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How gestational diabetes mellitus

affects the microcirculation and vasomotion of newborns and their mothers

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TABLE OF CONTENTS

1. INTRODUCTION

1.1. Definition of gestational diabetes

The World Health Organization (WHO) defines gestational diabetes as a

"hyperglycemia of varying severity diagnosed during pregnancy (without previously known diabetes) and usually (but not always) resolving within 6 weeks of delivery." (Organisation, 2013)

The German Diabetes Guidelines describe it as

"an impaired glucose tolerance diagnosed for the first time during pregnancy with a 75g oral glucose tolerance test under standardized conditions and a quality assured glucose measurement of venous plasma". (2011)

There exists no threshold value, but a flowing transition between a normal glucose tolerance during pregnancy and gestational diabetes (Bantle et al., 2008).

1.2. Epidemiology of gestational diabetes in Germany

For the year 2014, the Aqua Institute reported 30,889 cases of women with gestational diabetes in Germany, which represents 4,47% of 690,618 pregnant women giving birth in Germany and covers 99.8% of all childbirths in 745 hospitals in 2014 in Germany (GmbH, 2015).

1.3. Diagnosis

The American Diabetes Association (2011) stated for the diagnosis of gestational diabetes:

- Women with risk factors should be screened at the first visit. Elevated fasting and postprandial sugar can also show a preexisting diabetes mellitus, which should be taken into consideration, especially concerning the follow-up examinations

- A screening for GDM in women not known to have diabetes should be taken at 24-28 weeks using an OGTT with a 75g glucose drink and measuring after 1 and 2 hours the fasting plasma glucose and plasma glucose (see appendix)

- The test is taken in the morning after an overnight fast of at least 8 hours
- GDM is diagnosed if any of the following values is exceeded:
  Fasting ≥92 mg/dl (5.1 mmol/l)
  1 h ≥180 mg/dl (10.0 mmol/l)
  2 h ≥153 mg/dl (8.5 mmol/l)

- Women with GDM should be screened 6-12 weeks after delivery to eliminate a persistent diabetes

- For women who have had GDM, a screening for diabetes should be done at least every 3 years

1.4. Risk factors

Different risk factors for women to develop gestational diabetes have been evaluated over the last decades (Major CA. et al., 1998, Getahun et al., 2010, Hedderson et al., 2010). The significance of risk factors, however, seems to vary between studies (Kim et al., 2007). According to the AWMF guidelines (German Diabetes Guidelines, 2011), a risk for developing GDM was stated for the following factors:

1. Diabetes in first degree relatives
2. Obesity with a BMI ≥ 25 (depending on the study)
3. Diseases associated with Insulin resistance
4. Medication use with negative influence on glucose metabolism (beta blocker, glucocorticoids, antidepressants)
5. Proven temporary glucose intolerance in medical history
6. Age of mother varying between >25 years to >30 years
7. Ethnic group: women from the Middle East, South- and East-Asia, Africa, Central America
8. GDM in medical history (risk increasing with number of affected pregnancies) (Getahun et al., 2010))
9. More than 3 abortions in medical history based on the theory that high glucose during the conception period leads to a higher abortion rate in pre-gestational diabetic patients (Rosenn et al., 1994, Combs and Kitzmiller, 1991)
10. Previous birth of a child with > 4500g or malformations (high glucose during the conception period leads to malformations in the child (Combs and Kitzmiller, 1991, Rosenn et al., 1994)

1.5. Pathophysiology of gestational diabetes

GDM is based on a pancreatic ß-cell dysfunction similar to diabetes type 2. The underlying mechanisms are, however, not yet completely identified. There are currently three different explanations that include (Metzger et al., 2007):

A. autoimmune ß-cell dysfunction

B. impaired insulin secretion caused by genetic conditions

C. chronic insulin resistance

In the third trimester of pregnancy, the normal insulin sensitivity in the mother is physiologically reduced in order to ensure the glucose supply via the umbilical vein to the fetus (Diedrich et al., 2007). In GDM, a preexisting reduced insulin sensitivity cannot be balanced anymore by the insulin production, which, in turn, leads to a "relative insulin deficiency" (Kautzky-Willer et al., 1997). Regarding the underlying mechanism, pregnant women seem to have a dysfunction in the insulin signaling cascade in the advanced pregnancy. To be specific, they show a reduced insulin receptor substrate 1 expression. In contrast to women without a diabetic metabolism during pregnancy, women with GDM show in addition a defect in the insulin receptor ß-unit with a reduced tyrosine phosphorylation (Catalano et al., 2003, Friedman et al., 1999). Tyrosine phosphorylation also increases the up take of glucose to the cell. Thus, as a result of the additional deficiency, women with GDM have a 25%-decreased glucose transport activity in muscle compared to pregnant women without diabetes (Catalano et al., 2003).

Furthermore, changes in the placental adipokine secretion have been discovered. In particular, these are increases in Leptin and TNFα and decreases in adiponectin (Worda et al., 2004, Kirwan et al., 2002). Adiponectin plasma levels are also negatively associated with obesity and insulin resistance (Worda et al., 2004).

1.6. Genetics of GDM

GDM seems to be positively correlated with glucose tolerance disturbance or diabetes in the parental medical history (Jang et al., 1998, McLellan et al., 1995), demonstrating also an increased prevalence of diabetes mellitus in mothers of women with GDM (Martin et al., 1985). No clear monogenetic mutations have been identified in GDM yet. However, in 2 % of the cases, a MODY 2 mutation can be the underlying cause for a glucose tolerance disturbance hidden behind the false diagnosis of GDM (Saker PJ. et al., 1996, Andrew T. Hattersley et al., 2000). The discussion about how gestational diabetes can be correlated with other forms of diabetes led to gene examinations, which show a significant association between some gene variations in diabetes type 2 and GDM. Further research in gene sequences is needed to observe the diversities and commonalities of GDM and other forms of diabetes (Kwak et al., 2012, Watanabe et al., 2007).

1.7. Consequences of gestational diabetes for the child

1.7.1. Direct consequences

The complete picture of a badly managed GDM can be summarized as "fetopathia diabetica". In such a case, hyperglycemia of the fetus activates the adipogenesis and thereby results in a macrosomia (weight at birth > 4000g). Macrosomia itself comes with a higher risk for a shoulder dystocia (Ju et al., 2009, Tsur et al., 2012). Premature birth is also known as a risk for children of mothers with gestational diabetes (Cordero et al., 1998). Additionally, in the postnatal period, hypoglycemia and hyperbilirubinemia are commonly observed, whereas polycythemia and hypocalcemia are possible problems but not as frequently occurring (Cordero et al., 1998).

According to the AWMF guidelines (2011), fetal hyperglycemia is associated with an increased erythropoietin level (Salvesen et al., 1993, Madazli et al., 2008). It is also associated with an elevated number of nucleated fetal red blood cells (Daskalakis et al., 2008) leading to polycythemia.

Further observed malfunctions are cardiac defects caused by a storage of glycogen in the heart muscle cells (Oberhoffer 1997 EK IIb; Kozák-Bárány 2004 EK-IIb)(IH., 1996)(DGG). In some cases a correlation of a diabetic metabolism of the mother during pregnancy and the caudal regression syndrome in their children has been identified (Al Kaissi et al., 2008, Perrot et al., 1987).

The respiratory distress syndrome is a common problem in the neonatal period of children of mothers with gestational diabetes (Cordero et al., 1998). Experiments in fetal rat lungs have

shown a minimized surfactant synthesis under high glucose levels (Sugahara et al., 1994, IH., 1996)

1.7.2. Long-term consequences

Compared with children not exposed to a diabetic metabolism during pregnancy, the children of mothers with GDM tend to suffer more frequently from an impaired glucose tolerance and obesity in their childhood and adolescence (Vääräsmäki M et al., 2009). They are also more likely to develop a metabolic syndrome with hypertension and dyslipidemia in the first twenty years of their lives (Schaefer-Graf et al., 2005) (Silverman et al., 1995, Silverman et al., 1991, Silverman et al., 1998, Cho N.H. et al., 2000, Boney et al., 2005, Wright et al., 2009) or a diabetes type 2 (Clausen et al., 2008, Pirkola J. et al., 2010). Wright et al. (2009) found that the obesity and the systolic blood pressure are moderately higher at the three years of age, assuming an aggravation over time. In contrast to these studies, Pettitt et al. (2010) discovered that the blood glucose level at 28 gestational weeks does not have a significant influence on the obesity of two-year old children (Pettitt DJ. et al., 2010).

1.8. Consequences for the mother

1.8.1. Direct consequences

Regarding the complication for women with gestational diabetes compared to women without a diabetic metabolism, preeclampsia (Fadl et al., 2010, HAPO Study Cooperative Research Group et al., 2008) and hypertension (Shand et al., 2008) can occur with a higher risk during pregnancy. Moreover, women with gestational diabetes have a higher likelihood to give birth prematurely (Shand et al., 2008). During the birthing process, women with gestational diabetes are more likely to have (1) a caesarean section (Fadl et al., 2010, HAPO Study Cooperative Research Group et al., 2008, Shand et al., 2008), or a natural birthing with a higher risk for (2) shoulder dystocia (Fadl et al., 2010, HAPO Study Cooperative Research Group et al., 2008) and (3) 3rd to 4th perineal tear (Shand et al., 2008).

After delivery, women with GDM tend to suffer more often from severe postpartum hemorrhage requiring blood transfusion than women without GDM (Shand et al., 2008).

1.8.2. Long-term consequences

The risk of developing a glucose tolerance disturbance for women with GDM after pregnancy changes depending on the study. According to the AWMF guidelines (2011), after 10 years, 35-60% of the women will be glucose intolerant. Distinguishing between a manifest diabetes

and a disturbed glucose tolerance, in a Danish follow-up study 10 years post-partum 40% of the 481 examined women had diabetes and 27% had an impaired glucose tolerance. O'Sullivan et al. (1989) observed that 73% of the women with GDM suffered 25 years post-partum from diabetes type 2. 60% of obese women and 40% of women with a normal weight developed a glucose tolerance disturbance, which suggests that the risk is positively associated with the bodyweight. Overall, a meta-analysis of 20 studies identified that women with GDM bear a seven times higher risk (RR: 7,43; 95% CI: 4,79-11,51) for developing diabetes type 2 (Bellamy et al., 2009).
The risk for developing a diabetes type 1, five to ten years post-partum, varies between 2.3-10.0% (German Diabetes Guidelines, 2011).

Common risk factors for a subsequent GDM are as following (German Diabetes Guidelines, 2011):

- BMI >30 kg/m²
- Number of pregnancies
- GDM diagnosis in the previous pregnancy before 24 gestational weeks
- Insulin therapy
- Interval <24 months during pregnancies
- Weight gain >3kg during pregnancies
- Elevated fasting glucose 2 months post-partum

1.9. Treatment

Treatment of GDM improves the outcome of the newborn infant (Crowther et al., 2005). The German and American Diabetes Association stated different options for the therapy depending on the severity of the disease and on the glucose levels.

1.9.1. Self-monitoring of blood glucose

Capillary blood glucose should be measured with a finger stick every day and listed in a protocol. According to the Fourth and Fifth International Workshop Conference on GDM, capillary concentrations should be maintained at 65-95 mg/dl mg/dl (3.6-5.3 mmol/l) in the fasting state, <140 mg/dl (<7.8 mmol/l) at 1 h, and <120 mg/dl (<6.7 mmol/l) 2 h after beginning of the meal (calibrated for blood plasma) (Metzger et al., 2007).

At the beginning, the patient should be measured four times a day, in the fasting state in the morning, after breakfast, after lunch and after dinner. This protocol should be followed for one

to two weeks. If two measured values are increased in two following days, the protocol must be extended to measurements before lunch and before dinner so that the mean blood glucose can be calculated based on the six measured values. However, self-measured values have to be controlled in regular intervals and need to be compared to laboratory results. If mean blood glucose is increased over 100 mg/dl, an insulin therapy should be considered (German Diabetes Guidelines, 2011).

1.9.2. Dietetic treatment

The treatment should be personally discussed with the patient and the dietetic plan should be individually designed for her. Ensuring a normal growth of the fetus is one of the most important aims in dietetic treatment of GDM. Therefore, hypoglycemic phases and ketoacidosis have to be prevented, as standard blood glucose levels (see appendix) need to be maintained (Bantle et al., 2008).

The weight gain should be as recommended in normal pregnancy. Depending on the respective BMI of the woman, the calorie requirements can be calculated. For underweight (BMI < 18,5 kg/m²) an up-take of 35-40kcal/kg body weight is recommended, for normal weight (BMI 18,5- 24,9 kg/m²) 30-34 kcal/kg body weight, for over weight (BMI 25 - 29,9 kg/m²) 25-29 kcal/kg body weight and for obesity (>30 kg/m²) ≤ 20 kcal/kg body weight.
However, a calorie up-take of 1600-1800 kcal/day should be covered (Reader, 2007, Knopp RH. et al., 1991).
Regarding the percentages of nutrients, the recommended up-take includes 40-50% of carbohydrates, 20% of proteins and 30-35% of fat. The consumption of carbohydrates should not fall under a limit of 175g/day. Considering a higher Insulin resistance during the morning, the glucose up-take should be less at breakfast compared to the other meals. Aiming to maintain normal levels of post-prandial glucose, meals should be divided into three small- to medium sized portions and two to four snacks during the day (Bantle et al., 2008).

