Diplomarbeit

Consequences of foetal and neonatal hyperinsulinism on different organ systems

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Eidesstattliche Erklärung

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Zusammenfassung

EINLEITUNG: Die Inzidenz des fetalen und neonatalen Hyperinsulinismus ist steigend und damit auch die Notwendigkeit sich mit den Konsequenzen auseinanderzusetzen. Makrosomie und erhöhter Körperfettgehalt wurden bereits mit erhöhten kindlichen Insulinspiegeln in Zusammenhang gebracht. Da Insulinrezeptoren jedoch im Körper weit verbreitet sind liegen weitere Konsequenzen nahe.

ZIELSETZUNG: Ziel dieser Arbeit ist es einen Überblick über den aktuellen Wissenstand bezüglich morphologischer oder funktioneller Veränderungen der verschiedenen Organsysteme aufgrund von fetalem oder neonatalem Hyperinsulinismus zu präsentieren.

METHODEN: Dafür wurde eine systematische Literaturrecherche in den Datenbanken PubMed und Google Scholar durchgeführt. Untersucht wurden ausschließlich Daten zu Humanstudien und davon jene, die in Englischer Sprache verfügbar waren.

RESULTATE: Neben den bereits bekannten Veränderungen im kindlichen Körperfett, konnte vor allem ein Einfluss auf die Entwicklung und Funktion des Nervensystems, vornehmlich des Gehirnes, festgestellt werden. Des Weiteren zeigen sich Beeinträchtigungen der pulmonalen Oxygenierung und des erythrozytären Metabolismus im kindlichen Organismus. Ebenso ist auch Muskelgewebe, speziell im Bereich der Herzmuskulatur, von Veränderungen im Glucose Stoffwechsel betroffen. Weitere Auswirkungen zeigten sich zudem auf die hepatische Entwicklung und in der Plazenta. In vereinzelten Studien und Fallbeschreibungen wurden Veränderungen in weiteren Organsystemen, wie zum Beispiel der Haut oder inneren Organen, wie Nieren oder Milz, beschrieben.

KONKLUSION: Insulin scheint auf nahezu jedes Organsystem direkten oder indirekten Einfluss zu haben. Für die Ausprägung der Veränderungen im kindlichen Organismus ist allerdings nicht der Schweregrad von Bedeutung, vielmehr sind der Zeitpunkt und die Dauer der erhöhten Insulinspiegel im Blut sind entscheidend. In weiteren Studien bleibt zu klären, welche Langzeitkonsequenzen fetaler und neonataler Hyperinsulinismus mit sich bringen und in welchen Ausmaß die Zusammensetzung der mütterlichen Ernährung Einfluss nimmt.

Abstract

BACKGROUND: The increasing incidence of foetal and neonatal hyperinsulinism mainly due to the increase of pregnancies complicated by diabetes mellitus requires a more detailed understanding of the consequences on the foetal and neonatal organism. Macrosomia and excessive accumulation of overall body fat are well known conditions caused by elevated levels of insulin in the offspring. Insulin receptors are found all over the foetal and neonatal organism, therefore additional impact at other body sites is likely.

OBJECTIVE: The aim of this thesis is to gain an overview of current available knowledge regarding morphological or functional changes in different organ systems due to hyperinsulinaemia within the foetal and neonatal organism.

METHODS: A systematic literature review with literature search within the databases PubMed and Google Scholar was performed. Studies with human data and available in English language were considered for this review.

RESULTS: Besides altered body composition, infants with hyperinsulinism showed significant changes in the nervous system and neurodevelopment as well as changes in oxygenation and red blood cell metabolism. Furthermore, muscle tissue, especially cardiac muscle tissues, is susceptible for impaired glucose metabolism. In addition, effects on hepatic morphology and placental appearance are described. Singular studies and case reports are discussing further changes, i.e. in the skin or internal organ systems like kidneys or spleen.

CONCLUSION: It seems like almost any tissue can be affected by increased insulin levels, directly or on an indirect way. For the outcome the severity of hyperinsulinaemia is not as significant as the duration and time of onset of elevated insulin levels. Further studies are necessary to investigate the long term consequences of hyperinsulinism lasting into childhood and adulthood. Another aspect of further research is dietary care in pregnancy, which might have an even greater impact than currently thought.

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Abbreviations

AC	Abdominal circumference
AD	Abdominal diameter
AFT	Abdominal wall fat thickness
ATP	Adenosin Triphosphate
BG	Blood glucose
BMI	Body mass index
BPD	Biparietal diameter
BWS	Beckwith Wiedemann Syndrome
C	Carbon
C- peptide	Connecting Peptide
CHI	Congenital hyperinsulinism
DM	Diabetes mellitus
EEG	Electroencephalogram
EGG	Electrogastrogram
FDM	Foetus of diabetic mother
fMEG	Foetal magnetencephalogram
GA	Gestational age
GDH	Glutamate dehydrogenase
GDM	Gestational diabetes mellitus
GLUT	Glucose transporter
HbA_{1c}	Glycosylated Haemoglobin
HC	Head circumference
HI	Hyperinsulinism
HOCM	Hypertrophic obstructive cardiomyopathy
IDM	Infant(s) of diabetic mothers
INDM	Infants of non- diabetic mothers
IR	Insulin receptors
LASF	Left atrial shortening fraction
LGA	Large for gestational age
MRI	Magnet resonance imaging
OGTT	Oral glucose tolerance test
PET	Positron emission tomography

PG	Phosphatidylglycerol
PHHI	Persistent hyperinsulinaemic hypoglycaemia of infancy
PHI	Persistent hyperinsulinism
PP	Pancreatic peptide
RBC	Red blood cell(s)
RDS	Respiratory distress syndrome
rER	Rough endoplasmic reticulum
SGA	Small for gestational age
SGLT	Sodium-glucose linked transporter
SP- A	Surfactant protein A
TCB	Transcerebellar diameter
THI	Transient hyperinsulinism
UV	Umbilical vein

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1. Introduction

Foetal and consequently neonatal hyperinsulinism (HI) is a repeatedly discussed issue. Not only because of remaining difficulties in defining the level of hyperinsulinism requiring therapy, which is suitable for all infants, but also is the number of 'infants at risk' increasing over the years due to lifestyle changes in general population. Some consequences, like macrosomia, are relatively well studied compared to other effects, which are rarely known. This thesis aims to give an overview of the consequences of foetal and neonatal hyperinsulinism, exceeding excessive fat accumulation. For better understanding a short review of essential information is given first.

1.1. Glucose metabolism

Glucose plays a central role for energy supply in human cells. In a biochemical process including various steps it is degraded and converted to ATP, a molecule high in energy and fundamental for cellular functioning.

Especially for the foetus glucose is essential for energy supply, about 80% of the foetal energy supply is provided by glucose molecules. The foetal glucose utilisation runs at a higher rate compared to the adult's metabolism. Observed rates in foetuses ranged between 5-7mg/kg per minute compared to 2-3mg/kg per minute in adults. Under normal conditions the foetus is provided with glucose by the mother via the placenta. Maternal glucose levels are within a range of 63 to 100 mg/dl in fasting state and foetal levels are at 54 mg/dl, which accounts for 70-80% of the maternal values. (1,2).

After birth the glucose level of neonates drops to a nadir, especially in the first hour after birth. In 2000 a study showed that the type of delivery does not have a significant impact on the blood glucose. Furthermore, newborns with very low blood glucose of 25 mg/dl, did not show any symptoms of hypoglycaemia (3). This might suggest that the physiological values for blood glucose shortly after the delivery might even be as low as 25 mg/dl. Several studies showed similar results in term babies, with a normal APGAR score and no maternal or neonatal complications (1).

This drop of the glucose level is essential for the neonate to adapt to the altered metabolic situation. As the maternal supply of nutrients to the foetus in disrupted at birth, the newborn's metabolism has to maintain the blood glucose by gluconeogenesis, glycogenolysis and break down of fatty acids. Therefore, the hormonal situation adjusts to the feeding- fasting cycles (4).

1.2. Insulin

For a better understanding of the following thesis it is important to keep the basic knowledge about insulin synthesis, secretion and function in mind.
Insulin is the most important anabolic hormone. It is produced and stored in the pancreatic β-cells. So far, insulin is the only hormone known to be able to decrease the level of blood glucose. This is a highly coordinated process, including an increase of glucose uptake into the cells, intracellular breakdown of glucose, and conversion of glucose into glycogen, proteins and lipids for storage (5).

1.2.1. Insulin synthesis

Insulin is synthesised in the β- cells of the pancreas, which represent 70-80% of the cells in the Langerhans islets. The rest consists of α-cells (20%) and δ-cells (5%), which produce glucagon and somatostatin. Up to 2% of islet cells produce the Pancreatic Polypeptide (PP), a hormone inhibiting the pancreatic enzyme- and bile secretion (6).

The insulin peptide is synthesised in the rough endoplasmic reticulum (rER) and further processed in the Golgi apparatus of the pancreatic β- cells. Insulin is composed of two peptide chains, the α-chain consisting of 21 amino acids and the β-chain of 30 amino acids. Those peptide chains are joined by two disulphides bonding cysteine molecules of the α- and β- chain (5).

The insulin mRNA encodes a precursor protein of insulin named preproinsulin, which is translated in the rER. After modifications of the translated peptide taking place in the cisternae of rER, where the protein is cleaved by a peptidase and folded, disulphide bonds are added and finally pro-insulin is generated. Pro-insulin is build

up by the α- and β-chain and the linking part named “connecting peptide” (C-peptide). Due to the rapid degradation of pro-insulin, further modifications in the Golgi apparatus are necessary. There and partly also in early secretory granules the C-peptide is cleaved by a protease and stored in vesicles together with mature insulin (7). The C- Peptide is used for diagnostic reasons (i.e. in DM), since it reflects the remaining endogenous insulin production in the pancreas.

1.2.2. Insulin secretion

In general, the insulin secretion depends on the extracellular concentration of glucose. The pancreas starts secreting at a blood glucose level of 36-54 mg/dl (2-3 mmol/l) and reaches its maximum at about 270 mg/dl (15 mmol/l). At an adequate metabolism the glucose level does not fall below 72 mg/dl, resulting in a continuous secretion of insulin. This basal insulin secretion maintains the glucose supply of other body tissues than the pancreas (6).

In the foetus besides glucose, another important stimulus for β- cells to secret insulin are amino acids and fatty acids, even if to a lesser degree than glucose (8). Not only are amino acids a stimulus itself, but can also raise glucose- induced insulin secretion. Different amino acids stimulate secretion by different intensities. For example, does the combination of glutamine and leucine increase insulin secretion to a higher degree than glutamine on its own (9).

In the human organism insulin is secreted on a basal level and adapts according to nutritional intake. Glucose as the main stimulant leads to a five- time higher insulin secretion compared to fat or amino acids. Additionally, insulin secretion is affected on a cellular level by intracellular energetic level in pancreatic cells and hormonally, i.e. by oestrogen, melatonin or leptin amongst others (9).

1.2.3. Foetal insulin receptors

Insulin receptors (IR) are present all over the foetal cells with a greater number and a higher affinity to glucose than in adult cells. Especially insulin receptors on hepatic and pulmonic cells as well as erythrocytes and monocytes show a higher affinity to insulin than others (10,11). Animal studies showed a response of insulin

receptors to altered insulin levels. Whereas in adults high insulin levels usually lead to a down- regulation of insulin receptors in numerous tissues, foetal insulin receptors remained unchanged despite increased levels of insulin, especially in neuronal tissue. In early embryonic state a down- regulation of insulin receptors in neuronal tissues and a hyperinsulinaemic state might still be cause of various neurological defects (12). In human foetuses increased binding capacity of insulin in the liver was observed throughout pregnancy. Similar results were obtained for monocytes in newborn infants, where a greater binding capacity was shown in comparison to adults. Not only a higher affinity for insulin was found, but also a higher number of receptors, with even a greater increase in number of IR in hyperinsulinaemic foetuses (13).

1.2.4. Function of Insulin

Insulin is an anabolic hormone and responsible for the storage of energy substrates. Therefore, under influence of insulin carbohydrates and lipids are stored and protein is synthesised. Insulin has different impact on the various body tissues. In the liver, fat tissue and skeletal muscles, insulin suppresses catabolic pathways, like lipolysis or gluconeogenesis, and catalyses glycolysis and glycogen synthesis. In times of excessive energy supply (after meal intake) carbohydrates and fatty acids are transformed into triacylglycerol in the liver and adipose tissue and stored in peripheral cells or fat cells. In the skeletal muscles insulin stimulates glycogen synthesis (5). In foetal period insulin is important for growth of numerous tissues (14).

1.2.5. Glucose transport

For glucose uptake, cells express special glucose transporters (GLUT). So far there are fourteen transporters known to be encoded on the human genome, GLUT1 to GLUT14. Each of them has a specification in function and hence also can be found in particular cells or organs. Some of them transfer glucose according to the glucose concentration, others are dependent on insulin (15). Further information about glucose transporters is given in tables 1.

Nevertheless, glucose transporters are not only specified on glucose molecules. Especially GLUT5 and GLUT11 mainly carry fructose and GLUT7 i.e. shows a high affinity to glucose and fructose molecules (15).

