), mainly supplying the coronary arteries and the brain. Through the "via dextra", deoxygenated blood from the superior and inferior vena cava flow to the right atrium, right ventricle, pulmonary trunk, ductus arteriosus and descending aorta (23, 37).

The highest oxygen saturation in the fetal circulation is in the UV and is reported to be 80%. The lowest saturation is 30 – 35% in the IVC and PV (41). The left liver lobe is thus perfused by highly oxygenated UV blood, from which the left liver of fetal lamb extracts only 10 – 15% of the available O_2 (42). Flow from the left and medial hepatic vein is therefore another source of oxygen, and this blood is preferentially streamed into the "via sinistra", with some spillover to the right side of the atrial septum. This reduces the difference in oxygen saturation in fetal lamb,

between the "via sinistra" (SaO_2 55 – 60%), and the "via dextra" (SaO_2 40 – 45%) (37, 43).

Distribution of fetal venous flow

In humans, the DV shunt fraction (proportion of UV blood shunted through the DV) is 30% in week 20 and decreases to 20 % from week 30 of pregnancy (32, 44). Consequently, 70 – 80% of the UV blood perfuses the liver, illustrating the high priority of this organ under physiological conditions (45).

Similar to the UV and DV flows, LPV flow velocity steadily increases from gestational week 20, reaching a plateau in week 36 (36). The PV flow also increases during the last half of pregnancy and the PV fraction (contribution of PV flow to total venous liver flow) rises from 14% at mid-gestation to 20% near term (45, 46).

Regulation of venous flow

Pressure, viscosity and vascular resistance influence the flow and distribution of UV blood. The umbilico-caval pressure gradient, the difference in pressure between the UV and the IVC, drives the blood flow through the DV and the liver vasculature. Viscosity has a larger impact in the low velocity liver flow than in the high velocity DV flow, because low velocity gives higher viscous resistance. Fluid dynamics thus attribute to increased DV shunting at low pressures and when hematocrit is high, like in situations of fetal hypoxia. And contrary, with a higher umbilico-caval pressure gradient, relatively more UV blood perfuses the liver (47). Respiratory movements influence the central venous pressures and thus the umbilico-caval pressure gradient. Fetal respiratory movements thereby partly regulate fetal hemodynamics (48), and flow measurements should therefore be performed during fetal quiescence.

The existence of an anatomical DV sphincter has been controversial, but some degree of functional responsiveness and sensitivity to hormones is recognized (49); vasoconstriction occurs in response to α-adrenergic substances, and β-adrenergic stimulation induces vasodilation (50-52). The DV dilates during hypoxia in fetal sheep (53), but in human growth restricted fetuses the effect of DV dilatation may be moderate (54-56). Since also the hepatic vasculature is sensitive to neural and

hormonal signals, this is important in the regulation of venous flow (57, 58). Tchirikov *et al.* showed that the response to neurohormonal signals is more pronounced in the liver vasculature than in the DV (50). Given the large cross sectional area of the liver vasculature, small changes in liver resistance may contribute substantially to changes in venous flow, including the DV shunting (23).

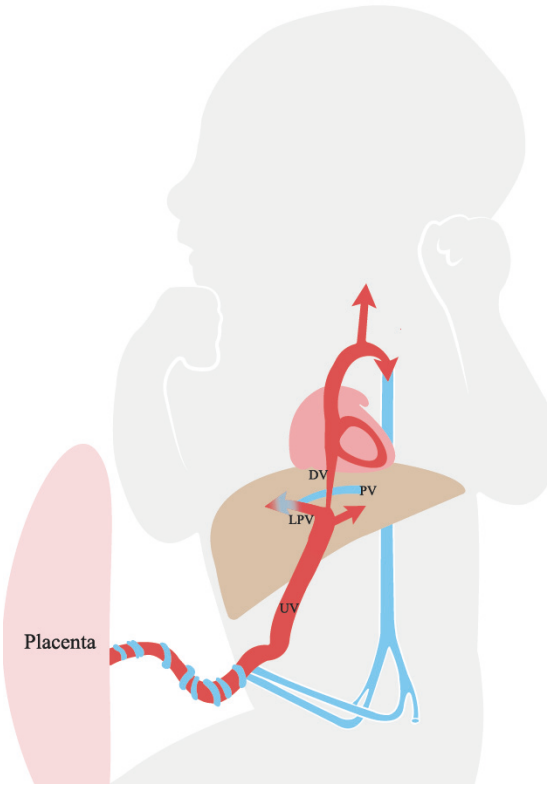

Figure 4 ***Diagrammatic representation of the fetal circulation***, showing flow of nutrient rich and highly oxygenated blood (red) from the placenta through the umbilical vein (UV). Ductus venosus (DV) bypasses the liver to supply the heart and brain with UV blood. The left liver lobe receives UV blood (red). The right liver lobe is mainly perfused with UV blood through the left portal vein (LPV) mixed with portal venous (PV) blood lower in oxygen-and nutrients (blue) (see also Fig. 3).

Fetal liver, venous flow and growth

The fetal liver has vital functions, such as hematopoiesis, nutrient metabolism, detoxification of venous blood, protein synthesis, fat storage and glucose homeostasis. The development of the fetal liver parenchyma is influenced by the

distribution of UV blood; the left- and right liver lobes have different architectures and functionality (59). It has been postulated that the pattern of UV blood distribution, to the brain and the liver, may have long-term consequences to the metabolism and body composition (60).

The distribution of UV blood to the fetal liver is an important mechanism in the regulation of fetal growth (61, 62). Experimentally increasing the UV blood flow to the liver, leads to increased cell proliferation, in the liver, heart, skeletal muscle and kidneys (63). If the flow of UV blood to the liver is high, the liver nutrient supply is excessive. A strong relation has been demonstrated between umbilical venous liver flow and infant fat mass at birth and at 4 years age (60, 64).

In non-diabetic pregnancies with macrosomia, UV flow is increased, including when normalized for fetal weight (65). Also, the total venous blood flow is augmented in macrosomia and a relatively higher proportion of UV blood perfuse the left liver lobe, while the PV fraction is decreased (62).

1.2.2 Fetal hypoxemia and venous flow

The fetal hemodynamic changes observed in pregnancies with placental compromise illustrate the physiological responsiveness in the fetal circulation. Experiments on fetal sheep have shown that there is a considerable increase in DV shunting when the fetus is exposed to hypoxia (53, 66), prioritizing highly oxygenated blood to the heart and brain. This has been confirmed in ultrasound studies of human pregnancies with severe growth restriction (54, 55). Compromised UV flow has a graded effect on the DV shunting (35 - 57%) (54), and in extreme cases the LPV flow can be reversed (55). When DV shunting is increased, the PV fraction is augmented. In cases of critical placental compromise, the right liver lobe can be perfused by deoxygenated PV blood only (67). Assessment of the fetal hemodynamics through Doppler velocimetry of the fetal circulation (umbilical artery, middle cerebral artery and DV) is commonly utilized in the diagnosis and surveillance of pregnancies with placental insufficiency (or/and anemia) (68-70).

1.2.3 Pregestational diabetes mellitus

Early fetal development

Through a series of experiments, Freinkel and Metzger developed the concept of "fuel mediated teratogenesis". They postulated that increased levels of nutrients, most importantly glucose, can affect fetal development, with short- and possibly long term consequences (71). Hyperglycemia during early embryogenesis may induce oxidative stress and through mechanisms partly unknown, cause congenital anomalies (72-74). Pre-pregnancy care has therefore long been a part of routine in diabetes counselling, but several studies show no improvements in the risk of anomalies during the last two to three decades (15, 18, 75, 76). The risk of congenital anomalies in a Norwegian PGDM population was 5.7% (OR 2.1) (1999 – 2004) (18), and a Danish study showed that 74% of congenital heart defects were attributable to PGDM (75).

Fetal growth and macrosomia

According to the Pedersen hypothesis, high maternal blood glucose results in fetal hyperglycemia, pancreatic cell overstimulation and hyperinsulinemia, and this is a causal pathway to fetal overgrowth in diabetic pregnancies (77). Macrosomia is an adverse outcome by itself, and is associated with events like stillbirth, fetal distress, operative delivery, shoulder dystocia and neonatal hypoglycemia (76, 78). In Sweden the risk of macrosomia (at term birthweight >4500g) or large for gestational age (LGA) (birthweight >90th gestational age specific percentile) was twelvefold in type 1 DM pregnancies compared with a background population (16). Of infants from women with type 1 diabetes, 32 – 57% were LGA (16, 20, 79), and Persson *et al.* reported that this incidence was increasing (16). In type 1 DM populations with adequate HbA_{1C}, nearly half of the infants were born LGA (79), with 3rd trimester HbA_{1C} and birthweight showing linear relations (80, 81).

Gestation induces maternal peripheral insulin resistance and larger insulin fluctuations (82). In pregnant women with PGDM, normally able to cope with their chronic disease, these metabolic changes make optimal glucose control difficult to accomplish (83, 84). Although the use of continuous glucose monitors has shown

promising results in PGDM pregnancies, the prevalence of macrosomia and LGA in groups using such monitors was 31% and 53% respectively in two recent studies (84, 85).

The fetal liver

The fetal liver development is affected by the levels of hormones and growth factors, such as insulin and insulin like growth factors (86, 87). In diabetic rats, uncontrolled severe diabetes led to reduced fetal liver size and growth restriction (88). Insulin treatment in diabetic pregnancies was associated with reduction of fetal liver insulin receptors, hyperinsulinemia and macrosomia (89). Also in rats, studies have found decreased insulin sensitivity in peripheral tissues and livers of adult PGDM offspring, suggesting some long term consequences of PGDM for later health (90). In pigs, diabetes induced fetal liver hyperplasia and increased glycogen reserves (91).

Boito *et al*. assessed fetal liver volume by ultrasound in human pregnancies with PGDM, finding a strong correlation between liver size and maternal HbA_{1C}, as well as a positive association between liver volume and fetal weight (92). In human stillborn neonates of PGDM pregnancies, hepatic steatosis is prevalent and more severe than in stillborn of non-diabetic pregnancies (93).

Maternal – and fetal circulation, and the placenta

In pregnancies with diabetes, studies report contradictory results, and fetal circulatory adaptations typical to PGDM have not been identified (94, 95). Maternal pregestational vasculopathy is related to abnormal uterine artery pulsatile index (PI) and adverse neonatal outcomes (96). There is evidence that the risks of stillbirth and fetal distress are related to a state of chronic fetal acidemia and hypoxemia in diabetic pregnancies (97-101). This could be due to reduced materno-placental oxygen supply and/or increased fetal oxygen demand. Maternal vascular complications in mothers with diabetes can cause reduced arterial oxygen saturation (102), and in diabetic pregnancies reduced utero-placental blood flow has been reported (103). Doppler of the uterine arteries has prognostic value in pregnancies with hypertensive

complications, but no additional predictive value has been shown for in pregnancies with diabetes (104-106).

No correlations were found between resistance in the umbilical- and middle cerebral arteries and maternal glucose levels in PGDM pregnancies (107, 108). Whether maternal diabetes affects the umbilical artery velocity waveforms is unclear (92, 95, 109). Maruotti *et al*. showed that in pregnancies with type I diabetes, lower pulsatility index in the umbilical artery was associated with macrosomia (110). The authors related this to typical changes found in PGDM placentas (111).

Distinct structural and functional changes of the placenta are associated with diabetes in pregnancy. The placenta is commonly heavier, the placental-/fetal weight ratio is increased (112-114) and typical histological findings are villous immaturity and enhanced angiogenesis (111). Nutrient transport across the placenta is regulated by a range of hormonal and metabolic stimuli and may contribute to fetal nutritional oversupply in the presence of PGDM (115).

Few studies report on fetal venous flow in diabetic pregnancies. Olofsson *et al.* examined the blood flow distribution in PGDM pregnancies and found; larger UV flow early in the third trimester, increased flow to the lower extremities and reduced flow to the viscera (116). Boito *et al*. found no difference in UV volumes in pregnancies with insulin dependent diabetes, but UV flow adjusted for fetal weight was reduced compared to a low risk group (92). Stuart *et al.* showed that the DV pulsatility index was commonly higher in diabetic pregnancies than in low-risk populations and this positively correlated with HbA_{1C} (117). Still, the sensitivity of DV velocimetry to predict adverse perinatal outcomes in PGDM pregnancies remains unclear, with positive- and negative predictive values of 32% and 88% respectively (118, 119).

Excess glucose metabolism caused by hyperglycemia and hyperinsulinemia accelerates the fetal oxygen consumption in diabetic pregnancies (120, 121). In addition, larger body mass in LGA fetuses increases the oxygen demand. Reduced materno-placental oxygen supply and increased fetal oxygen consumption results in

upregulation of placental growth factors and leptin, possibly affecting the fetal body composition (122-124). In diabetic pregnancies the risks related to hypoxemia are increased, but the placentae or fetuses do not exhibit the ultrasound and Doppler signs typically present in pregnancies with placental insufficiency and intrauterine growth restriction. Thus our clinical tools to identify fetuses at risk come in short in pregnancies with PGDM.

Continuous focus on pre-conception counselling, compliance and glucose control can possibly reduce the burden of complications in pregnancies with PGDM (15). Still, almost 50 years after the Pedersen hypothesis was published, the mechanisms causing increased risks in pregnancies with PGDM are partly unknown.

Maternal glycemic control and fetal glucose

Glycated haemoglobin, $HbA_{1C,}$ is used as an indicator of long-term glycemic control during the preceding two to three months (125). Although HbA_{1C} does not give a complete picture of maternal hypo- or hyperglycemia, it is recommended as a secondary clinical measure of glycemic control in pregnancy, in addition to self- or continuously monitored glucose (126). HbA_{1C} is lower in healthy pregnant than in non-pregnant women; the upper normal limit in late pregnancy is 5.8% (40 mmol/mol) (127). The Norwegian clinical guidelines for PGDM pregnancies recommends measuring HbA_{1C} every four weeks; pre-pregnancy HbA_{1C} should be <7.0% (53mmol/mol), and second and third trimester HbA_{1C} <6.0% (42 mmol/mol) (128). Lowering the HbA1C further may cause more frequent episodes of hypoglycemia and thus the targets for women with PGDM are not set to the normal levels of HbA_{1C}.in pregnancy (126).

Glucose is the main energy substrate for the fetus, and since the fetus probably has no significant gluconeogenesis it depends on glucose transfer over the placenta (129, 130). To ensure fetal glucose availability, the maternal physiology adapts during normal pregnancy, by peripheral insulin resistance and increased hepatic glucose production (82, 131). The fetal glucose concentration is dependent on several factors; the maternal-fetal glucose gradient, placental morphology and transport, the placental

and fetal blood flows, the placental glucose metabolism and the fetal hormones and metabolism (77, 132-134). Through "the fetal glucose steal phenomenon", early establishment of fetal hyperinsulinemia and consequently lower fetal glycemic levels, in addition to maternal hyperglycemia, creates a higher glucose flux gradient across the placenta (135, 136). Exaggerated "fetal glucose steal" in diabetic pregnancies may partly explain why the risk of large for gestational age offspring remains high in pregnancies with seemingly good glycemic control.

1.2.4 **Maternal weight and weight gain in pregnancy**

Maternal gestational weight gain varies considerably among women and is attributable to the uterus and its contents, larger breasts and the increased blood- and extracellular volumes. In sum, the placenta, fetus and amniotic fluid comprise approximately 35% of the total gestational weight gain (137). In addition, the deposition of new fat and protein make up the maternal reserves.

IOM guidelines

The Institute of Medicine (IOM) has provided a guideline for gestational weight gain (GWG) that is widely accepted (138, 139), also by the Norwegian health authorities (140). The IOM guideline supplies GWG recommendations for each category of pre-pregnancy body mass index (BMI); underweight, normal-weight, overweight and obese, and discusses in detail the challenges in pregnancy care created by "the obesity epidemic" (Table 1) (138). The guideline does not specify any GWG recommendations in pregnancies with diabetes, and the committee encourages further research on this topic.

Table 1 Body mass index categories and gestational weight gain recommendations

Pre-pregnancy body mass index (kg/m^2)	Total gestational weight gain (kg)	Weekly gestational weight gain (kg/week)
Underweight (<18.5)	12.5 – 18.0	0.44 – 0.58
Normal weight (18.5 – 24.9)	11.5 – 16.0	0.35 – 0.50
Overweight (25 – 29.9)	7.0 – 11.5	0.23 – 0.33
Obese (>30)	5.0 – 9.0	0.17 – 0.27

Institute of Medicine (IOM) recommendations for total weight gain during pregnancy and weekly gestational weight gain (2^{nd} and 3^{rd} trimester), by pre-pregnancy Body Mass Index (BMI) (138).

Numerous studies report that pregnant populations are becoming increasingly overweight and obese, and that these are factors associated with adverse pregnancy outcomes. In a Norwegian population-based study (1999 – 2009) 22.3 % of women were overweight and 9.1% were obese in pregnancy (141). The maternal BMI and weight gain in pregnancy were positively associated with birthweight and BMI of the child at 3 years of age (142). The Medical Birth Registry reported pre-pregnancy BMI in 74% of all births in Norway in 2017; 4.3% of women were underweight, 62.4% normal weight, 21.7% overweight and 11.6% obese (143).

BMI, weight gain and the fetal liver

Increased birthweights suggest that the trans-placental transport of fuels, such as glucose and fatty acids, is increased in pregnancies with obesity and excess GWG. In early pregnancy fetal subcutaneous fat is not yet developed and it has been proposed that the fetus therefore must utilize the liver for the storage of excess energy (144). Neonates of obese mothers with GDM have 68% higher hepatocellular lipid levels, and in GDM pregnancies there is a positive association between pre-pregnancy BMI and neonatal hepatic fat in both normal-weight and obese women (145).

In a population of uncomplicated pregnancies, Haugen *et al.* found that in mothers with low skinfold thickness, the fetal umbilical liver flow was increased, and introduced the concept of "fetal liver-sparing". This strategic adaptive response increase the offspring fat stores in preparation for postnatal conditions with restricted nutrient supply (146), and such liver-sparing is thought to have consequences for later health risks (60). Low pre-pregnancy BMI was not associated with umbilical venous flow distribution in the longitudinal study constituting our low-risk reference group (45). However, GWG had an impact on the venous flow distribution in the fetal liver, with relatively high flow of UV blood to the right liver lobe in women with high GWG.