Furthermore, it has been observed that a diet with low fiber and a high glycemic load comes with a 2.15-fold (95% CI 1.04–4.29) higher risk of GDM (Zhang et al., 2006). Thus, a consumption of at least 30g/d of fiber is recommended (German Diabetes Guidelines, 2011).

Regarding the post-partum period, lactation is also suggested for women with GDM, but needs to be considered in the diet plan or insulin treatment, as more carbohydrates are required (Bantle et al., 2008).

Physical activity for 30 min/day is recommended as along as movements are being avoided that could injure the child (Metzger et al., 2007).

1.9.3. Insulin Treatment

Several criteria, if fulfilled, suggest an insulin treatment.
First, capillary blood glucose measurements under a dietetic treatment need to be observed and combined with results of fetal ultrasound over a period of two weeks.
According to the German Diabetes Guidelines (2011), capillary glucose concentrations should be maintained at 65-95 mg/dl (3.6-5.3 mmol/l) in the fasting state, <140 mg/dl (<7.8 mmol/l) at one hour after the beginning of the meal, and <120 mg/dl (<6.7 mmol/l) two hours after the beginning of the meal (calibrated for blood plasma) (Metzger et al., 2007).

Time of measurement	Glucose concentration	
	in mg/dl	In mmol/l
Fasting state	65 - 95	3.6 - 5.3
1 hour p.c.	< 140	< 7.8
Mean glucose concentration 1 hour p.c.	90 - 110	5.0 - 6.1
Mean glucose concentration 2 hours p.c.	80 - 100	4.4 - 5.6

Table 1 – Blood glucose measurement under diabetic treatment

Calculating the mean blood glucose level out of three pre-prandial measurements, and either three measurements one hour or two hours after the meal can help reducing aberrations in the trend estimation of the blood glucose level. In case the measured glucose concentrations are exceeding their mean-range on two consecutive days, an insulin therapy should be started.

Furthermore, if either in one week more than 50% of the blood glucose measurements exceed the threshold limits, the glucose level is higher than 110 mg/dl (6.1 mmol/l) in the fasting state, or above 162 mg/dl (9.0 mmol/l) at any time point, insulin treatment should be started without the fetal ultrasound.

To adjust the insulin therapy if needed, the target blood glucose level should be set according to the fetal growth, which should be measured via ultrasound every two to four weeks.
In the second and third trimenon the abdominal circumference (ac) can provide predictive information on whether a child is likely to be “large for gestational age” (LGA) or whether hyperinsulinism of the child is likely to occur (German Diabetes Guidelines, 2011).

However, an overtreatment with insulin comes with the risk of growth retardation for the child.

If the mean blood glucose level is < 90 mg/dl (calculated with the one hour postprandial value), insulin doses are too high and should be adjusted.

For therapy of women with GDM, Neutral Protamine Hagedorn insulin (NPH-insulin) as a basal insulin is recommended in the morning and evening. It can be complemented with short-acting human insulin before meals. If an alternative for short-acting human insulin is required, Insulin-Lispro and Insulin-Aspart can be used, as they do not cause teratogenic side effects during pregnancy.

Oral antihyperglycemic agents are not recommended for therapy of women with GDM as no sufficient data are available that confirm a utilization without damaging side effects for the fetus (German Diabetes Guidelines, 2011).

1.10. Microcirculation

1.11. Definition

The microcirculation is a complex system consisting of a structure of smaller vessels (diameter <100µm), which are the arterioles (diameter 20µm), venules (diameter 15-30 µm) and capillaries (diameter 6-12µm) (Welsch, 2010). The microcirculation provides the tissue with oxygen, nutrients, immunological factors and delivers medication to the cells (Ince, 2005).

The arterioles branching from the arteries are responsible for the regulation of the blood flow to the capillaries. The venules accumulate the blood from the capillary bed and transport it to the veins where it is gathered and transferred back to the heart (Welsch, 2010).

The capillaries play an essential role for the microcirculation, as they build a network in the tissue in which the oxygen interchange occurs (Ince, 2005, Welsch, 2010). The capillaries' vessel wall consists of flat endothelial cells, basal lamina and pericytes.
With over >3500cm² (Welsch, 2010) the capillary network represents the largest endothelial surface of the human body and is therefore well suited for oxygen delivery (Verdant and D., 2005). The reduced flow velocity in the capillaries and the high permeability of their vessel walls are essential for the exchange of substances. The percentage of perfused capillaries can be increased depending on the requirements of the organ (Ince, 2005, Speckmann et al., 2008).

The cell types participating in the microcirculation are endothelial cells, muscle cells, erythrocytes, leukocytes and plasma components. Whereas capillaries consist of endothelial cells, arterioles are also equipped with muscle cells and hence can influence the capillary blood flow via constriction. Driving pressure, hemorheology and capillary patency additionally influence the capillary blood flow (Ince, 2005).

The microcirculation uses different regulation systems to control the tissue perfusion. These are (1) **myogenic** reaction to strain and stress, (2) **metabolic** response to O2, CO2, Lactate and H+ and (3) **neurohumoral activation** based on the autonomic nervous system (Ince, 2005).

Studies by Vallet (2002) and Segal (2005) have shown that endothelial and muscle cells interact within a complex communicating network. Capillaries give signals to up-stream arterioles, inter alia, to adapt the blood flow to required levels. If more oxygen extraction is needed, the proximal arterioles will dilate in order to open new capillaries in the tissue (Segal, 2005, Vallet, 2002).

Due to the velocity difference between erythrocytes and plasma, cross sections in vessels of the microcirculation show a decreased hematocrit. Pries et al. (1986) described this phenomenon as the “Fahreus effect” in blood vessels. This holds particularly true in the distal branch points of the capillary network (Pries et al., 1986). Hence, the oxygen delivery changes with the respective section of the microcirculation (Verdant and D., 2005, Ince, 2005).

1.12. Microcirculation in newborn infants

The skin of newborn infants already possesses a protecting sensing function and is able to regulate the temperature, hydration and hormonal production of the body (Fluhr et al., 2010).

At birth, the skin of a newborn infant is supplied with many blood vessels and shows a disorderly pattern of the microcirculation with no capillary loops, except for the palms, soles and nail folds. After the first week, papillary loops can be observed as small superficial dilations or buds. A fully organized pattern with a vertical plexus, as it can be seen in adults, is not developed until the 14th to 17th week (Perera et al., 1970).

1.13. Microcirculatory dysfunction

Microcirculatory dysfunction is defined as an imbalance between non-perfused capillaries and capillaries, which show a normal to high blood flow (Bateman et al., 2003, De Backer et al., 2002). This can be explained by "functional shunting", a process during sepsis which limits the blood flow in particular parts (den Uil et al., 2008, Buwalda and C., 2002, Ince and Sinaasappel, 1999).

Ince (2005) stated that the evaluation of the microcirculation could reveal pathologic variations in sepsis prior to macrocirculatory parameters. Since then, the interest in the clinical assessment of the microcirculation has increased significantly.

Macrocirculation is, inter alia, defined by temperature, heart rate and blood pressure. Spronk et al. (2004) stated that the dysfunction in the microcirculation represents a central point in sepsis by reducing the blood flow to the tissue and thus limiting the oxygen delivery to the cells, which can result in organ failure (Ince, 2005). Furthermore, Sakr et al. (2004) showed that microcirculatory vis-a-vis macrocirculatory parameters can also provide valuable information for a prognosis on the outcome of the patient after 24 hours of shock, underlining the importance of microcirculatory evaluation (Sakr et al., 2004).

An impairment of the microcirculation, which is commonly defined as an imbalance between vasodilatation and vasoconstriction, originates from a dysfunction of the endothelial cells (Vallet, 2002). The endothelium, which lines the inner side of the vessels is responsible for the vessel tone, the regulation between coagulation and fibrinolysis, as well as the inflammatory response in the vessel wall and adhesion of leucocytes. In order to perform these tasks, the endothelium produces several factors such as NO (nitric oxide), vWF (von Willebrand factor), t-PA (tissue-type plasminogen activator), PAI-1 (plasminogen activator inhibtor-1), ET-1 (endothelin-1), Ang 2 (angiotensin 2), prostanoids, adhesion molecules and cytokines.
NO plays an important role in the function of the endothelium as it is a vasodilator and an anti-platelet, anti-proliferative, permeability-decreasing, anti-inflammatory factor. To see if the microcirculation is impaired, the endothelial function can be measured by its vasodilating properties. However, it is not clear how other factors may influence the vessel function and, thus, the results of the test could not only be interpreted as a consequence of an impairment of the endothelium (Schalkwijk and Stehouwer, 2005).
In order to improve the microcirculatory situation, vasodilating therapies based on this knowledge have been further examined (Buwalda and C., 2002). As shown in the following studies diabetes can also lead to an impairment in the microcirculation.

1.14. The microcirculatory dysfunction in diabetes

Studies in the past have revealed a strong association between microcirculatory dysfunction and a diabetic metabolism. For example, Padilla et al. (2006) discovered that for laboratory rats with diabetes type 2 the delivery of oxygen to the muscles is diminished as the percentage of red-blood-cell-perfused capillaries is significantly reduced.
Looking at large clinical trials (UKPDS, 1993) with patients suffering from diabetes type 1 or type 2, hyperglycemia was shown to be strongly related to the risk of microangiopathy, a microvascular dysfunction and a well-known pathology in diabetic patients.
Heimhalt-El Hamriti et al. (2013) observed the skin microcirculation of children with type 1 diabetes[1] and measured an endothelial dysfunction in one out of four patients. Furthermore, patients with late onset of type 1 diabetes show a reduced skin microvascular reactivity (Neubauer-Geryk et al., 2013). Microcirculatory dysfunction was also observed in the coronary vessels of diabetic patients (Nitenberg et al., 2000, L'Abbate, 2005).
The Microcirculatory dysfunction in diabetes can on the one hand be explained based on the complex relationship with the hormone insulin, which was examined by different research groups (Eringa et al., 2002, Eringa et al., 2004, Potenza et al., 2005, Kim et al., 2006). However, the different factors and pathways associated with insulin are various and not yet all discovered. The following part deals with the most important findings explaining the endothelial dysfunction in diabetic patients in association to insulin.

First, insulin can influence the microcirculation via two main different pathways. First, insulin can induce the activity of Phosphatidylinositide (PI) 3-kinase which leads to vasodilatation via a rise in NO production (Eringa et al., 2002). This results in a recruitment of new capillaries and an increased blood flow as well as glucose-uptake in the tissue (Vincent et al., 2003, Vincent et al., 2004).

Second, insulin stimulates the Mitogen-activated protein (MAP) kinase pathway. The MAP kinase pathway is also responsible for protein synthesis, enzyme activation and inactivation as well as gene expression. A vasoconstriction can be caused by endothelin 1 (ET-1) produced via the MAP kinase pathway. This balancing of vasoconstriction and vasodilatation demonstrates the ability of Insulin to regulate the blood flow and thus the microcirculation in the tissue (Kim et al., 2006).

[1] Diabetes type 1 being present at least for 1 year

Insulin resistance in a diabetic metabolism shows a defective PI 3-kinase pathway resulting in a decreased NO production, whereas the MAP kinase pathway remains intact. The disbalance between the two systems leads to vasoconstriction, impaired blood flow and missing recruitment of capillaries (Kim et al., 2006, Potenza et al., 2005).

On the other hand, acute hyperglycemia was examined as a factor to cause direct damage to the glycocalyx. The glycocalyx is constructed as a protective layer to the endothelial cells of the vessel wall, thus, damaging the protection means impairing the function of the vessel (Nieuwdorp et al., 2006). Changes in the microcirculation associated with a loss of glycocalyx were shown for the first time in children with diabetes type 1 before clinical symptoms appear (Nussbaum et al., 2014).
In addition, impaired microcirculation in children with diabetes type 1 without clinical symptoms could also be found in others studies (Khan et al., 2000, Babar et al., 2011), however, without examining an association to the glycocalyx.