For insulin secretion GLUT1 is fundamental, as it is an insulin independent glucose transporter and found on a broad spectrum of different cell types. Thus, GLUT1 activity is proportional to the blood glucose level. Likewise is the influx of glucose in pancreatic β- cells, regulated by GLUT1. Consequently, the intracellular rate of glycolysis rises, leading to an increase of intracellular ATP. The ATP binds to the sulphonylurea receptor, which is part of the potassium (K^+) channel. This results in closure of the K^+ channels and depolarisation of the cell membrane, Ca^{2+} channels open and Ca^{2+} enters the cells. This sequence of steps induces the fusion of the insulin containing vesicles inside the pancreatic β- cells with the plasma membrane, which leads to the release of insulin (6).

	GLUT transporter	Insulin dependency	Sites of expression
Class 1	GLUT 1	independent	ubiquitous
	GLUT 2	independent	β- cells, liver, kidneys, small intestine
	GLUT 3	independent	ubiquitous: i.e. brain, placenta, kidneys
	GLUT 4	dependent	skeletal muscles, adipocytes, heart
	GLUT 14	independent	testis, similar in structure to GLUT3, testis
Class 2	GLUT 5	independent	jejunum, testes, kidney
	GLUT 7	independent	small intestine, colon, testis, prostate
	GLUT 9	independent	kidney, liver, placenta
	GLUT 11	independent	adipose tissue, heart, kidney, skeletal muscles

	GLUT transporter	Insulin dependency	Sites of expression
Class 3	GLUT 6	independent	brain, spleen, peripheral leukocytes
	GLUT 8	independent	brain, testis
	GLUT 10	independent	brain, skeletal muscle, heart, lung, placenta, kidney, liver, pancreas
	GLUT 12	dependent and independent, contingent on cell type	adipose tissue, skeletal muscle, placenta, small intestine
	GLUT 13	independent	adipose tissue, kidneys, brain (cerebellum, hippocampus, hypothalamus, brain stem)

Table 1: facilitated glucose transport – GLUT (15–18)

Besides GLUTs, sodium-glucose linked transporter (SGLT) represent a second major class of glucose transporting molecules. These transporters perform a symport of glucose and sodium ions, meaning the two molecules are transported in the same direction. SGLTs are localised mainly in the small intestine, where they are in charge of nutrient resorption and in kidney tissue for reabsorption of glucose. In contrary to GLUTs SGLTs are independent on insulin and neither on glucose, however the glucose transport is based on a sodium concentration gradient (15).

1.3. Glucose homeostasis in fasting state

Whilst insulin is the only hormone to decrease the blood glucose levels, it has various antagonists. By complex interactions numerous hormones stabilize the glucose values in fasting periods at a range between 60-100 mg/dl. This concentration accounts for normal fasting glucose throughout human life, i.e. for infants, children and adults (1).

In case of deficient energy supply antagonizing hormones of insulin, like glucagon or epinephrine, are responsible for a rise of plasma glucose levels via gluconeogenesis and glycogenolysis (5).

During a fasting period the secretion of insulin is lowered and hormones with adverse effects rise in concentration. Hence, alternative energy supply is provided by the liver, adipose tissue and skeletal muscles. Ketonbodies and glycogen is mobilized within the tissues and used by peripheral cells due to the lack of carbohydrate supply. Additionally to glucagon and epinephrine, which are secreted in primarily, cortisol and growth hormones are responsible for long term glucose homeostasis (5,19).

After birth hormonal adaptions to short term changes in metabolism are observed to maintain energy supply of the cells. In contrast to this, during foetal period changes in hormones result from long term disturbances. Therefore, changes in insulin and glucagon secretion are observed in foetuses of diabetic mothers with an increase of insulin secretion and a suppression of glucagon secretion (10).

1.3.1. Glucagon

Glucagon, a polypeptide, is synthesised and secreted by the α- cells of Langerhans islets in the pancreas. Its secretion is stimulated by low blood glucose, amino acids and epinephrine (19). As one of the main opponents of insulin, glucagon elevates the blood glucose level by stimulating glycogenolysis, gluconeogenesis as well as β-oxidation and ketogenesis in the liver and muscle (5). In foetal development glucagon is detected at around 15 weeks of gestation, with a maximum in concentration at 24 to 26 weeks of gestation (10).

Besides low glucose levels, glucagon secretion is stimulated by amino acids and fatty acids. Other than glucose, which stimulates insulin secretion only, do amino acids and fatty acids stimulate both, glucagon and insulin secretion (20).

1.3.2. Epinephrine

Together with glucagon epinephrine is responsible for acute elevation of blood glucose. As so called stress hormone, it rapidly raises the blood glucose concentration for short term. In favour of this, epinephrine activates the release of glucagon and stimulates glycogenolysis as well as gluconeogenesis in the cells of liver and skeletal muscles (5,19).

1.4. Embryologic aspects of insulin secretion

The pancreas develops out of two endodermal buds, a ventral one beneath the liver primordium and one dorsal bud cranial to the liver primordium. The buds can already be distinguished at day 30 of pregnancy, when the embryo is about 5 mm in length. The buds continuously grow, until they fuse (21). Already at week 10 to 12 of gestation, the Langerhans islet cells can be distinguished from the pancreatic epithelium. At the end of the first trimester insulin can be detected in the foetal blood (14). Pancreatic development and insulin secretion start early, but progressive development continues up to the age of 2 years (22).

1.5. Foetal and neonatal hyperinsulinism

G.F. Laidlaw (23) was one of the first to describe the symptoms of hyperinsulinism and introducing the term ‘nesidioblastoma’ in 1938. In his article, published in the ‘American journal of Pathology’, he described his findings as ‘adenomas of the islets of Langerhans’ consisting of ‘nothing but gigantic islets of Langerhans’. By now the terms ‘persistent hyperinsulinaemic hypoglycaemia of infancy’ (PHHI) and ‘congenital hyperinsulinism’ (CHI) are more common.

An insulin level below 5 mU/l is considered to be normal (24) and more than 10 mU/l is referred as hyperinsulinaemia (25). However, setting insulin in relation to the glucose level seems more adequate for specification of hyperinsulinism. Therefore an insulin/glucose ratio of 0.4 is considered to be hyperinsulinaemic at least for most of the (25)

1.5.1. Causes and risk factors of hyperinsulinism

It is highly recommended to screen neonates at risk for hyperinsulinism. Therefore, a complete history of the mother has to be taken. Any information about previous cases of hyperinsulinism within the family is crucial. Despite genetic factors also a holistic history of course of pregnancy might give a hint for possible hyperinsulinaemia in the infant. Risk factors for the foetus include maternal diabetes mellitus (gestational or not), pre-eclampsia/ eclampsia, LGA or SGA newborns, premature or beyond term delivery, as well as birth deformities (26).

In case of maternal diabetes mellitus (DM) in early pregnancy the foetus is exposed to hyperglycaemia early in development. This leads to hypertrophy of the islets in the pancreas and hyperplasia of the β- cells in foetus and neonates. Additionally, the maturation of the insulin- producing- cells is accelerated. As a consequence, not only higher amounts of insulin are observed, but also foetal insulin is detected earlier in pregnancy (27,28).

Referring to the case report by West and Thorpe in 2011, also maternal nutrition has a great impact on the foetus. A high glucose intake by the mother, can lead to hyperinsulinism of the newborn later on. In less severe cases the hyperinsulinism is transitional and resolves spontaneously (24).

1.5.2. Maternal Conditions

Maternal diabetes mellitus and obesity are the main risk factors, besides genetics, for the infant to develop hyperinsulinism. Poorly controlled DM can lead to (transient) hyperinsulinism in the foetus and neonate. Therefore, diagnostic guidelines with tight therapeutic measures need to be followed.

1.5.3. Diabetes Mellitus, pre- existing

The prevalence of diabetes is increasing, mainly caused by changes in diet and lifestyle. It is estimated to affect about 430 million people by 2030, compared to

371 million back in 2012 and 80 million in 1985. There are different forms of diabetes, which need to be differentiated (5).

Type 1 is an absolute deficiency of insulin due to autoimmune destruction of the pancreatic β- cells. Affected individuals are usually younger than 35 years of age at onset of the disease and are dependent on external administration of insulin in terms of therapy (5).

Type 2 DM is a relative insulin deficiency, usually occurring in adipose patients of advanced age (>40 years). For Type 2 DM two components need to be considered, on the one hand peripheral insulin resistance and on the other hand impotent synthesis of insulin by the islet cells. Most important therapeutic measures are lifestyle changes with focus on diet and physical exercise. Patients suffering from Type 2 DM account for approximately 90% of all diabetic patients (5).

1.5.4. Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is limited to pregnancy and of special interest regarding the development of foetal and neonatal hyperinsulinism. It develops during pregnancy and usually resolves with giving birth. The pregnant women are tested at 24 to 28 weeks of gestation with an 'Oral-glucose-tolerance-test' (OGTT). To exclude pre-existing DM the blood glucose level as well as HbA_{1c} are commonly tested after an overnight fast at the first gynaecological visit in the first trimester, as GDM is defined with an onset beyond 24 weeks of gestation. The OGTT is performed with 75g of glucose dissolved in 300 ml water. Blood glucose is measured at the beginning after 8 hours fasted state, 1hour after the intake and 2 hours after the intake, treshold values for pathologic results are demonstrated in table 2. Blood is drawn of a peripheral venous site. In case of blood glucose levels at fasted state ≥126 mg/dl the diagnosis of Diabetes Mellitus can be set without an OGTT (29,30).

Time of sample taking	Venous blood sampling
Fasting	> 92 mg/dl
1 hour	>180 mg/dl
2 hours	>153 mg/dl

Table 2: OGTT threshold values (29)

Depending on the initial blood glucose level, measured before 24 weeks of gestational age, the German diabetes association offers following approach for high risk patients in two phases (30):

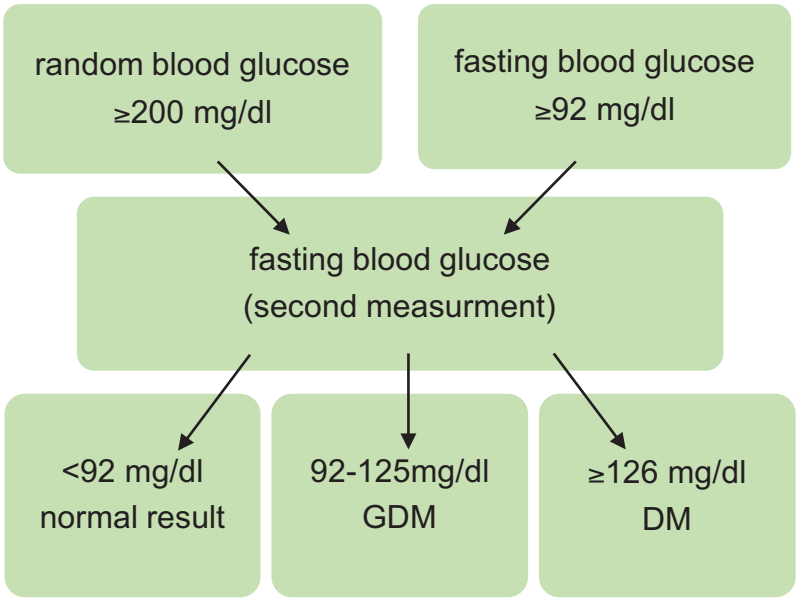

Figure 1: Approach high risk pregnancies for DM before 24 weeks of gestation

1.5.5. Maternal obesity

Infants born to obese women show a higher prevalence of overgrowth and macrosomia. Maternal obesity is often referred to as a pre- diabetic condition, which might be undiagnosed by the OGTT but still lead to moderate hyperglycaemia in the foetus. Besides overgrowth infants of obese mothers show an increase in perinatal death and congenital malformations. The neurological and cardiovascular system are the ones to be mainly affected in the neonates (31,32).

1.5.6. White Classification

In 1949 Priscilla White (33) published a study with data collected from 439 infants of diabetic mothers. Besides stillbirths and early neonatal death, she described cases of skeletal malformations, congenital heart malformations, anencephaly and some more. Based on the foetal and maternal findings, White established an often cited classification. Classes from A to F consider many different risk factors, whereas A to E refer to foetal conditions, F refers to maternal risk.

Class A	Patients had a slightly abnormal GTT, few dietary changes are necessary, no insulin required; this class comes with the highest survival rate for the foetus	5%
Class B	Onset of DM ≥20 years of age, duration < 10 years and no vascular disease	29%
Class C	Onset of DM 10-19 years of age or duration 10-19 years or minimal vascular disease	44%
Class D	Onset of DM <10 years of age or duration ≥20 years or signs of advance vascular disease (retinitis, transitory albuminuria or transitory hypertension)	14%
Class E	Pelvic artery calcifications demonstrated on x-ray	7%
Class F	All participants diagnosed with nephritis	1%

Table 3: White Classification, percentages referring to the study- population of White, 1949 (33)

1.5.7. Incidence and prevalence of hyperinsulinaemia

Knowledge of hyperinsulinism is scarce due to the lack of existing data. It is estimated to affect 1 infant of 40 000 to 50 000 in the general population, with an increase up to 1 of 2500 in populations with high consanguinity (34).

Various papers show that male infants seem to be more affected. Avatapalle et al. (35) reported about 78% male infants with CHI in a cohort of 67 infants examined in terms of neurodevelopmental delay. For cardiomyopathy or myocardial hypertrophy is estimated to appear in 25- 30% of all IDM and macrosomia in about 25% of infants born to a diabetic mother (36,37).