BMI and weight gain in PGDM pregnancies

Maternal BMI is generally higher in type 1 DM pregnancies (16). In an unselected Danish population, 43% of the pregnant women with type 1 DM were overweight or obese, and 54% had excessive GWG defined by the IOM criteria (147). In PGDM pregnancies, high BMI is associated with increased risk of cesarean section, congenital heart malformations, preterm birth, LGA and admission to neonatal intensive care (148), and excessive GWG is an independent risk factor for LGA (147).

1.3 Ultrasound

1.3.1 History

The Doppler principle has been exploited in technology since its discovery by Christian A. Doppler in 1843. Ian Donald and co-workers were able to produce static ultrasonographic images and published in 1958 on the use of ultrasound in obstetrics. The group of FitzGerald and Drumm is recognized as the first to publish on the clinical use of fetal Doppler in1977(149). Surely, grey scale imaging and Doppler ultrasound have led to important advances in fetal medicine since (150).

1.3.2 Gray scale ultrasound

Sound waves with frequency above 20 kHz are called ultrasound and are above the range of human hearing. The images displayed on the screen are recorded reflections of the ultrasonic waves directed from a probe towards tissues. The strength of the reflections from different tissues is displayed as graded brightness in the images created.

Penetration and resolution affects the quality of the displayed ultrasound images. Impedance is the loss of ultrasonic wave energy due to tissue resistance and is dependent on tissue density. The penetration is affected by both the tissue impedance and frequency of the ultrasound. High penetration can be obtained by reducing the frequency. Thus, in obstetric ultrasound imaging, low frequencies (2.5 -3.5MHZ) are commonly used to visualize the fetus, deep in the abdomen. However, low frequency reduces the resolution of ultrasound images and the sonographer should be aware of this accommodation.

Image quality also depends on the surface of the tissues in contrast to the surrounding organs. The fetal vessel walls are smooth structures yielding a strong reflection. Small vessels, like the DV, can be clearly defined when insonation of the ultrasonic beam is kept close to perpendicular to the vessel walls (Fig: 6A, 7A and 7C).

1.3.3 Doppler

The Doppler effect is the change in wave frequency when the transmitter and reflector move relative to each other. If the reflector, i.e. blood cells in a vessel, moves toward the ultrasound source, the ultrasound waves will be compressed, and the frequency increased. This phenomenon is exploited in Doppler ultrasound, to measure blood flow direction and velocity.

For optimal measurements of velocity, the angle of insonation should be aligned with the vessel (angle of 0°). To illustrate, if the angle is perpendicular to the flow direction (90°), the flow velocity will be estimated to zero. The effect of the insonation angle diverting from 0° is expressed by a cosinus function, thus a 30° angle imposes only 6% error. An angle of insonation within the range of 0 – 30 ° is

therefore commonly accepted in velocity measurements for clinical and scientific purposes (151).

Continuous wave (CW) Doppler is extensively used for external fetal heart rate detection, but does not provide information on blood flow velocities. In pulsed-wave (PW) Doppler, short bursts of ultrasound waves are utilized to determine distance and this allows targeting a small area, or sample volume, with high resolution. Information from the Doppler analysis can be presented in color imposed upon the grey scale image. Color Doppler gives a rough visualization of speed and flow direction.

Aliasing, or the Nyquist effect, occurs if the maximum shift in wave frequency registered by the Doppler exceeds half the pulse repetition frequency of the PW and color Doppler system. The distorted color image that occurs can be useful for identification of the DV, and by adjustments of the baseline and pulse repetition frequency, the PW Doppler yields a blood flow analysis.

1.3.4 Blood flow calculations

In this thesis, blood flow refers to volumes (mL/min). In the calculation of blood flow (Q), the distribution of blood velocity is assumed to have a parabolic profile (Fig. 5) in the vessel lumen, and flow is calculated by the formula:

$$Q=\pi\cdot(D/2)^2\cdot h\cdot\mathrm{TAMXV}$$

D is vessel diameter, h is the velocity profile factor and TAMXV is the time average maximum flow velocity. The velocity profile factor is an expression of the parabolic shape of the blood velocity across the vascular lumen. The blood velocity profile is partly blunted in the DV and h is therefore higher than in the low velocity veins (152-154); h=0.5 for UV and PV, and h=0.7 for DV (23).

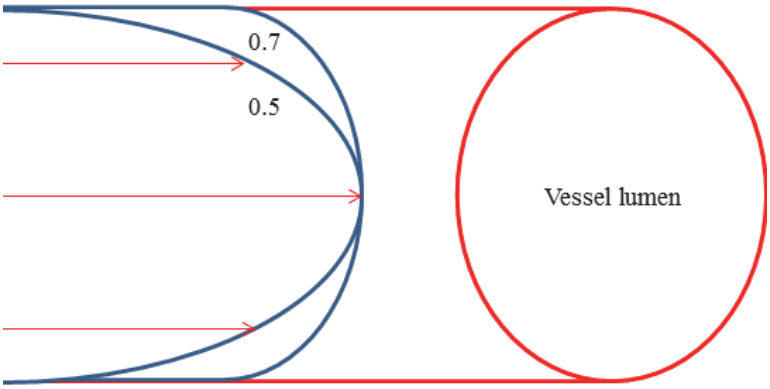

Figure 5 ***Blood flow in the vessel lumen*** (red arrows). The velocity profile (blue) is partially blunted in the ductus venosus corresponding to a ratio of 0.7, in the umbilical vein and portal vein the flow is parabolic with a ratio of 0.5.

The inner vessel diameter (*D*) is measured and squared in the equation, thus the diameter has a larger impact on the flow calculated, than the velocity. In order to minimize measurement error repeated diameter measurement is recommended. When *D* measurements in the UV, PV and DV are repeated (≥3 times), it has been shown that flow calculations in fetal vessels during the second half of pregnancy, are reproducible and valid (46, 155-157).

1.3.5 Safety

Gray scale ultrasound in the low intensity range used for fetal assessment is generally considered safe (158). However, the number of ultrasound scans per pregnancy is probably increasing, and methods other than grey scale imaging have become more available. Thus, the safety of fetal ultrasound should be under constant evaluation (159, 160).

Systematic reviews report no harmful effects of fetal ultrasound in humans (161, 162). However, in some epidemiological studies on biological effects of prenatal ultrasound, the scanners had lower acoustic outputs compared with modern apparatuses. Animal studies have shown effects on neuronal migration and reversible liver apoptosis at output energies used in obstetrics (163, 164). It has been argued that the probe-to-organ distance, organ size and the length of gestation make the

relative ultrasound power difficult to compare between these experimental animal studies and human pregnancies (159). In one randomized controlled trial of low-risk pregnancies, a group exposed to continuous Doppler of fetal vessels (five sessions), was compared to a control group examined by grey scale ultrasound in gestational week 18 only (165). No beneficial effects of assessing blood velocities were demonstrated, but a significantly higher risk of growth restriction was found in the intervention group. Another randomized fetal Doppler study did not demonstrate a similar increased risk of fetal growth restriction (166). Although fetal diagnostic ultrasound has been used extensively during the last 3 decades, the only consistent outcome is a weak association between ultrasound screening and left-handedness in boys (167).

The ALARA principle states that exposure time and acoustic output should be kept as low as reasonably achievable consistent with obtaining diagnostic information. To assess the risks during an examination, the thermal index (TI) and mechanical index (MI) are recommended indicators (160).

The MI is an expression of the non-thermal bio-effects of ultrasound, most importantly the expansion and collapse of bubbles, a phenomenon called cavitation. This has not been demonstrated in humans, nevertheless, in all diagnostic ultrasound the MI should be kept below 1.0 (158).

The energy of ultrasound waves can convert to heat depending on the tissue properties and the ultrasound exposure. TI is defined as the ratio of the power used and the power needed to increase tissue temperature by 1°C. TI does not take into account the exposure time or maternal body temperature. Mineralized bones have the highest energy absorption, and beyond the 10^{th} gestational week TI for bone is the recommended index that the user should be aware of (168). Exposure that produces no more than a 1.5°C temperature rise (given normal body temperature at 37°C) is considered safe (169). Users should remain aware of the MI and TI indices during scanning and make sure guidelines are followed.

According to the Norwegian guidelines for PGDM pregnancies, first trimester ultrasound to measure CRL, a routine scan at 18 weeks and ultrasound for fetal biometry in week 24, 28, 32, 36 and 38 is usually performed, comprising a minimum of 7 sessions (170).

2. Aims of the study

The overall aim of the thesis was to compare the longitudinal development of the venous liver blood flow in PGDM pregnancies with reference values from a low-risk population.

More specifically, the aims were to describe the development in PGDM pregnancies of:

Paper I

- Umbilical venous flow
- Ductus venosus flow
- Ductus venosus shunt fraction
- Relation between DV flow and maternal glycemic control (HbA_{1C})

Paper II

- Left portal vein velocity
- Portal venous flow
- Portal venous fraction of total venous liver flow
- Total venous liver flow
- Umbilical venous liver flow
- Associations between venous liver flow and maternal glycemic control (HbA_{1C})

Paper III

- Effect of venous liver flow on birthweight
- Influence of BMI on venous liver flow
- Influence of weekly GWG on venous liver flow
- Association between BMI, GWG and birthweight

3. Material and methods

The study design was a prospective longitudinal observational study and the protocol was approved by the Regional Committee for Medical and Health Research Ethics (REK Vest 2011/2030).

3.1 Study population

In our region with 454,000 inhabitants, Haukeland University Hospital had 5169 (mean) deliveries yearly during the study period (171). All pregnant women with PGDM were referred our clinic for multidisciplinary follow-up, as soon as pregnancy was confirmed. Between August 2013 and May 2016, all referred patients with PGDM (82 women) received written invitation to participate. Of these, 12 had early fetal demise and in two pregnancies twins were detected at the first ultrasound examination. This left 68 women invited, three women were unable to consent because of a language barrier, and 13 declined the invitation. There was no protocol to register the individual reasons for those who declined the invitation. However, several women reported this unencouraged; four lived geographically remote, four did not have time, three had concerns about ultrasound safety, one had a psychiatric disorder and one did not mention any cause for declining participation. Three patients with type 2 DM withdrew after inclusion, leaving 49 PGDM in pregnancies in this study; 44 had type 1 DM and five women had type 2 DM. Three women with type 1 DM participated in two consecutive pregnancies (Table 2).

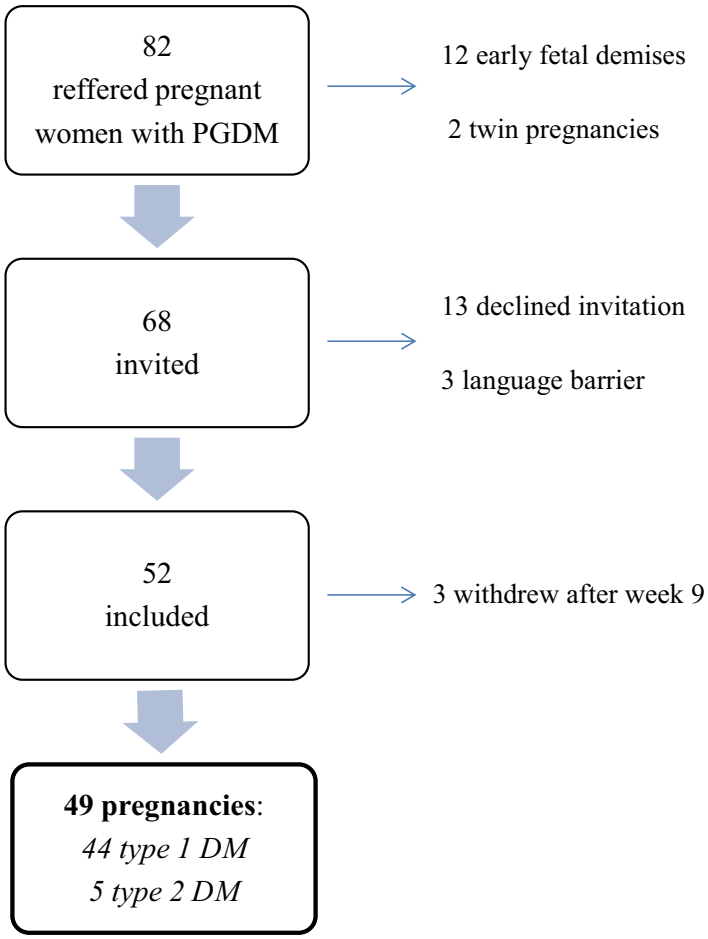

Table 2 All women with pregestational diabetes mellitus (PGDM) referred to our institution during the study period were identified, all women that met the inclusion criteria were invited, 52 women (76% of the invited) agreed to participate, 49 pregnancies were followed longitudinally in the study**;** DM, diabetes mellitus.

3.2 Measurements

At the first visit around week 9 (median GA 9.4, range 6.7 – 20.1), background and health information was collected systematically, describing ethnicity, education, employment, duration of diabetes, diabetic complications, co-morbidity, medication,

menstrual cycle and obstetric history. Gestational age (GA) was determined by measuring the crown rump length (172) with a vaginal probe (Vivid 7, GE Healthcare Vingmed Ultrasound, E8C, 8 MHz).

Blood samples were collected at each visit and in the present study we used the results from the HbA1c analyses at inclusion. The first HbA_{1C} in pregnancy was collected at median GA 8.6 weeks (range 2.0 – 12.6). For HbA_{1C} values in the 2nd and 3rd trimester, the means for each trimester were used in multilevel regression analyses (Paper I; Fig. 4 and Discussion).

Information on the sex of the neonate, birthweight, mode of delivery, Apgar score, cord-blood gases and transfer to the neonatal ward was collected from clinical records. Neonatal blood was collected from the heel 1 hour after delivery and the hematocrit (EVF) from this analysis is reported (Paper I and II; Table 1).

3.2.1 Maternal weight and weight gain

Maternal weight was measured at inclusion and in each trimester using a Tanita Body Composition Analyzer (BC-418). The measured weights are discussed only briefly in this thesis. Since equivalent data on measured weight in the first trimester were not available from the reference population, self-reported pre-pregnancy weight and height were used in the analyses. The body mass index (BMI) was calculated by the formula BMI = weight (kg)/height $(m)^2$.

Weekly GWG was calculated by subtracting pre-pregnancy weight from the weight last measured before delivery, divided by gestational age at the last weighing. Weekly GWG was categorized according to pre-pregnancy BMI and the IOM guideline as; insufficient; appropriate; excessive (139) (Paper III).

3.2.2 Ultrasound and Doppler

The ultrasound examinations were performed at gestational weeks 9, 20, 24, 28, 32, and 36 using an ultrasound system (Vivid 7, GE Healthcare Vingmed Ultrasound, Horten, Norway) with an abdominal transducer (M4S, 2.0–4.3 MHz). All ultrasound measurements were performed by three observers; 193 sessions by A.L, 20 by J.K.

and 11 by C.E. During the first 6 months of the study, all Doppler and diameter measurements performed by A.L. were supervised by J.K. and during the whole study period J.K. or C.E. were consulted if assessments were difficult to obtain. Measuring the UV, DV and LPV was prioritized if all examinations could not be performed within the time limit. Each session lasted no more than 1 hour and the TI was kept below 1.0.

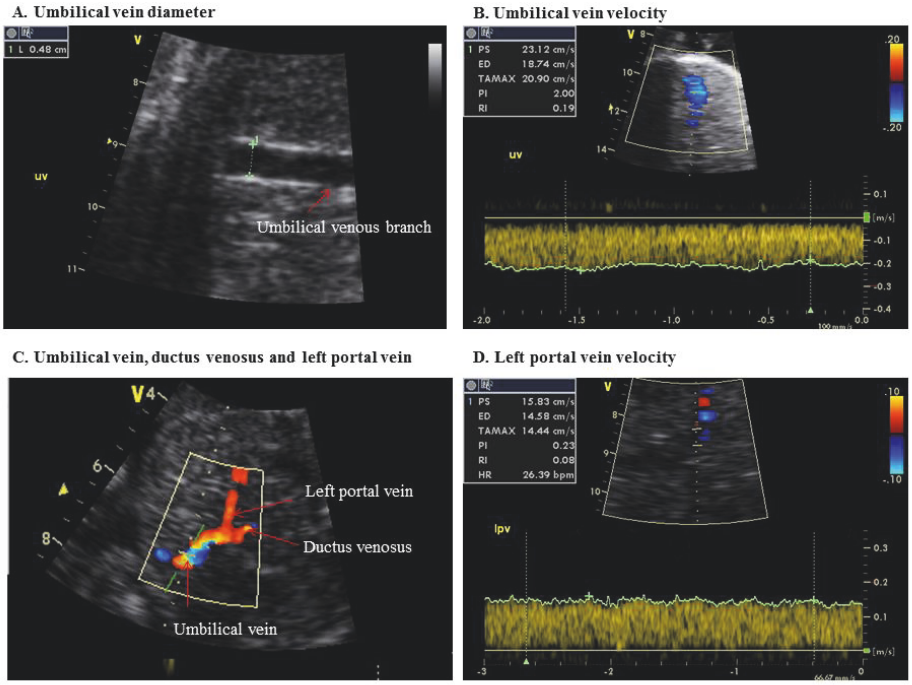

Figure 6 A. The inner diameter of the umbilical vein was measured minimum three times, intra abdominally and before any branching. B. Perpendicular to this umbilical vein velocity was measured. C. The anatomy of the umbilical vein, ductus venosus and left portal vein. D. Left portal vein velocity

The time-averaged maximum blood velocity (TAMXV) was measured in the umbilical vein, ductus venosus, left portal vein and portal vein (Fig. 6 and 7). The angle of insonation was kept as small as possible, not exceeding 30° (in all vessels median angle correction was 0, range 0 - 30°). At the same site perpendicular to the vessel wall, the inner vessel diameter (*D*) was measured at least three times (median

3, range 3 – 5 times) in the umbilical vein, ductus venosus and portal vein, with the mean of these measurements used for the analyses. *D* was measured in magnified images, with color Doppler turned off, after identification of the vessel. When analyzing the data, all outliers (defined as ± 2 SD or identified visually in scatter plots) were reexamined by A.L. and J.K. in ultrasound images, to make sure the right vessel was identified and that the correct velocities and *D* were used for analyses.