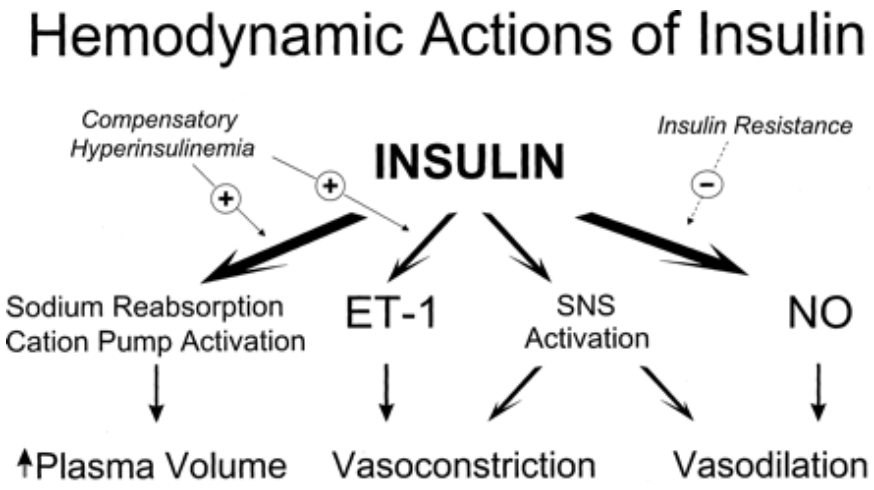

Figure 1 – hemodynamic actions of insulin, Source: (Kim et al., 2006)

1.15. Role of microcirculatory dysfunction in gestational diabetes

Examining the microcirculatory dysfunction in gestational diabetes, one must consider the fetoplacental unit as the exchange system between mother and fetus. Trying to explain the underlying mechanisms of gestational diabetes and its consequences for the fetus, research groups (Guzman-Gutierrez et al., 2013, Guzmán-Gutiérrez et al., 2011, Westermeier et al., 2011) examined the fetoplacental unit as it plays a central role in the development of the fetus.

1.15.1. The fetoplacental unit – a unique system

The fetus is connected to the mother and her metabolism via umbilical cord and placenta. The complete placenta is developed in the 13th week after fertilization. At birth, it is 2-3 cm thick, 20 cm long and weights 500g. The umbilical cord consists of two arteries and one vein, which are built in a spiral system and protected by Wharton's jelly. The umbilical cord is not directly connected to the uterine wall, but the vessels go over into the placenta.
The umbilical vein transports oxygenated blood, nutrients and hormones to the fetus, whereas the umbilical arteries transport deoxygenated blood and waste products from the fetus to the mother.
The exchange process of the fetal and maternal blood takes place in the fetoplacental unit. However, due to the barrier function of the placenta, blood does not intermingle between mother and fetus (Welsch, 2010).

The fetoplacental unit also provides the fetus with immunological factors and hormones thereby regulating growth and development of the child (Holzgreve et al., 2007). The placenta produces progesterone for the maintenance of the pregnancy as well as leptin for fetal growth and glucocorticoids for its maturation (Lewis et al., 2006).

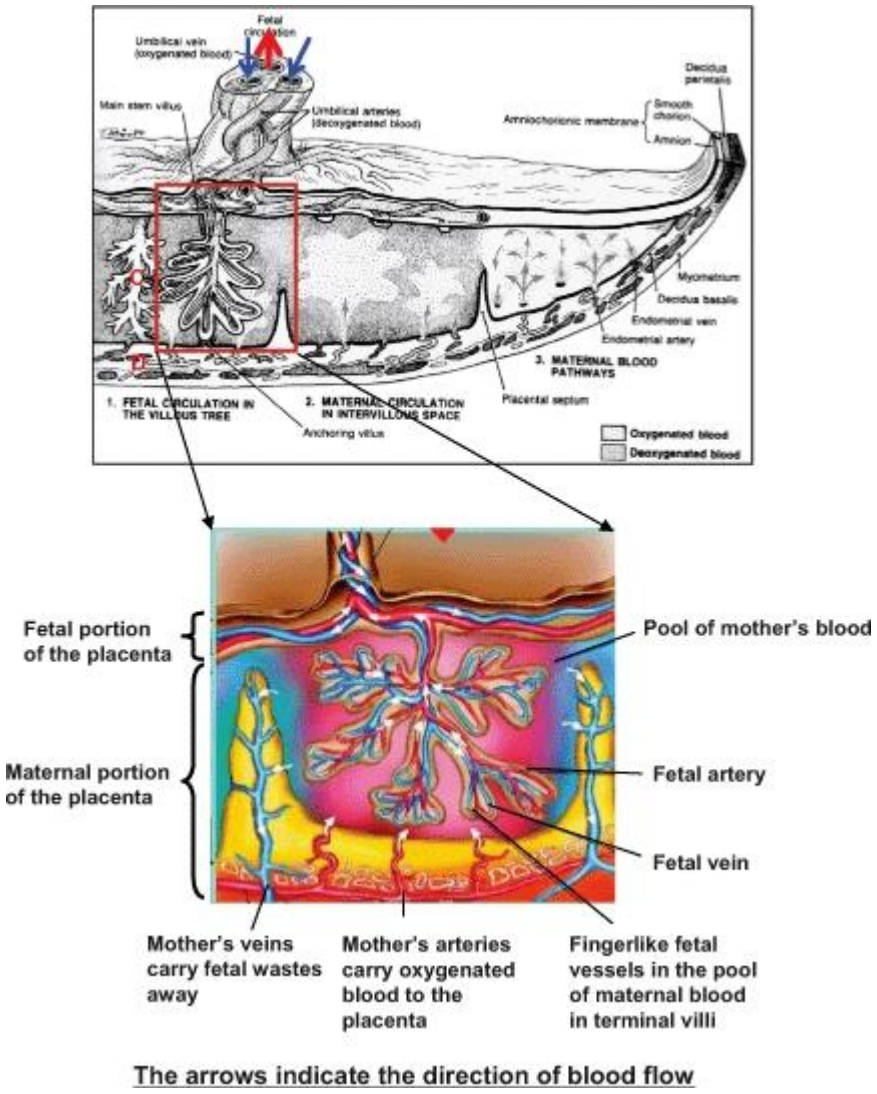

Figure 2 – the fetoplacental unit (Wang Y and S., 2010)

1.15.2. The fetoplacental unit in gestational diabetes

Mordwinkin et al. (2013) assessed the microcirculation of women with gestational diabetes and their fetuses and found that endothelial dysfunction concerns both the woman and the fetus and is associated with the same molecular mechanisms as assumed to cause oxidative stress in diabetes type 2 patients.

Gestational diabetes studies (Guzmán-Gutiérrez et al., 2011, Westermeier et al., 2009, Hiden et al., 2009, Guzman-Gutierrez et al., 2013) found out that the fetoplacental unit shows a defective insulin signaling. The defective insulin signaling possibly eases the insulin-induced vasodilatation and thereby facilitating the blood flow in the umbilical vein.
Gestational diabetes alters the uptake of adenosine (a vasodilator), l-arginine (a metabolite, which is needed for the production of NO), as well as the NO synthesis (Vasquez et al., 2004,

Westermeier et al., 2009). The umbilical vein shows therefore an increased level of adenosine (Westermeier et al., 2011, Westermeier et al., 2009).

Insulin may stimulate angiogenesis in the placenta by increasing the expression of insulin receptors and receptors for endothelial growth factors (Hiden et al., 2009).

Summarized, several studies implicate that an endothelial dysfunction in the fetoplacental unit can lead to an altered metabolic state, which could influence the health condition of the fetus also in its later life.

1.2. Methods of evaluating the microcirculation

As discussed earlier in the section, macrocirculatory markers are considered as insufficient for an evaluation of the tissue perfusion (Sakr et al., 2004). The capillary refill time is a standardized parameter to evaluate the tissue perfusion of children. Yet it might have limited value for the intensive care, as only a poor correlation with the hemodynamics has been identified (Tibby et al., 1999, Top et al., 2011). Increased lactate levels, as a result of an anaerobic metabolism, do not necessarily give proof of oxygen deprived tissue perfusion, as alternatively they could have been caused by renal- and liver diseases (KOCH et al., 2001). There exist several methods to evaluate the microcirculation (Christ et al., 2002, De Backer et al., 2012):

Venous occlusion plethysmography (VOP)

VOP is an approved method for the measurement of the tissue blood flow in the upper or lower limb via mechanical occlusion of the venous system. The increase in volume due to the remaining arterial inflow is measured distal to the cuff. A disadvantage of this method is the occurrence of movement artifacts.

Laser Doppler Fluxmetry (LDF)

LDF is a non-invasive method to evaluate a mean erythrocyte flux by using the Doppler shift. Monochromatic light is emitted into the tissue, reflected and scattered back by erythrocytes. The differences of the signals, caused by moving erythrocytes and based on the Doppler shift, are translated to a mean flux.

LDF is easy to use and produces results within minutes. Disadvantages are the production of artifacts, as well as the limited penetration of the tissue and the missing absolute value.

Near Infrared Spectroscopy (NIRS)

The NIRS method measures the oxygen saturation non-invasively in deep tissue layers by working with near infrared light. It is used for the observation of metabolic changes in the tissue in different clinical settings. A limitation of this technique is its inability to differentiate between vessel types.

Intravital invasive microscopy

This method is often used in research settings creating a very high quality image of the microcirculation. As a fluorescent dye is needed, the method remains limited to cell experiments or animal models.

Intravital non-invasive microscopy

Orthogonal Polarization Spectral (OPS) - and Sidestream Dark Field (SDF) imaging allow non-invasive and in-vivo measurements of the microcirculation without fluorescent dyes. Images can be taken with an optical handheld device on the bedside of the patients. The technical background will be further explained in 4.2.. The method is susceptible to pressure and movement artifacts.

Development of Orthogonal Polarization Spectral imaging

Groner et al. introduced 1999 a handheld device to monitor microcirculation non-invasively based on a method using two orthogonal polarizers developed by Slaaf et al. (1987). Since then, the method has been enhanced and tested in several clinical settings.

Test series on animals, as in the dorsal skin fold chamber of hamsters (Groner W. et al., 1999) or in the colon of mice with inflammatory bowel diseases (Biberthaler et al., 2001) showed that the microcirculation measured with OPS imaging could deliver comparable results to Intravital microscopy using fluorescent dyes.

Following up on these studies, Mathura et al. (2001) demonstrated that the method can also be used in-vivo in human organs, with even better image quality compared to conventional capillary microscopy (Mathura et al., 2001).

Genzel-Boroviczény et al. (2002) introduced OPS as a new tool to measure the microcirculation in children non-invasively. Due to the characteristics of their skin, newborns can be measured at the inner upper arm (Genzel-Boroviczeny et al., 2002) or in addition at the ear conch (Alba-Alejandre et al., 2013). Adults, however, need to be measured on a mucosa surface. Most frequently, the sublingual area was used in several studies (Boerma et al., 2007, De Backer et al., 2002).

In other studies using this method, microcirculation parameters have been presented as early markers for sepsis in preterm (Weidlich et al., 2009) and term children (Alba-Alejandre et al., 2013). Several research groups showed changes in the microcirculatory flow of adult patients with sepsis (Boerma et al., 2005, De Backer et al., 2002).

Sakr et al. (2004) demonstrated how the small vessel perfusion works as a prognostic value for the death of patients with a septic shock (Sakr et al., 2004). Moreover, an assessment of antiangiogenic treatment in tumors (Pahernik et al., 2002) and the topography of the oral squamous cell carcinoma has been described (Lindeboom et al.,

2006). The method has also been used for the examination of the microcirculation in burn wounds (Langer et al., 2001) as well as for the assessment of cardiac interventions (Erol-Yilmaz et al., 2007).

In 2005, Ince et al. introduced the Sidestream Dark Field (SDF) imaging, which represents an improved version of the OPS imaging with better image quality, especially regarding the smaller capillaries (Ince, 2005). SDF is based on the Dark Field Illumination technique introduced by Sherman et al. (Sherman et al., 1971). This new technique was then also used in newborns for measurements at the ear conch (Hiedl et al., 2010). Technical details are explained in 4.2..

We have used the SDF imaging for measuring the microcirculation in our study, as it produces by now the images with the highest quality.

1.3. Goals of the dissertation

GDM is one of the most common diseases in pregnancy and is associated with severe consequences for mother and child. The microcirculation and vasomotion were, to our knowledge, never evaluated in newborn infants who were exposed to a diabetic metabolism during pregnancy. An association between an impaired microcirculation and diabetes was already shown in several studies. We wanted to know, if and how GDM affects the microcirculation of the mothers and their newborn children. Based on this interest, we developed several research questions:

1. Is there a difference of the microcirculation parameters between women with GDM compared to women without GDM?

2. Is there a difference of the microcirculation parameters between newborns of mothers with GDM compared to newborns with mothers without GDM?

3. How does the vasomotion behave in newborns?

4. Is there a difference of the vasomotion in newborns with exposition to GDM compared to newborns without such exposition, and if so, which kind of differences can be observed?

2. METHODS

2.1. Study design and study group

The single-center, prospective, observational study was conducted at the newborn care unit of the Ludwig-Maximilians-University in Munich, Germany. The newborns were examined on their 2nd to 3rd day of life, along with their mothers. The period of examination started in September 2010 and ended in October 2011. The experiments were performed after the parental approval was given. The study was authorized by the ethics committee of the medical faculty of the Ludwig-Maximilians University Munich.

Inclusion criteria for the control group included:

- Gestational age between 37+0-42+0 weeks
- Birth weight > 2000g
- Written parental consent

Inclusion criteria for the patient group included:

- Gestational age between 37+0-42+0
- Birth weight >2000g
- Gestational diabetes of the mother or LGA >95%

Exclusion criteria included:

- severe congenital defects or acquired diseases
- clinical signs of infection
- CRP values > 0.5 mg/dl in the first 3 days of life

Diagnosis of gestational diabetes

The diagnosis was made during pregnancy by the responsible gynecologist with the 75-g 2-h oral glucose tolerance test (OGTT).