At the moment no clear data for overall prevalence of foetal and neonatal hyperinsulinism has been collected. An increase over the last decades is suspected as the prevalence of gestational diabetes mellitus has increased. The worldwide prevalence of hyperinsulinaemia is estimated to be at 7% today, compared to 0.9% back in 1980, but ranges from 1% to 14% depending on the geographical region. Numbers change with different populations especially with high consanguinity and with advanced pregnancy. Highest prevalence was found in studies with multiple measures in second and third trimester. Due to the increasing numbers of GDM it is recommended to perform an oral glucose tolerance test (OGTT) within 24 to 28 weeks of gestation, especially in women with high risk. Typical characteristics are obesity, history of GDM or family history of (gestational) DM. For women meeting all of the following criteria a OGTT is not necessarily recommended, as they are considered to be in low risk for GDM: (1) <25 years of age, (2) normal weight before pregnancy, (3) low prevalence of GDM within the related ethnic group, (4) no first-degree family members with DM, (5) no history of suspect glucose testing and (6) no history of adverse pregnancy outcome. At the moment a 50g oral glucose testing is recommended primarily for women at risk, with this approach about 80% to 90% of women with GDM are detected. A 100g oral glucose testing brings more accurate results. But also the week of gestation in which the OGTT is performed seems to have an impact on the accuracy of the result (38,39).

For developed countries overweight and obesity increased over the last decades. In the United States, i.e., in 1999 28.4% of women aged 20-39 were obese. Until 2010 this number increased to 34.0% (31). In 2008, the overall age adjusted prevalence for overweight and obesity was about 64.1% among women (40).

On the one hand, the overall increasing numbers might be due to clearer definition of GDM and increasing diagnostic measures. On the other hand, the underlying cause could be the lifestyle changes within the last decades, with decreased physical activity and more affordable processed food.

1.5.8. Types of hyperinsulinism

One classification of hyperinsulinism is made by the duration of present hyperinsulinaemia. In this case, three types of hyperinsulinism can be differentiated – (1) transient, (2) prolonged and (3) persistent.

(1) **Transient** hyperinsulinism usually is secondary and related to maternal diseases or events during labour. Maternal related conditions leading to transient hyperinsulinism are for example maternal diabetes or maternal drug intake, i.e. sulphonylurea. Gestational as well as insulin- dependant maternal diabetes are risk factors for hyperinsulinism (41).

Beside these, events happening during labour do have influence on the newborn. Therefore, birth asphyxia and glucose infusions during delivery can lead to transitional hyperinsulinism (41).

(2) Hyperinsulinaemia is considered to be **prolonged**, if it lasts for several weeks. Influencing factors are perinatal- stress, like maternal pre-eclampsia, birth-asphyxia or prematurity. Additionally, prolonged forms are associated with hypopituitarism and Beckwith- Wiedemann- Syndrome. Like the transitional form, prolonged hyperinsulinism resolves spontaneously within weeks after birth (42).

(3) On the other hand, **congenital and persistent** hyperinsulinaemic hypoglycaemia mostly occurs due to genetic abnormalities. Congenital hyperinsulinism furthermore can be subdivided into channelopathies, enzyme defects or transcription error; whereas the latter plays a minor role compared to the others (41).

The most common and severe form belongs to the channelopathies. ATP-sensitive potassium channel hyperinsulinism is due to mutations in ABCC8 and KCNJ11 genes, encoding subunits (SUR1 und Kir6.2) of the K_{ATP} – Channel. This mutation leads to non functioning or absence of the K^+- channel in the β- cells and consequently, the cell membrane is depolarised and the insulin secretion deregulated (43).

The second most common form of persistent hyperinsulinism is an enzymatic error. The hyperinsulinism- hyperammonaemia syndrome, also known as glutamate-dehydrogenase- hyperinsulinism, is the consequence of a mutation in the GLUD 1 gene. This dominantly inherited mutation leads to changes in the mitochondrial glutamate dehydrogenase (GDH) activity. GDH catalyses the deamination of glutamate resulting in a α-ketoglutarate and ammonia. The increased level of α- ketoglutarate leads to an increased activity of the citric acid cycle and consequently the ATP/ADP ratio in β-cells rises. As an effect of the increased ATP/ADP ratio in the cells higher amounts of insulin are secreted. Therefore, the insulin level and hence the hypoglycaemia is connected to the protein intake (41,44).

Glucokinase- hyperinsulinism is a less common, dominantly inherited form of CHI. Cause of the hyperinsulinism is a higher affinity of the enzyme glucokinase to glucose. Glucokinase acts as a glucose sensor in the pancreatic β- cells. Hence, the glucose dependent insulin secretion is impaired. Due to the high affinity of the mutated gucokinase levels of plasma glucose as low as 27mg/dl occur instead of normal levels of about 90 mg/dl. The high intracellular levels of glucose lead to closure of the K_{ATP}- channel and insulin secretion despite low levels of plasma glucose (42).

1.5.9. Histological forms of hyperinsulinism

Additionally, depending on the genetics different histological forms develop. Diffuse, focal and atypical forms of hyperinsulinism are differentiated by distribution of the β- cells.

A homozygote mutation in both alleles results in **diffuse** disease of the β- cells. The diffuse type is distributed over the pancreas and characterised by overactive cells. Up to 5% do have enlarged nuclei and abundant cytoplasm (41,43). In **focal** adenomatosis of the β- cells heterogenic genes are found. The cells of the focal form resemble those of the diffuse form, again they are increased in size with a lot of plasma and enlarged nuclei. Small regions of mutated cells are found within the pancreas, with 2.5 to 10 mm in diameter (41). The **atypical type** is not completely understood yet, histologically it shows characteristics of both, focal and diffuse,

types. Genetically in some patients the atypical form can be explained by mosaicism (41).

1.5.10. Diagnosis of hyperinsulinism

The diagnosis of hyperinsulinaemic hypoglycaemia is made on clinical and biochemical characteristics. To identify the type of hyperinsulinism genetic screening and pancreatic imaging is performed.

Following biochemical parameters appear in case of hyperinsulinism (25,45):

- ➔ hypoglycaemia, hypoketonaemia and hypofattyacidaemia, allegeable by the effects and counter regulatory effects of insulin
- ➔ inappropriate levels of insulin (>10mU/l)
- ➔ insulin to glucose ratio of 0.4
- ➔ demand of high glucose infusion rate to attain normoglycaemia (20mg/kg/min)

In some cases CHI presents without detectable hyperinsulinism, but shows an inappropriate suppression of insulin secretion, therefore the ratio of insulin to glucose as a diagnostic criteria can be taken into account (42).

Pancreatic imaging is performed by PET scan, mainly to distinguish focal from diffuse hyperinsulinism. Although the symptoms in both forms remain the same it is still important to distinguish, as the final treatment methods are different.

An uptake of DOPA in pancreatic β- cells was shown. This is now used to distinguish the focal and diffuse mutations of the β- cells. The children are sedated and under general anaesthesia. At PET scans flurorine-18 l-3,4-dihydroxyphenylalanine (^{18}F-fluroro-L- DOPA) is injected intravenously and 10 minutes after the injection examination starts. The imaging takes several hours to detect with signals captured at 10 minute intervals. During this period of time, blood glucose is maintained at about 70mg/dl via infusion. For preparation any glycaemic medication is discontinued days before the imaging (46,47).

A holistic investigation with biochemical parameters, genetic screening and pancreatic imaging is crucial for further treatment.

1.5.11. Management of hyperinsulinism

For an adequate treatment of infants with hyperinsulinaemia a multidisciplinary approach is crucial. First step is to raise blood glucose level and stabilize at a minimum of 70mg/dl, as glucose is the main source of energy, especially in early infancy. To maintain glucose level infusions as high as 30 mg per kilogram and minute might be necessary (42).

First line medical treatment in hyperinsulinaemic hypoglycaemia is diazoxide. Diazoxide is a K_{ATP}- channel inhibitor, leading to prolonged opening of the channel. The inhibitor has a high responding rate in patients with hyperinsulinism. In case of non responding patients after 4 days, a K_{ATP}- channel mutation must be suspected, since diazoxide needs functioning potassium channels to unfold its pharmaceutical effects. In case of no improvement of the situation, despite rising dosages of diazoxide, genetic screening is indicated. An effective second line treatment is the somatostatin analogue octreotide. Stabilizing the K^+- channel, it reduces the entry of calcium and inhibits the insulin secretion. Further research on medical treatment, like Glucagon-like-peptide 1 receptor antagonists, is performed (42,48). One of the most common adverse effects of diazoxide therapy is hirsutism and in case of long term therapy diabetes mellitus is commonly reported (49).

Second line treatment is octreotide, as somatostatin analogue it has insulin inhibitory effects. It is used as single therapy or in combination with diazoxide, depending on the severity of hyperinsulinaemic hypoglycaemia. Different studies showed an increased risk for necrotising enterocolitis (NEC) in infants with hyperinsulinism treated with octreotide. Reaching from 0.9% to 2% of cases with NEC. Most cases are reported with an onset following few days after initiating octreotide therapy, presenting with diarrhoea and vomiting (50,51).

As a third therapy alternative sirolimus is frequently investigated. Sirolimus is a mTOR inhibitor, suggesting to have a positive effect on β- cell proliferation and insulin production. In a study published 2014 in the NEJM sirolimus is mentioned as an alternative therapy to pancreatectomy. The use should be considered, when HH is unresponsive to maximal dosages of diazoxide or octreotide. Sirolimus successfully led to stabilisation of blood glucose level in single therapy in 3 out of 4

cases. The fourth infant required a combinatory treatment of sirolimus and octreotide. No severe side effects of sirolimus have been reported (52).

In case of unsuccessful medical treatment surgery can be performed as final action. Antecedent distinction of focal and diffuse HI is essential to determine the required type of surgery. Focal HI can easily be treated by removal of the focal lesion, whereas the diffuse HI requires a nearly total pancreatectomy (95- 98% of pancreatic tissue). Nevertheless, some patients, who underwent pancreatectomy still had frequent episodes of hypoglycaemia (42,48,52).

1.6. Hypoglycaemia in neonates

Hypoglycaemia is the immediate consequence of hyperinsulinism in neonates. Nevertheless, to define a specific level of blood glucose as ‘too low’ is difficult. Some newborns are without symptoms at a level as low as 25 mg/dl (3). This is one of the reasons why a clear definition for hypoglycaemia is hard to find. The threshold for brain associated symptoms is depending on the individual. Thus, it is not possible to set a universally appropriate value. Additionally the result of measuring the glucose level can differ depending on the method used (26).

The brain utilises most of the available glucose, therefore low glucose levels lead to neurologic symptoms like confusion, coma or seizures. Furthermore, adrenergic symptoms, like tremor and anxiety, and cholinergic symptoms, such as sweating and hunger, are early but unspecific symptoms of hypoglycaemia (26). Further symptoms are shown below in table 4. Typically patients show the ‘Whipple triad’ consisting of documented hypoglycaemia, with suitable symptoms and recovery of the symptoms after carbohydrate intake or rather increase of blood glucose levels (25,26). In case of hyperinsulinaemic hypoglycaemia infants do not show and adequate raise of blood glucose and symptoms, like those mentioned below, are persisting.

▪ Coma	▪ Poor feeding
▪ Seizures	▪ Lethargy
▪ Change of consciousness	▪ Muscular hypotonia
▪ Sweating	▪ Apnea
▪ Tremor	▪ Hypothermia

Table 4: Symptoms of hypoglycaemia in neonates (20,47)

Nevertheless, during labour the infants blood glucose is about 70-80% of the maternal BG level. Within the first hour of life the neonatal glucose level physiologically falls to a minimum of 20-25 mg/dl, but will rise again in the following hours and days (53).
A glucose level below 30 mg/dl within the first 24 hours of life is generally referred to hypoglycaemia. After this time period the threshold is set at 45 mg/dl (54).

Due to the fact that the symptoms of hypoglycaemia are unspecific, neonates at risk should be screened for hyperinsulinism and blood glucose level needs to be measured regularly. Figure 4 gives an overview of the main risk factors for hypoglycaemia and those infants, who should be screened.