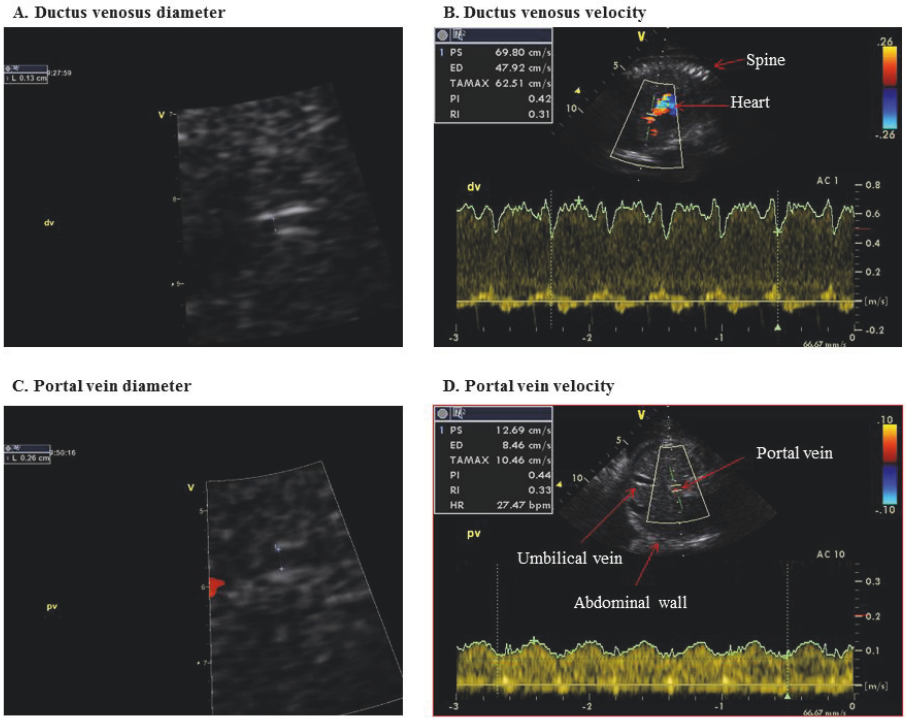

Figure 7 A and C. The inner vessel diameters in ductus venosus and portal vein respectively, measured at least three times, in magnified images with the color Doppler turned off. B. Sagittal view angled from the neck towards the flow direction of ductus venosus. D. Portal vein velocity with a typical pulsatile flow pattern

Blood flow (Q, mL·min^{-1}) was calculated by the formula $Q = \pi \cdot (D/2)^2 \cdot h \cdot$ TAMXV. The velocity profile parameter was $h = 0.5$ for the umbilical vein (UV) and the portal vein (PV) (46), $h = 0.7$ for the ductus venosus (DV) (152, 153). Umbilical venous liver flow (UV_{liver}) was calculated as $Q_{UV\,liver} = Q_{UV} - Q_{DV}$, total

venous liver flow as $Q_{\text{liver}} = (Q_{\text{UV}} - Q_{\text{DV}}) + Q_{\text{PV}}$ and PV fraction (F_{PV}) of the total venous supply to the liver was $F_{\text{PV}} = 100\% \cdot Q_{\text{PV}}/Q_{\text{liver}}$.

3.2.3 Estimated fetal weight

Flow was normalized based on estimated fetal weight (EFW) as Q/EFW (mL·min^{-1}·kg^{-1}). Birthweight z-score was used to calculate the intrauterine estimated fetal weights at the time of examination, by extrapolation according to the longitudinal reference curve for estimated fetal weight (45, 173).

3.2.4 Reference values

Reference curves for the fetal flow variables had been created in a separate project describing the normal venous blood supply development of the fetal liver (36, 45, 46, 174, 175). In this longitudinal study, 160 women with low-risk pregnancies were included during the period August 2004 – July 2005. Reasons for exclusion were twins, fetal malformations, chronic maternal disease (including diabetes) or complicated obstetric history. No women were excluded during or after pregnancy and thus no selection occurred after inclusion. .

The methods for calculation of estimated fetal weight, pre-pregnancy BMI and weekly GWG were identical in the present study population and the reference population. In the previously published reference curves, the association to GWG was not categorized by the IOM guideline (45), in contrast to Paper III of this thesis.

3.3 Statistics

3.3.1 Power

Since the effects of PGDM on the outcome variables were not known, no power calculations were performed to calculate sample size for the present study. Instead the sample size was based on previous studies of pregnancies with fetal growth restriction and fetal macrosomia (62, 67), demonstrating significant associations between fetal growth patterns and variation in the venous liver circulation in populations of 29 and 25 pregnancies, respectively. We allowed for a lower success

rate and possibly smaller effects in the present study protocol, and increased the number of participants to 50 women.

3.3.2 Statistical methods

The mean curves were modeled according to gestational age using multilevel regression analysis (176). In addition, *z*-scores for outcome variables in the PGDM and reference groups were compared using the independent-samples *t*-test with a significance cutoff of $p \leq 0.05$. The relations between maternal first-trimester HbA_{1c} and *z* –scores for DV flow velocity, DV flow volume and DV shunt fraction (Paper I; Fig 4), and of LPV velocity and PV fraction (Paper II; Fig 6), were assessed by multilevel regression analysis. To test differences between independent subgroups within each population by BMI, GWG and flow tertiles, ANOVA was used (Paper III). Also, log-likelihood was performed to assess whether adding BMI or GWG categories improved the model for fetal flow by gestational age. The statistical analyses were done in the Statistical Package for the Social Sciences (version 24, SPSS, Chicago, IL) and the MLWin program (version 2.35, Centre of Multilevel Modeling, University of Bristol, UK).

4. Results

4.1 Maternal characteristics

The characteristics of the study population are described in Table 3.

There were no smokers in the study group. Participants defined their ethnic identity by their own terms; in the type 1 DM group 39 were Norwegian, four from other European countries and one Norwegian with parents from Chile. In the type 2 DM group one was Japanese-American, one Chilean-Norwegian, one from the Philippines and two Norwegian. The level of education was categorized as ≤12 years, 13 – 16 years or ≥17 years; three, 22 and 20 women reported this respectively. This information was missing in four pregnancies.

Maternal BMI and GWG categories in the reference and PGDM populations are presented in Paper III; Supplementary Table 1. The categories were not similarly distributed in the two populations (Fig 8).

No adverse effects caused by participation in the study were registered, although some experienced pelvic- or back pain or a transitory fall in blood pressure, during the ultrasound examination.

Table 3 Maternal characteristics in 49 pregnancies with pregestational diabetes mellitus

	Number	**Percent**
Para 0	20	40.8
Para1+	29	59.2
Retinopathy	9	18.4
Nephropathy	1	2.0
Hypothyroidism	9	18.4
Chronic hypertension	7	14.3
Preeclampsia	3	6.1
Continuous glucose monitoring	14	28.6
Pregnancy planned pre conception	20	40.8
	Median	**Range**
Maternal age at inclusion (years)	31	23 - 42
Gestational week at inclusion	9.4	6.7 – 20.1
Duration of diabetes (years)		
Type 1 DM	17	1 – 37
Type 2 DM	5.5	4 - 15
Pre-pregnancy weight (kg)	70	57 – 113
Pre-pregnancy BMI	24.86	19.82 - 44.14
Maternal weight gain	15.8	–5.0 - 33.1
HbA_{1c} at inclusion (%)	6.70	4.90 – 12.00
Mean HbA_{1C} 2^{nd} trimester (%)	5.87	4.33 – 7.57
Mean HbA_{1C} 3^{rd} trimester (%)	6.08	4.90 – 8.46

DM, diabetes mellitus; BMI, body mass index

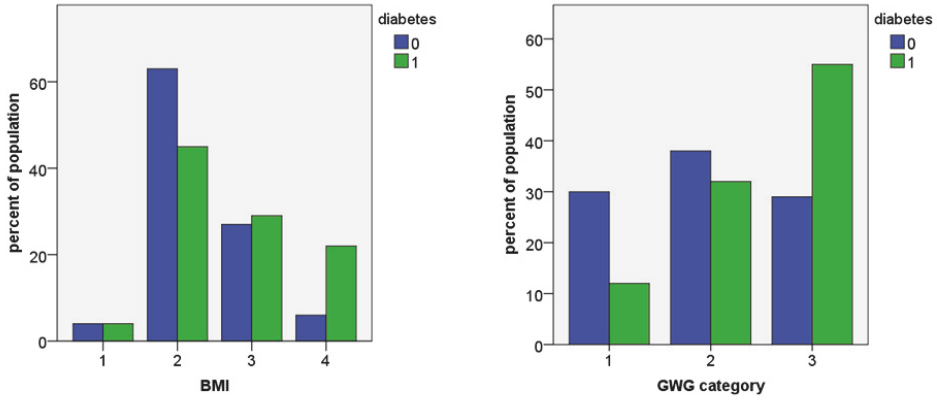

Figure 8 Percent of participants in the body mass index (BMI) and gestational weight gain (GWG) categories in the low-risk reference population (blue) and pregestational diabetes (PGDM) population (green); The number of participants in each category is summarized in Paper III, Supplementary Table 1.

4.2 Pregnancy outcomes and neonatal characteristics

The pregnancy outcomes and neonatal characteristics of the PGDM population are presented in Paper I-III, Tables 2. The mean birthweight *z*-score was -0.06 in the reference population and 1.05 in the PGDM population (mean difference 1.11, $p<0.001$).

The mean gestational age at delivery was 40.3 weeks in the reference population and 37.8 weeks in the PGDM population ($p<0.001$ tested by independent sample T-test). In the reference group 9.4% were delivered by Cesarean section compared to 44.9% in the PGDM population (175).

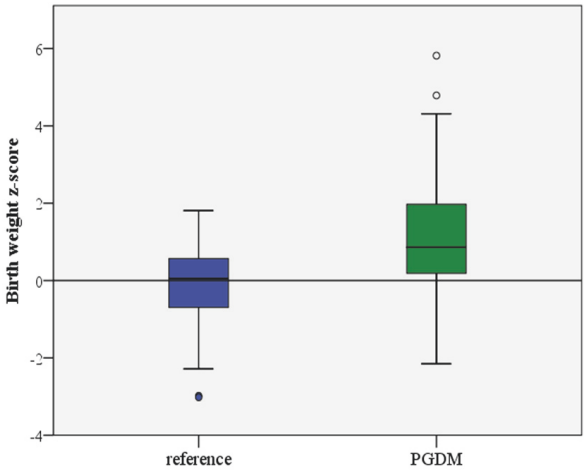

Figure 9 ***Birthweight z-scores boxplot*** in the low-risk reference population (blue) and the pregestational diabetes PGDM population (green)

4.3 Main results

4.3.1 Umbilical vein

The UV flow was higher in PGDM pregnancies, and this was due to larger UV diameters in the PGDM compared with the reference group (Paper I; Tables 3 and S1). Normalized UV flow was smaller, with a blunted development curve near term (Paper I; Fig. 1b).

4.3.2 Ductus venosus

The DV flow, normalized DV flow and DV shunt fraction were reduced in PGDM pregnancies (Paper I; Table 3, Fig 2 and 3). The DV velocity reference curve has been published earlier (174), while the mean DV diameter for the reference population was calculated for the present study (Paper I; Appendix) (1). The DV diameters were larger and the DV velocities lower in the PGDM group, compared with the reference. However, at 36 weeks of gestation, the mean DV diameter z-scores were similar between the populations. Thus, the reduced DV flow in the PGDM population near term was mainly due to lower DV flow velocities (Fig. 10).

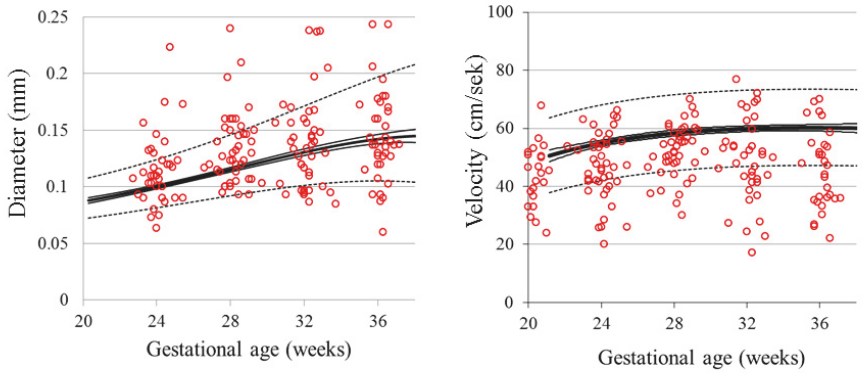

Figure 10 ***Longitudinal observations of the ductus venosus*** diameter (mm; millimeter) and time averaged maximum velocity (TAMXV) in 49 pregnancies with pregestational diabetes (red circles) plotted on top of the reference curve; low-risk (black lines; mean (thick line), confidence interval (thin lines) and 5-95 percentiles (dotted lines))

4.3.3 Venous liver flow

The umbilical venous flow to the liver ($Q_{UVliver}$), total venous liver flow (Q_{liver}) and left portal vein (LPV) blood velocity (TAMXV) were higher in the PGDM group. Normalized PV flow and normalized total venous liver flow were lower in PGDM pregnancies after 30 gestational weeks compared with the reference values (Paper II; Table 3).

4.3.4 BMI, gestational weight gain and fetal liver flow

In PGDM pregnancies, the overweight BMI category had higher umbilical venous liver flow flows than in the normal- and obese weight categories. BMI had a smaller impact on fetal flows in the reference population (Fig. 11a and Paper III; Table 2).

Weekly gestational weight gain category was associated with altered fetal flow developments in the PGDM population only. There was a graded positive response to GWG, and those with excessive GWG had the highest umbilical venous liver flow (Fig. 11b and Paper III; Table 3).

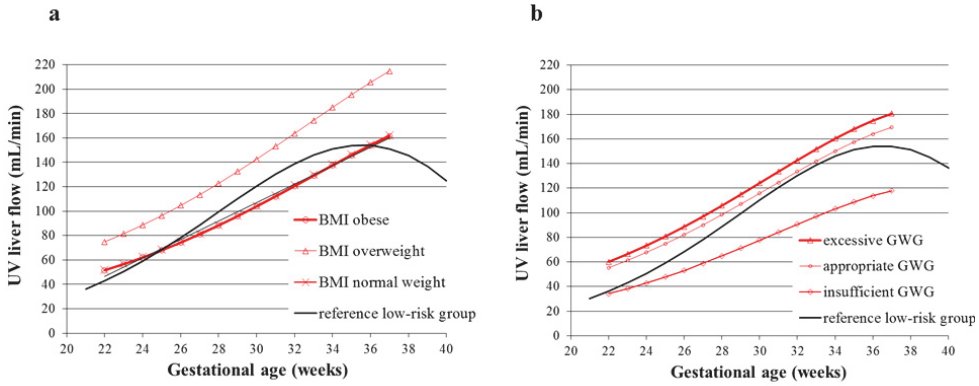

Figure 11 ***Longitudinal observations of the umbilical venous liver flow***, umbilical venous liver flow (mL/min), during the second half of pregnancy in a low-risk population (black) and a population with pregestational diabetes (red), analyzed by log-likelihood test for **a)** BMI categories; normal weight, overweight and obese and **b)** Gestational weight gain (GWG) categories; insufficient, appropriate and excessive

4.3.5 Fetal flow and birthweight

Umbilical venous flow distribution was associated with birthweight in both the reference and PGDM populations, but the effect was more pronounced in pregnancies with PGDM. There was a graded positive relation between LPV velocity, UV flow, total venous liver flow, umbilical venous liver flow and birthweight (Paper III; Table 1).

4.3.6 Glycated hemoglobin, HbA_{1C}

In PGDM pregnancies, first trimester HbA_{1C} was positively related to LPV velocity and negatively related to DV velocity, DV flow, DV shunt fraction and PV fraction of the total venous liver flow (Fig 12).

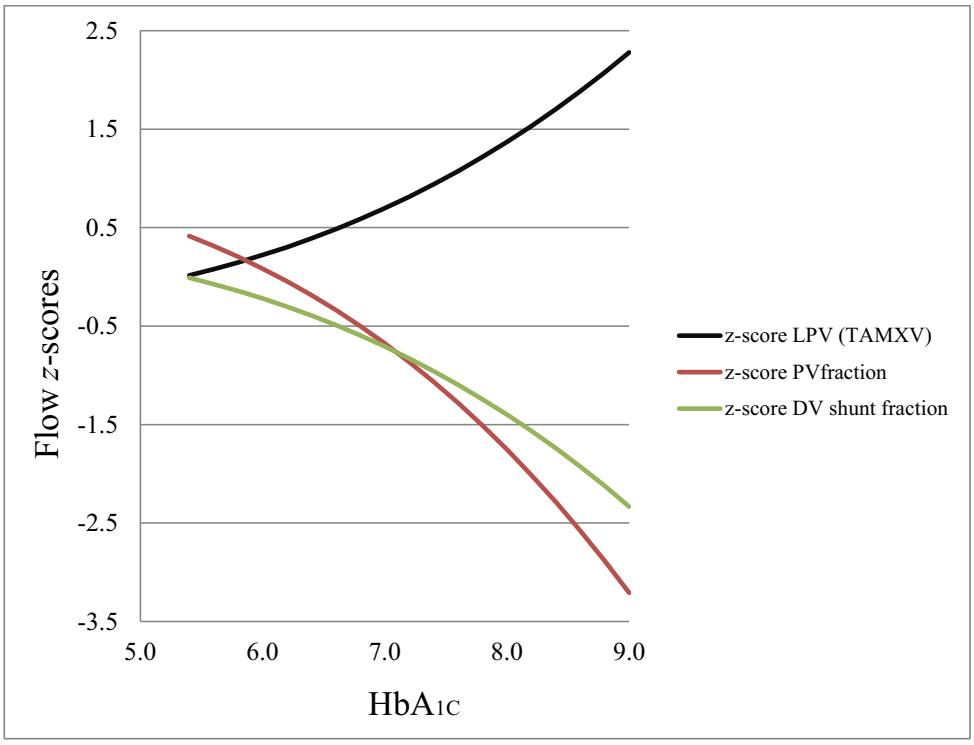

Figure 12 ***Fetal blood flow and relation to HbA$_{1C}$.*** Relations between z-scores of the ductus venosus (DV) shunt fraction, time-averaged maximum left portal vein (LPV) flow velocity (TAMXV) and portal vein (PV) fraction, and first-trimester HbA$_{1c}$ in the study population.