Treatment

Being included in the study did not change the treatment of the newborn infants. Children of the gestational diabetes group were treated according to the German Guidelines for Pregnancies complicated by diabetes (AWMF, 2010) with blood glucose measurements 2 h post prandial after feeding the newborn at 30 min after birth. Following measurements were taken at 6h, 12h and eventually 24h and 48h depending on the blood glucose levels. If necessary supplementary feedings, preferably with breast milk, were given depending on the blood sugar level as per recommendation.

2.2. Assessment of the microcirculation

2.2.1. SDF imaging

Sidestream Dark Field imaging is an advancement of the Orthogonal Polarization Spectral imaging. The technique is based on green light (530nm), produced by peripheral light-emitting diodes (LEDs), passing through the tissue. As the hemoglobin in the erythrocytes absorbs the light, the cells appear dark, flowing in the stream of the microcirculation on the video image of the camera. Building different channels for the reflected and illuminated light in the Sidestream Dark Field imaging, the visualization delivers a higher degree of detail than in the Mainstream Dark Field imaging. Illuminating the tissue from the side, disturbing superficial reflections are prevented. Hence, SDF can deliver a better quality image, especially of the smaller capillaries (Ince, 2005).

Sketch of SDF imaging technique **Hand-held device**

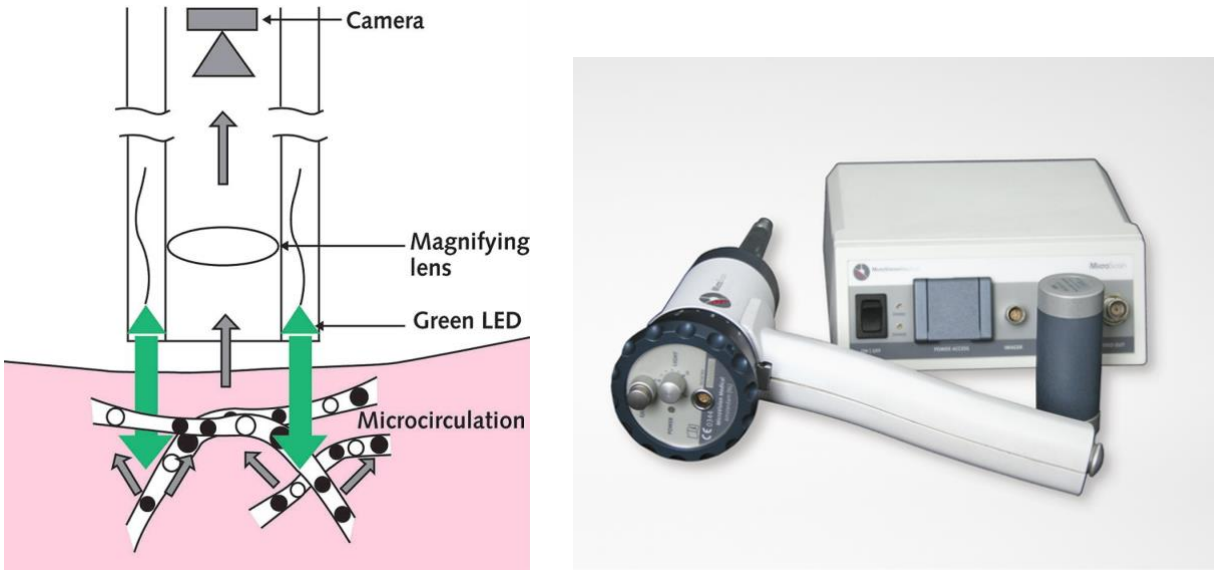

Figure 3 – Sketch of Sidestream Dark Field (SDF) imaging technique and hand-held device Source: (MicroVisionMedical).

2.2.2. MicroScan™

We measured the microcirculation non-invasively with SDF imaging using the MicroScan™ microscope by MicrovisionMedical, a portable handheld device which can be connected easily to a laptop. The video sequences can be saved directly on hardware for analysis.

Microscan can be used with a 5- or 10-time magnifying lens. We used a 5-time magnifying lens which transfers the light on a video camera and produces an image of the microcirculation on the screen. A 5-time magnifying lens is suggested by De backer et al. (2007) to measure the microcirculation in humans to provide a larger window of observation and is not as susceptible for movement artifacts as a 10-time magnifying lens (De Backer et al., 2007a).
The instrument remains sterile by using different plastic caps to put onto the lens for every patient.
(MicroVisionMedical User Manual, 2007).

The MicroScan™ system includes:

- MicroScan™ Battery Unit
- MicroScan™ Imaging Unit
- Connecting cables

The analog information received from the MicroScan™ imaging unit is transferred to an analog digital converter (ADVC110 Canopus Co., San Jose, CA, USA) which transforms it into digital information. The Canopus itself connects to the laptop. The video sequence can be directly seen on the screen of the laptop at the bedside and afterwards saved on the hard drive.

2.2.3. Sites for SDF measurements

For our study, we used two different sites, ear conch and inner upper arm, for measurements in the children groups. As already described by Prof. Dr. Genzel-Boroviczény and Co-workers (2004) the ear conch is a good site for measurements of the microcirculation in newborn infants. It shows fewer lanugo hair, which can otherwise cause artifacts, and is easy accessible for the examiner (Alba-Alejandre et al., 2013).
However, to obtain images of the microcirculation and in order to analyze the vascular reaction, e.g. vasomotion, during blood pressure measurements, we used the upper inner site of the arm, before the antecubital fossa underneath the blood pressure cuff.
Sublingual Measurements were not performed in the children groups as they are not easy to obtain in newborns due to movement artifacts.

For mothers, we used the sublingual site and the buccal site for measurements. Sublingual measurements were already performed in different studies (Ince, 2005, Boerma et al., 2007) and have shown good quality images in adults. Buccal measurements (den Uil et al., 2008, Wiessner et al., 2009) have also presented good quality images, however they were not used as often as the sublingual site.

2.2.4. Performance of measurements

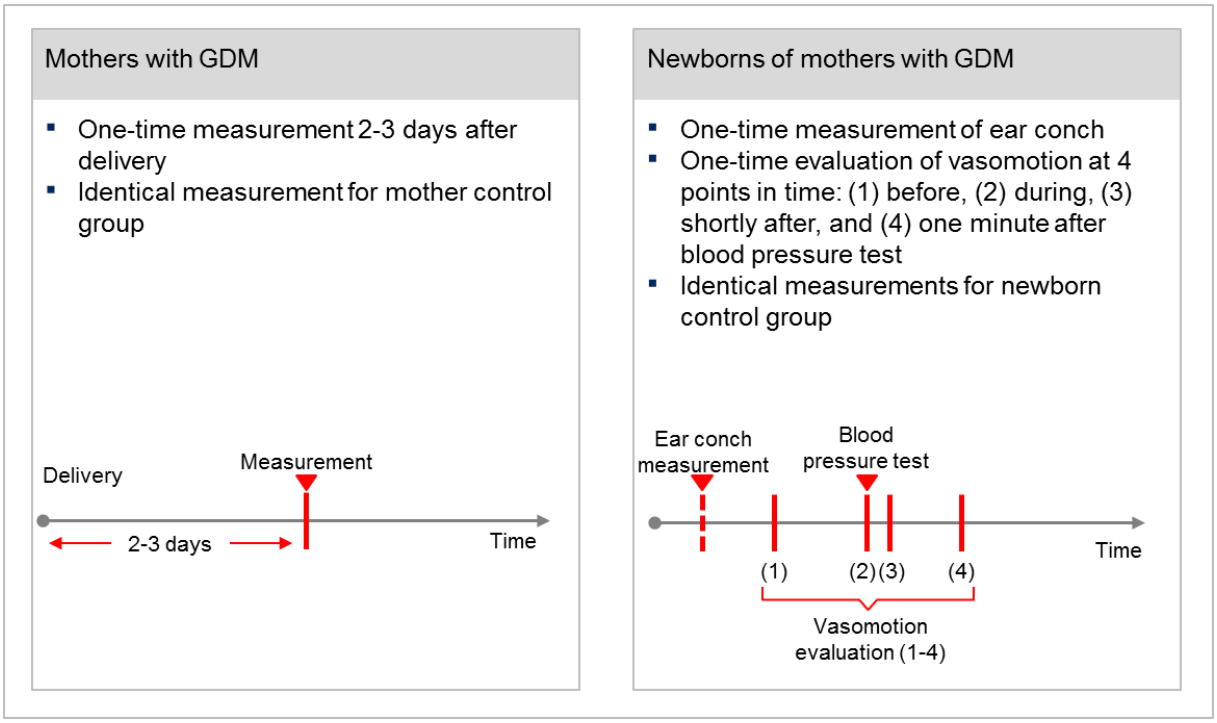

Figure 4 – Measurement procedure of microcirculation and vasomotion

Infants

The measurements on the ear conch were obtained taken first. Three different video sequences, each of them minimum 10 seconds long, were taken on different parts of the ear conch.

For the measurement, the arm was undressed and the blood pressure cuff brought onto the left arm in the right position. To improve image quality, a water drop was placed between the optical device and the skin. The MicroScan device was held at a 60° angle to the skin surface and lowered slowly to avoid pressure artifacts and to ensure correct measurements. Children were measured on day 2 to 3 of their life. It was a single-time measurement with 5 different sequences and 2 different measurement sites. Measurements took place at the perinatal care center at the Frauenklinik Maistraße of the Ludwig-Maximilians-University.

For a good quality video sequence, it is important to avoid movements of the camera. Therefore, the children were measured asleep or after breastfeeding. For the measurement of the ear the babies could remain dressed, whereas for the blood pressure measurement, the left arm had to be undressed to put on the cuff. They were laid on the dressing table at a normal room temperature. Pressure artifacts can be seen as a lower or intermittent flow. The optical device must be then lifted slowly to see if the reason for the intermittent flow is caused by a false handling by the examiner or is a real and valid observation of the microcirculation. Therefore, the examiner has to be familiar with the video microscope and its handling.

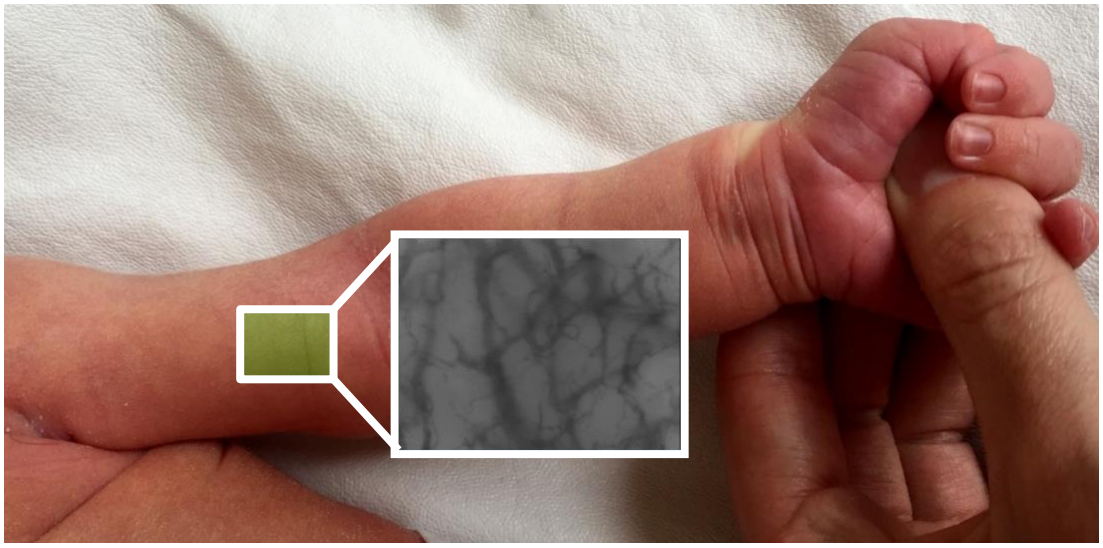

Figure 5 – Microcirculation measured in newborns with OPS imaging

Vasomotion and Blood flow during blood pressure measurement

In order to analyze the vascular reaction, e.g. vasomotion, of the microcirculation, we captured sequences at the arm at four points of time: before, during, shortly after and 1 minute after blood pressure measurement. The cuff would interrupt the microcirculation and allow us to compare the reaction of the limited blood supply on the vessels, e.g. the vasomotion, in the two groups.

Three video sequences on the inner upper left arm were taken before blood pressure measurement. In the following, we started the automatically blood pressure measurement and three sequences were captured during, as well as three 30 seconds after the measurement. Additionally, three sequences were taken 1 minute after the blood pressure measurement was finished. As we wanted to measure the microcirculation exactly during blood pressure measurement, only one sequence was taken at this point for analysis. Regarding the other points of measurement, the mean value of three sequences was calculated and used for statistical evaluation.

Mothers

The mothers were measured on the same day as their children. As sites of measurement we used the lower lip and the oral mucosa of the buccal region. Each time, multiple measurements, each 10 seconds minimum long, were taken and three in good quality of them chosen and their mean value taken for statistical analysis.