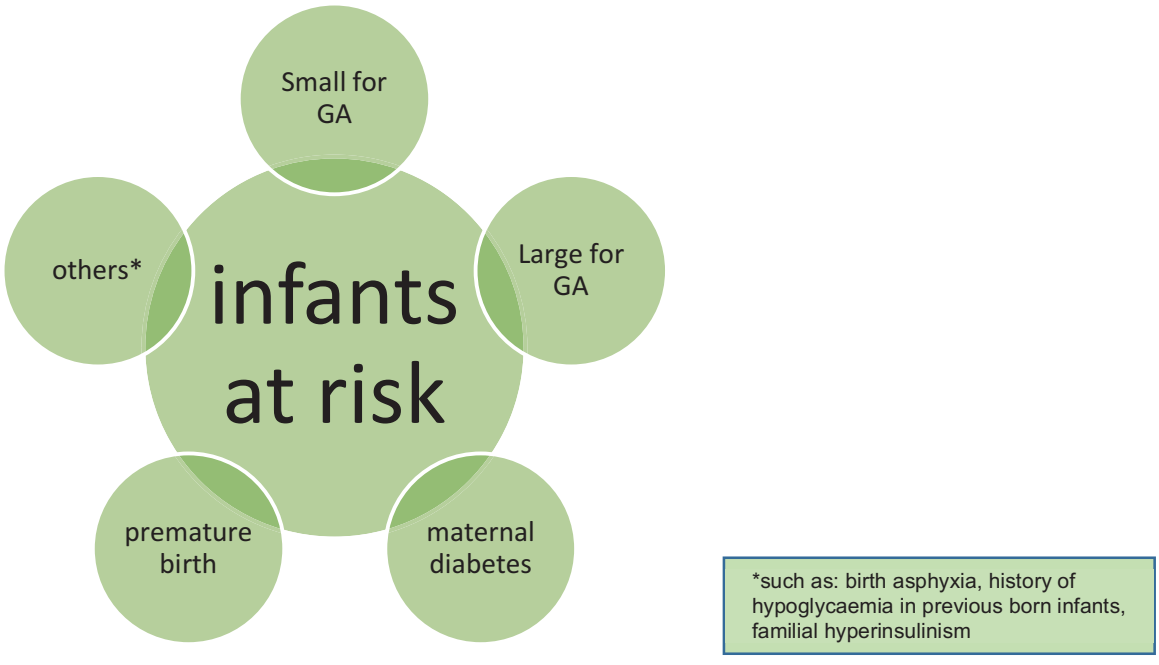

Figure 2: Main risk factors for hypoglycaemia in infants (55,56)

A study of 2012 showed that approximately half (51,9%) of the babies identified to be at risk actually developed hypoglycaemia. In this research the threshold for the diagnosis of hypoglycaemia was set at 45 mg/dl (55). Years before, Pildes et al. presented their study with an incidence of 6% of neonatal hypoglycaemia in infants with low birth weights, using a threshold of 20 mg/dl (57). McGowan in 1999 claimed neonatal hypoglycaemia to reach an incidence of 8% in LGA infants, mainly born to diabetic mothers (56).

As no clear definition is found yet and and thresholds are variable in individuals, the diagnosis of hypoglycaemia is a combination of measured blood glucose levels and the infant's symptoms.

2. Methods

The aim of this thesis is to give an overview of the consequences of foetal and neonatal hyperinsulinism. In favour of this a systematic literature review was the method of choice. Literature was obtained from the Medical University's library in Graz and of various online data bases.

In the end suitable sources were taken of books and following databases:

- PubMed
- Google Scholar

Including criteria for literature search for the review were:

- human data only
- English or German language
- Insulin levels or insulin to glucose ratio measured
- Infants at high risk for hyperinsulinism
- Any group size >5 participants for organ specific review
- Case reports for overall changes

For discussion additionally animal studies were included.

Following search terms including all possible variations and combinations were used:

- foetal
- neonatal
- congenital
- persistent
- transient
- hyperinsulinism/hyperinsulinaemia
- hypoglycaemia
- PHHI
- consequences
- maternal diabetes mellitus
- enlargement
- large for gestational age
- hormones, endocrine system
- glucocorticoids, adrenal gland
- diabetic foetopathy
- placenta
- organs
- lung, respiratory system
- heart
- neurodevelopment, brain
- body composition/fat
- macrosomia
- liver
- gastrointestinal (dysmotility)
- kidney, urinary system
- thyroid
- spleen
- dysfunction
- haematology, erythropoiesis
- hypoxia
- immune system
- Cord blood

Those terms were connected in modified terms by AND as well as OR for literature search in PubMed and Google Scholar.

Resources were mainly collected from November 2017 to July 2018, and were extended by single papers afterwards, extracted from list of references of included studies.

To gain an overview the primary search terms on PubMed were “congenital hyperinsulinism” with 788 human specified items found and “foetal hypoglycaemia” with 1451 human specified items found. Limited to the past 5 years, from 2017 backwards, 231 articles were left. The search for “neonatal hypoglycaemia” with the identical criteria (5 years, human only) showed 715 results. Without limitation for date of publication “neonatal hyperinsulinism” showed 2063 results, whereas the search for “neonatal hypoglycaemia” resulted in 4406 human specified items. The search term for “congenital hyperinsulinism” resulted in 871 items on PubMed, with similar results if “PHHI” was used.

In total 63 appropriate resources were considered for reviewing, after eliminating those with imprecise information about the study protocol or data of subjects and those with additional conditions to hyperinsulinism. Those studies ranged from a population group with a minimum of 10 individuals to a maximum population group of 829 individuals. One study used clinical data of 21142 patients for control parameters. Studies discussing additional conditions, such as congenital malformations or maternal hypertension, were excluded as too many factors affect the infants condition and a clear cause for infantile changes could not be detected in most reports. In that case it mostly is an interaction of several different comorbidities and symptoms can not be assigned to hyperinsulinism solely. In the end, including animal studies, 72 studies were used to discuss the consequences of foetal and neonatal hyperinsulinism.

3. Results

The impact of hyperinsulinism is allegeable due to the combination of a hyperglycaemic and anabolic state in foetal period and hypoglycaemia in neonatal period. The lack of available glucose in neonates is intensified by suppression of alternative fuels, like fat breakdown, due to excessive levels of insulin and therefore inhibiting the secretion of katabolic hormones. This situation leads to an overall deficiency of possible energy supplements in the infant.

Bennewitz in 1826 was the first one to describe a case of a pregnant women with diabetes mellitus. In his report he described an abnormal appearance of the infant. For a long period of time pregnancy with diabetes was lethal for the mother and/or the child. A liveborn baby of a mother with diabetes was regarded as “divine lifesaving grace” (58,59).

3.1. Nervous system

As glucose is the main source of energy for the neurological system, severe consequences of hypoglycaemia can be observed. Following studies show that neonatal onset of hypoglycaemia has more severe consequences than onset beyond neonatal period. But changes were already observed in foetal period.

As early as 1973, Pildes (60) described in a paper published in 'The New England Journal Of Medicine' changes in the EEG and sleeping- pattern in IDM, suggesting a neurodevelopmental immaturity at an early age.

3.1.1. Changes in foetal period

In 2015 Linder et al. (61) stated the hypothesis that "the foetal brain response to maternal oral glucose load is prolonged in women with gestational diabetes mellitus compared to healthy pregnant women".

The tests to affirm this theory were performed using foetal magnetencephalography (fMEG), a non invasive method to measure intrauterine foetal neuronal activity directly. It is a method to quantify foetal heart activity, spontaneous brain activity and evoked response brain activity to auditory and visual stimulations. Evoked response can be described by amplitude and latency. Whereas the amplitude depends on the position of the sensor and the foetus, latency does not. Therefore, latency is taken for comparison of two individuals (62).

Linder et al. (61) analysed the data from 40 women, 12 of them diagnosed with GDM. Foetal response is detected by fMEG. After an over night fasting period all women underwent a 75g- OGTT. Blood samples were taken before the glucose load, 60 minutes and 120 minutes after the intake. The fMEG measurements were performed right after the blood samples were taken. The foetal auditory evoke response to a 500 Hz- tone was measured. The tone was produced by a speaker and transmitted to a plastic bag placed between the maternal abdomen and a sensor. Results of this study showed a prolonged reaction time of IDM compared to the control group. In the control group, infants showed a faster response to the tone at the 60- minute measurement after glucose intake compared to the response at the beginning of the test right after a fasting period. Whereas, the infants in the diabetic group showed a prolonged reaction time after glucose intake. This suggests that maturation of the brain and thus cognitive brain function is impaired already in intrauterine period. This especially accounts for postprandial period. No difference between the two groups were observed at reaction time after the fasting period. In this study, Linder and colleagues postulated that the prolonged response latency might be due to increased glucose levels interact with the increased insulin levels.

Alternatively, due to the consistent hyperinsulinaemia insulin resistance might occur and hence weaken the stimulating effects of insulin.

A case study including four neonates diagnosed with transitional hypoglycaemia, three due to hyperinsulinism, used ultrasound, electroencephalogram (EEG) and MRI to investigate brain structure and abnormalities. Examinations were performed several times within the first week of life. MRI imaging revealed restricted diffusion in the cortex and white matter of the occipital lobe, corpus callosum and optic radiations. Additionally, abnormal signals in the posterior parieto- temporal lobe and cerebral cortex were detected at least in half of the patients. The follow- up of 3 infants showed a generalized atrophy of the occipital lobe. One infant diagnosed with hyperinsulinaemic hypoglycaemia showed microcephaly and visual impairment at the follow- up at 12 months of age. The changes in brain structure seen as early as in the first days after birth indicate abnormal brain development already in foetal period (63).

3.1.2. Changes in neonatal period

A retrospective study by Menni et al. (64) in 2001 with 90 patients diagnosed with PHHI showed the consequences of hypoglycaemia on neurological development., characteristics of the cohort are shown below in Table 5. Out of the 90 patients 54 had neonatal onset of hyperinsulinaemia and consequently fell into a hypoglycaemic state. Including criteria were hypoglycaemia with an inappropriate high level of insulin. Patients with transient hyperinsulinism or syndromes associated to hyperinsulinaemia were excluded from the study. Mainly hyperinsulinism was diagnosed due to first episodes of symptoms of hypoglycaemia, such as seizures or generalized hypotonia. In this study patients presented with intermediate to severe psychomotor retardation and epilepsy as the most common consequence of PHHI with neonatal onset. Moreover, patients requiring surgery had more severe symptoms. Seven patients suffered from major retardation, 6 of those had neonatal onset and again 6 patients with major retardation showed symptoms within the first hours of life. This results are concordant to previous studies, where patients with onset early in neonatal period had a worse outcome compared to onset in infancy. Also retardation was more

severe in patients requiring surgery than those treated medically. The population requiring surgery was to small for further division into subgroups for diffuse and focal hyperplasia, thus significant results in statistical analysis could not be obtained for the different forms of hyperplasia. Of those seven patients three had frequent seizures and three different patients developed generalized hypotonia. For patient 2 and 6 hyperinsulinism was not detected right away and they developed seizures at the age of 5 weeks and status epilepticus at the age of 9 months (patient 2), due to hypoglycaemia. Patient 7 remained free of symptoms up to the age of 5 months, when he lost consciousness multiple times. At the follow up all of them showed a psychomotor delay and three started walking at the age of 2 to 4 years, the remaining four patients needed assistance with sitting at the age of 15 to 24 months. In this subgroup four infants were diagnosed with microcephaly, one with strabismus and one patient suffered from loss of vision. In the group of patients with intermediate impairment no statistically significant difference in age of onset or type of hyperinsulinism was found. Again four participants were diagnosed with microcephaly, one with strabismus and two patients were found to be deaf. Epilepsy was diagnosed in four severely retarded patients (57%), in seven of intermediated disabled patients (58%) and in 6% of the patients with normal neurological development. In total 16 patients were diagnosed with epilepsy, thirteen with neonatal onset and three with infancy onset.

	n	Group 1	Group 2	Group 3
All patients	90	74%	18%	8%
Neonates	54	68%	21%	11%
Infants	36	82%	15%	3%
Diffuse form	34	75%	14%	9%
Focal form	29	68%	22%	10%
Medical treatment	27	80%	16%	4%

Table 5: Infants with hyperinsulinism; Group 1- normal development, Group 2- intermediate disability, Group 3- severe psychomotor retardation (64)

Meissner et al. (65) published results in 2003, which are contrary to the findings of Menni and colleagues (64) in 2001. They included 114 Patients

diagnosed with hyperinsulinism, with onsets in neonatal period (48h after birth), infancy (12 months) or childhood (>12 months). Whereas Menni mentioned a more severe neurological impairment in children with neonatal onset, Meissner found developmental retardation to be more severe in patients with infancy onset.

Also, the study group of Burns et al. (66) in 2008 performed research on the neurological development in patients with hypoglycaemia. They included 35 patients with symptomatic hypoglycaemia in neonatal period and performed an MRI for changes in the brain substance. As a control group they had 229 healthy, term patients. Including criteria were a gestational age of 36 completed weeks at birth and at least one episode of hypoglycaemia (≤47mg/dl) associated with acute neurologic dysfunction within the first week after birth. As third study entry criteria the performance of an MRI scan in the first 6 weeks of life was defined. Exclusion criteria where any chromosomal abnormalities, intrapartum infections, hypoxic-ischaemic encephalopathy or any other kind of malformations. Of those 35 infants, one was diagnosed with hyperinsulinaemic hypoglycaemia and several showed at least transient hyperinsulinaemic hypoglycaemic episodes. In average the MRI scan was done on the 9th day after birth. Only two neonates had normal MRI scans, both had transient hypoglycaemia on day one. For the other 94% of study subjects the MRI investigations showed white matter abnormalities including haemorrhage, infarction and asymmetrical distribution. Fourteen infants showed different kinds of abnormalities in the basal ganglia/thalami and eighteen patients (51%) cortical abnormalities. In twenty- six percent of the neonates' MRI scans a loss of cortical markings due to infarction was found. Only three study subjects had findings on other sites such as brainstem, cerebellum and a small haemorrhagic lesion. No correlation of severity or duration of hypoglycaemia was found. Besides changes on the MRI scan, neurodevelopmental impairment was noticed at the follow up examinations at the age of 18 to 24 months. Including delayed walking or sitting, vision problems, and delay in development of speech and language.