4.4 Measurement success rate

In addition to the 49 examinations at inclusion (at mean gestational week 9.4), a total of 224 sessions were performed in gestational weeks 20, 24, 28, 32 and 36; median 5 sessions per participant, range 2 – 6. Due to preterm deliveries, 9 women were not examined in week 36.

Missing values for the ultrasound variables are summarized in Table 4. There were no extra missing values for the normalized flows since birthweight and gestational age at examination was registered in all pregnancies. The reasons for missing values were not collected systematically, but common causes were the time limitation

(maximum one hour of fetal ultrasound), discomfort or hypoglycemia during examination, fetal movements and difficult examination conditions.

Table 4 Missing values for each ultrasound variable from a total of 224 sessions

	Diameter (millimeter)		Velocity (TAMXV)		Flow (mL/min)		Fraction (%)	
	n	% missing	n	% missing	n	% missing	n	% missing
UV	15	6.7	22	9.8	32	14.3		
DV	66	29.5	33	14.7	86	38.4	101	45.1
LPV			22	9.8				
PV	124	55.4	94	42	130	58	148	66.1
Q_{liver}					148	66.1		
$Q_{UVliver}$					101	45.1		

UV, Umbilical vein; DV, ductus venous; LPV, left portal vein; PV, portal vein; Q_{liver}, total venous liver flow; $Q_{UVliver}$, umbilical venous liver flow; n, number of missing values; TAMXV, time average maximum flow velocity

When the study population was divided into two groups (by missing and non-missing values) and compared by independent sample *t*-test, maternal BMI at inclusion in the missing data group was significantly higher than in the non-missing group (Tab. 5). HbA_{1C} at inclusion was not different in the missing vs. non-missing groups for any of the flow variables.

Table 5 BMI and HbA1C in the missing vs. non-missing data groups

		n	Mean BMI	Mean BMI difference*	*p* BMI difference	Mean HbA_{1C} difference	*p* HbA_{1C} difference*
UV flow	Missing	32	29.5	2.07	*0.044*	0.03	0.817
	Non-missing	192	27.5				
DV flow	Missing	86	28.7	1.63	*0.028*	0.14	0.176
	Non-missing	138	27.1				
LPV velocity	Missing	22	29.0	1.44	0.236	-0.11	0.512
	Non-missing	202	27.6				
PV flow	Missing	130	28.6	1.98	*0.007*	-0.04	0.715
	Non-missing	94	26.6				

*Difference of the means tested by independent sample *t*-test. Umbilical vein, UV; ductus venous, DV; left portal vein, LPV; portal vein, PV; total venous liver flow

4.5 Type 1 and type 2 diabetes mellitus

Since type 1DM and type 2DM are different diseases, we repeated the analyses with the results from pregnancies with type 2 DM excluded, to test if this would significantly change our findings. The main outcomes were explored (independent sample *t*-test between type 1 DM pregnancies and the low-risk group) and the mean DV flow z-score was no longer significantly different between these groups (Table 6) (week 24 – 36). When DV flow after gestational week 34 was analyzed, the mean z-score was significantly lower in the type 1 DM group (mean *z*-score difference -0.64, *p*=0.009) compared with the reference group.

Table 6 Umbilical venous and fetal liver blood flow in pregnancies with type 1 DM compared with reference values from a low-risk population.

	Population	n	Mean *z*-score (95% CI)	*p*
Umbilical venous flow (mL/min)	Reference	574	-0.003 (-0.09 – 0.08)	0.001
	PGDM	174	0.44 (0.18 – 0.65)	
Ductus venosus flow (mL/min)	Reference	543	-0.003 (-0.08 – 0.10)	0.081
	PGDM	123	-0.21 (-0.53 – 0.11)	
Left portal vein velocity (cm/sek)	Reference	553	0.005 (-0.08 – 0.09)	<0.001
	PGDM	179	0.583 (0.29 – 0.87)	
Umbilical venous liver flow (mL/min)	Reference	555	-0.027 (-0.12 – 0.06)	<0.001
	PGDM	111	0.38 (0.10 – 0.77)	
Total venous liver flow (mL/min)	Reference	525	-0.002 (-0.09 – 0.08)	<0.001
	PGDM	69	0.595 (0.14 – 0.92)	

n, number of observations; Mean z-score, mean of all flow measurements week 24 – 36; CI, confidence interval

5. Discussion

5.1 Principal findings

The development of the UV, DV and venous liver flow were different in pregnancies with PGDM compared with low-risk pregnancies; the DV shunting was reduced near term and UV flow to the liver was increased. Although the UV and total venous liver flows were increased before 30 gestational weeks, the total venous liver flow did not match third trimester fetal growth in pregnancies with PGDM. First trimester HbA_{1C} was positively related to the umbilical venous liver flow, and negatively to the DV shunting. Maternal BMI and GWG had a greater impact on fetal venous liver flow, and umbilical venous liver flow had a larger effect birthweight, in PGDM- compared with low-risk pregnancies.

5.2 Methodological considerations

5.2.1 Ethical aspects

Participation was voluntary and withdrawal possible at any time. Examinations were time consuming and some women commented this. No adverse effects were registered, and in case of time limits or discomfort, the ultrasound examination was halted. Although three women withdrew after the examination in week nine, all other participants remained in the project until birth. The low withdrawal rate probably indicates that participation was acceptable to the women.

Information about objectives, procedures and safety of the study was accessible online (177). Participants could leave feedback by email any time during the study period. These measures were taken to ensure that the consent was informed, and that patient involvement was possible.

The protocol was up to date with publications and guidelines on safety (158, 160, 162). Current knowledge supports that this study was safe. However, possible long-term effects of fetal ultrasound have not been studied prospectively. Thus the

ALARA principles for fetal ultrasound were followed strictly. The duration of the examinations never exceeded one hour.

5.2.2 The study and reference populations - selection, representativeness and generalizability

The terms *reference population* and *reference values* are used in the present study although the collection of the reference data was done as a separate project and during a different time period (2004-2005) (36, 45, 46). It may represent a limitation that the reference study was performed approximately 11 years before the present PGDM study. However, the reference study was conducted in the same hospital and by the same research group. Also, the examination techniques were identical, which are strengths that reduce the risk of variability due to differences in technique.

The reference study aimed for recruitment of low-risk pregnancies. No women were excluded after inclusion to avoid selection by pregnancy outcomes. The distribution of pre-pregnancy BMI in the reference group was in concordance with reports form the Medical Birth Registry of Norway at the time of our PGDM study (2013 – 2016)(178), and the distribution of birthweight in the reference group was also similar to the background population (179). Pre-pregnancy BMI, weekly GWG and birthweights, as well as the flow variables, were normally distributed in the reference population. Thus, the reference values were considered representative for low-risk pregnancies in Norway at the time of our study and suitable for comparison with the PGDM study group.

The study population was an unselected group of PGDM pregnancies from our department. Data from the women who declined the invitation were unavailable. Thus some degree of selection bias cannot entirely be ruled out. However, 75 % of the invited women participated and we did not identify any systematical reasons for declining participation. We therefore conclude that t study group is representative of PGDM pregnancies in Norway.

The study population received clinical follow up according to Norwegian and internationally accepted guidelines (170). This substantiates that the results are

generalizable in similar clinical settings. In our clinic the overall rate of cesarean deliveries is generally low (12.7% during the study period) (180). However, the cesarean frequency in our PGDM study group was 44.9% and similar to 46% in a large Swedish population (16). Thus, the management of labor in the PGDM study group was probably not different from clinics that are relevant for comparison.

There were more missing data in the study- compared with the reference population (Table 4)(175). Within the study group there was a selection by BMI, with higher BMI in the missing-data group (Table 5). This was discussed in Paper I-III and interpreted as a possible selection bias towards data from a leaner PGDM population. Such a selection would likely lead to less significant differences between the populations. Nevertheless, this selection may represent a weakness because the study population is then less representative of all PGDM pregnancies.

HbA_{1C} did not influence the examination success rate and thus a selection by HbA_{1C} was unlikely (Table 5).

Women with type 1 and type 2 DM were invited. Only 5 women with type 2 DM participated in the study while the rest of the participants had type 1 DM (Table 3). Glycemic profiles of women with type 1 DM and type 2 DM are different, and when studied with continuous glucose monitoring, women with type 2 DM spent less time hyper- and hypoglycemic during pregnancy compared with type 1 DM (181). Still, epidemiological studies have shown that the risk of adverse outcomes are similar in pregnancies with type 1 DM and type 2 DM (182). One may argue that the optimal design would be to study pregnancies with type 1 DM and type 2 DM as separate groups. However, we chose not to exclude women with type 2 DM, and the main results did not change when measurements from the type 2 DM subgroup were excluded from the analysis (Table 6).

5.2.3 Validity and reliability of fetal flow

Validity refers to whether a study measures what it aims to measure. Reliability is a measurement of error, and reflects the difference between the observed value and the "true" value. The reliability is high when measurement errors are small and different

observed values are optimally a reflection of genuine differences between measurements, not due to random or systematic errors (183).

Measurements should be repeatable and reproducible. This can be evaluated by estimating the intra – and inter-observer variation. This was done in the studies that established the measurement techniques and constructed the reference values; For the ductus venosus and LPV velocities, the intra and inter-observer variations were low (36, 174). For the portal vein, intra-observer variation was low for both velocity and diameter (46).

The calculation of blood flow is based on flow velocities and vessel diameter. Since experimental invasive procedures in human fetuses are not ethically acceptable, models have been used for validation of the Doppler ultrasound technique to calculate fetal flow (184, 185). In sheep they found no differences between triplex mode ultrasound and steady-state diffusion measurement of umbilical venous flows. In addition, Babera *et al* studied the intra- and inter-observer variability for these measurements in human pregnancies and concluded that valid and reliable measurements of umbilical vein blood flow can be produced by this technique.

Doppler ultrasound is widely used for fetal blood velocity measurements in research as well as clinical settings.

The fetal vessel diameters are not commonly measured in clinical settings, and since the diameter is squared in the blood flow equation, the accuracy of this value is crucial. To reduce measurement error, each vessel diameter was measured at least three times, and the mean of the measurements was used (155, 186). Supervision by J.K. during the first 6 months, consulting ultrasound experts from the research group in case of difficult examinations, and the reassessment of all outlier values, were measures taken to secure the validity of data.

The number of missing values reflects that optimal measurements could be difficult to obtain in our study group. But the fact that calculated flow in the study population, examined mostly by one examiner (A.L), did not deviate more from the reference

curves, indicates that the data are reliable. The confidence intervals were larger in the study group than in the reference group, and this is probably partly due to the smaller study population. It is also possible that measurement errors were larger in the study group, due to participant characteristics that made measurements technically more challenging (Table 4).

The techniques applied are described in publications as methods to measure fetal flow (23, 45, 46, 157, 175). It should be emphasized that the present study was performed in a scientific setting and that we do not suggest the methods for clinical use. This is of importance since we have not studied the diagnostic value of calculating fetal venous flow in PGDM pregnancies (183).

5.2.4 Glycated hemoglobin as a measure of glycemic control

We used HbA_{1c} as an expression of maternal glycemic control, and analyzed the relation between HbA_{1C} and the distribution of umbilical venous flow. The umbilical venous liver flow (expressed by LPV velocity) related positively to HbA_{1C} (Figure 12). The effect of augmented umbilical venous liver flow was higher birthweights (Paper III). We did not find that HbA_{1C} differed between the BMI and GWG categories, although birthweights did (Paper III, Supplementary Table 2).

This complies with studies showing increased risk of macrosomia in PGDM pregnancies, despite HbA_{1C} within the recommended levels (79, 147). Although HbA_{1C} is a measure of glycemic control in women with PGDM, it does not fully reflect the glucose variability in women with PGDM (126, 135, 181).

5.2.5 Body mass index and gestational weight gain in pregnancies with diabetes mellitus

The IOM guidelines use pre-pregnancy BMI categories to advice maternal weight gain in pregnancy. These criteria are widely adopted and have been used in publications on diabetes in pregnancy (138, 147, 148, 187). There are some weaknesses however, as BMI does not reflect the fat percentage, fat distribution, the muscle mass or oedema. Rasmussen *et al* emphasize that the evidence for the IOM recommendations regarding weight gain in pregnancies is not strong (138).

5.2.6 Comparison with other studies

Fetal venous flow in pregnancies with PGDM has been studied by Olofsson *et al.* and by Boito *et al* (92, 116). The former showed that in women with diabetes in pregnancy, UV flow was higher in gestational weeks 26-34, but not different than their reference population from week 35. This is in concordance with our present study as discussed in Paper I. Furthermore, Olfosson *et al.* found that flow to the lower extremities increased from 35 gestational weeks in the diabetic group. We did not examine flow to the extremities, but the reduced PV flow found in our study showed that flow to the viscera was low after 30 weeks of gestation, and this was even more pronounced when PV flow was normalized for fetal weight (Paper II, Figure 1). Prioritized flow to the lower extremities, at the expense of the visceral flow, corroborates with these findings.

Boito conducted a cross-sectional PGDM study (gestational week 18 – 36), and reported no difference in the UV flow. In contrast, we found larger mean UV flow in PGDM compared with low-risk pregnancies, with non-overlapping CI in weeks 28 – 34. The differences in design of Boitos study and the present can explain the different results. However, both studies describe significantly reduced UV flow when normalized for fetal weight (Paper I) (92).

The larger liver volume found by Boito *et al* could relate to higher venous liver flows as described in our Paper II.

5.3 Psychological aspects

With permission, one participant with type 1 DM is quoted: *Worries for the child's well-being and development, for my own health through pregnancy and for the possible complications from birth – complications that my efforts might not prevent - increased the stress and this could contribute to less optimal regulation.*

The study was not designed to describe the emotional stress that women with diabetes might experience during pregnancy. Nevertheless, 410 hours were spent during the study period consulting the women (mean 8.5 hours per pregnancy). The impression

from these consultations was that PGDM is challenging to cope with. Diabetes is associated with lower psychological well-being during pregnancy, and women with diabetes in pregnancy carry a higher risk of post-partum depression symptoms (188). However, in a Danish study of PGDM, women with lower GWG and HbA_{1C} had a slight improvement in mental quality of life during pregnancy (189) .

The risk of increasing stress should be considered when counselling women with PGDM. How to communicate knowledge about lifestyle, weight gain, glucose control, pregnancy outcomes and the possible Developmental Origins of Health and Disease (DOHaD) effects, without causing anxiety, should be discussed and studied further.

6. Conclusion and future aspects

This longitudinal study adds new knowledge by describing the development of fetal venous liver flow during the last half of PGDM pregnancies,

In pregnancies with PGDM, the reduced DV shunting near term may pose an augmented risk during hypoxic challenges in late pregnancy and during labor. We thus suggest that further studies explore the usefulness of DV Doppler measurements to identify fetuses at risk in PGDM pregnancies.

The finding of increased umbilical venous liver flow might partly explain the increased risk of macrosomia, even in well-regulated patients (79). However, unlike non-diabetic fetuses, venous liver flow did not match fetal growth in PGDM pregnancies. The possible consequences of altered venous liver flow for neonatal body composition and postnatal growth, is a relevant subject to study further.

We observed that blood flow changes accelerated during the third trimester. The last measurements in the study were performed around gestational week 36. Future research should investigate the blood flow development close to delivery.

Our study identifies the umbilical venous liver flow as an important mechanism by which maternal BMI and gestational weight gain influence birthweight in PGDM pregnancies. Measures to decrease the risk of macrosomia in PGDM pregnancies are called for. Future research could further explore the effects of restricted weight and weight gain on the fetal hemodynamic development in pregnancies with PGDM.

The present study is part of a larger research project, established and organized by our research group. Maternal anthropometrics, nutritional intake, salivary cortisol and blood samples were collected at each visit. Blood was also drawn for bio-bank preservation in every trimester, and fetal ECG Holter monitoring was performed twice. Placental and umbilical cord tissue, and umbilical venous blood for our bio-bank, was collected shortly after birth. The placentas were examined by perinatal pathologists, and histology was described. A neonatal echocardiography was

performed within 4 days after delivery. Approximately 6 months after birth, blood samples were drawn from the mother and child for bio-banking and an Alberts Infant Motor Scale (AIMS) test assessed the child's motor development. This large amount of systematically collected data, combined with a detailed characterization of the fetal and pregnancy developments, permits for future projects to further explore the relation between maternal health, fetal development and later health in PGDM pregnancies.

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Maternal diabetes alters the development of ductus venosus shunting in the fetus

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Conflict of interest
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Abstract

Introduction. Despite adequate glycemic control, the risks of fetal macrosomia and perinatal complications are increased in diabetic pregnancies. Adjustments of the umbilical venous distribution, including increased ductus venosus shunting, can be important fetal compensatory mechanisms, but the impact of pregestational diabetes on umbilical venous and ductus venosus flow is not known. *Material and methods.* In this prospective study, 49 women with pregestational diabetes mellitus underwent monthly ultrasound examinations from gestational week 20 to 36. The blood velocity and the mean diameters of the umbilical vein and ductus venosus were used for calculating blood flow volumes. The development of the umbilical venous flow, ductus venosus flow and ductus venosus shunt fraction (% of umbilical venous blood shunted through the ductus venosus) was compared with a reference population, and the effect of HbA_{1c} on the ductus venosus flow was assessed. *Results.* The umbilical venous flow was larger in pregnancies with pregestational diabetes mellitus than in low-risk pregnancies ($p < 0.001$) but smaller when normalized for fetal weight ($p = 0.036$). The distributional pattern of the ductus venosus flow developed differently in diabetic pregnancies, particularly during the third trimester, being smaller ($p = 0.007$), also when normalized for fetal weight ($p < 0.001$). Correspondingly, the ductus venosus shunt fraction was reduced ($p < 0.0001$), most prominently at 36 weeks. There were negative relations between the maternal HbA_{1c} and the ductus venosus flow velocity, flow volume and shunt fraction. *Conclusions.* In pregnancies with pregestational diabetes mellitus, prioritized umbilical venous distribution to the fetal liver and lower ductus venosus shunt capacity reduce the compensatory capability of the fetus and may represent an augmented risk during hypoxic challenges during late pregnancy and birth.