2.2.5. Analysis

All measurements were performed by one investigator. The video sequences have first been newly organized with a random number system, blinded to the investigator, and afterwards been analyzed with the Automated Vascular Analysis (AVA) 3.0 software package (MicroVision Medical, Amsterdam, The Netherlands).

Working with AVA 3.0

The software allows for semiautomatic measurement of detected vessel length, functional vessel density, vessel surface and distribution of vessel diameter. The program was calibrated first manually by the investigator by defining how many micrometers conform to pixels on the screen.

The program is then able to search for the vessels via pattern recognition algorithm and define their Functional Vessel Density (FVD) as well as the vessel diameters. The quality of the blood flow was in our study evaluated by the investigator as can be read below.

By using "Reference Frames" under "Average Tool", one image was produced constructing it from all sequences of the video.

The program first stabilizes the picture and thereby reduces movement artifacts before constructing a working image with the help of "Reference Frames". Afterwards it is able to detect the different kind of vessels organized after their diameter and according to them marks them with different colors. It measures the microcirculatory parameters to present them in a "microcirculatory report" with all data needed for statistical evaluation.

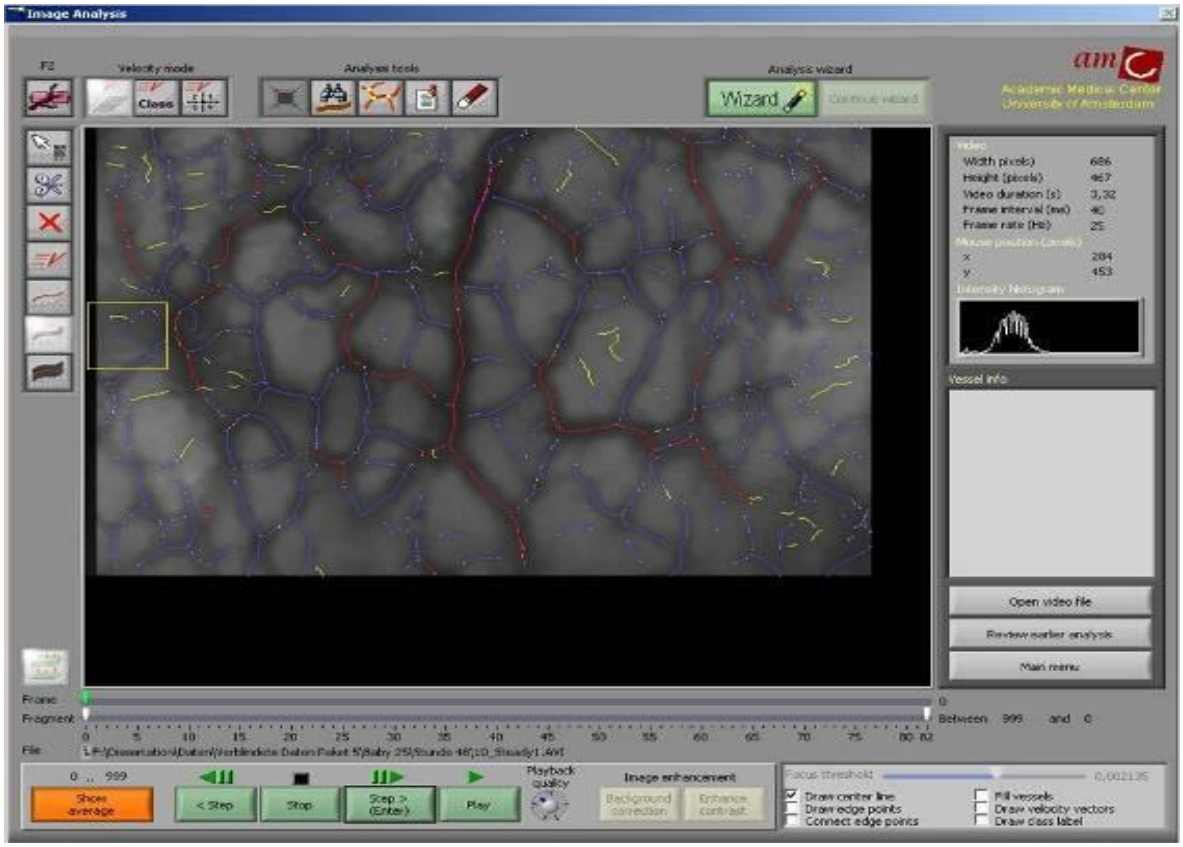

Figure 6 – Visual evaluation of microcirculation using AVA 3.0 Source: MicroVision Medical

For this study, we defined the vessels as following:

- small vessels (0-10µm)
- medium vessels (10-20µm)
- large vessels (20-100µm)

Microcirculatory parameters:
1. Detected Vessel Length (DVL): length of the total detected vessel in mm
This parameter was determined and statistically analyzed, but excluded later from the results as it holds no additional information for the research

2. Vessel Density (VD), also known as Functional Vessel Density (FVD): the length of the detected vessels in mm/mm² per captured image section

3. Vessel density distribution
Vessel Diameter Distribution (VDD): percentage of a vessel diameter (small, medium or large) compared to the overall vessel length in %

4. Vessel Surface (VS): the percentage of the captured image section covered with vessels in (mm²/ mm²) x 100 %

5. De Backer score: Calculated vessel density. For the determination of the score, three equidistant horizontal and three equidistant vertical lines are drawn on the screen. Vessel density can be calculated as the number of vessels crossing the lines divided by the total length of the lines. The flow is then categorized by the investigator in continuous, intermittent or absent flow. Afterwards, the proportion of perfused vessels can be calculated with the formula: 100 x ((total number of vessels) - (no flow + intermittent flow))/ total number of vessels.
For our study, we did not use this score, as it also holds some disadvantages. It does not take the velocity of red blood cells into account. Moreover, the method can be inaccurate when stabilizing the picture as the length of the lines can differ from the original image. Instead we used the Microvascular Flow Index.

6. Microvascular Flow Index (MFI): semi quantitative measurement of Blood flow quality

For measuring the blood flow, the investigator proceeded as following.
The vessels were subdivided into small, medium and large vessels by the program.

The screen was then divided into four equal quadrants and each quadrant was evaluated separately by the investigator. The mean of the blood flow of the four quadrants was taken for statistical analysis.
Artifacts such as hair were removed independently by the investigator. The video was then started to evaluate the moving cells i.e. the blood flow.

The blood flow was classified as:

- no flow: 0
- intermittent flow: 1
- sluggish flow: 2
- continuous flow: 3
- hyperdynamic flow: 4

2.2.6. Statistical evaluation

The data were analyzed using GraphPad 4.0 (GraphPad Software Inc., La Jolla, California). Data are presented with their mean values and 95% confidence Intervals. The level of significance was set at $P < 0.05$. In the following, statistical tests were performed depending on whether the results were parametric or non-parametric.
For the comparison between the patient and the control group the unpaired and paired t-test and the Mann-Whitney test were utilized. For the comparison between different points of time during the blood pressure measurement within one group, the Friedman test and the repeated measures test were conducted.

Column Statistics: generates median, mean value and confidence interval of a population
Normality test: tests the Gaussian distribution of a population
Unpaired t-test: comparison between two different unpaired populations with Gaussian distribution
Paired t-test: comparison between two different paired population with Gaussian distribution
Mann-Whitney U test: comparison between two different unpaired populations with a non-normal distribution
Friedman test: non-parametric test within one group over multiple test attempts
Repeated measures ANOVA test: test within one group for repeated measures over time.

3. RESULTS

3.1. Clinical data

Clinical data of newborns

	Patient group	*Control group*
Birth weight (g)	3561 (3295-3827)	3544 (3377-3712)
Birth length (cm)	51.6 (50.3-52.9)	51.8 (51.1-52.5)
Gestational age (weeks)	39.2 (38.6-39.8)	40.2 (39.5-40.8)
APGAR Score min 1	9.0 (9.0-10.0)	9.0 (9.0-10.0)
APGAR Score min 5	10.0 (10.0-10.0)	10.0 (10-10.)
APGAR Score min 10	10.0 (10.0-10.0)	10.0 (10.0-10.0)
Umbilical cord (pH)	7.30 (7.26-7.34)	7.30 (7.26-7.33)
O2 Saturation (%)	99 (99-99)	99 (99-100)
Heart rate (bpm)	119 (114-125)	111 (106-117)
Blood pressure systolic (mmHg)	78 (74-82)	80 (72-88)
Blood pressure diastolic (mmHg)	54 (48-60)	51 (45-58)

Table 2: clinical data of newborns - control and patient group

(mean 95% CI, median 95%CI for the APGAR score)

3.1.1. Patient collective

This study' sample group consists of 30 term infants who were in the 40th +/- 2 gestational week. The patient group comprises 15 newborns of mothers with gestational diabetes who were in the same age range. Similarly, the control group includes 15 newborns whose mothers did not have gestational diabetes. In addition, images of the inner site of the lip were taken of 15 mothers of the control group and 13 mothers of the patient group. Later in the study, measurements of the buccal region were added and images could be captured of eight mothers in each group.

No statistical differences between the two groups were found concerning the birth weight and birth height. The gestational age of the 15 patients was, however, within the age range, significantly lower than the one of the control group (n=15) (mean gestational age, 39.20 weeks; 95% CI, 38.6- 39.8 versus 40.15 weeks; 95% CI, 39.5-40.8 (p=0.0307).

3.1.2. Birth modus

In the patient group, three of the 15 children were born by caesarean section, two by vacuum extraction and ten spontaneously. In comparison, in the control group, two newborns out of 15 were delivered via caesarean section, three via vacuum extraction and ten were born spontaneously. There was no significant difference in the birth modus between the two groups.

3.1.3. APGAR score

Every child of the two groups was evaluated by the APGAR score in the first, fifth and tenth minute of life. Comparing the two groups, no significant statistical difference could be observed.

3.1.4. Macrocirculatory parameters and O2 saturation

The blood pressure, the mean blood pressure, the heart rate and the O2 saturation were measured for 13 children in the control group and 15 children in the patient group. The results were not statistically different between the two groups.

3.1.5. Therapy

The mothers of nine newborns of the patient group (=n 15) received insulin treatment in addition to their diet during pregnancy whereas six out of 15 were not treated with insulin but

had to keep to a diet. The mothers of the newborns in the control group received no insulin treatment, nor did they follow a diet plan.

3.2. Microcirculatory parameters of the children

The microcirculation was measured at the left upper arm and the ear conch on the second or third day of life in the newborns of mothers with gestational diabetes and compared to a control group (c).

3.2.1. Functional Vascular Density (FVD)

Comparison between the two groups

Comparing the children of the patient group with the children of the control group, no statistical difference in the FVD could be observed in the video sequences of the ear conch. The same can be stated for the images taken at the inner upper arm.

During blood pressure measurement at the arm till one minute after, no statistical difference in the FVD could be shown between the two groups.

Comparison within one group

Comparing the four images recorded at the inner upper arm before, during, and after blood pressure measurement within the control group, no statistically significant difference was observed. Regarding however the patient group, the FVD is significant lower during blood pressure measurement than the one at the arm before, shortly after and 1 minute after blood pressure measurement (Friedman test, p=0,0392).

3.2.2. Vessel surface

Comparison between the two groups

Looking at the sequences measured at the ear conch, there was no observed statistical difference in the vessel surface. The same could be noticed for the video sequences captured at the arm before till 1 min after blood pressure measurement.

Comparison within one group

Looking at the images before, during and after the test series at the arm, no statistical difference could be detected within the control or the patient group.

3.2.3. Percentage of small, medium and large vessel diameter

Comparison between the two groups

Comparing video sequences captured at the ear conch of the patient group with the control group, no statistical difference could be presented regarding the small, medium or large

diameter percentage. The images taken at the arm before, during all states of measurement and afterwards show no statistical difference between the two groups.

Comparison within one group

Looking at the percentage of the small, medium and large vessel diameter no significant difference could be observed between the 4 sequences in the patient group. This also applies for the control group.