Similar results showed the study of Avatapelle et al. (35), where they performed MRI scans on 15 children with transient or persistent hyperinsulinaemia. In total the study included 67 children aged 2.5 to 5 years diagnosed with persistent or transient neonatal hyperinsulinism. MRI scans were not performed routinely, but

according to the clinical signs. Therefore, imaging was not essential for the diagnosis of hyperinsulinism or the consequences, mainly it was performed due to informative reasons. The MRI scans of 73% of the patients showed substantial changes like occipital lobe atrophy and high signal intensities, as shown in Figure 3 below. Furthermore, gliosis, periventricular leukomalacia, changes in the basal ganglia, white matter atrophy, infarctions or sagittal sinus thrombosis were found.

Mainly the children presented with abnormal motor neurodevelopment, seizures and severe cognitive dysfunction at the follow up. In this cohort, 39% had an abnormal neurodevelopment, 8 patients had mild impairment and 18 had severe abnormal conditions. In both categories, mild and severe abnormalities, patients had a developmental delay in motor function, speech and language. Visual abnormalities, infantile spasms and lower limb weakness were reported in severe abnormal neurodevelopmental patients only.

In the same study, two children with PHI developed autism, but did not show any changes at the MRI scan. Despite expectations there was no significant difference in the brain scans of patients with THI or PHI. Summarized, 41 patients had hyperinsulinism but had a normal neurodevelopmental outcome. On the contrary, 26 patients with hyperinsulinism had abnormal development. Whereat the percentage of affected infants with impaired neurodevelopment due to THI and PHI was at an equal level.

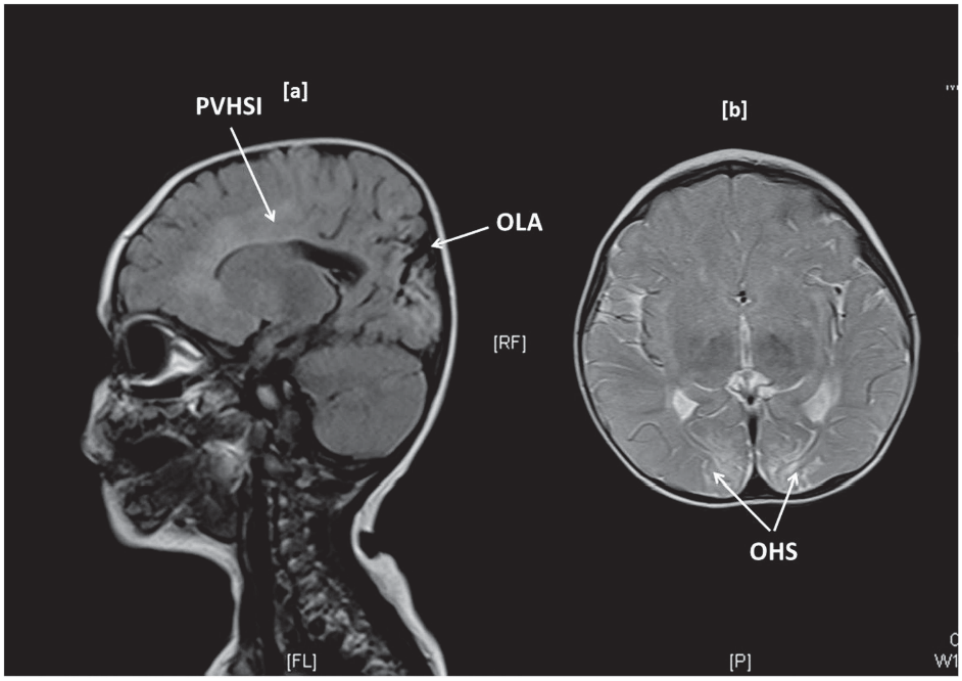

Figure 3: MRI scan of an 1- year- old diagnosed with transient CHI showing occipital lobe atrophy and periventricular high signal intensities (35)

Another study performed retrospectively on MRI images of 50 infants with symptomatic hypoglycaemia at a median age of 4 days, showed that time of hypoglycaemic injuries might affect the anatomical dispersion of brain damage. MRI images of 50 patients were present, of those 33 were diagnosed with CHI. The remaining either had a disorder of glycogen or fatty acid metabolism. Images used for research were all taken at follow- up investigations more than a month after the initial episode of symptomatic hypoglycaemia. In general, patients presented with seizures and epileptic attacks to the point of coma. MRI scans showed brain injuries in 28 patients. Lesions of white matter at the parieto- occipital region, basal ganglia and cortex were the three dominant findings. White matter and basal ganglia lesions were either affecting one or two hemispheres. In four patients with white matter lesions multicystic formations were additionally observed and haemorrhage in two. All patients with white matter lesions were aged under 6 months, with a median age of 2 days, when they suffered symptomatic hypoglycaemia for the first time. Regarding lesions of basal ganglia patients were at a median age of 12 months (6-22 months) and temporo- parietal cortical injuries affected infants with an age range of 20 months to 5 years of symptomatic hypoglycaemia. Of 33 patients with hyperinsulinaemia, 15 showed brain injuries at the MRI scan, all 15 had white matter injuries in the posterior area. Whereas, infants with HI did not show lesions at basal ganglia or cortex, white matter is not solely affected by HI but also in other conditions leading to hypoglycaemia. In comparison to the infants with fatty acid or glycogen storage disorder, neonates with HI developed symptoms at an earlier age with a narrower age range. According to the findings the authors postulated that the age at which first episodes of symptomatic hypoglycaemia occur is determining the location of lesions, whereas the glucose level or the aetiology of hypoglycaemia do not account for the topography of lesions (67).

A case report by Jahanara Begum- Hasan (68) published in 2014 described the development of a late preterm female baby with severe hypoglycaemia. The girl was born at a gestational age of 36+5, the pregnancy was without any complications and the mother had no history of gestational diabetes. Within the first hour of life the neonate presented with tachypnea. The following glucose measurements showed severe hypoglycaemia (20mg/dl). In the following hours the multidisciplinary team performed multiple attempts to increase the glucose level of the baby. Initial formula

feedings failed to increase blood glucose. Therefore, an intravenous infusion of glucose with a rate of 5 mg/kg/min was started. Due to persistent low blood glucose levels, despite glucose infusions, formula feeding was continued and after a while the infusion rate was raised to 7 mg/kg/min. By the time the neonates respiratory rate increased and oxygen supply was required, a septic event could be excluded successfully by negative blood cultures. Investigations at the initial hospital remained without results, hence the newborn was transferred to a tertiary care centre. Short time improvement was observed, the baby was active at the transfer, within a normal glucose level and respiratory rate. On the following days the glucose levels dropped again (i.v. glucose rate at 15 mg/kg/min) and the newborn developed seizures. Therefore, the doctors performed a CT scan of the head and detected large intraventricular haemorrhage (grade 4). The CT scan showed bilateral intraventricular haemorrhage with intraparenchymal extension at the parieto-occipital region. Based on the facts that the pregnancy and delivery were without any complications and nearly term with APGAR scores after birth of 8 and 9, the haemorrhage is referred to severe hypoglycaemia due to hyperinsulinism.

At the age of one month a US-EEG revealed an enlarged left ventricle and several cystic formations. The cerebral changes were accompanied by prolonged jaundice, cholestasis and cardiomyopathy, which resolved spontaneously within 1,5 months after delivery. After a period without any seizures, she had abnormal activity despite normal blood glucose levels, thus she was diagnosed with epilepsy. Regarding her growth parameters (height/weight/head circumference) she was within normal range, but she was globally delayed at her development. At the follow-up with 25 months of age, she was at 63rd and 68th percentile regarding height and weight, but at 20th percentile for her head circumference. Blood glucose levels stabilized with diazoxide, so it could be tapered off slowly by the mother. The girl was diagnosed with developmental delay, microcephaly and impaired cortical vision.

The pathologies of this case coincide with the study results of various studies. The detailed pathophysiology of haemorrhage due to hypoglycaemia is not understood yet, but an increase of incidence was observed. The study of Jarjour et al. (69) including children diagnosed with insulin- dependent- diabetes- mellitus showed an increased intracerebral blood flow at a hypoglycaemic state, which could

be one reason for intracerebral haemorrhages. Haemorrhagic lesions were also associated with hypoglycaemia in another study. Also microcephaly is often linked to hyperinsulinism (64,66).

John M. Anderson et al. (70) observed 6 patients with hypoglycaemia in neonatal period. Case 1 was a male, term born (39^{th} week of gestation) neonate and without any complications during pregnancy. Seventeen hours after birth he showed first symptoms of hypoglycaemia, like abnormal movements and jitteriness, the symptoms kept getting worse up to the point where the newborn was lethargic and carried out little movements; 51 hours after birth they performed a lumbal puncture, where cerebral pressure was within normal values and fluid was clear, but the glucose level was at 6mg/100ml in the cerebral fluid and at 8mg/100ml in the blood samples. Although they immediately gave glucose infusion the baby died 55 hours after birth. Case 2 was also a male patient born premature and small for gestational age, which indicates severe intrauterine malnutrition. Despite several feedings the baby remained hypoglycaemic. In both patients, severe degeneration of nerve and glia cells in the central nervous system was reported and both died of untreated hypoglycaemia.

Overall developmental delay is a frequent complication observed in patients with hyperinsulinism and consequently hypoglycaemia. The severity of neurodevelopmental impairment does not correlate with the severity of hypoglycaemia; it seems to be dependent on the individuals limit of tolerance.

Also the period of onset to the point of initiation of therapy plays a main role for the outcome. Therefore, the duration of hypoglycaemia is important to be reduced to a minimal time period.

3.2. Cardiovascular system

Within the cardiovascular system especially the heart is known to be sensitive for insulin and glucose. From DM in adults i.e. microangiopathies are known as a long term consequence of disturbances in the glucose metabolism. Hence an impact on the infants' cardiovascular system needs to be considered.

3.2.1. Changes in foetal period

The effects of insulin are diverse in different types of tissues. As mentioned above, GLUT4 is an insulin dependent transporter and mainly found on muscle cells, including cardiac muscle cells. The combination of high levels of insulin and intrauterine increased quantity of available glucose leads to increasing protein, glycogen and fat synthesis in the myocardial cells and consequently to hypertrophy of the heart. Among others, this was described already in 1973 by R.S. Pildes, where enlargement of specific organs, especially liver and heart, in infants of diabetic mothers was mentioned. In this study, cardiomegaly was observed in approximately 30 percent of the infants with hyperinsulinism (60).

Especially the interventricular septum is susceptible for asymmetrical hypertrophy, due to a high amount of insulin receptors (71). However, the free walls of the left and right ventricle may be affected too, with a higher incidence of the left ventricle being involved. Due to left ventricular- hypertrophy the end- diastolic pressure in the left ventricle is increased, leading to impairment of the left atrial dynamics and pulmonary distress (36).

As the foetal heart has a unique anatomical structure not only left and right ventricular function is involved in the cardiac output. Among other factors the mobility of the flap valve of the foramen ovale, named septum primum, can be assessed. Zilinsky et al. (36) hypothesized that the amplitude of movement of the septum primum correlates with the left atrial pressure and dynamics. For evaluation of cardiac impairment, they used the ‘excursion index’ (EI), which is defined by the maximum displacement of the valve and the left atrial diameter measured in four-chamber view at the echocardiography. They found a significant inverse correlation between the EI of the septum primum and the septal hypertrophy. Septal hypertrophy came along with decreased compliance of the left atrium and increased intra- atrial pressure during diastole, consequently this might lead to impairment of the physiological movement of the flap valve. In comparison, the EI in third trimester foetus without myocardial hypertrophy and those of euglycaemic mothers did not show any changes in the excursion index.

3.2.2. Changes in neonatal period

Similar results showed the study of Gandhi et al. in 1995 (72). The sample size consisted of 23 women with diabetes, one with gestational, two with type 1 diabetes and 20 were diagnosed with type 2 diabetes. According to the gestational age they were divided into three groups. For comparison, non- diabetic pregnant women with normal sized foetuses and similar gestational age were recruited and split into three groups for control. On examination with ultrasonography and echocardiography, in two- dimensions and with M-mode, they found cardiac hypertrophy of the interventricular septum as well as hypertrophy of the left and right ventricle wall in the foetuses of diabetic mothers.

In contrast to the results of Zielinsky et al. (36), who described an increased ris for left ventricular hypertrophy, the study group of Gandhi found a higher risk for the right wall to be hypertrophic. Moreover, the measurements showed a greater right ventricular shortening fraction compared to the control group in late gestation, which indicates hypercontractility of the right ventricle in FDM (72).

In their study Zielinksy et al. (36) sonographically investigated the left atrial shortening fraction (LASF) by using M-mode values and following formula – (end-systolic diameter- end-diastolic diameter)/ end- systolic diameter. They described a lower LASF in IDM with myocardial hypertrophy compared to control groups. In synopsis with other studies they concluded that diastolic dysfunction also occurs in non hypertrophic hearts as measured by LASF and correlates with a ventricular compliance.

In most cases cardiac hypertrophy resolves within 6 months after birth, presupposed normal insulin levels and euglycaemia are reached. Only few cases are reported where the foetus died due to cardiomyopathy.