Abbreviations: DM, diabetes mellitus; DV, ductus venosus; EFW, estimated fetal weight; PGDM, pregestational diabetes mellitus; TAMXV, time-averaged maximum flow velocity; UV, umbilical vein.

Introduction

Pregnancies complicated by pregestational diabetes mellitus (PGDM) are associated with increased risks of perinatal mortality, congenital anomalies, macrosomia, preterm birth, and fetal distress (1,2). According to the Pedersen

Key Message

In pregnancies with pregestational diabetes the umbilical venous flow to the liver is increased at the expense of ductus venosus shunting, possibly increasing fetal vulnerability near term.

hypothesis, fetal hyperglycemia and hyperinsulinemia cause higher rates of macrosomia (3). Although strict glycemic control can reduce mortality and morbidity (4), the risk of adverse outcomes is higher than in non-diabetic pregnancies (5).

Identifying at-risk fetuses in diabetic pregnancies remains challenging due to the inadequacy of diagnostic tools (6). Umbilical artery Doppler ultrasound is widely used in pregnancies with placental insufficiency, and evaluation of the ductus venosus (DV) flow velocity waveform has become an integral part of surveillance (7,8). The DV pulsatility index is commonly higher in diabetic pregnancies than in low-risk populations (9), but its usefulness in monitoring diabetic fetuses remains unclear. Typical circulatory adaptations in PGDM pregnancies have not been identified (10,11), with the exception of one study finding that the umbilical vein (UV) flow was larger early in the third trimester and decreased toward term (12).

The distribution of the blood flow through the UV and DV develop differently in fetuses with normal growth, growth restriction, and macrosomia without maternal diabetes (7,13). This suggests that the umbilical supply to the liver plays a role in regulating fetal growth (13,14) and fat deposition (15), with possible lifelong consequences (16). The DV shunts oxygenated UV blood to the fetal heart and brain, bypassing the liver (17), and increased shunting serves as a protective mechanism (18,19). In fetal lamb and humans with gestational diabetes, hyperglycemia leads to accelerated metabolism and oxygen consumption, causing chronic fetal hypoxemia (20,21). However, the UV blood distribution and DV flow pattern in pregestational diabetic pregnancies have not been reported previously.

We hypothesized that the fetal distribution of the UV and DV flow is altered in PGDM pregnancies compared with a reference population and that this is influenced by the degree of maternal glycemic control. Thus, the aim of this study was to describe the development of the UV flow and its distribution to the fetal liver and DV in diabetic pregnancies, and to examine whether the distribution of UV blood through the DV correlates to the maternal glycemic control.

Material and methods

Subjects

All pregnant women with PGDM [type 1 or type 2 diabetes mellitus (DM)] in our region are referred to Haukeland University Hospital, a tertiary center for multidisciplinary follow up. All women with PGDM and singleton pregnancies who presented at our hospital between August 2013 and October 2016 were invited to participate in this prospective longitudinal observational study. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (REK vest 2011/2030), and 52 women (74% of those invited) gave written consent: 43 participants had type 1 DM, eight participants had type 2 DM, all of these received gestational insulin treatment. Three participants with type 2 DM withdrew after the first visit leaving a total of 49 PGDM pregnancies for statistical analyses. Gestational age was determined by the crown–rump length measurements using a vaginal transducer (E8C, 8 MHz) at the first visit at approximately nine gestational weeks (22). Second-trimester routine scans did not reveal any fetal malformation in the study population. Information on maternal HbA_{1c}, neonatal sex, birthweight, mode of delivery, Apgar score, cord-blood gases, and transfer to a neonatal ward was collected from clinical records. The study group was compared with reference ranges established in a longitudinal study on 160 low-risk pregnancies (564 observations for the UV and 536 observations for the DV) during the second half of pregnancy (23,24).

Ultrasound examinations

The ultrasound examinations were performed at gestational weeks 20, 24, 28, 32, and 36 (at 20 weeks only the UV was measured). Each session lasted no more than one hour, and the thermal index was kept below 1.0. All ultrasound measurements were performed by three observers (A.L., J.K. or C.E.) using an ultrasound system (Vivid 7, GE Healthcare Vingmed Ultrasound, Horten, Norway) with an abdominal transducer (M4S, 2.0–4.3 MHz).

The time-averaged maximum flow velocity (TAMXV) was measured in the intraabdominal part of the UV and in the DV during fetal quiescence, with the angle of insonation kept as small as possible, not exceeding 30° (median angle correction was 0, range 0–30°). The inner vessel diameter (D) was measured at least three times at the same site perpendicular to the vessel wall, with the mean of these measurements used for the analyses. D was measured in magnified images, with color Doppler turned off, after UV and DV identification. Blood flow volume (Q, mL·min^{-1}) was calculated by the formula $Q = \pi \cdot (D/2)^2 \cdot h \cdot \text{TAMXV}$. Velocity profile parameter h was 0.7 for DV and 0.5 for the UV. The DV shunt fraction (%) was calculated as $100 \cdot Q_{DV}/Q_{UV}$. Flow volume was normalized based on the estimated fetal weight (EFW) as Q/EFW (mL·min^{-1}·kg^{-1}) (25). The techniques applied are identical with those used to establish the reference ranges, and are described in detail elsewhere (23,26).

Statistical analyses

The sample size was based on previous studies (13,27) demonstrating significant associations between fetal growth patterns and variation in the venous liver circulation. We allowed for a lower success rate and possibly smaller effects when planning the present study, and increased the number of participants from 30 to 50.

The mean and standard deviation values for the outcome variables in the diabetic group were modeled according to gestational age using multilevel regression analysis. The absence of overlap in the 95% confidence intervals of the mean indicates a statistically significant difference between the study and reference (24) populations. In addition, *z*-scores for outcome variables in the PGDM and reference groups were compared using the independent-samples *t*-test with a significance cutoff of $p \leq 0.05$. The relations between maternal first trimester HbA_{1c} and DV flow velocity, DV flow volume, and DV shunt fraction *z*-scores were assessed by multilevel regression analysis. The statistical analyses were performed with the Statistical Package for the Social Sciences (version 24; SPSS, Chicago, IL, USA) and the MLWin program (version 2.35; Centre of Multilevel Modeling, University of Bristol, Bristol, UK).

Results

The characteristics of the study population are described in Tables 1 and 2. The median gestational age at birth was lower and birthweights were higher in the study group than in the reference population (24) (Tables 1 and 2). In the study group, 39% of the neonates were macrosomic (birthweight >90th percentile for gestational age) (25). The UV and DV flow velocities and diameters were successfully measured in 85.8% and 77.2% of 225 examination sessions, respectively. The DV shunt fraction could be calculated for 69.5% of the sessions.

The UV flow velocities in the study group did not differ significantly from those in the reference group (Appendix S1). However, the mean UV diameter was larger in the PGDM group (mean *z*-score = 0.48, $p < 0.005$), resulting in a larger UV flow volume in the study group (Table 3), primarily during gestational weeks 25–31 (Figure 1a). When normalized for EFW, the UV flow was smaller compared with that in the reference population (Table 3), and in PGDM pregnancies the normalized UV flow exhibited a significant blunting at the end of the third trimester (Figure 1b).

The DV diameter was larger (mean *z*-score = 0.35, $p = 0.034$) while the DV TAMXV was smaller (mean *z*-score = −1.20, $p < 0.005$) in the study group than in the reference (Appendix S2). However, at week 36 there was no difference in the DV diameters (mean *z*-score = 0.31, $p = 0.40$) but the DV TAMXV remained smaller in the study group (mean *z*-score = −1.87, $p < 0.005$). In the reference population, the DV flow volume increased steadily from mid-gestation until term, whereas it was smaller in the diabetic group (Table 3) and became progressively blunted beyond 30 weeks of gestation (Figure 2a). The normalized DV flow volume was significantly smaller in the study group after gestational week 32 (Table 3, Figure 2b).

In PGDM pregnancies, the degree of DV shunting was significantly smaller during the second half of pregnancy (Table 3). In the reference population this reached a minimum of 20% at 30 weeks of gestation (23), whereas it was smaller in diabetic pregnancies both before and after 30 weeks, which constituted a strikingly different pattern of development (Figure 3a).

There was a borderline significant negative linear relation between the DV shunt fraction at 36 weeks of gestation and the lactate concentration in the umbilical artery at birth ($B = -0.19$, $p = 0.051$; Figure 3b).

Table 1. Maternal characteristics and outcomes in 49 pregnancies with pregestational diabetes mellitus.

	n	%
Type 1 DM	44	89.8
Type 2 DM	5	10.2
Maternal diabetic complications or disease		
Retinopathy	9	18.4
Nephropathy	1	2.0
Hypothyroidism	9	18.4
Chronic hypertension	7	14.3
Preeclampsia	3	6.1
Preterm birth	15	30.6
Induction of labor	30	61.2
Normal delivery	20	40.8
Operative vaginal delivery	7	14.3
Cesarean section	22	44.9
Elective	9	18.4
Acute[a]	13	26.5

	Median	Range
Maternal age at inclusion, years	31	23–42
Prepregnancy weight, kg	70	57–113
Maternal weight gain	15.8	−5.0 to 33.1
Prepregnancy BMI	24.86	19.82–44.14
HbA_{1c} at inclusion	6.70	4.90–12.00
Individual mean HbA_{1c}[b]	6.12	4.86–8.24

Preterm birth, gestational age <37 weeks.
DM, diabetes mellitus.
[a]Acute cesarean section during labor.
[b]Mean of all HbA_{1c} measurements throughout each pregnancy.

The DV TAMXV, DV flow volume, and DV shunt fraction were negatively related to the first trimester HbA_{1c} (Figure 4). The multilevel regression analyses were also performed for second and third trimester HbA_{1C}, with similar results for DV flow and shunting (data not shown).

Excluding T2DM participants from the PGDM population did not significantly change the results for the *z*-score mean flow variables UV flow, normalized UV flow, normalized DV flow or DV shunt fraction (univariate analysis of variance), but the mean DV flow *z*-score was no longer significantly different for the T1DM population alone compared with the reference; −0.212 [95% confidence interval (CI) −0.424 to 0.000, *p*-value = 0.071].

Table 2. Neonatal characteristics and outcomes in pregnancies with pregestational diabetes mellitus.

	Median	Range
Gestational age at delivery (weeks^{+days})	38^{+4}	27^{+6} to 40^{+5}
Birthweight (g)	3695	990–5990
Birthweight *z*-score	0.93	−2.15 to 5.82
Umbilical cord acid-base data		
Umbilical artery		
pH	7.24	6.92–7.34
pCO_2 (kPa)	7.88	5.80–12.40
pO_2 (kPa)	2.19	1.16–3.47
Base deficit (mmol·L^{-1})	−2.11	−13.36 to 1.00
Lactate (mmol·L^{-1})	4.70	2.00–14.40
Umbilical vein		
pH	7.30	6.89–7.44
pCO_2 (kPa)	6.10	4.20–15.30
pO_2 (kPa)	3.29	0.25–5.68
Base deficit (mmol·L^{-1})	−2.36	−10.98 to −0.15
Lactate (mmol·L^{-1})	3.50	1.80–12.80
Erythrocyte volume fraction	0.63	0.52–0.76

	n	%
Male sex	25	51
Operative delivery for intrapartum fetal distress	13	26.5
Metabolic acidosis at birth[a]	1	2
5-min Apgar score <7	1	2
Transfer to neonatal intensive care ward	20	40.8
Perinatal death[b]	1	2
Malformation[c]	2	4

[a]Metabolic acidosis defined as an umbilical arterial pH of <7.0 and a base deficit of >12.
[b]Intrauterine fetal death at gestational week 36. Autopsy showed UV thrombosis and signs of acute asphyxia.
[c]One neonate with sagittal craniosynostosis and one with congenital heart defect (anomalous left coronary artery from the pulmonary artery).

Discussion

This study found that the distribution of UV blood was significantly altered in PGDM pregnancies, with a smaller fraction of the UV blood directed through the DV and relatively more to the fetal liver. This distorted development of the UV distribution in PGDM pregnancies was evident from mid-gestation but was particularly prominent during the last weeks of pregnancy (Figures 1–3).

Our findings are partly in contrast with those of Boito et al. (28) showing unaffected UV flow volumes in PGDM pregnancies, but smaller normalized UV flow volume, in line with our present study. Olofsson et al. found larger normalized UV flow in the early third trimester of diabetic pregnancies, but like in our study group the normalized UV flow decreased during the last weeks of pregnancy (12). The discrepancies between the studies might be due to differences in methods and study populations.

The portocaval pressure gradient between the UV and the vena cava inferior drives the perfusion of the fetal liver and flow through the DV, and the DV blood velocity directly reflects this pressure (29). A reduced resistance to flow in the portal system relative to the DV would contribute to preferential UV distribution to the liver (30,31). Greater UV distribution to the liver in PGDM pregnancies could be related to increased liver size

Table 3. Umbilical venous (UV) and ductus venosus (DV) blood flow *z*-scores in pregnancies complicated by pregestational diabetes mellitus (PGDM) compared with reference values from a low-risk population.

Parameter	Population	Mean *z*-score	95% CI		No. of observations	*p*
UV flow (mL·min^{-1})	Reference	0.000	−0.097	0.097	562	<0.001
	PGDM	0.356	0.191	0.521	192	
Normalized UV flow (mL·min^{-1}·kg^{-1})	Reference	0.005	−0.096	0.106	562	0.036
	PGDM	−0.230	−0.414	−0.046	191	
DV flow (mL·min^{-1})	Reference	0.005	−0.101	0.110	532	0.007
	PGDM	−0.332	−0.539	−0.125	138	
Normalized DV flow (mL·min^{-1}·kg^{-1})	Reference	0.005	−0.104	0.114	532	<0.001
	PGDM	−0.614	−0.818	−0.410	137	
DV shunt fraction (%)	Reference	0.000	−0.0821	0.0904	524	<0.0001
	PGDM	−0.510	−0.8119	−0.2006	125	

CI, confidence interval; DV shunt fraction (%), (DV flow volume/UV flow volume)·100; reference values from low-risk population (23).

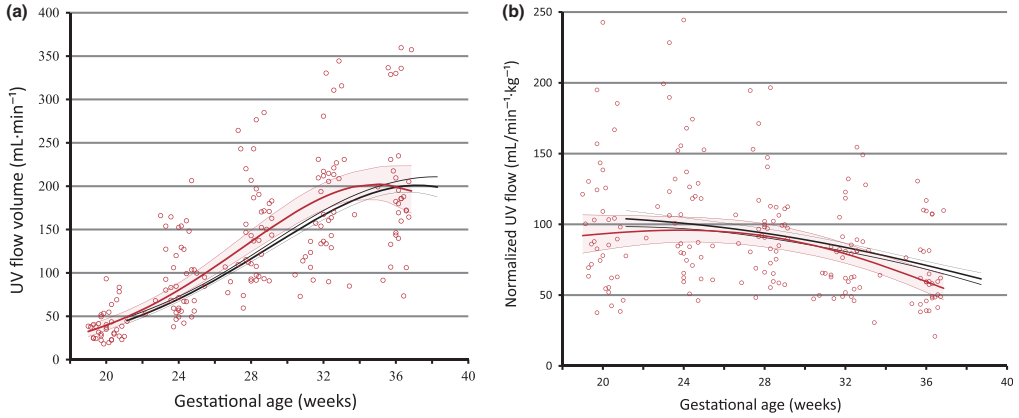

Figure 1. Umbilical venous flow. Longitudinal observations of umbilical vein (UV) flow volume (a) and normalized UV flow volume (b) in 49 pregnancies with pregestational diabetes mellitus (red circles and lines) compared with a reference population (black lines). Mean (thick lines) and 95% confidence interval (thin lines) values are shown. [Color figure can be viewed at wileyonlinelibrary.com].

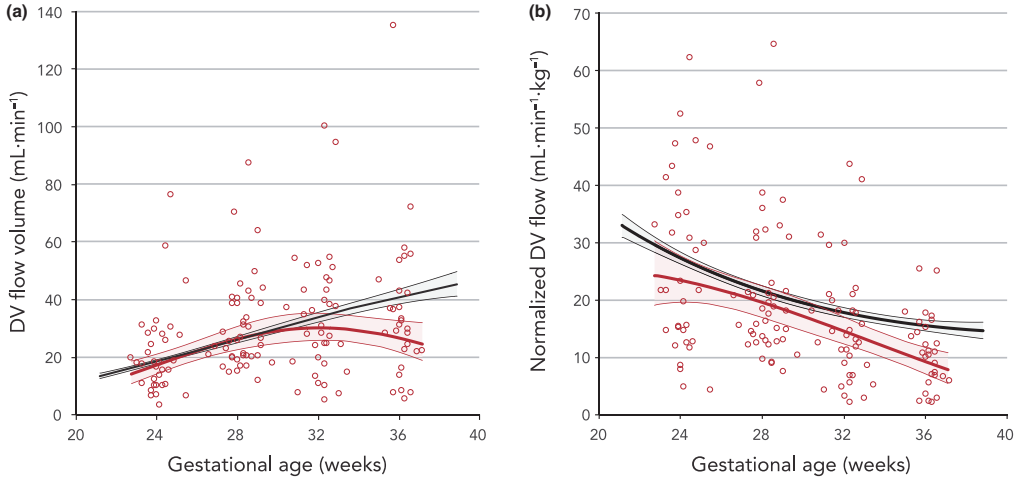

Figure 2. Ductus venosus (DV) flow. Longitudinal observations of DV flow volume (a) and normalized DV flow volume (b) in 49 pregnancies with pregestational diabetes mellitus (red circles and lines) compared with a reference population (black lines). Mean (thick lines) and 95% confidence interval (thin lines) values are shown. [Color figure can be viewed at wileyonlinelibrary.com].

(28) and a larger vascular cross-section of the portal system. A larger viscous resistance in the liver vasculature due to polycythemia (Table 2) was expected (32), and this would shift blood flow from the liver to the DV, but the opposite situation was found in the present diabetic pregnancies (Figures 2 and 3). This suggests that the impact of fetal hyperglycemia on the circulatory regulation of the fetal liver overpowers the physiological mechanisms that would otherwise operate.