Microcirculation parameters - Patient group and Control group in comparison

		Ear patient group	*Ear control group*	*Arm patient group*	*Arm control group*	*RR patient group*	*RR control group*	*ARR patient group*	*ARR control group*	*1 min patient group*	*1 min control group*
Functional Vessel density (mm/mm²)		14.1 (13.0-15.2)	13.7 (12.8-14.6)	16.7 (15.9-17.5)	16.1 (15.2-17.0)	15.2 (13.9-16.5)	14.2 (13.0-15.4)	16.8 (16.0-17.6)	16 (14.8-17.1)	16.6 (15.8-17.4)	16.3 (15.1-17.5)
Vessel diameter (%)	*small (<10 µm*)	47.5 (41.9-53.1)	48.1 (43.4-52.9)	49.2 (44.0-54.0)	47.9 (41.6-54.1)	49.4 (42.8-56.1)	46.2 (37.7-54.8)	52.6 (47.8-57.5)	47.7 (38.3-57.1)	49.9 (45.4-54.3)	51.8 (42.4-61.1)
	medium (10-20µm)	41.6 (38.5-44.7)	39.2 (36.3-42.1)	44.7 (41.1-48.3)	45.6 (41.1-50.2)	43.2 (38.6-47.9)	44.7 (39.3-50.0)	42.7 (38.9-46.4)	44.2 (38.0-50.3)	42.2 (35.1-49.2)	41.9 (35.9-48.0)
	large (>20µm)	11.0 (8.0-13.9)	12.6 (9.0-16.3)	6.2 (4.0-8.4)	6.5 (4.0-9.0)	7.4 (3.2-11.6)	9.1 (4.6-13.7)	4.7 (3.0-6.5)	8.2 (3.7-12.6)	5.1 (3.4-7.0)	6.3 (2.4-10.2)
Vessel surface (mm2/mm2x100%)		24.0 (22.7-25.3)	23.8 (22.2-25.3)	26.2 (25.0-27.4)	25.6 (24.4-26.8)	25.0 (23.2-26.8)	24.1 (22.0-26.2)	25.4 (24.1-26.8)	25.7 (23.4-28.1)	25.9 (24.6-27.2)	25.2 (23.4-27.0)

Table 3: Microcirculation parameters (mean, 95% CI) in the patient group and control group at the ear (Ear) and the arm before (Arm), during (RR), shortly after (ARR) and one minute (1 min) after blood pressure measurement

3.3. Microvascular flow index of the children

The microvascular flow index was measured at the ear conch and at the arm on the second or third day of life in the newborns of the patient and the control group as described in 5.2.5.

Microvascular flow index at the ear

No significant difference in the microvascular flow index could be observed between the two groups regarding the small, medium and large vessels at the ear.

Microvascular flow index and vasomotion of the children at the arm

As described in 4.2., not just to observe the blood flow of the newborns, but also to analyze the vasomotion, the blood flow was evaluated under blood pressure measurement at the arm in different time slots.

Microvascular flow index - patient group

	arm	RR	*ARR*	*1 min*
Small vessel (<10µm)	2.6	**1.1**	2.8	3
	(2.2-3.0)	**(0.5-1.6)**	(2.5-3.1)	(2.7-3.2)
Medium vessel (10-20µm)	2.5	1.3	2.6	2.8
	(2.2-2.8)	(0.7-1.9)	(2.3-2.9)	(2.5-3.1)
Large vessel (>20µm)	2.6	**1.2**	2.6	2.8
	(2.3-2.9)	**(0.7-1.7)**	(2.3-2.9)	(2.5-3.0)

Table 4: Microvascular flow index (Mean, 95%CI) in the patient group at the arm before (arm), during (RR), shortly after (ARR) and one minute after (1 min) blood pressure measurement

Microvascular flow index - control group

	Arm	RR	*ARR*	*1 min*
Small vessel (<10µm)	2.5	**2.1**	2.8	2.7
	(2.2-2.8)	**(1.7-2.6)**	(2.4-3.2)	(2.3-3.0)
Medium vessel (10-20µm)	2	1.9	2.6	2.4
	(2.0-2.6)	(1.6-2.3)	(2.1-3.0)	(2.1-2.8)
Large vessel (>20µm)	2.4	**2.1**	2.7	2.9
	(2.0-2.7)	**(1.7-2.5)**	(2.4-3.1)	(2.3-3.5)

Table 5: Microvascular flow index (Mean, 95% CI) in the control group at the arm before (arm), during (RR), shortly after (ARR) and one minute after (1 min) blood pressure measurement

As can be seen in table 5 and 6, the results show a significant lower blood flow or higher percentage of intermittent flow in the small vessels during blood pressure measurement at the patient group compared with the control group (Mann Whitney test, p=0,0063).

Moreover, a significant lower microvascular flow index can be found in the large vessels during blood pressure measurement at the patient group compared with the control group (unpaired t-test, p=0,0094)(see figure 7).

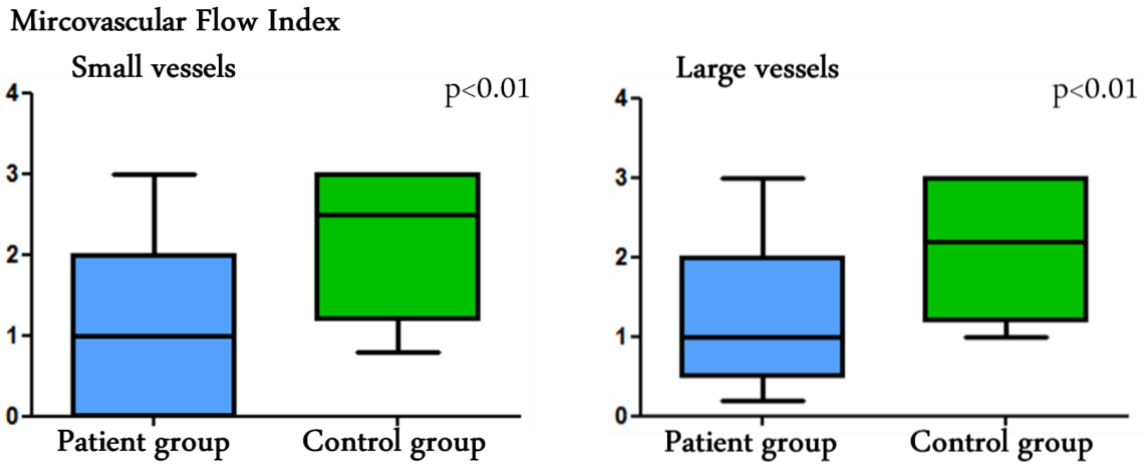

Figure 7: Mean Microvascular Flow Index in small and large vessels in patient and control group. 0= No flow, 1=intermittent, 2= sluggish, 3= continuous, 4= hyperdynamic

There is also a diminished blood flow in the medium vessels at the arm during blood pressure measurement between the groups, which is, however, not significant.

Looking at the 4 sequences within the patient group, there is a highly significant lower Boerma flow in the small, medium and large vessels during the blood pressure measurement than before, shortly after and 1 minute after blood pressure measurement (Repeated measures test, p<0,0001).

Whereas within the control group, there is a significant lower blood flow in the medium vessels compared to the other three sequences (Repeated measures test, p=0,0166). The blood flow of the small and large vessels is also decreased during blood pressure measurement but does not show a significant difference.

In the patient group, comparing the blood flow of the vessels between the ear and the arm, a significant difference can be found looking at the small (Mann Whitney test, p=0,0056) and the

medium vessels (unpaired t-test, p=0,0008). The flow was significant lower at the arm compared to the ear in both types of vessel.
However, there is no significant difference comparing the Boerma flow of the large vessel of the ear against the one at the arm within the patient group.

In the control group, the Boerma flow in the small (unpaired t-test, p=0,0368), medium (unpaired t-test, p=0,0086) and large (unpaired t-test, p=0,0134) vessels was significant lower at the arm than at the ear.

The microcirculation and blood flow quality of the mothers

The mothers with gestational diabetes and the mothers of the control group were measured on the second or third day postpartum at the inner sight of the lip and at the mucosa of the buccal region as described in 5.2..

Microcirculation parameters - mothers

		Patient group lip	*Control group lip*	*Patient group buccal*	*Control group buccal*
Functional Vessel density (mm/mm2)		12.1 (11.0-14.0)	12.1 (10.3-13.9)	9.4 (8.5-10.2)	9.1 (7.7-10.4)
Vessel diameter (%)	*small (<10µm)*	40.8 (34.1-47.4)	46.2 (37.7-54.8)	30.1 (24.0-36.1)	26.4 (22.8-30.1)
	medium (10-20µm)	45.8 (42.3-49.2)	46.3 (40.5-52.1)	**49.3 (43.0-55.6)**	**56.3 (52.8-59.8)**
	large (>20µm)	13.7 (9.3-18.2)	13.0 (6.4-19.6)	20.7 (9.7-31.6)	17.3 (14.1-20.5)
Vessel surface (mm2/mm2x100%)		21.9 (19.8-23.9)	21.4 (18.7-24.0)	19.0 (15.8-22.1)	18.5 (15.3-21.6)

Table 6: Microcirculation parameters (Mean, 95% CI) of the mothers in the patient group and the control group, lip measurements vs. buccal measurements

Functional vessel density

The measurement of the functional vessel density at the lip or the buccal region cannot reveal significant difference between the mothers of the patient group compared with the mothers of the control group.

Vessel surface

There was no observed statistical difference between the two groups regarding the measurements of the vessels surface regarding the images captured at the inner sight of the lip, nor was there a statistical difference observed at the buccal region.

Percentage of small, medium and large vessel diameter

Regarding the video sequences of the buccal region, the mothers of the patient group have a significant lower percentage of medium vessels in comparison with the mothers of the control group (unpaired t-test, p= 0,0425).

No significant difference, however, can be revealed looking at the inner site of the lip.

Microvascular flow index

Microvascular Flow Index - Buccal region

	Patient group	*Control group*
Small vessels (<10µm)	2.9	2.77
	(2.7-3.1)	(2.3-3.2)
Medium Vessels (10-20µm)	2.6	2.6
	(1.7-3.5)	(2.3-3.0)
Large Vessels (>20µm)	2.5	2.8
	(1.7-3.3)	(2.5-3.1)

Table 7: Microvascular flow index (Mean, 95% CI) in the control group and the patient group at the buccal region of the mothers

	Patient group	*Control group*
Small vessels (<10µm)	1.9	1.6
	(1.3-2.6)	(1.1-2.1)
Medium Vessels (10-20µm)	1.7	1.6
	(1.3-3.0)	(1.2-2.0)
Large Vessels (>20µm)	1.9	2.0
	(1.5-2.3)	(1.6-2.5)

Table 8: Microvascular flow index (Mean, 95% CI) in the control group and the patient group at the lip of the mothers

Looking at the microvascular flow index measured at the lip and the flow index measured at the buccal region compared between the mothers of the patient group with the mothers of the control group shows no significant difference

4. DISCUSSION

4.1. Overview

The goal of the study was to examine the microcirculation, the blood flow, e.g. Microvascular flow index and the vasomotion of the children and their mothers with GDM. The children were measured on the second or third day of life and compared to a control group of newborns and their mothers without a diabetic metabolism during pregnancy. The blood flow was examined before, during, shortly after and 1 minute after the providing arteries were constrained with the help of a blood pressure cuff. The experiment allowed us to indirectly measure the vasomotion. The results of the study show that the newborns of the patient group display a significant lower blood flow or a higher percentage of a sluggish or intermittent flow pattern in the small and large capillaries during the testing than the newborns of the control group. These findings suggest a missing response to the limited blood supply during compression of the providing arteries in the vessels of children who were exposed to a diabetic metabolism. The microcirculation parameters show no significant difference in the children group or in the testing of their mothers. The results will be critically discussed in the following section.

4.2. Study design

The study was an observational prospective study. The tests were performed on the second or third day of life in the newborns. Additionally, we also measured the microcirculation of the mothers in the patient group as well as in the control group. The inclusion in the study had no influence on the therapy.
Measurements were taken at the upper ear conch as well as at the upper arm before, during, shortly after and one minute after limiting the blood flow of the providing arteries via blood pressure cuff.

The measurements of the microcirculation in the mothers were first only performed at the inner site of the lip. To compare the quality of the images, buccal measurements were included during the study and measured additionally in 8 mothers in both groups. One limitation of the study is the reduced number of participants. Due to the high time consumption of the method and analysis, in two years 15 children of mothers with GDM and 15 children of the control group could be measured as well as 15 mothers of the control group and 13 mothers with GDM. Therefore, the results of the study have to be seen in relation to the small number of the surveyed group and further studies should be performed to validate the results. Moreover, the

investigation is only a one time measurement and includes no follow-up measurements. Therefore, the results can only be related to this point of time and holds no longtime prediction.

4.3. Method

The microcirculation was measured with the handheld Microscan device with a technique based on the Sidestream Dark Field Imaging. The Sidestream Dark Field Imaging is an improvement of the Orthogonal Polarization Spectral Imaging. Studies (Harris et al., 2002, Harris et al., 2000) comparing OPS to fluorescence microscopy showed in animal models that the technique is suitable to evaluate the microcirculation. In addition to that, the method can be applied to measure the microcirculation of newborn infants non-invasively, as validated in several studies (Genzel-Boroviczeny et al., 2002, Genzel-Boroviczeny et al., 2004, Hiedl et al., 2010, Alba-Alejandre et al., 2013).

A round table conference (De Backer et al., 2007b) developed guidelines for microcirculatory measurements suggesting that a measurement should be conducted at least at three sites, e.g. three sequences of every organ. We followed this rule for every site of measurement: for the ear conch and the arm of the newborn infants, as well as for the inner site of the lip and the buccal area for the mothers. However, when testing the blood flow and indirectly evaluating the vasomotion of the newborns, only one site could be analyzed given the short time frame for each measurement. This constraint on the range of available sites should to be taken into consideration when evaluating the results.

We also fulfilled further measurement criteria defined by De Backer et al. such as the elimination of secretions, contrast adjustment and a sufficiently high recording quality. We used a 5-time magnifying lens to obtain an adequate focus with an optimal magnification.