As described in the case report of Sardesai et al. (73) from 2001, they found intrauterine cardiac failure at the very end of the pregnancy (>36 weeks of gestation). On examination they found mural thrombi leading to myocardial necrosis, caused by ventricular dilatation and hypocontractility of the foetal heart.

A different case report by Bulbul et al. (74) in 2010 showed similar findings in a neonate requiring observation at the neonatal intensive care unit. The newborn

suffered from severe hypoglycaemia, a respiratory rate of 76/min and systolic murmurs. At further examinations they found severe left ventricular hypertrophy, subaortic stenosis, mitral regurgitation as well as dysrhythmia. Although normal glycaemic levels could be reached after near total pancreatectomy, the infant died on the 70th postnatal day due to sudden heart failure and ventricular dysrhythmia.

Most of the studies showed consistent results regarding long term outcome. Hyperinsulinism may lead to cardiac hypertrophy in foetal period and consequently to impairment of the cardiac function as the compliance is decreased. However, with adequate postnatal treatment targeting physiologic blood levels of glucose and insulin a spontaneous regression to normotrophic heart conditions within a few months is likely.

3.3. Body composition

The human body weight is composed of lean body mass and fat mass. Insulin is an anabolic hormone, which promotes the storage of glucose in terms of glycogen and fat. Different tissues store more or less amounts of glycogen, for which reason it is imperative if and to which degree insulin has an impact in early age development.

3.3.1. Changes in foetal period

An increased foetal weight gain could be observed in infants with hyperinsulinism. Especially in third trimester an elevated accumulation of body fat is common. This change in IDM was one of the first to be noticed, followed by a lot of research.

One of the first studies about foetal fat distribution was published by Poissonnet and colleagues (75) in 1984. In a cohort of 488 human aborted foetuses with a gestational age of 1 to 41 weeks they investigated at what point of pregnancy fat synthesis occurs. They took tissue samples all over the body at 18 different sides, such as from the cheeks and chin, neck, at four different sites of lower and upper limb each and the abdomen. Tissue samples were processed and examined by light

microscopy. Adipogenesis was first observed at the abdominal wall at 14 to 15 weeks of gestation. Also perirenal fat tissue as well as fat tissue at neck and cheeks occurred as early as 14 to 15 weeks of gestation. No difference in sex regarding fat distribution was described. Based on their results, the study group postulated that the second trimester plays a main role in adipogenesis. They recommended to think about special diet for obese and diabetic women, especially from 14 to 25 weeks of gestation to potentially decrease excessive fat accumulation in the infants.

In synopsis of the findings about initial adipogenesis and the start of insulin secretion at the end of the first trimester a close connection of both seems likely.

In 1992 Rosati's group (76) investigated the prediction value of sonography for infants to be macrosomic. They included 732 pregnant women with 35 to 42 weeks of gestation. For evaluation they used Gagliadi's italian growth curve, whereof the 90^{th} percentile was set as a border for infants to be large for gestational age (LGA).

In this study following parameters were obtained sonographically and compared to each other:

Abdominal circumference (AC)	Biparietal diameter (BPD)
Femur length	Head circumference (HC)
Humerus length	Abdominal diameter (AD)

Rosati and his group compared the data of ultrasound examinations to birth weights by applying Pearson's correlation coefficient. On this basis they concluded, that for intrauterine estimation of the foetal birth weight mean abdominal circumference and abdominal diameter were the most accurate predictive values. Compared to the other parameters, AC and AD showed the greatest sensitivity for detection of macrosomia in infants (76).

Based on this information and with the techniques of Bernstein et al., Larciprete and colleagues (77) performed a study including 303 pregnant women, whereof 28% were diagnosed with GDM. The aim of this study was to compare subcutaneous tissue thickness of the two groups and to provide reference parameters for foetal body composition in pregnancies complicated by GDM.

Women considered to be at high risk for GDM were included. The authors set following risk factors: (1) first degree family member with DM, (2) increased BMI (≥27 kg/m^2), (3) glycosuria >600 mg/L, (4) history of macrosomic birth weight in previous pregnancies (≥4500g), (5) polyhydramnion, (6) GDM in previous pregnancies and (7) ≥37 years of age. Besides standardised foetometry, with measurements of HC, AC and BPD, they measured mid-arm lean mass and fat mass, mid- thigh fat mass, cross section of the abdomen and at the subscapular site. Measurements were taken from two different operators three times each. At each point of measurement, starting at a GA of 20 to 22 weeks, infants in the diabetic group had significant elevated parameters compared to the non- diabetic group.

Consistent with the findings of Poisonnet and colleagues are the study results of Carpenter et al. (78) in 2001. They showed a correlation of elevated insulin levels in the AF in infants with the birth weight. They analysed the data of 247 pregnant women, who underwent amniotic fluid sampling at 14 to 20 GA for foetal karyotyping. An 50g- oral glucose testing was performed routinely at 24 to 30 weeks of gestation, which was repeated in 60 women with a 100g OGTT because of values >7.2 mmol/l. A correlation between increased insulin levels in the AF and OGTT could not be described within a significant value. Nevertheless, increased AF levels of insulin already in early second trimester showed a strong correlation to macrosomic infants at birth. Elevated insulin levels correlated with an up to fivefold increase in risk for macrosomia at birth (birthweight >90^{th} percentile).

3.3.2. Changes in neonatal period

In 1954 Pedersen (79) designed a study with 244 pregnant women to investigate the difference in body weight in infants of diabetic mothers compared to infants of non- diabetic mothers. For most accurate comparison he defined two cohorts, diabetic and non- diabetic, which were identical in as many parameters as possible. He found 122 infants of diabetic mothers and 122 infants of non- diabetic mothers, who matched in foetal age, maternal obesity, parity and gender. After analysing the data, Pedersen described a significant difference between the two cohorts. The IDM appeared to have increased body weight compared to the infants in the non- diabetic group, but were quite similar in length. He described a similar

weight distribution within the groups, but the IDM weight distribution curve was on an increased weight level, which is demonstrated in Figure 4. The last six to seven weeks of gestation he measured the blood sugar levels in both groups and found increased levels in the diabetic group. Therefore, he concluded that the increased blood sugar levels of the mother and consequently in the foetal blood lead to an increased insulin secretion in the foetus. As one of the first ones he set up the theory of increased insulin level functioning as growth stimulating agent, known until now as the 'Pedersen hypothesis'.

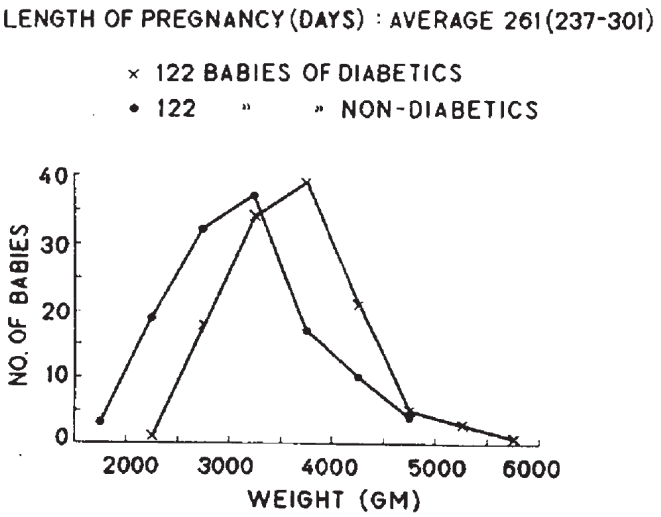

Figure 4: Distribution Curve of weight of infants in diabetic and normal pregnancies (79)

The purpose of the study of Richard L. Naeye (80) in 1965 was to investigate the body composition of infants born to a diabetic mother on an anatomical morphological basis. For this purpose, he examined the bodies and autopsy material of 56 infants, who were stillborn or died in the first two weeks of life. The material of 26 infants could not be used for further investigations, for 10 the material was not preserved well enough, 4 had congenital malformations and 14 were born premature, before 36th week of gestation. At the end, there were 21 infants with more than 20% above their predicted value for weight and 4 with 20% less than their predicted value. Unfortunately, there is no information about the remaining 5 infants. R.L. Naeye rose data for comparison from 14 infants, which were within the range of 20% of the predicted weight value. For predictive values he referred to a study of Schulz and colleagues (81).

Within this cohort the body weight of the 21 overweight infants of diabetic mothers was 141% of the values of the control group, heart weights were up to 174% and liver weights even 179%. Whereas, the cardiac changes in the overweight infants might be due to a higher number of myocardial cells and thicker fibres, the changes in the liver were referred to be mainly due to an increase in the cytoplasm, but also a higher number of cells. Nevertheless, also underweight babies had 60% increased cell plasma compared to the control group. Additionally, underweight and overweight infants showed more erythropoietic precursor cells in comparison to the infants of non diabetic mothers (80).

Similar results showed the study of Fee and Weil in 1963, where they differentiated an increase of lean body mass (LBM) to fat tissue. Ten infants of diabetic mothers and thirteen infants of non diabetic mothers, who were stillborn or died within 14 days after the delivery, were weighed for total body weight and later on organs were weighed separately. They described a two- fold increase of body fat in IDM compared to non IDM. Only one infant was born premature at 28^{th} week of gestation (59).

In a different study published in 1993, 50 infants born with a gestational weight above 95^{th} percentile for gestational age and sex were compared to 32 infants born adequate for gestational age (within 25^{th} to 75^{th} percentile). The data of both groups were corrected for maternal height. Including criterium in this study was a normal glucose challenge test (GCT) result at 24 to 28 weeks of gestation. Therefore, the authors were about to investigate hyperinsulinism in macrosomic infants of non-diabetic mothers. A significant difference in insulin levels in the cord plasma was found in 17 macrosomic infants, whereas hyperinsulinaemia was defined as -/+ 2 standard deviations from the control group levels. In more than 40% of infants in the macrosomic group high levels of insulin were measured. Based on their results, they postulated that not only high insulin levels are contributing to foetal weight gain, but also other factors as socioeconomic status or foetal sex may have a significant influence. Overall, a strong correlation of macrosomia and foetal hyperinsulinism could be affirmed by this study. This theory can also be encouraged by reverse studies with pregnant women with strictly controlled glucose levels by (prophylactic) insulin therapy. In this case the infants are less likely to be born LGA (82,83).

In 1996, Kehl (84) and his study group rose results consistent with previous described studies. They included 38 women, all of them diagnosed with gestational or pregestational diabetes. Including criteria were a diagnose of diabetes mellitus made before the 32nd week of gestation, non smoking women, no other medical or obstetrical issue present and singleton pregnancies. Initially they got dietary instructions to avoid insulin therapy, if possible. During pregnancy the foetus got checked via ultrasound every three weeks. Biparietal diameter (BD), head circumference (HC), abdominal circumference (AC) and femur length (FL) were measured as a standard at the performing institution. For this study specifically transcerebellar diameter (TCB), longitudinal liver and kidney length as well as subcutaneous fat of the abdominal wall (AFT) and tight (TF) were additionally obtained. The infants body composition was examined by electrical conductivity, related on previous studies LBM has a greater electrical conductivity due to a higher amount of electrolytes dissolved in water compared to fat tissue. By subtracting LBM of the total body weight the amount of fat tissue was identified. To summarise, Kehl et al. did not find a significant difference in lean body mass between diabetic and non- diabetic pregnancies. Nevertheless, they could proof an accelerated growth in insulin- sensitive tissues, such as the liver, abdominal fat tissue, leading to abdominal circumference, and tight fat. In total 14 babies were born large for gestational age. Those 14 presented with 27% more weight compared to non- LGA. In LBM they found increase of 17% in LGA infants, whereas in fat mass it was up to 99% of increase compared to non- LGA infants.

Also Pildes and Avatapelle observed an excessive increase in body weight, mainly based on an increase in fat tissue. Pildes additionally described enlargement of certain organs and a greater skeletal growth. The study of Avatapelle et al. was primarily to investigate neurodevelopmental outcome. In this context they described body weights up to 5.5 kg, with a mean bodyweight of 3.3 kg and 19% prematurely born infants (35,60).

Similar data were risen in a study performed to investigate oxygenation and erythropoietin levels in foetuses of insulin dependent diabetic mothers. The children were born via planned C-section. Compared to the control group they were born 10 days earlier in mean and still showed a significant increase in birth weight (z score) (85). Another study doing research on neonatal haematocrit levels found a

significant difference in body weight data of the diabetic group and the control group. The infants in both groups were born at 37.9 ± 1.7 weeks of gestational age. The infants of diabetic mothers appeared to have in mean 276g more body weight than those of non diabetic mothers (86).

3.4. Body length

In contrast to body weight the results about changes in the infants' body length were not as consistent.

3.4.1. Changes in foetal period

The study group of Roberts in 1994 (87) showed a significant increase of femur length in IDM in comparison to the control group. Indicating an impact of insulin on the growth of long bones. They included 104 pregnant women in total, of which 80 were diagnosed with DM Type 1 or 2. The data of 24 obese pregnant women were used as a control references. Within this population they found amongst other values an increase of femur length in the diabetic subjects. An early accelerated growth is shown, but approaching normal values at advanced pregnancy. Nevertheless, a significant difference at term is found.

Rosati et al. (76) investigated a possible correlation of different parameters with foetal body weight for a more accurate detection of macrosomia. Within this study they could not find an adequate relation of humerus or femur length to the later birth weight of overweight infants.