In fetal lamb, occluding the DV and forcing all UV blood to perfuse the fetal liver, leads to increased liver weight, cell proliferation, and differential fetal organ growth (14). Up-regulation of the UV blood distribution to the liver is believed to be an important mechanism underlying the development of macrosomia and increased fat accretion (15), and the present study has demonstrated this flow pattern in PGDM pregnancies. However, in macrosomia without DM, the UV flow volume

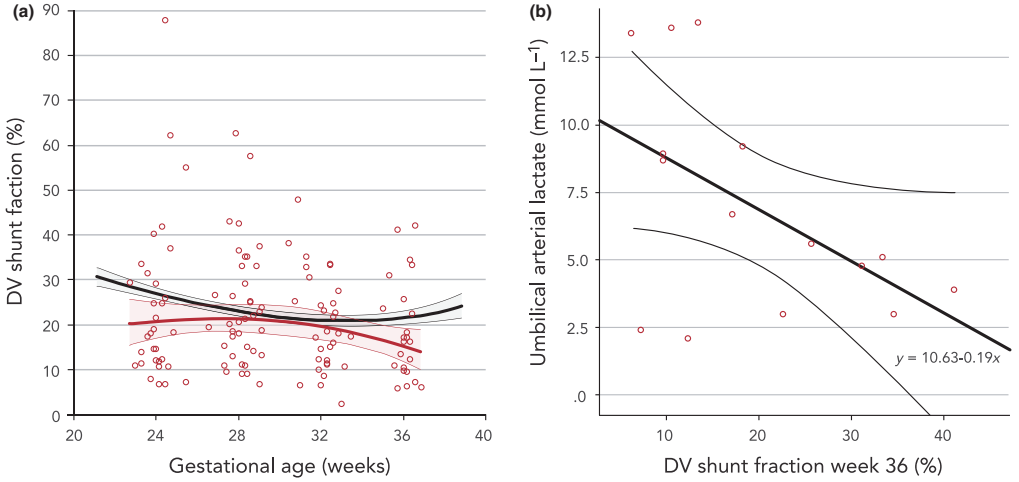

Figure 3. Ductus venosus (DV) shunt fraction. (a) Longitudinal observations of DV shunt fraction in 49 pregnancies with pregestational diabetes mellitus (red circles and lines) compared with a reference population (black lines). Mean (thick lines) and 95% confidence interval (thin lines) values are shown. (b) Relation between umbilical arterial lactate at birth and DV shunt fraction *z*-score at gestational week 36 (n = 15). Missing data for week 36 were due to premature delivery (n = 18), unsuccessful cord-blood lactate measurements (n = 13), and/or missing measurements for calculating the DV shunt fraction (n = 20). [Color figure can be viewed at wileyonlinelibrary.com].

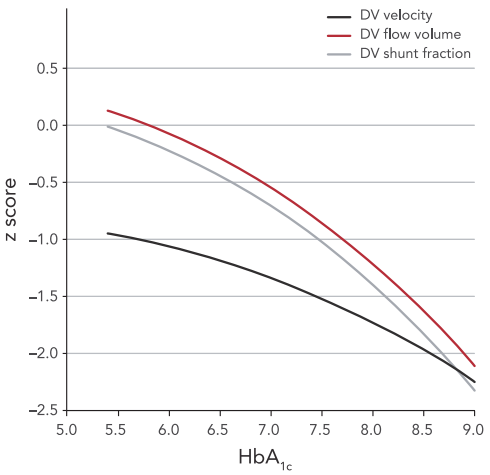

Figure 4. Ductus venosus (DV) shunt fraction and HbA_{1c}. Relations between the time-averaged maximum DV flow velocity (TAMXV), DV flow volume, and DV shunt fraction and first trimester HbA_{1c}. DV flow velocity mean *z*-score = −0.6867 to 0.0010 (95% CI −0.00194836 to −0.00001008) $HbA_{1c}{}^3$ ln(HbA_{1c}), DV flow volume mean *z*-score = 0.5760–0.0017 (95% CI 0.00008183–0.00328055) $HbA_{1c}{}^3$ ln(HbA_{1c}), and DV shunt fraction mean *z*-score = 0.4529–0.0017 (95% CI −0.00300435 to −0.00047375) $HbA_{1c}{}^3$ ln(HbA_{1c}). [Color figure can be viewed at wileyonlinelibrary.com].

accelerates during the third trimester, supporting the increased growth velocity, whereas the DV flow volume remains unchanged (13). This is at variance with the distorted UV distribution in our PGDM group, in which UV return from the placenta decreased (Figure 1a) and DV shunting was reduced near term (Figure 3a). The UV flow volume normalized for EFW was strikingly low during late gestation, possibly signifying a relative discrepancy between fetal demand and substrate availability (Figure 1b) in our study group (13).

Fetuses of mothers without diabetes, consuming a diet defined as imprudent, exhibits "liver-sparing" UV blood distribution, with a larger UV flow to the liver and smaller DV shunting at week 36 (33). Larger fetal abdominal circumference was found to be associated with increased UV flow after oral glucose loading in a low-risk population (34). The negative relation between HbA_{1c} levels and the DV shunt fraction (Figure 4) in our study supports the assumption that maternal metabolic factors and glucose promote UV flow distribution to the fetal liver. Even within the range of clinically acceptable glycemic control (maternal first trimester HbA_{1c} <7%), the DV shunt fraction decreased significantly with increasing HbA_{1c} (Figure 4). Although the use of HbA1c in pregnancy may have limitations, the association between HbA1c and plasma glucose is linear in most individuals (35). First trimester HbA1c measurement gives information about

maternal glycemic control at conception and in early pregnancy and the relation between HbA1c and DV flow underlines the effect of the periconceptional environment on later fetal and neonatal health. This is in line with other studies where less optimal glycemic control in the first trimester (HbA_{1c} >6.5 mmol·L^{-1}) was associated with accelerated fetal growth (36), and the levels of biomarkers for placentation and placental growth factor in the first trimester of PGDM pregnancies had an impact on birthweight (37).

Growth-restricted fetuses shunt a larger fraction of the UV blood through the DV (18,19), graded according to the severity of placental compromise, ranging from 25 to 57% (18). The PGDM fetuses in the present study had a mean DV shunting of only 18% (Figure 3), mainly caused by the reduction in DV flow velocity, and in spite of a distended DV diameter (Appendix S2). It appears that this blood distribution to the liver is driven by metabolic factors, mainly as reduction of the liver vascular resistance. This corroborates the finding that the liver vasculature is more sensitive to endocrine regulation compared with the DV (31).

The diabetic intrauterine environment can induce chronic fetal hypoxemia (21,38), but the mechanisms that protect the fetus during hypoxemia (18) seem less developed in PGDM fetuses near term. This is supported by the finding of reduced DV shunt fraction in our study, and a tendency toward lactacidemia at delivery in those with low capacity for DV shunting (Figure 3b). Furthermore, this pattern is augmented according to the degree of glycemic control in the first trimester (Figure 4). The level of Hb_{A1C} influenced the fetal venous circulation, supporting the clinical focus on tight periconceptional glucose and dietary control in women with DM.

The strengths of this study are the prospective longitudinal design involving an unselected group of PGDM pregnancies and identical methods applied to the study and reference populations. Low intra- and interobserver variation has been demonstrated earlier for DV flow velocities (23) and almost identical results for UV flow were achieved by different investigators using the same technique for ultrasound measurement and blood flow calculation (26,39). Measurement success rate was lower in the PGDM group than in the reference study, probably explained by the higher BMI in the study population (24), causing challenging examination conditions (Table 1). Since the intergroup BMI was higher in the missing compared with the non-missing PGDM data group (data not shown), this may have introduced a selection towards a leaner study population, more similar to the reference. However, such a selection is expected to reduce rather than augment the differences between the study group and the reference population. Technical challenges related to the angle and depth of insonation could also introduce systematic errors by underestimating flow velocities, but in the lower gestational ages the measured velocities did not differ from the reference population. The increasing difference in flow velocity with gestational age is thus physiologically plausible and supports the validity of the results.

The objective of the present study was to assess the fetal circulatory physiology in PGDM pregnancies and the study was not powered for subgroup analysis related to the mother (for example diabetic vasculopathy) or the fetus (intrapartum hypoxia). Nonetheless, the results warrant further research in this section of the circulation to develop clinical tools for identifying PGDM fetuses at risk.

Epidemiological studies indicate that the increased risk of adverse outcomes in PGDM, including stillbirth, is limited to the last weeks of pregnancy (2). Our findings in these PGDM pregnancies suggest that prioritizing UV flow to the liver at the expense of DV shunting could increase fetal vulnerability in late pregnancy and during labor.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Reference population (1), gestational age (GA) in weeks.

Appendix S2. Study population, pregnancies with pregestational diabetes, gestational age (GA) in weeks.

Appendix S1

Reference population (1), gestational age (GA) in weeks.

Umbilical vein (UV) diameter, mean and variance

Mean diameter was transformed to the 0.382 power.

Transformed mean **=**

$0.48101595044136+0.0000758466339902953 \cdot GA^3-0.0000190573191503063 \cdot GA^3 \cdot \ln(GA)$

Variance =

$0.00109042716212571-2.56153676048144\ E-08 \cdot GA^3 + 7.80816600612355E-13 \cdot GA^6$

Umbilical vein velocities, mean and variance

Mean velocity was transformed to the -0.166 power.

Transformed mean =

$0.64591223+263.12112427 \cdot GA^{-2}-90.02454376 \cdot \ln(GA) \cdot GA^{-2}$

Variance =

0.00033238372998312

Ductus venosus (DV) diameter, mean and variance

Mean diameter was transformed to the -0.266 power.

Transformed mean = μ

$= 2.0811376572-0.0000838075 \cdot (GA)^3+0.0000209877 \cdot \ln(GA) \cdot GA^3$

Variance =

$= 0.003764423-1.48793E-08 \cdot GA^3+1.90734E-12 \cdot GA^6$

Appendix S2

Study population, pregnancies with pregestational diabetes, gestational age (GA) in weeks

UV flow volume

Mean UV flow volume was transformed to the 0.282 power.

Transformed mean =

$1.162891865+0.00090542360^{GA3}-0.00023297807^{GA3}\cdot\ln(GA)$

Variance =

0.208563655614853

Normalized UV flow volume, mean and variance

Mean normalized UV flow volume was transformed to the -0.119 power.

Transformed mean =

$0.5998132229-0.000014567\cdot GA^3+0.0000041554GA^3\cdot\ln(GA)$

Variance =

$0.0004220234-0.00000000752759\cdot GA^3+0.0000000000001261475\cdot GA^6$

DV flow volume

Mean flow volume was transformed to the -0.025 power.

Transformed mean=

$0.6633474231 + 1.2373400927\cdot GA^{-0.5} + 0.0000011074\cdot GA^3$

Variance =

0.000199967034859583

Normalized DV flow volume, mean and variance

Mean Normalized DV flow volume was transformed to the 0.383 power.

Transformed mean=

$2.32871628+0.12603378\cdot GA - 0.00349011\cdot GA^2$

Variance = σ^2

= 0.45418781

DV shunt fraction

Mean DV shunt fraction was transformed to the 0.302 power.

Transformed mean =

$2.1142778+0.000216869GA^3-0.000059556598GA^3 \cdot \ln(GA)$

Variance =

0.191669091582298

Reference

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II

PLOS ONE

RESEARCH ARTICLE

Altered development of fetal liver perfusion in pregnancies with pregestational diabetes

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Data Availability Statement: The combination of detailed clinical information in our study could enable identification of specific participants. Therefore, data sharing must be approved by our ethics committee, even if the data are de-identified. The Regional Committee for Medical and Health Research Ethics (REK Vest) can be contacted referring to the number REK vest 2011/2030; post@helseforskning.etikkom.no. The rules and procedures can be found here: https://helseforskning.etikkom.no/reglerogrutiner/

Abstract

Background

Pregestational diabetes is associated with fetal macrosomia, and umbilical perfusion of the fetal liver has a role in regulating fetal growth. We therefore hypothesized that pregestational diabetes alters fetal liver blood flow depending on degree of glycemic control.

Methods

In a prospective study, 49 women with pregestational diabetes underwent monthly ultrasound examinations during 24–36 gestational weeks. Blood flow was determined in the umbilical vein, ductus venosus and portal vein, and blood velocity was measured in the left portal vein, the latter reflecting the watershed between splanchnic and umbilical flow. The measurements were compared with reference values by z-score statistics, and the effect of HbA_{1c} assessed.

Results

The umbilical venous flow to the liver (*z*-score 0.36, p = 0.002), total venous liver flow (*z*-score 0.51, p<0.001) and left portal vein blood velocity (*z*-score 0.64, p<0.001), were higher in the study group. Normalized portal venous flow was lower (*z*-score -0.42, p = 0.002), and normalized total venous liver flow tended to be lower after 30 gestational weeks (*z*-score -0.54, p = 0.047) in the diabetic pregnancies compared with reference values from a low-risk population. The left portal vein blood velocity was positively, and the portal fraction of total venous liver flow negatively correlated with first trimester HbA_{1C}.

Conclusions

In spite of increased umbilical blood distribution to the fetal liver, graded according to glycemic control, the total venous liver flow did not match third trimester fetal growth in pregnancies with pregestational diabetes, thus contributing towards increased perinatal risks and possibly altered liver function with long-term metabolic consequences.

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Introduction

Pregnancies complicated by pregestational diabetes mellitus (PGDM) are associated with increased perinatal morbidity and mortality [1], and fetal macrosomia is related to these adverse neonatal outcomes [2]. Fetal hyperglycemia and hyperinsulinemia can cause accelerated fetal growth [3] even with HbA_{1C} levels within the recommended range [4], and this makes clinical surveillance in diabetic pregnancies challenging [5].

The liver has been called "the metabolic brain" of the fetus [6], controlling the distribution and utilization of nutrients from the placenta. Nutrient access and fetal liver blood flow act both independently and together to influence fetal growth and body composition [7, 8]. The fetal liver has two sources of venous supply; well-oxygenated blood from the placenta through the umbilical vein being the main source, and low-oxygenated blood from visceral organs through the portal vein. The distribution of the nutrient rich umbilical venous blood to the liver has been suggested to be a mechanism for regulation of fetal growth [9]. This is based on experimental studies showing that increasing liver flow from the umbilical vein leads to higher cell proliferation in the liver, heart, skeletal muscle and kidneys in fetal lamb [9]. In addition, studies of human low-risk pregnancies have shown that larger fetal size is associated with higher umbilical venous liver flow as a response to maternal glucose intake [10]. Also, higher umbilical venous flow to the liver is associated with newborn adiposity [11].

In studies of macrosomic fetuses in non-diabetic pregnancies, umbilical- and total venous liver flow was higher during the 2nd and 3rd trimester, including when normalized for estimated fetal weight [7, 12]. This indicates that increased umbilical venous flow led to augmented fetal growth in pregnancies without diabetes. In low-risk pregnancies, the portal venous contribution to the liver increases throughout gestation, and the same pattern is observed in macrosomic non-diabetic fetuses [7]. However, although the fetal liver is larger [13] and macrosomic growth is frequent in diabetic pregnancies [4], umbilical venous flow normalized for fetal weight, is lower [13, 14].

Fetal liver gene expression in baboons is different in the left and right liver lobes [15], and this is ascribed to the specific venous perfusion pattern during fetal life. Thus, fetal hemodynamic development might influence liver function and be part of a pathway regulating intrauterine growth, with possible long-term consequences [7, 12].

In diabetic pregnancies, fetal liver size measured by ultrasound is greater than in low-risk pregnancies and liver volume positively correlates with maternal HbA_{1C} [13]. Experimental studies in pigs showed that diabetes induces fetal liver hyperplasia [16], the fetal liver protein synthesis and glycogen reserves increase [16], and total body fat percentage is higher than in non-diabetic controls [17]. In human stillborn neonates of diabetic mothers, hepatic steatosis is prevalent and more severe than in stillborn of non-diabetic pregnancies [18].

Fetuses of women with PGDM have greater risk of later diabetes independently of genetic factors [19], possibly mediated through epigenetic mechanisms. It has been suggested that the human fetal strategy to prioritize fat deposition for neonatal survival evolved under conditions where high glycemic diets were not available; but with their currently widespread consumption, these mechanisms enhance fetal fat deposition [20]. As both diabetes [21] and chronic liver disease [22] are becoming increasingly prevalent, and there is currently much interest in the developmental origins of these conditions, studies of factors such as maternal diabetes on fetal liver development are called for as a basis for informing preventive strategies [23]. We therefore aimed to determine the fetal liver blood flow in PGDM pregnancies in a prospective longitudinal study and present the longitudinal development of venous liver blood flow during the second half of PGDM pregnancies.

Materials and methods

The present prospective longitudinal observational study is part of a larger project investigating fetal hemodynamics in pregnancies with PGDM. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (REK vest 2011/2030). We have reported the development of the umbilical venous and ductus venosus flows in this population [14]. Here we present data on the development of the venous supply to the fetal liver in PGDM pregnancies. We have used the left portal vein blood velocity as a marker of the watershed between the portal and umbilical venous contributions (Fig 1).