Measuring the mothers, we tested other sites than the sublingual area, which has been tested before in adults (Boerma et al., 2007, De Backer et al., 2002). To our knowledge, we are the first to take video sequences of the inner lip. As we had problems to obtain good quality images with the lip measurements without discomfort for the women, buccal measurements were included during the study and measured in eight mothers of each group as well. Buccal measurements were easier to obtain and more comfortable for the mother, as this area of measurement did not produce an unpleasant dryness of the lip. We had to film several video sequences to obtain a sufficient quality without bubbles. To preempt inter-observer variability, all measurements were taken by the same investigator.

For the study, ear conch scans as well as arm scans were taken to measure the newborns. Ear conch scans have the advantage to be easily accessible and the babies could sleep during the tests, which reduced movement of the child and made the filming easier. However, testing the blood flow and indirectly evaluating the vasomotion, a larger area had to be taken. It was necessary to reduce the macrocirculatory blood flow and to measure the microcirculation in an area downstream at the arm to prevent the video quality from being impaired by direct pressure artifacts resulting from the inflated arm cuff. Arm scans were already proven suitable to measure the microcirculation in newborns (Alba-Alejandre et al., 2013). In our study, the images of the arm showed a good quality and contained a rich field of vessels.
However, a new study (van den Berg et al., 2015) showed that measurements of the microcirculation in newborns are well reproducible in the buccal region but not in the cutaneous region. Even if a difference was only tested and shown in this singular study, it has to be taken into account when discussing the relevance of our results.

4.4. Analysis

The video sequences organized in a new random number system and blinded to the investigator have been afterwards analyzed with the Automated Vascular Analysis (AVA) 3.0 software package (MicroVision Medical, Amsterdam, The Netherlands).
The software allows for semiautomatic measurement of detected vessel length, functional vessel density, vessel surface and distribution of vessel diameter. The program was calibrated first manually by the investigator by defining how many micrometers conform to pixels on the screen. The program is then able to search for the vessels via pattern recognition algorithm and define their Functional Vessel Density (FVD) as well as the vessel coverage and the vessel diameters.

Artifacts as hair which have been incorrectly detected as vessels can be corrected by the user. Furthermore, vessels which have not been detected, can be added to the analysis. However, it was decided on a round table conference (De Backer et al., 2007b) that no further vessels should be added as it would depend on the different user of the program how many vessels are traced and therefore allow bias.

In order to analyze the quality of the blood flow, the image can be divided into four quadrants according to Boerma (De Backer et al., 2007b). Each quadrant can be analyzed individually and the flow of vessels evaluated according to their group of small, medium and large vessels. The flow can be evaluated as:

0=no flow
1=sluggish
2=intermediate
3=continuous
4=hyperdynamic

The analysis including the blood flow was evaluated by the same investigator to ensure that the same interpretation of the scale was used for every sequence.
A point of critical discussion could be that the blood flow quality is arranged in an ordinal scale from 0 to 4 and is discrete. For example, two quadrants of one picture showing a hyperdynamic flow (= 4), and two a sluggish flow (= 2) would result in an average continuous flow (= 3), which does not represent the reality in any of the four quadrants. This means that pathologically low and high blood flows would lead to a “normal” continuous flow. This weakness in measurement is, however, only of marginal relevance for our study, as we do not interpret the single average of the measurements. We exclusively evaluate the reaction of the vessels to the limited blood flow, assessing whether the reaction of the patient group is significantly different from the reaction in the control group. Therefore, we focus on the differences of the means in the two groups and not on the actual number of the mean in each group.

Furthermore, when measuring the microcirculation and the Boerma flow, in most of the studies the measuring scale ranges from 0 to 3 excluding the hyperdynamic flow. Including the hyperdynamic flow, widens the range and is in some way more accurate but can also lead to further incorrect interpretation of the resulting averages as described above. However, as we only concentrate on the differences in means between the patient and the control group, using a 0 to 4 scale instead of a 0 to 3 scale does not distort our results.

There are some restrictions to the study. The off-line analysis is still a time-consuming process and therefore only a limited number of patients could be included in the study. The results need to be validated in a larger prospective clinical cohort study.

4.5. Is there a difference of the microcirculation parameters between women with GDM compared to women without GDM?

To our knowledge, it was the first time that the microcirculation of women with GDM was measured. The group of mothers with GDM tested in the study has received different therapies from their attending physicians according to their blood sugar profile. Nine out of ten mothers with GDM received insulin treatment in addition to their diet, whereas six only received a dietetic treatment. The mothers of the control group did not receive any kind of treatment for diabetes. Being included into the study did not change the therapy.

It is known that alterations in the microcirculation leading to microangiopathy and causing retinopathy and nephropathy (Stehouwer et al., 1997, Flyvbjerg, 2000) may occur as consequences of diabetes type 1 or 2.

Moreover, diabetes was shown to impair the vascular function regarding the vasodilatation (Steinberg and Baron, 2002), especially in women with diabetes type 2 before the menopause (Steinberg et al., 2000).

GDM seems to have the same underlying molecular mechanisms as diabetes type 2 (Mordwinkin et al., 2013). A long-term study (O'Sullivan 1989) showed that 73% of women with GDM could be diagnosed with diabetes type 2, 25 years post-partum.

Considering these findings, we wanted to know if alterations in the microcirculation could also be found in women with GDM.

In the results of our study, however, no significant difference except for the higher percentage of medium vessels in the buccal measurements control group was found in the microcirculation parameters between the mothers with GDM compared to the mothers without GDM. We also looked at the different therapies, comparing mothers who were treated only with diet against mothers treated with insulin. However, the group size of mothers treated exclusively with diet was too small to compare the group with a statistical test.

Looking at the setting of the study in a university hospital, the question arises whether the good glycemic control of the mothers has perhaps already prevented alterations in the microcirculation. Tests (Jaap et al., 1995) with diabetes 2 patients have shown how a good glycemic control over 8 months can improve the vasodilatation in vessels. Moreover, large longitudinal cohort studies (Group., 2003, Group, 1993) in diabetes 1 patients have demonstrated how a nearly normal blood glucose level can improve the clinical outcome underlining the importance of a good therapy.

However, no statement can yet be made concerning the vasomotion. As the adult’s layer of skin is too thick for the microcirculation to be measured with SDF imaging, the test must be performed on the mucosa. We performed our test for the comparison of the microcirculation on the buccal region and the inner lip of the mothers. Testing the reaction to a limited blood flow on the vessels reliably is far more complicated in such an area compared to the test

performed on the skin of the arm in the newborns. To perform this kind of test in adults, a more accessible area layered with mucosa would need to be found, which would to this point only be possible in organs, i.e. invasively.

4.6. Is there a difference of the microcirculation parameters between newborns of mothers with GDM compared to newborns with mothers without GDM?

To our knowledge, the microcirculation has not yet been examined in children of mothers with GDM. Studies (Nussbaum et al., 2014, Khan et al., 2000, Babar et al., 2011, Heimhalt-El Hamriti et al., 2013) of children with diabetes type 1 showed alterations in the microcirculation which led us to the question if these alterations can be seen in newborns who have been under a diabetic metabolism during pregnancy. However, no statistically significant differences could be found between the two groups regarding the Functional Vessel diameter, the Vessel coverage or the Vessel percentage. The microcirculation parameters need to be distinguished from the microvascular flow index, e.g. blood flow quality, which will be outlined in 6.8..

Several points need to be considered for a correct interpretation of these findings. First, it may be possible that the time period under evaluation was not sufficient to allow for changes in the microcirculation. In comparison to the studies (Nussbaum et al., 2014, Babar et al., 2011, Heimhalt-El Hamriti et al., 2013, Khan et al., 2000) measuring children with diabetes type 1, the period of time in which the developing fetus is under the influence of a diabetic metabolism is limited. The screening test for GDM is performed at the 24th week of pregnancy. Therefore, we can normally assume 16 weeks of a diabetic influence on the newborns.

Only Nussbaum et al. (2014) also used the SDF imaging and the AVA 3.0 analysis program to evaluate the microcirculation under a diabetic influence. Like us, they have been a team of the research department of the University Children's Hospital of the Ludwig-Maximilians-University. Next to our study, the vessel diameter was only measured in the study by Nussbaum et al. (2014) and an accurate comparison can only be made between the two studies considering the microcirculatory parameters. Nussbaum et al. (2014) examined the microcirculation of children who were diagnosed with diabetes type 1 for a minimum of one year. The results of the study showed, next to the reduction of the glycocalyx, a significantly higher percentage of large vessels in children with diabetes type 1.

Other research groups worked with Laser Doppler Flowmetry (Khan et al., 2000, Heimhalt-El Hamriti et al., 2013) or Flow-mediated dilatation (Babar et al., 2011) and did not examine the

FVD, VC or VD. They measured alterations in the blood flow and, thus, will be of further importance in part 6.8..

Finally, it is possible that the glycemic control in the therapy of the mothers was too strict to allow high glucose levels in the blood to be delivered to the fetus via the placenta. The therapy may thereby have prevented further diabetic effects in the newborns.

4.7. How does the vasomotion behave in newborns?

The vasomotion is described as the reaction to the blood flow by vessels in the capillary bed. There are different theories when and how vasomotion occurs.

Vasomotion as well as the microcirculation is often understood as a "network" of vessels, which is influenced by (1) **myogenic** reaction to strain and stress, (2) **metabolic** response to O_2, CO_2, Lactate and H^+ and (3) **neurohumoral activation** based on the autonomic nervous system (Ince, 2005). The different signals seem to communicate in the system adapting the blood flow in order to regulate the oxygen delivery in the tissue.

To our knowledge, the vasomotion has not yet been examined in newborns. We wanted to understand how it behaves under normal conditions as compared to situations of vasomotoric stress.

As reported by other studies, vasomotion seems to occur when metabolic stress is present or can be caused by a limited blood supply (Pradhan and Chakravarthy, 2011), i.e. a limited oxygen delivery, which was produced by an inflated blood pressure cuff in our study.

Looking at the results, the blood flow in the control group did not change significantly between the different points of measurement, except for the flow in the medium vessels. Within the patient group, the blood flow changes in all types of vessels significantly during the limited macrocirculation compared to the other sequences.

One explanation could be that the up-stream vessels in the newborns of the control group show a reaction to the limited blood supply so that the blood flow in the area is not impaired. This assumption can be supported by different studies (Segal, 2005, Vallet, 2002), which have shown that microcirculation works as a complex system in which capillaries transmit signals to up-stream arterioles in order to manage the blood flow and adapt it to the required oxygen level. If more blood supply is needed, proximal arterioles can dilate.

Thus, a functioning vasomotion could be the cause of the continuous blood flow in the newborns of the control group as the blood flow is not impaired in the control group under a decreased blood supply from the providing arteries. Proximal vessels might influence the blood supply in the tissue by not only opening the pre-capillary sphincters, but by diminishing the resistance using dilatation (Secomb and Pries, 2002).

The blood flow, which was examined in the other sequences was continuous in both groups. The difference between the two groups only occurs when the vessels are exposed to stress. In conclusion, the reaction to the limited blood flow might be different, which indirectly supports the theory that vasomotion, e.g. oscillation in the microcirculation, is only present when a change of vessel tone is needed to provide the tissue with oxygen and nutrients.

De Backer et al. (2007) stated that the homogenous flow is better for the oxygenation in the tissue, but the impact of the speed of flow is not yet discovered.

Finally, there are different concepts about microcirculation functionalities. Even if by the current state of scientific knowledge the microcirculation is defined as a network of communicating vessels, it is yet unknown how the physiological flow is designed (Pradhan and Chakravarthy, 2011).

4.8. Is there a difference of the vasomotion in newborns with exposition to GDM compared to newborns without such exposition, and if so, which kind of differences can be observed?

Between the two groups of newborns, we found a statistical difference in the blood flow quality and vasomotion in the microcirculation under a limited supply of blood flow in the macrocirculation.

The results show an impaired blood flow in the microcirculation in newborns of mothers with gestational diabetes whereas the control group showed no sign of limited blood flow in the same sequence.

As described above, vessels are normally able to adapt to the current blood flow with vasodilatation and vasoconstriction. If the blood flow is limited, vessels can widen to ensure a continuous blood flow in the peripheral zone.

In the group of newborns exposed to GDM, however, there seems to be a limited response in the vessels i.e. missing vasomotion, as the blood flow is different in the time of the blood pressure measurement in comparison to the newborns of mothers without gestational diabetes. Here, the blood flow does not change significantly even when the same change in the blood flow supply was made.

In addition to vasomotion, the blood flow might be influenced by other factors which have to be considered carefully in order to find the causes for the altered blood flow in newborns of mothers with GDM.

First, a higher temperature can improve the microcirculation (Genzel-Boroviczeny et al., 2007) and thus lead to a better quality of blood flow. However, both groups of newborns had no need of incubators and were measured at the same room temperature.