3.4.2. Changes in neonatal period

Pedersen (79) stated a significant difference in weight and length of IDM compared to his control group. He observed a difference of 1.5 cm at 38 weeks of gestation. In average he described IDM to reach normal birth weight and height already 3 weeks prior to the calculated due date.

Concordant with these findings, described Naeye (80) an increased body height of 12% in overweight IDM compared to the control group. In contrast to Pedersen, who excluded dead infants for the data of body height, Naeye's study population consisted of dead infants only.

Inconsistent results are described by Catalano et al. (88), where no significant difference in height between diabetic and non diabetic pregnancies was found. Although Kehl et al. (84) found an increase of mean values of femur length compared to his control group, results remained without significance difference.

A possible confounding factor here are the different body sites used for measurement. Whereas some studies measured the complete body length, others only included measurements of single bones and again others measured from crown to rump. Still a strong correlation could not be found. Whereas Rosati et al. (76) described a correlation of increase in femur length in overweight infants, Kehl et al. (84) described no significance in femur length in his study population (76,84).

As in animal studies no difference in crown to rump length was found, a possible explanation for increase in body length would be an impact of insulin on long bones (89).

3.5. Lung development and oxygenation

Besides a normally developed anatomy of the lungs, the pulmonary surfactant is the second most important parameter for adequate function; consisting of a variety of lipids and proteins its synthesis is regulated and impaired by different hormones.

The active characteristics is primarily due to dipalmitoyl phosphatidylcholine, which accounts for 45% of the surfactant's weight, approximately 10% are phosphatidylglycerol (PG). The quantity of both substances keeps increasing during the last trimester of pregnancy, therefore they are used to estimate foetal lung maturity. Up to 10% of the surfactant is made up by proteins with surfactant protein A (SP-A) as the most abundant one. Furthermore, inhibitory effects of TGF-β and insulin on SP-A synthesis are reported, which suggests that the respiratory distress

in newborns of diabetic mothers is related to foetal hyperinsulinism and an impaired surfactant synthesis (90).

3.5.1. Changes in foetal period

This was subject of interest in the study by Piper et al. (91) conducted in 1993, where they investigated the effects of maternal diabetes on the maturation of the foetal lung. From 1986 to 1992, 1000 women underwent amniocentesis to investigate the foetal lung maturity. After eliminating those with inadequate samples, incomplete data, preeclampsia or maternal hypertensive disorders 829 women remained. Split into different groups by the womens condition there were 289 pregnancies with diabetes, of those 106 with pregestational and 183 with gestational diabetes, and 540 cases without diabetes or hypertension as a control group. The diabetic pregnancies were again divided into well- controlled (140 cases) and poorly- controlled diabetes (149 cases). Well- controlled was defined as a median glucose level of ≤105 mg/dl and consequently poor-controlled diabetes was defined as a median glucose level of ≥105 mg/dl. The foetal lung maturity was indicated by the presence of phosphatidylglycerol in the amniotic fluid, which was detected as early as 0.5μg/ml. For comparison the results of the amniocentesis were categorised by the gestational age. Significant differences regarding foetal maturity of the lung could be found in the poorly controlled group compared to the non-diabetic group at the gestational age of 34 to 36.9, 37 to 37.9 and above 39 weeks. A significant difference between the well and poorly controlled diabetic groups could only be seen at 37 to 37.9th weeks of gestation. Moreover, an impaired lung maturity <34 weeks in the diabetic groups compared to non- diabetic women was observed, with a higher incidence of hyaline membrane disease. This again underlines the theory of maternal diabetes delaying foetal lung maturity. But, if well controlled, maternal diabetes does not have a significant impact on pulmonary maturity.

3.5.2. Changes in neonatal period

Bourbon et al. (92) recorded information about the connection of respiratory distress syndrome (RDS) and maternal diabetes mellitus in 1985. Besides risk

factors as foetal sex, mode of delivery or maternal hypertension they also found maternal diabetes with hyperglycaemic episodes to be a relevant risk factor for RDS in the newborn. An incidence of RDS in IDM to reach 37% is mentioned. After correction of the results for better comparison, the risk for acute respiratory distress syndrome in babies of diabetic mothers remained to be 5.6 times higher as in the control group. Figure 5 shows a possible mechanism of the influence of maternal DM on foetal respiratory function.

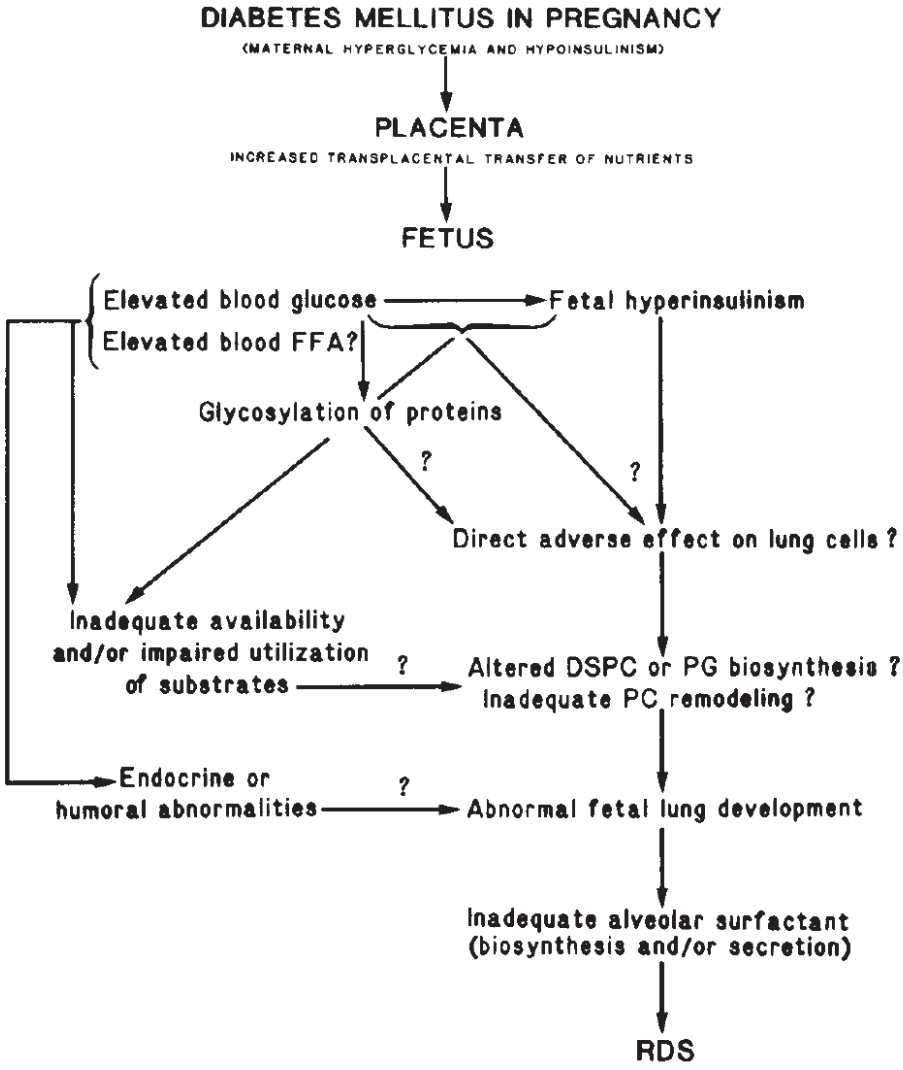

Figure 5: Possible mechanism of RDS in IDM (92)

From 1958 to 1968 Robert and colleagues (93) collected data of 820 women pregnant women admitted to the Joslin Clinic in Boston; all of them diagnosed with gestational or pregestational diabetes mellitus. For those 805 IDM, they had >10.000 INDM for comparison. The diabetic group was subdivided according to the

White- Classification: Class A included 2.2% of women within the diabetic group, Class B 21.0%, Class C 22.4%, Class D 44.0%, Class E 0.12% (data collection was discontinued) and Class F 10%. After considering all confounding factors Robert et al. found a nearly 6- fold increased risk for RDS to occur in IDM compared to the control group. Despite increased risk for RDS the mortality rate in the diabetic group (23%) was lower compared to the infants of non diabetic mothers (35%).

Consistent with the findings of Robert et al. are the study results of Allen and colleagues (94) published in 1981. They compared the outcome regarding RDS in pre- diabetic and diabetic women to non diabetic pregnancies. An increased risk for RDS in IDM was shown in this study. In total the results of 35 diabetic pregnancies, 114 pre- diabetic pregnancies and 21142 control infants were compared to each other. They found a significant increased risk in insulin dependent women with the diagnosis of RDS in 31% of the infants (see Table 6). But only a slightly increase of RDS cases in pre- diabetic women, with 2.6% of the infants.

maternal group	total births, n	respiratory distress	
		n	%
diabetic mother	35	11	31
prediabetic mother	114	3	2.6
control mother [7, 8]	21,142	98	0.46

Table 6: Respiratory distress in infants of diabetic and pre-diabetic mothers (94)

The increased risk of RDS found in infants born via C- section and in infants born before 38 weeks of gestation was tought to be mainly due to prematurity of the infants. Furthermore, an increased incidence of RDS was found in infants with a maternal gestational weight gain of more than 11 kg, which remains unexplained by the authors. Additional risk factors for RDS are listed in Table 7 (94).

	insulin-dependent diabetic mothers			prediabetic mothers		
	number of babies	with respiratory distress		number of babies	with respiratory distress	
		n	%		n	%
birth weight (g)						
<2,500	5	2	40	5	1	20
2,500-3,100	7	0	0	22	2	9
>3,100	23	9	39	87	0	0
gestational age by date (weeks)						
≤34	8	2	25	6	1	17
35-37	23	9	39	6	0	0
≥38	4	0	0	102	2	2
delivery						
vaginal	17	2	12	107	3	3
cesarean	18	9	50	7	0	0
toxemia						
yes	1	0	0	11	2	18
no	34	11	32	103	1	1
weight gain by mother during pregnancy (kg)						
≤11	23	4	17	78	2	2.6
>11	12	7	58	36	1	2.8

Table 7: Further risk factors for RDS in infants (94)

Although the risk is decreasing by advanced gestational age, there is still a slightly increased risk for term born babies to suffer from RDS.

3.6. Erythropoiesis and hyperbilirubinaemia

Maternal hyperglycaemia is suspected to affect the foetal erythropoiesis and pH value. Several studies investigated maternal glycaemic control and foetal hyperinsulinism in correlation with foetal erythropoiesis.

Hyperinsulinism also shows indirect effects on red blood cells. Due to increased body mass the foetus of a diabetic mother shows a higher demand of oxygen. This leads to increased synthesis of erythropoietin and consequently to an increase in red blood cells (95).

3.6.1. Changes in foetal period

In 1986, Mimouni et al. (86) paired 34 diabetic pregnant women with 34 non-diabetic pregnant women in a prospective study investigate the haematocrit levels of infants. All women within the control group had an oral glucose testing at 28 weeks of gestation with physiological results. Both groups were matched regarding gestational age (± 1 week), mode of delivery, time of cord clamping, neonatal asphyxia, site of blood sampling and time of sampling to reduce the confounding factors to a minimum. Polycythaemia was present in 29.4% of the diabetic neonatal blood samples with 13 ± 22 nucleated red blood cells per 100 with blood cells. On the contrary only 5.9% of the subjects within the control group had polycythaemia with a mean of 3 ± 4 nucleated red blood cells per 100 white cells. The high amount of nucleated red blood cells in the diabetic group may indicate an increased erythropoiesis as hypothesized before. Furthermore, the authors found no correlation of increased haematocrit with maternal glycaemic levels or the infants body weight, but with neonatal hypoglycaemia. Based on animal studies they brought up the theory of maternal hyperglycaemia leading to foetal hyperinsulinism and this again leading to arterial hypoxia, which then would be a cause of increased erythropoiesis in the infant.

The study group of Salvesen (96) included 31 pregnant women with pre-existent or gestational diabetes mellitus treated with insulin to analyse the effect on the foetal erythropoiesis. For investigations umbilical cord blood was drawn by cordocentesis up to 24h before the delivery. The mean age at delivery was 38 weeks of gestation. Analysis of blood samples showed an increase in mean values of pCO_2, lactate, erythropoietin and erythroblasts as well as haemoglobin concentrations compared to normal values for the same gestational age. Furthermore, a significant correlation between foetal erythropoietin and erythroblasts as well as between erythroblasts and haemoglobin concentrations in the foetal blood. The initial theory of tissue hypoxia leading to increased erythropoietin levels could not be confirmed. With the data collected in this study they postulated that the increase of foetal haemoglobin is due to premature or elevated hepatic erythropoiesis either due to erythropoietin or hyperinsulinism. Pathways for both theories are shown in Figure 6. In contrast to other studies,

dealing with hypoxia in the foetus, abnormal pO_2 values could not be detected and was only found in one foetus, with values below 5th percentile.