Subjects

All women in our region, with PGDM in pregnancy, are referred to our tertiary center at Haukeland University Hospital for multidisciplinary follow-up. All women with singleton pregnancies and PGDM who presented at our clinic between August 2013 and May 2016 were invited to participate. Fifty-two women (74% of those invited) gave written consent: 44 participants had type 1 diabetes mellitus (DM) and 8 had type 2 DM of which all received gestational insulin treatment. Three participants with type 2 DM withdrew, leaving a total of 49 PGDM pregnancies for statistical analyzes. Gestational age (GA) was determined using a vaginal probe (Vivid 7, GE Healthcare Vingmed Ultrasound, E8C, 8 MHz) at the first visit (around week 9), by measuring the crown rump length [24]. No fetal malformations were revealed by

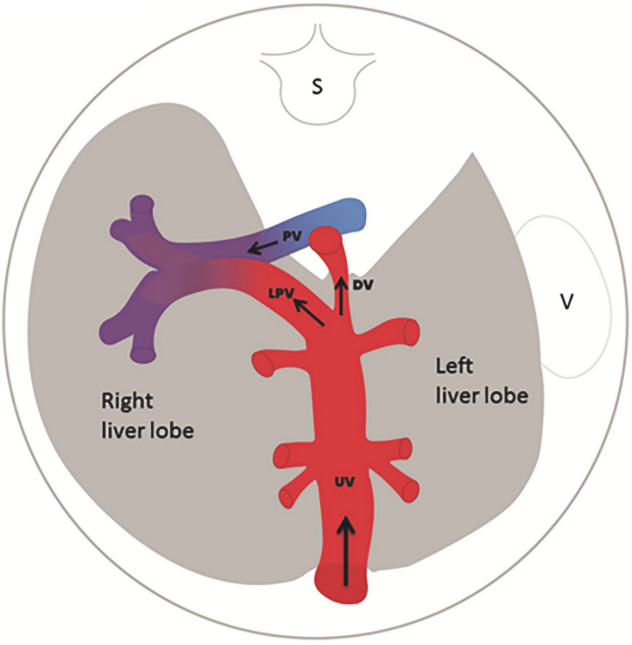

Fig 1. Venous supply to the fetal liver. Cross section of the fetal abdomen with black arrows indicating physiological blood flow directions in the fetal liver (grey). Typically, well-oxygenated umbilical blood (red) blends in with deoxygenated portal blood (blue) to feed the right liver lobe; UV, umbilical vein; DV, ductus venosus; LPV, Left portal vein; PV, portal vein; S, spine; V, stomach.

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second-trimester routine scans in the study population. Information on first trimester maternal HbA_{1C}, neonatal sex, birthweight, mode of delivery, Apgar score, cord-blood gases, and transfer to the neonatal ward was collected from clinical records. The results from the study group were compared with reference ranges established in the same research unit using identical methods, in a longitudinal study of 160 low-risk pregnancies [25, 26].

Measurements

The ultrasound examinations were performed in each pregnancy at gestational weeks 24, 28, 32, and 36. All ultrasound measurements were performed by three observers (A.L., J.K. and C. E.) using an abdominal transducer (M4S, 2.0–4.3 MHz) ultrasound system (Vivid 7, GE Healthcare Vingmed Ultrasound, Horten, Norway). The sessions lasted maximum one hour and the thermal index was kept below 1.0.

The time-averaged maximum blood velocity (TAMXV) was measured in the umbilical vein, ductus venosus, left portal vein and portal vein (Fig 1). The angle of insonation was kept small, not exceeding 30˚ (median angle correction was 0, range 0–30˚). At the same site perpendicular to the vessel wall, the inner vessel diameter (D) was measured at least three times in the umbilical vein, ductus venosus and portal vein. The mean D was used for the analyses (Fig 1). After identification of the vessel, the color Doppler was turned off and D was measured in magnified images. The techniques applied are described in detail elsewhere [25, 26].

Blood flow (Q, $mL{\cdot}min^{-1}$) was calculated by the formula $Q = \pi \cdot (D/2)^2 \cdot h \cdot \text{TAMXV}$. The velocity profile parameter was $h = 0.5$ for the umbilical vein (UV) and the portal vein (PV) [26], $h = 0.7$ for the ductus venosus (DV) [27, 28]. Flow was normalized based on the estimated fetal weight (EFW) as Q/EFW ($mL{\cdot}min^{-1}{\cdot}kg^{-1}$) [29]. Umbilical venous liver flow (UV_{liver}) was calculated as $Q_{UV\ liver} = Q_{UV} - Q_{DV}$, total liver flow as $Q_{liver} = (Q_{UV} - Q_{DV}) + Q_{PV}$ and PV fraction (F_{PV}) of the total venous supply to the liver was $F_{PV} = 100\% \cdot Q_{PV}/Q_{liver}$.

Statistics

The sample size was based on our previous studies in non-diabetic pregnancies, demonstrating significant associations between fetal growth patterns and variation in the venous liver circulation [7, 30]. We allowed for lower measurement success rates and possibly smaller effects in the PGDM group by increasing the number of participants from 30 to 50. It was not possible to perform a formal sample size calculation since there were no earlier reports on the effects of PGDM on fetal liver flow.

Multilevel regression analysis was used to model the mean and standard deviation values for the outcome variables according to gestational age. The absence of overlap of the 95% confidence intervals of the mean indicated a statistically significant difference between the PGDM group and the reference values [25, 26, 31]. In addition, z-scores for means of outcome variables in the study population were compared with the reference group using the independent-samples t-test, with a significance cutoff of $p \leq 0.05$. The populations were also stratified for gestational age (GA $</\geq$ 30 weeks), and independent sample t-tests comparing mean z-scores were performed to test differences between PGDM and low-risk pregnancies before and after 30 weeks of gestation. The relations between maternal first-trimester HbA_{1c} and left portal vein flow velocity, portal venous flow, and portal venous shunt fraction z-scores after 30 gestational weeks were assessed using multilevel regression analysis. The statistical analyses were performed with the Statistical Package for the Social Sciences (version 24, SPSS, Chicago, IL) and the MLWin program (version 2.35, Centre of Multilevel Modeling, University of Bristol, UK).

Results

The characteristics of the study population are described in Tables 1 and 2. The median gestational age at birth was lower and birthweights were higher in the study group than in the reference population [31]. In the study group, 19 (39%) of the neonates were macrosomic (birthweight >90th percentile) and 3 (6%) were small for gestational age (<10th percentile) [29] (Tables 1 and 2).

The left portal vein and portal vein blood velocity, and the portal vein diameters, were successfully measured in 94.4%, 70.9% and 55.9% of 179 examination sessions, respectively. Further, portal venous flow was calculated in 52.5%, total venous liver flow (Q_{liver}) in 42.5%, and the portal venous fraction of the total venous liver flow in 42.5% of the sessions. The success rate for the umbilical vein and ductus venosus measurements have been published earlier [14].

The mean left portal vein flow velocity in the PGDM group was significantly higher than the reference values, both before and after 30 weeks (Table 3, Fig 2A).

The mean portal venous flow in the PGDM pregnancies was not significantly different from the reference values over the study period as a whole, but was significantly higher for the period before 30 weeks of gestation, and the development after 30 weeks was blunted compared with the reference values (Fig 3A). When normalized for EFW, the overall mean portal venous flow was significantly smaller in PGDM, mainly due to reduced flow after 30 weeks of gestation (Table 3 and Fig 3B).

Table 1. Maternal characteristics and outcomes in 49 pregnancies with pregestational diabetes mellitus.

	Number	Percent
Type 1 DM	44	89.8
Type 2 DM	5	10.2
Maternal diabetic complications or condition		
- Retinopathy	9	18.4
- Nephropathy	1	2.0
- Hypothyroidism	9	18.4
- Chronic hypertension	7	14.3
Preeclampsia	3	6.1
Preterm birth	15	30.6
Induction of labor	30	61.2
Normal delivery	20	40.8
Operative vaginal delivery	7	14.3
Cesarean section	22	44.9
- Elective	9	18.4
- Acute	13	26.5
	Median	**Range**
Maternal age (years)	31	23 to 42
Pre-pregnancy weight (kg)	70	57 to 113
Maternal weight gain	15.8	-5.0 to 33.1
Pre-pregnancy BMI	24.9	19.8 to 44.1
HbA_{1c} at inclusion (%)	6.7	4.9 to 12.0
Individual mean HbA_{1c}† (%)	6.12	4.9 to 8.2

DM, diabetes mellitus; Preterm birth, gestational age <37 weeks

*acute cesarean section during labor

†mean of all HbA_{1c} measurements throughout each pregnancy

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Table 2. Neonatal characteristics and outcomes in pregnancies with pregestational diabetes mellitus.

	Median	Range
Gestational age at delivery (weeks+days)	38+4	27+6 to 40+5
Birthweight (g)	3695	990 to 5990
Birthweight *z*-score	0.93	−2.15 to 5.82
Umbilical artery		
- pH	7.24	6.92 to 7.34
- pCO_2 (kPa)	7.88	5.80 to 12.40
- pO_2 (kPa)	2.19	1.16 to 3.47
- Base deficit (mmol·L^{-1})	−2.11	−13.36 to 1.00
- Lactate (mmol·L^{-1})	4.70	2.00 to 14.40
Umbilical vein		
- pH	7.30	6.89 to 7.44
- pCO_2 (kPa)	6.10	4.20 to 15.30
- pO_2 (kPa)	3.29	0.25 to 5.68
- Base deficit (mmol·L^{-1})	−2.36	−10.98 to −0.15
- Lactate (mmol·L^{-1})	3.50	1.80 to 12.80
Erythrocyte volume fraction	0.63	0.52 to 0.76
	Number	**Percent**
Male sex	25	51%
Operative delivery for intrapartum fetal distress	13	26.5%
Metabolic acidosis at birth*	1	2%
5-min Apgar score <7	1	2%
Neonatal intensive care	20	40.8%
Perinatal death†	1	2%
Malformation‡	2	4%

* Metabolic acidosis defined as an umbilical arterial pH of <7.0 and a base deficit of >12.

† Intrauterine fetal death at gestational week 36. Autopsy showed UV thrombosis and signs of acute asphyxia.

‡ One neonate with sagittal craniosynostosis and one with congenital heart defect (anomalous left coronary artery from the pulmonary artery)

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The total venous supply to the fetal liver (Q_{liver}) was larger in PGDM pregnancies (Table 3), mainly due to high volumes in the second trimester (Fig 4A). When normalized for EFW, the overall mean total venous liver flow in the PGDM group did not differ from that of the reference values but was significantly smaller after 30 weeks and with a different trajectory of the mean curve (Table 3 and Fig 4B).

In the study group, the mean portal venous fraction for all observations through pregnancy did not differ from the low-risk group (Table 3). However, the curve describing mean portal venous fraction had an inverted U-shape in PGDM fetuses, the opposite of that in the reference group, where the portal venous fraction increased after week 33 (Fig 2B).

The overall mean umbilical venous liver flow ($Q_{UV\ liver}$) was higher in PGDM pregnancies compared with the reference, mainly due to the high flows before 30 weeks of gestation (Fig 5A). However, when normalized for EFW, the overall mean umbilical venous liver flow was not different from low-risk pregnancies (Table 3), but the trajectory of the flow development tended to be different in PGDM pregnancies with borderline significantly lower flow after 30 weeks (Table 3, Fig 5B).

The *z*-scores for left portal vein blood velocity were positively related to first trimester HbA_{1C} and correspondingly, the *z*-scores for portal venous fraction were negatively related to

Table 3. Fetal venous liver blood flow in pregnancies complicated by pregestational diabetes mellitus compared with reference values from a low risk population.

Parameter	Popu-lation	n	Mean *z*-score (95% CI)	*p*	*GA <30 Mean z-score* (95% CI)	*GA<30p*	*GA≥30 Mean z-score* (95% CI)	*GA≥30p*
LPV velocity (cm/s)	Ref.	537	0.003	<0.001	0.011	<0.001	-0.005	<0.001
			(-0.09–0.09)		(-0.11–0.13)		(-0.12–0.12)	
	PGDM	201	0.639		0.675		0.575	
			(0.49–0.79)		(0.46–0.89)		(0.29–0.86)	
PV flow ($mL \cdot min^{-1}$)	Ref.	547	0.011	0.052	0.016	0.005	0.006	0.131
			(-0.09–0.11)		(-0.10–0.13)		(-0.12–0.13)	
	PGDM	93	0.272		0.796		-0.466	
			(0.03–0.52)		(0.26–1.32)		(-1.07–0.14)	
Normalized PV flow ($mL \cdot min^{-1} \cdot kg^{-1}$)	Ref.	547	0.007	0.002	0.022)	0.821	0.009	0.002
			(-0.10–0.11)		(-0.09–0.13		(-0.14–0.12)	
	PGDM	93	-0.418		0.089		-1.132	
			(-0.67 - -0.17)		(-0.49–0.67)		(-1.80 - -0.46)	
Total venous liver flow, Q_{liver} ($mL \cdot min^{-1}$)	Ref.	514	-0.005	<0.001	-0.008)	0.001	-0.001	0.881
			(-0.10–0.09)		(-0.13–0.11		(-0.13–0.13)	
	PGDM	75	0.507		0.847		-0.045	
			(0.26–0.75)		(0.39–1.30)		(-0.63–0.54)	
Normalizedvenous liver flow ($mL \cdot min^{-1} \cdot kg^{-1}$)	Ref.	473	0.010	0.479	0.007	0.342	0.033	0.047
			(-0.09–0.11)		(-0.13–0.11)		(-0.11–0.17)	
	PGDM	75	-0.085		0.195		-0.538	
			(-0.33–0.16)		(-0.21–0.60)		(-1.08–0.01)	
PV fraction of total venous liver flow (%)	Ref.	511	0.004	0.645	-0.002	0.909	0.012	0.550
			(-0.09–0.10)		(-0.12–0.11)		(-0.12–0.14)	
	PGDM	75	-0.098		0.028		0.217	
			(-0.35–0.16)		(-0.49–0.54)		(-0.46–0.89)	
UV liver flow, $Q_{UV\ liver}$ ($mL \cdot min^{-1}$)	Ref.	558	0.00	0.002	-0.02	<0.001	0.01)	0.952
			(-0.09–0.10)		(-0.13–0.09)		(-0.11–0.14	
	PGDM	122	0.364		0.65		0.00	
			(0.16–0.57)		(0.23–1.06)		(-0.37–0.38)	
Normalized UV liver flow ($mL \cdot min^{-1} \cdot kg^{-1}$)	Ref.	558	0.004	0.229	-0.05	0.630	0.03)	0.049
			(-0.09 –-0.10)		(-0.17–0.07)		(-0.09–0.15	
	PGDM	122	-0.131		0.03		-0.33	
			(-0.33–0.06)		(-0.35–0.41)		(-0.67–0.00)	

PGDM, pregestational diabetes mellitus; Ref., low-risk reference group [25, 26]; n, number of observations; CI, confidence interval for the mean *z*-score; *p*, probability value; GA, gestational age (weeks)—before and after 30 weeks; LPV, Left portal vein; PV, portal vein; Q_{liver}, total venous liver flow; PV fraction (%) = (PV flow/Total liver flow)·100; $Q_{UV\ liver}$, umbilical venous flow to the liver

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first trimester HbA_{1C} (Fig 6). There was no relation between HbA_{1C} and portal venous or total venous liver flow.

Since 39% of the neonates in our PGDM group were macrosomic, we compared the development of the total venous liver, umbilical and portal flows in low-risk, non-diabetic macrosomic and PGDM pregnancies, to illustrate the different flow patterns (Fig 7).

We compared the mean *z*-scores in the T1DM group with the reference values for LPV velocity, PV flow, normalized PV flow, total venous liver flow, normalized venous liver flow and UV liver flow (S1 Table). Excluding T2DM participants from the PGDM population did not significantly change the results, except for PV flow which then became borderline significantly higher compared with the reference values ($p = 0.046$).

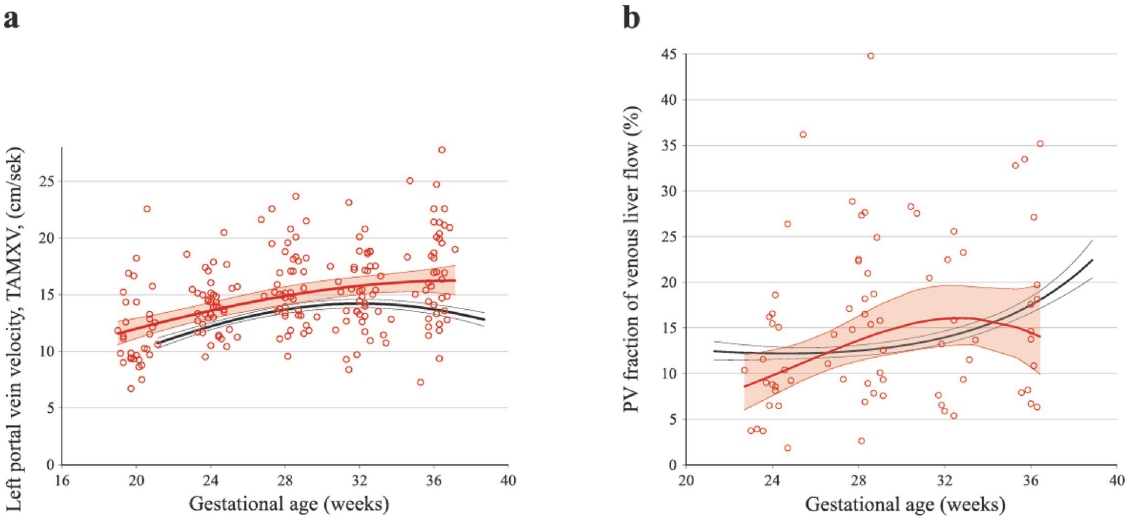

Fig 2. Longitudinal observations of left portal vein blood velocity and portal venous fraction in PGDM and low-risk pregnancies. Left portal vein blood velocity (TAMXV) as marker of the watershed between portal and umbilical contribution to fetal venous liver flow **(a),** and the portal fraction (%) of total venous volume **(b)** in 49 pregnancies with pregestational diabetes (PGDM; red circles and lines) compared with reference values from a low-risk population (black lines) presented with mean (thick lines) and 95% confidence interval (thin lines).