Furthermore, the gestational age could influence the microcirculation and is assumed to differ between preterm and term infants. The newborns of the patient group show a significantly lower age, but in a limited range of 40 +/- 2 gestational weeks which was set as a condition in the study design. That time range defines a newborn as a term infant and the difference in this range is therefore of minor importance to the interpretation.
Boys were proven to have a higher blood flow in the first hours of life (Stark et al., 2008). First, the study is based on the findings in preterm infants and our study consists of term infants. Second, the male to female quotient did not differ between the two groups in our study.
Moreover, a different blood pressure could eventually change the microcirculatory parameters, but neither the systolic or diastolic blood pressure, nor the medium arterial blood pressure showed any significant difference between the newborn groups.
While additional factors as mentioned above have been reported to influence blood flows, they are unlikely to have an impact on our findings due to the experimental set-up. Nonetheless, our results should still be interpreted with the caveat in mind that the number of participants in our study has been small and the slight difference between the groups is only significant at one point in time in our sequence of measurements.

The concept of Endothelial dysfunction in Diabetes

A study by Schalkwijk and Stehouwer (2005) has shown that vessel response is impaired in patients with diabetes suggesting as an explanation a loss of function in the vessel wall due to damage in the endothelial cells.
Several research groups examined the concept of endothelial dysfunction in relation to diabetes. To this point, multiple factors and ways were discovered, which could cause the damage of the vessel wall.

It was found that the endothelial dysfunction is already present in children with diabetes type 1 before any clinical symptoms appear (Babar et al., 2011, Khan et al., 2000). Khan et al. (2000) examined the vascular response in the microcirculation of the skin at the dorsum of the foot in children with diabetes type 1. They used laser doppler flowmetry and measured the vasomotion after stimulation with acetylcholine and nitroprusside – two known vasodilators. Acetylcholine acts depending on the endothelium, whereas nitroprusside functions endothelium independent.
The measurements were performed under 44°C of local heating, providing maximum vasodilating conditions for the skin. Children in the patient group showed a reduced vasodilatation after the stimulation with both substances, leading to the assumption of a loss of function in the vessel wall in diabetes.

Measuring a reduced response to both agents, Khan et al. (2000) assumed that the loss of function in the vessel wall must be caused by several factors.
One explanation includes the damage of the endothelial cells resulting in an impairment of the NO production. As the vessels react to NO by dilating and opening up, the response is reduced when NO is missing.
Furthermore, the relation of HbA1c to a good vasodilative response to acetylcholine was shown as inverse, leading the researchers to the conclusion that a poor glycemic control is associated with endothelial dysfunction.
This assumption can be supported by referring also to a study where good glycemic control for a year has already contributed to a better microvascular vasodilative response in patients with diabetes type 2 (Jaap et al., 1995).

As Khan et al. (2000) concluded, if the vasodilative answer to the endothelium-independent factor nitroprusside is also reduced, the answer could not only lie in a damage of the endothelial cells, but could perhaps be found in a direct negative influence on the activity of NO.

In addition, Khan et al. (2000) assumed that a structural loss of function in the vessel wall might also be contributive factor as it might explain the reduced microcirculatory response to local heating in the patient group of his study.

In our study, we found a reduced quality of blood flow in the microcirculation of newborns of mothers with GDM as compared to the control group under a short-term restricted blood flow in the macrocirculation.
As already discussed, and considering what we know about the vasomotion, it could be that the reduced quality of the blood flow is the consequence of a missing vasodilatation in the up-stream arterioles in the patient group. Upon comparing our findings with the results of other research groups, the impairment of vascular response might be caused by the same mechanism in both the newborns of mothers with GDM and the children with diabetes type 1.

As the two systems, the one of the fetus and the one of the mother, are connected via umbilical cord, a diabetic metabolism could affect the development of the vessel wall in the fetus.
However, there seems to be no altered recruitment of capillaries as the Vessel Coverage does not change, nor does the percentage of vessels between the two groups.
This could be explained by a difference between a long-term influence in children who have been diagnosed with diabetes type 1 for over a year or a short-term response in newborns of mothers with GDM.

Nevertheless, the subject of this study is to our knowledge very new to the field and the findings need to be further validated in additional studies.

Hemodynamic influence of insulin

In order to fully understand the possible causes for the findings in our study, one more aspect has to be added to the picture.

Insulin is known to have vasoactive properties, which can influence the blood flow and the up-take of glucose in skeletal muscle.

The underlying process includes an insulin-binding receptor on the surface of the endothelial cells and therefore the ability of the vessel wall to react to the release of insulin.

Insulin can stimulate the endothelium to produce NO, a vasodilator, or ET-, a vasoconstrictor. It is, however, not yet fully understood how the mechanism works (Schalkwijk and Stehouwer, 2005).

Studies (Kim et al., 2006, Potenza et al., 2005) showed how a resistance to insulin can result in an imbalance of vasoconstriction and vasodilatation. In this imbalance, vasodilatation is seen as the normal response, whereas an impaired vasodilatation is interpreted as the pathological or missing answer to insulin (Eringa et al., 2004).

Looking at our study, the lack of insulin, which occurs in a diabetic metabolism, might also contribute to the impaired quality of the blood flow in the group of newborns of mothers with GDM. However, the insulin resistance is present in the mothers and the diabetic metabolism only influences the fetus via the placenta.

It might be possible that the balance between the hormones is not yet established and therefore the reaction to stress is altered. It is yet unknown, if a lack of insulin could continue two days post-partum when the newborns were measured. Moreover, we cannot be certain about how the insulin metabolism works and how soon the babies are able to regulate the release of hormones themselves after birth.

However, it is necessary to consider a hormonal disbalance contributing to the impaired blood flow in the newborns of mothers with GDM.

What is more, research (Mordwinkin et al., 2013, Guzman-Gutierrez et al., 2013, Guzmán-Gutiérrez et al., 2011, Westermeier et al., 2011) showed a defective insulin-signaling in the fetoplacental unit. In these studies alterations in women with GDM as well as in their fetus were already present. It might be possible that a defective insulin-signaling could impact the microcirculation in the first period of the newborns' life. This might be an explanation for the altered response in the group of newborns of mothers with GDM.

4.9. Outlook

To this point, we know that diabetes can cause endothelial dysfunction leading to alterations in the microcirculation and vasomotion in adults as well as in children. The multiple causes for endothelial dysfunction in diabetes are not yet all discovered. However, there are ways and factors that have been demonstrated to contribute to an impaired endothelial function in the vessel wall.
Our study showed for the first time that alterations in the vasomotion also exist in children of mothers with GDM after birth. These findings might add to the knowledge about gestational diabetes mellitus and its consequences for the newborn.

Additional studies should further investigate the role of endothelial dysfunction in newborn infants of mothers with GDM. Glycocalyx as a major cause for altered microcirculation found in children with diabetes type 1 should also be measured in newborns of mothers with GDM.

Further research should also include an acute blood sugar status taken before the measurements of the microcirculation and vasomotion. With this addition, one could distinguish between the results of acute hyperglycemia, perhaps caused by therapeutic glucose infusions, and the influence of a diabetic metabolism during pregnancy on the child.

No alterations were found in the vasomotion or microcirculation of mothers with GDM. Larger clinical trial with more patients who had severe GDM should be conducted to see whether alterations can be found in this group of patients.

5. SUMMARY

Hyperglycemia can cause endothelial dysfunction leading to alterations in the microcirculation and vasomotion in adults with Diabetes Mellitus (DM) 1 or 2 as well as in children with DM 1. Gestational Diabetes Mellitus (GDM) is one of the most common diseases in pregnancy and can be associated with severe consequences for mother and child. The microcirculation and vasomotion were, to our knowledge, not yet observed in newborn infants who were exposed to a diabetic metabolism during pregnancy. Hence, the influence of the maternal diabetic metabolism on the microcirculation of the newborn is still unknown.

It was the aim of the study to analyze potential alterations in newborns' microcirculation and vasomotion with maternal Gestational Diabetes Mellitus (GDM) compared to healthy newborns with no history of GDM as a control group. Moreover, we wanted to know if and how GDM affects the microcirculation of the mothers.
Video sequences of 15 newborns with maternal GDM and 15 otherwise similar healthy newborns were captured using Sidestream Darkfield imaging. The microcirculation was measured at the newborns' ears and arms on the second or third day of life. In order to indirectly analyze vasomotion, the microcirculation was additionally measured at the arm before, during, shortly after (≤10 seconds) and 1 minute after the providing arteries were constrained with the help of a blood pressure cuff. Analyzed parameters have been i) quality of the blood flow (Microvascular Flow Index=MFI, 0= no flow, 1= sluggish flow, 2 = intermediate flow, 3 = continuous flow, 4=hyperdynamic flow), ii) Functional Vessel Density, iii) Vessel Surface Coverage and iv) Vessel Diameter.

MFI was significantly decreased in GDM infants in small vessels (<10 µm diameter) and in large vessels (> 20 µm diameter) indicating a more sluggish flow during compression of the providing vessels in the GDM group compared to the control group. The reason for this could be a reduced vasodilatation in the capillary system. Functional Vessel Density, Vessel Surface Coverage and Vessel Diameter distribution did not differ significantly between the two groups. There was no difference concerning the microcirculatory parameters between the mothers with GDM and the mothers without GDM.

Shortly after birth infants with maternal GDM show a reduced vasodilative answer. These findings could extend our understanding of gestational diabetes mellitus and its consequences for the newborn. If this vascular "stiffness" disappears later or persists is yet unknown.

Zusammenfassung

Hyperglykämien können eine endotheliale Dysfunktion verursachen, die wiederum zu Veränderungen der Mikrozirkulation und Vasomotorik bei Erwachsenen mit Diabetes Mellitus Typ 1 oder 2 führt. Gestationsdiabetes ist eine der häufigsten Erkrankungen in der Schwangerschaft und kann mit schwerwiegenden Konsequenzen für Mutter und Kind einhergehen. Die Mikrozirkulation und Vasomotorik bei Neugeborenen, die einer diabetischen Stoffwechsellage in der Schwangerschaft ausgesetzt waren, wurde unserem Wissensstand nach, bisher noch nicht untersucht. Der Einfluss des diabetischen Stoffwechsels der Mutter auf die Mikrozirkulation des Kindes ist daher bisher ungeklärt.

Es war das Ziel der Studie potenzielle Veränderungen in der Mikrozirkulation und Vasomotorik von Neugeborenen mit mütterlichem Gestationsdiabetes zu untersuchen, um sie mit gesunden Neugeborenen ohne mütterliche Anamnese eines Gestationsdiabetes als gesunde Kontrollgruppe zu vergleichen. Zudem wollten wir herausfinden, ob und wie Gestationsdiabetes die mütterliche Mikrozirkulation beeinflusst.

Video Sequenzen von 15 Neugeborenen mit mütterlichem Gestationsdiabetes und von 15 gesunden Neugeborenen wurden mit der Technik des "Sidestream Darkfield Imaging" aufgenommen.

Die Mikrozirkulation wurde am zweiten oder am dritten Lebenstag am Ohr und am Arm der Neugeborenen gemessen. Um die Vasomotorik indirekt zu messen, wurde die Mikrozirkulation zusätzlich am Arm bevor, während, kurz nachdem (≦10 Sekunden), und 1 Minute nachdem die zuführenden Arterien mittels einer Blutdruckmanschette komprimiert wurden, gemessen. Die untersuchten Parameter waren i) Qualität des Blutflusses (Microvascular Flow Index= MFI, 0=kein Fluss, 1=stagnierender Fluss, 2=intermittierender Fluss, 3=kontinuierlicher Fluss, 4=hyperdynamer Fluss), ii) funktionelle Gefäßdichte, iii) Gefäßoberfläche und iv) Gefäßdurchmesser.

Die Qualität des Blutflusses, der MFI, war signifikant reduziert in den Neugeborenen mit mütterlichem Gestationsdiabetes in den kleinen Gefäßen (<10 µm Durchmesser) sowie in den großen Gefäßen (> 20 µm Durchmesser). Dies deutet verglichen mit der Kontrollgruppe auf einen eher stagnierenden bis intermittierenden Fluss während der Kompression der zuführenden Gefäße hin. Grund hierfür könnte eine verminderte Vasodilatation der kapillären Gefäße sein. Die funktionelle Gefäßdichte, die Gefäßoberfläche sowie der Gefäßdurchmesser unterschieden sich nicht signifikant zischen den 2 Gruppen.

Ebenfalls bestand kein Unterschied in den Parametern der Mikrozirkulation zwischen den Müttern mit Gestationsdiabetes im Vergleich zu den Müttern ohne Gestationsdiabetes.

Kurz nach der Geburt zeigen Kinder mit mütterlichem GDM eine reduzierte vasodilatative Antwort. Diese Ergebnisse könnten unser Verständnis des Gestationsdiabetes und seinen

Folgen für das Neugeborene erweitern. Ob die vaskuläre "Steifigkeit" im fortschreitenden Alter verschwindet oder persistiert, ist bisher unbekannt.

6. LIST OF ABBREVIATIONS

DM	Diabetes Mellitus
DVL	Detected Vessel Length
FVD	Functional Vessel Density
GDM	Gestational Diabetes Mellitus
MFI	Microvascular Flow Index
NO	Nitric Oxide
OGTT	Oral Glucose Tolerance Test
OPS	Orthogonal Polarization Spectral Imaging
SDF	Sidestream Darkfield Imaging
VDD	Vessel density distribution
VS	Vessel Surface

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