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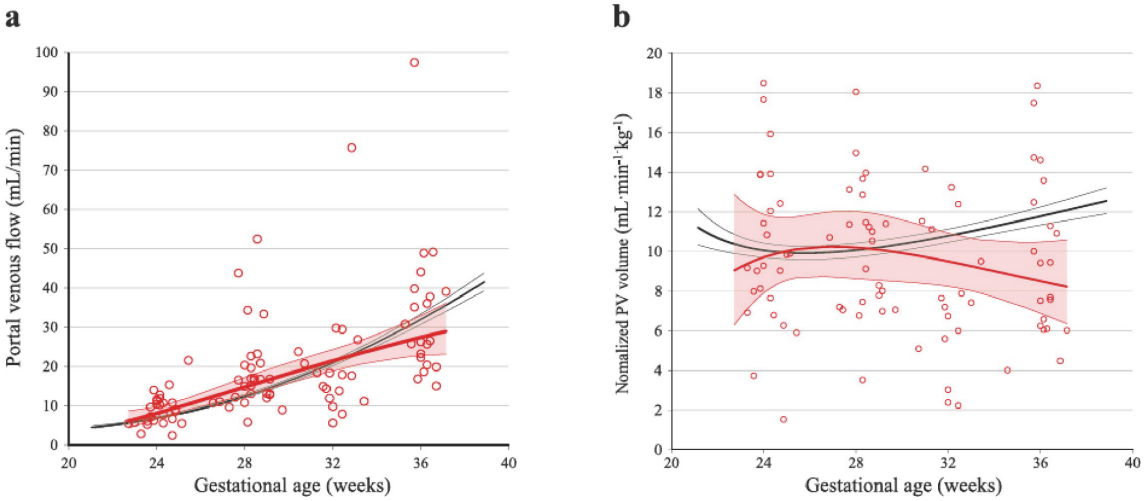

Fig 3. Longitudinal observations of portal venous flow in PGDM and low-risk pregnancies. Portal venous flow **(a)** and normalized portal venous flow **(b)** in 49 pregnancies with pregestational diabetes (PGDM; red circles and lines) compared with reference values from a low-risk population (black lines), with mean (thick lines) and 95% confidence-interval (thin lines).

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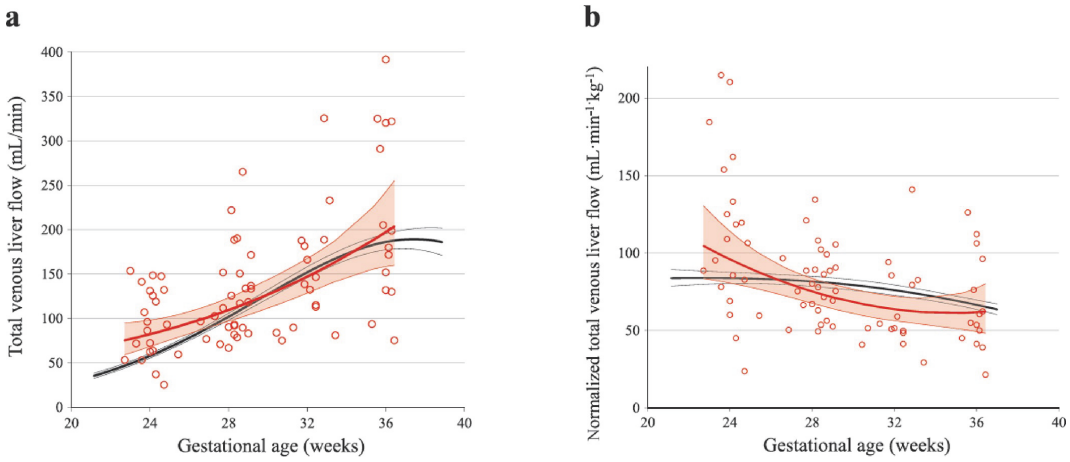

Fig 4. Longitudinal observations of total venous supply to the fetal liver in PGDM and low-risk pregnancies. Total venous liver flow **(a)** and the correspondingly normalized flow values **(b)** in 49 pregnancies with pregestational diabetes mellitus (PGDM; red circles and lines) compared with reference values from a low-risk population (black lines) presented with mean (thick lines) and 95% confidence interval (thin lines).

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Discussion

In pregnancies complicated with PGDM, the fetal liver perfusion with nutritious umbilical blood from the placenta was prioritized (Table 3, Figs 1 and 2A). This effect was graded according to the maternal HbA1c level (Fig 6) and was associated with correspondingly accelerated fetal growth during the 2nd trimester. However, the blunted umbilical flow development

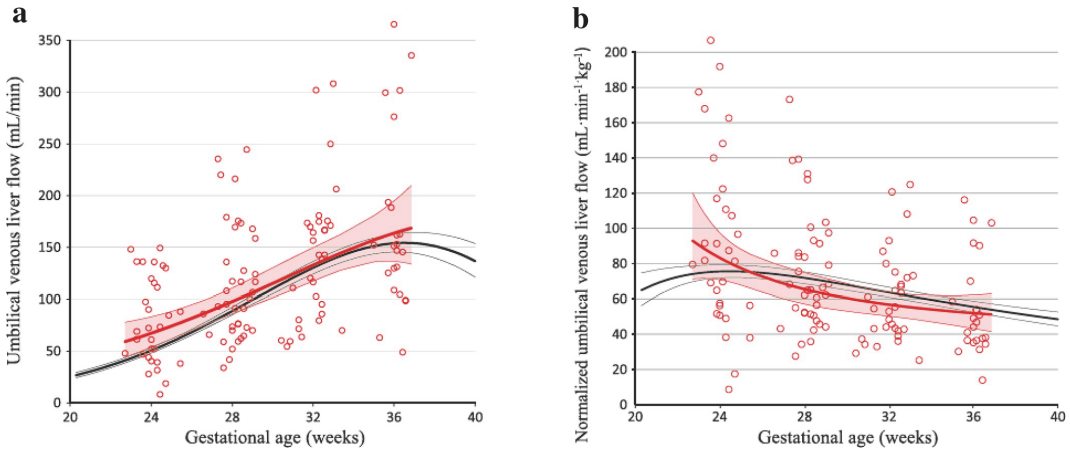

Fig 5. Longitudinal observations of the umbilical venous supply to the fetal liver in PGDM and low-risk pregnancies. Umbilical venous liver flow **(a)** and the correspondingly normalized flow values **(b)** in 49 pregnancies with pregestational diabetes mellitus (PGDM; red circles and lines) compared with reference values from a low-risk population (black lines) presented with mean (thick lines) and 95% confidence interval (thin lines).

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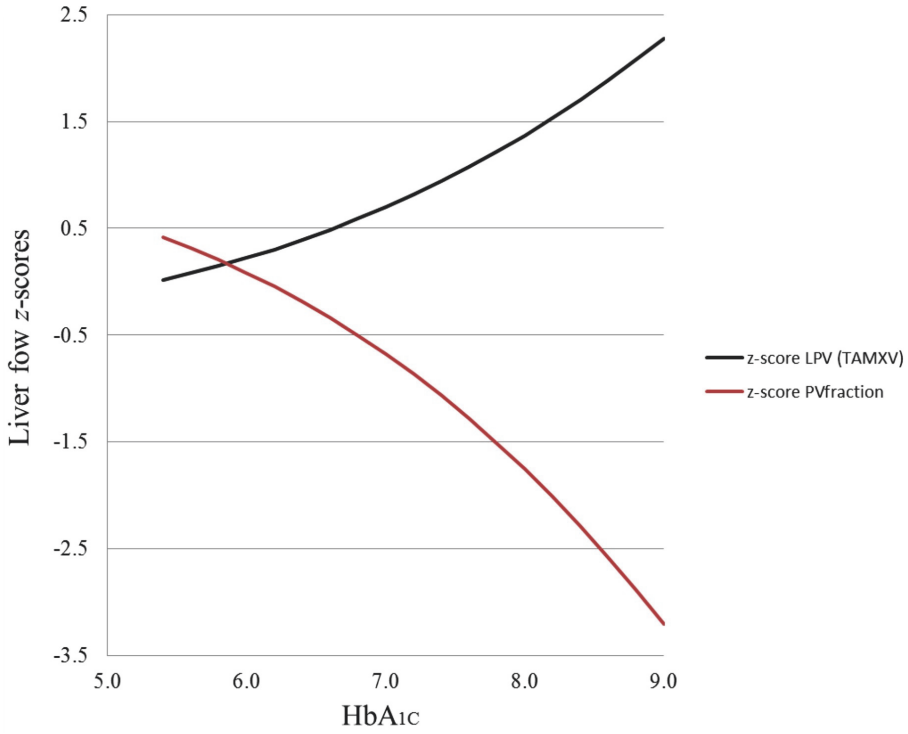

Fig 6. Fetal liver blood flow and relation to HbA_{1C}. Relations between z-scores of the time-averaged maximum left portal vein (LPV) flow velocity (TAMXV) and portal vein (PV) fraction, and first-trimester HbA_{1c}.

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during the 3rd trimester seemed to cause an increasing mismatch between growth and blood supply (Table 3, Figs 2–5).

The venous supply to the liver is radically different in fetal and postnatal life, with contributions from both the umbilical (≥80%) and the portal vein (≤20%) (Figs 1 and 7) [26]. Thus, the umbilical vein is the principal source of fetal liver blood supply [31], and this umbilical venous flow to the liver is augmented in diabetic pregnancies (Table 3, Fig 5A). Such increased delivery of oxygen and nutrient rich umbilical venous blood to the liver, is thought to be instrumental in the development of macrosomia [8].

The left portal vein connects the umbilical vein with the portal circulation and directs umbilical venous blood to the right lobe of the liver (Fig 1). Blood flow in the left portal vein is regulated by catecholamines [32] and maternal glucose levels [10]. Measurement of the left portal vein velocity alone provides a simple method for gauging the umbilical/portal watershed and for assessment of intrahepatic venous redistribution in compromised fetuses [25]. In the present study, the mean left portal vein velocity was higher in PGDM pregnancies than the reference values, throughout the second half of pregnancy (Fig 2A). This signifies increased prioritization of umbilical blood flow to the right liver lobe and is known to induce liver growth, increased production of IGF-1 and -2 and in turn, differential organ growth [8, 9].

The portal contribution to the venous liver perfusion was higher in PGDM than the reference group before 30 weeks (Table 3), but the portal venous flow did not keep up with fetal

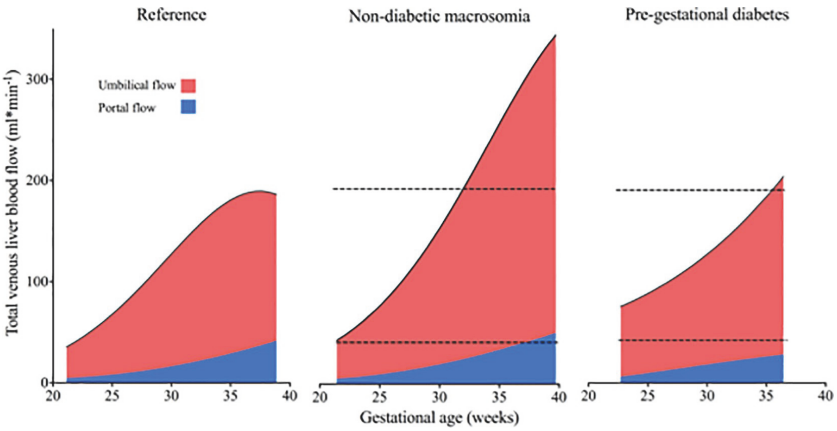

Fig 7. Fetal venous liver flow development in pregnancies with low risk, macrosomia and PGDM. The fetal venous liver flow in three different populations: a low-risk population (physiological venous liver flow during the last weeks of pregnancy; dotted lines), fetal macrosomic growth *without* maternal diabetes, and pregnancies with pregestational diabetes mellitus (PGDM, the present study population).

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growth later in pregnancy (Fig 3B). This corroborates a study of Olofsson *et al*., showing that blood flow to the lower extremities was prioritized at the expense of visceral blood flow during the third trimester, in pregnancies with type 1 DM [33]. In addition, lower portal venous return could be a result of reduced fetal swallowing or intestinal activity in diabetic pregnancies [34]. We hypothesize that the increased umbilical venous flow to the right liver lobe observed in our study, may induce accelerated fetal growth that is less supported by umbilical venous supply at the end of pregnancy, and without any increase of portal blood flow to the liver. Higher umbilical venous flow to the right liver lobe, as found in our study, could also influence liver gene expression [15], fetal body composition [11] and possibly later health [20].

In the present study, 39% of the newborns had a birthweight $> 90^{th}$ percentile for gestational age (Table 2). Fetal macrosomia in PGDM is different from that in non-diabetic pregnancies, with disproportionate fetal growth expressed as a higher ponderal index [35]. In a study of macrosomic fetuses *without* maternal diabetes, the umbilical venous perfusion [12], left portal venous flow, portal and total venous liver flow, were all increased during the second half of pregnancy [7], even when corrected for fetal weight. Similarly, in the present study, high umbilical venous flow [14], correspondingly low placental impedance [36] and increased portal blood flow permit an up-regulation of liver flow before 30 gestational weeks (Table 3, Figs 2–5). In contrast, after 30 weeks gestation, fetuses of diabetic mothers had reduced portal and total venous liver flow when normalized for fetal weight, while in non-diabetic macrosomic fetuses no restriction in venous blood flow to the liver was observed (Table 3, Fig 7).

It is known that during placental compromise associated with fetal growth restriction, shunting through the ductus venosus is prioritized at the expense of the umbilical venous liver flow [37, 38]. This leads to reduced liver size that increasingly depends on the low-oxygenated portal flow. In PGDM pregnancies however, the increased risk of chronic hypoxemia, acidosis, and perinatal death in the last weeks of gestation [39–41] follows relatively greater umbilical supply during the 2^{nd} trimester (Table 3). The liver received umbilical blood at the expense of flow through the ductus venosus [14]. Towards the end of pregnancy, PGDM fetuses outgrew their supply of umbilical venous blood and did not maintain portal flow corresponding to

their weight (Figs 2 and 3). Although being at risk of relative hypoxia, the re-distribution mechanisms well-known in fetal growth restriction did not seem to operate.

The strengths of this study are its prospective longitudinal design, involving an unselected group of PGDM pregnancies, and identical and validated ultrasound and Doppler methods applied to the reference population [42]. Low intra- and inter-observer variation has been demonstrated for measurements of ductus venosus flow velocities [43], and almost identical results for umbilical venous flow were achieved by different investigators using the same technique for ultrasound measurement and blood flow calculation [26, 44, 45]. The success rate for measurements varied from 93.4% for left portal vein blood velocity to 52.5% for portal venous flow, the latter being lower than in the reference population [26]. This was mainly due to difficult examination conditions caused by high BMI in our study group. Although there was no inter-group difference in liver flow between PGDM participants with BMI$<$ or $\geq$30, a selection bias cannot entirely be ruled out. Because the BMI was borderline significantly higher in the missing compared with the complete data group (tested by independent sample *t*-test, mean BMI *z*-score in the $Q_{UV\ liver}$ missing data vs. non-missing data groups were 1.47 and 1.13 respectively, $p = 0.07$) this could introduce selection of a leaner PGDM population for the estimation of umbilical venous liver flow. However, such a selection is expected to reduce rather than augment the differences between the study- and the reference populations. Also, wider confidence intervals in the study group compared with the reference group warrant a cautious interpretation of the findings.

Including women with type 1 and type 2 DM in one study group may represent a limitation, since these conditions differ in many respects. Our goal was however, to study fetal flow and growth in pregnancies with PGDM. Our population was not large enough to answer the question of whether fetal venous liver circulation is different in pregnancies with type 1 or type 2 DM. Nevertheless, when women with type 2 DM were excluded the findings remained significant in the type 1 DM group (S1 Table).

Conclusion

Maternal diabetes is associated with adverse consequences in the offspring [46], including macrosomia and metabolic syndrome [47], but the underlying mechanisms are not established. Fetal liver blood flow is linked to fetal growth, and we showed that flow is related to maternal blood glucose in the first trimester in PGDM pregnancies. However, the relatively greater liver perfusion in PGDM pregnancies before 30 weeks was not maintained in late gestation, possibly leading to mismatch between fetal growth and nutrient supply, and later effects on health.

Supporting information

S1 Table. Fetal venous liver blood flow in pregnancies complicated by type 1 diabetes mellitus compared with a low risk reference population. Ref., low-risk reference group; n, number of observations; CI, confidence interval for the mean *z*-score; *p*, probability value; LPV, Left portal vein; PV, portal vein; Q_{liver}, total venous liver flow; UV liver flow, umbilical venous flow to the liver.
(DOCX)

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Investigation: Agnethe Lund, Cathrine Ebbing, Jörg Kessler.

Methodology: Agnethe Lund, Cathrine Ebbing, Torvid Kiserud, Jörg Kessler.

Project administration: Agnethe Lund, Jörg Kessler.

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Visualization: Agnethe Lund.

Writing – original draft: Agnethe Lund.

Writing – review & editing: Agnethe Lund, Cathrine Ebbing, Svein Rasmussen, Torvid Kiserud, Mark Hanson, Jörg Kessler.

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Supporting information

S1 Table

Fetal venous liver blood flow in pregnancies complicated by type 1 diabetes mellitus compared with a low risk reference population

Parameter	**Population**	**n**	**Mean *z*-score (CI of mean)**	***p***
LPV velocity (cm/s)	Ref.	553	0.004 (-0.86 – 0.09)	*<0.001*
	Type 1DM	179	0.691 (0.29 – 0.87)	
PV flow ($mL \cdot min^{-1}$)	Ref.	558	0.017 (-0.87 – 0.09)	0.046
	Type 1DM	86	0.297 (-0.05 – 0.99)	
Normalized PV flow ($mL \cdot min^{-1} \cdot kg^{-1}$)	Ref.	558	0.011 (-0.09 – 0.08)	*0.002*
	Type 1DM	86	-0.450 (-0.88 – 0.26)	
Total venous liver flow, Q_{liver} ($mL \cdot min^{-1}$)	Ref.	525	-0.002 (-0.10 – 0.08)	*<0.001*
	Type 1DM	69	0.570 (0.14 – 0.92)	
Normalized venous liver flow ($mL \cdot min^{-1} \cdot kg^{-1}$)	Ref.	528	-0.012 (-0.09 – 0.09)	0.598
	Type 1DM	69	-0.087 (-0.48 – 0.21)	
UV liver flow, $Q_{UV\ liver}$ ($mL \cdot min^{-1}$)	Ref.	555	-0.006 (-0.12 – 0.06)	*<0.001*
	Type 1DM	111	0.435 (-0.09 – 0.76)	

Ref., low-risk reference group ; n, number of observations; CI, confidence interval for the mean *z*-score; *p*, probability value; LPV, Left portal vein; PV, portal vein; Q_{liver}, total venous liver flow; UV liver flow, umbilical venous flow to the liver

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at the University of Bergen

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(date and sign. of candidate) (date and sign. of faculty)

Errata

p. 18, Figure 4 changed to Figure 3

p. 21, Figure 5 changed to Figure 4

p. 32, Figure 3 changed to Figure 5

The text referring to the figures has been revised accordingly.

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