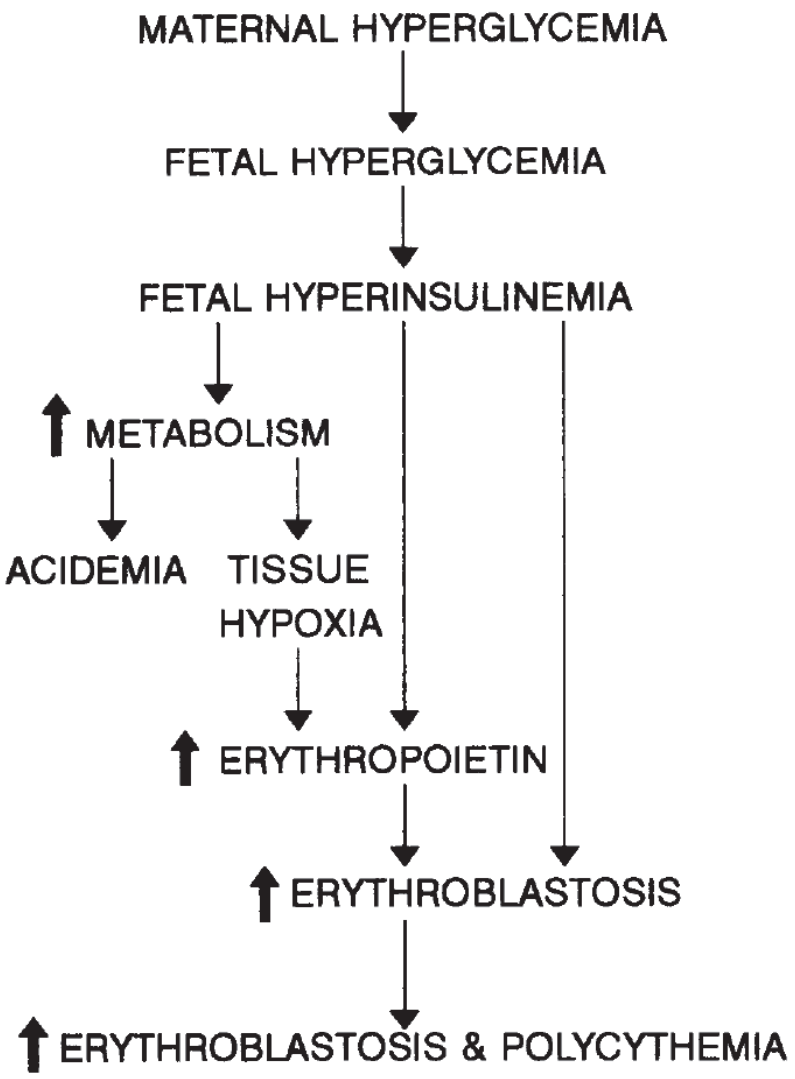

Figure 6: Possible mechanism for increased erythropoiesis (96)

In 1990, a study investigated chronic foetal hypoxemia linked to maternal antepartum glucose control in women with DM Type I (insulin- dependent). For the investigations 44 women with singleton pregnancies and Type I diabetes were compared to 23 non diabetic pregnancies. The distribution of age and ethnicity was similar in both groups. The maternal HbA1c levels were investigated six to four weeks prior to the C-section. There was no HbA1c testing in the control group. Foetal parameters like antepartum glucose, insulin and C-peptide were obtained from amniotic fluid at selective C- section. Indications for foetal hypoxemia was

detected indirectly by venous plasma erythropoietin level in the umbilical vein. Women with delivery through C- section were included only to eliminate the confounding factor of labour inducing foetal distress and consequently acute elevation of erythropoietin levels in some cases. In 45% of women with DM the HbA1c value was above 6.6% measured 4 weeks prior to the C- section. Biochemical and hormonal foetal parameters of the diabetic group were significantly increased at the time of delivery and are shown in the table 8. The mean value of C-peptide, indicating foetal endogenous insulin production, of the diabetic group was twice as high as those of the control group. Also the foetal erythropoietin and haemoglobin values taken from the umbilical vein showed a significant increase with a p- value of $p<0.001$, listed in table 9 (85).

Additionally, a subgroup of 28 foetuses was examined separately. In this subgroup any possible confounding factor as hypertension, maternal bronchiectasis or smoking (>10 cigarettes/day) in pregnancy were excluded. Again the data showed a significant difference in UV erythropoietin levels (85).

The diabetic subjects showed a significant correlation of amniotic fluid glucose, insulin, C-peptide and UV erythropoietin levels to one another. The amniotic parameters of the control group showed no correlation to UV erythropoietin. Due to the fact of correlation of foetal amniotic fluid parameters to maternal HbA1c and UV erythropoietin concentrations suggests foetal hyperglycaemia and consequently hyperinsulinaemia to be a causal factor for an increased erythropoietin synthesis (85).

	Control subjects	Type 1 (insulin-dependent) diabetic subjects
Number of subjects	23	44
Umbilical vein plasma erythropoietin (mU/ml)	23.8 ± 9.5 (9.8 to 50.3)	294 ± 976[a] (11 to 6130)
Amniotic fluid glucose (mmol/l)	1.03 ± 0.58 (0.39 to 2.11)	2.11 ± 1.68[a] (0.39 to 8.56) [43]
Amniotic fluid insulin (pmol/l)	48.4 ± 25.6 (13 to 133) [22]	309 ± 465[a] (40 to 2,533) [41]
Amniotic fluid C-peptide (pmol/l)	325 ± 142 (151 to 647)	791 ± 464[a] (167 to 2004)

mean ± SD; ( ) range. [ ] number of subjects, if different. [a] $p < 0.001$ compared to control subjects (Mann-Whitney U Test)

Table 8: Foetal biochemical and hormonal data at delivery (85)

	Control subjects	Type 1 (insulin-dependent) diabetic subjects
Number of subjects	23	44
Umbilical vein Hb (g/l)	146 ± 15.9 (125 to 185) [20]	168 ± 20.2[a] (121 to 209) [38]
Umbilical artery pH	7.30 ± 0.049 (7.20 to 7.41) [21]	7.25 ± 0.055[a] (7.14 to 7.36) [39]
Umbilical artery pO_2 (kP_a)	2.27 ± 0.71 (1.18 to 3.59) [20]	2.33 ± 0.68 (2.33 to 3.62) [39]
Umbilical artery pCO_2 (kP_a)	6.77 ± 0.88 (5.19 to 8.64) [21]	7.69 ± 1.00[a] (5.56 to 9.58) [39]
Umbilical artery base excess (mmol/l)	−2.2 ± 2.87 (−11.4 to +1.5) [21]	−3.1 ± 2.77 (−7.5 to +2.2) [39]

mean ± SD; ( ) range. [ ] number of subjects, if different. [a] $p < 0.001$ compared to control subjects (unpaired t test)

Table 9: Foetal Hb and blood gases at delivery (85)

In synopsis of the new gained information in this study and data from previous studies including animal studies the authors described a model to demonstrate possible pathways, like shown in the figure below, of maternal glucose management and foetal oxygenation. Theories also include increased need for oxygen due to an increased foetal metabolism caused by hyperinsulinaemia (85).

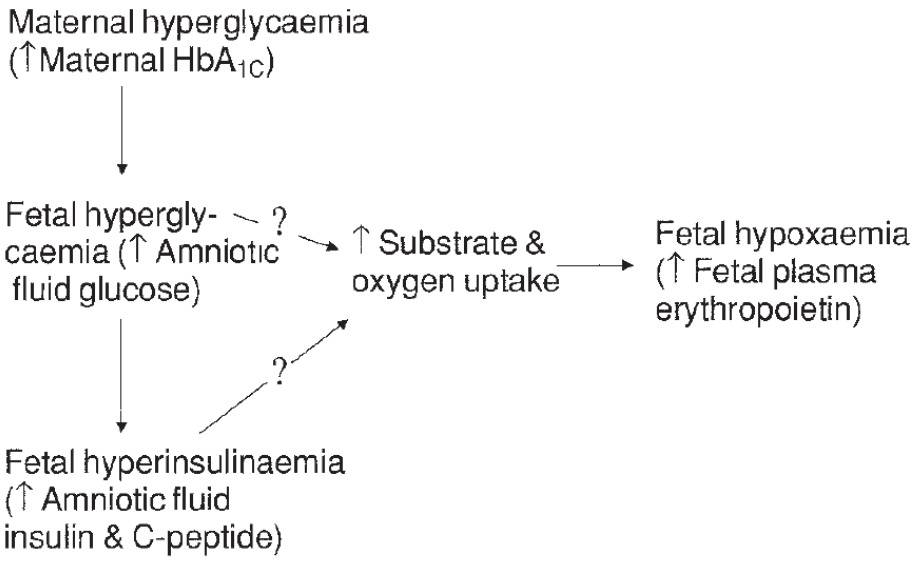

Figure 7: Schematic presentation of a possible pathway of foetal hypoxia (85)

A study published two years later in 1992 showed different results. They investigated the maternal HbA1 level of 32 diabetic mothers delivering in the years from 1979 to 1988. At the beginning they hypothesized that in IDM the neonatal haematocrit would correlate with maternal glucose control. The mean HbA1 level was measured at 36 weeks of gestation and at delivery. To eliminate confounding factor of increased foetal erythropoiesis following including criteria were set: (1) gestation not complicated by regular alcohol, drug or tobacco use, (2) delivery at term (≥37; ≤42 weeks of gestation) and (3) no signs of acute perinatal asphyxia defined by an APGAR- Score of <7 at 1 minute. At 36 weeks the mean value of HbA1 was at 7.6% ± 1% and at delivery 7.6% ± 1.1%. Foetal samples were taken 2 to 4 hours after delivery from a peripheral venous site, with a mean venous haematocrit of 59% ± 7%. In 1973 Mackintosh and Walker defined a normal value for venous haematocrit in a term newborn of <65%, as they found profound changes in viscosity above those limit. The results of the 32 women showed no correlation of maternal HbA1 level at 36 weeks GA, but a correlation was found at foetal haematocrit and maternal HbA1 levels at the time of delivery. Based on the results of this and previous studies including animal studies they concluded that foetal erythropoiesis is related to maternal glycaemic control. As maternal hyperglycaemia leading to foetal hyperglycaemia, hyperketonaemia and hyperinsulinaemia and consequently impairing foetal oxygenation (97,98). Nevertheless, insulin and insulin-like growth factor may also affect erythropoietin concentrations more directly, acting as growth factors for reticulocytes (99).

A different approach was chosen 1994 by Petry et al. (89), when they did research on the foetal iron metabolism in diabetic pregnancies. For comparison they investigated the transferrin receptors in placentas of 10 diabetic women and 10 non-diabetic pregnancies. All women were ≥ 36 weeks of gestation, screened for glucose control during pregnancy and haemoglobin levels. Women with assured iron deficiency or increased risk for foetal hypoxia, like hypertension, were excluded. Non diabetic women had to be without any gestational complication to be included. Cord blood samples of IDM showed a significant higher ferritin level, a higher calculated iron storage, increased non- heme iron concentrations and a greater amount of iron on red cell mass than the control samples, listed in the subsequent table.

Iron	IDM	Control
Serum ferritin, μg/l	31 ± 7*	216 ± 35
Calculated storage iron, mg/kg	4.0 ± 1.3*	21.2 ± 1.4
Placental nonheme iron, μg/g dry wt	285 ± 29†	375 ± 31
Calculated red blood cell iron, mg/kg	61.1 ± 4.4†	51.4 ± 1.2

Values are means ± SE. IDM, infants of diabetic mothers. No. of both IDM and control is 10. *$P < 0.0002$ between IDM and control; †$P < 0.05$ between IDM and control.

Table 10: Neonatal references of foetal iron status (102)

Furthermore, IDM had increased C- peptide, erythropoietin and haemoglobin levels in cord blood samples, concluding the foetus to be hyperinsulinaemic and hypoxemic. Storage iron levels correlated inversely with C- peptide levels, erythropoietin and haemoglobin concentrations. As mentioned previously, foetal hyperinsulinaemia can cause chronic hypoxemia in many cases. Therefore, erythropoietin synthesis and iron demand is increased in those infants. Beside reduced cord serum iron and ferritin levels, infants of diabetic mothers in a previous study showed a significant decrease of iron storage in the heart, liver and brain paired with polycythaemia. These results affirm the theory of iron being mobilised from storage and used for increased erythropoiesis in infants with hyperinsulinaemia (100,101). In response to increased iron demand the synthesis and activation of hypoxia- inducible- factor 1 α for increased iron uptake is elevated, which additionally leads to a higher amount of iron supply for erythropoiesis independent from erythropoietin (99).

Mimouni (86) and Green (97) used similar sampling methods. In both studies the samples were drawn 2-4 hours after birth, from cubital or wrist site and without a tourniquet. In terms of labour in both studies cord clamping was within 30 minutes after birth. In both studies the infants had an increased haematocrit. Green et al. had a better glycaemic control in their study group than Mimouni and colleagues, with HbA1 of 7.6% ± 1% and HbA1 of 10.6% ± 3.4% . Anyway Green could not found a correlation of the HbA1 level at 36 weeks of gestation, but at labour. In contrast, Widness et al. (85) found an increased maternal HbA1c in maternal

samples and increased erythropoietin levels in the amniotic fluid one month prior to birth.

Regarding red cell metabolism connected to GDM and foetal hyperinsulinism the studies showed concordant results. One factor leading to elevated erythropoiesis in the infant is chronic hypoxia caused by the increased metabolic rate of the infant. The other mechanism is insulin stimulating the synthesis of reticulocytes directly.

Polycythaemia in the infant then often leads to neonatal jaundice, caused by the high amount of red cell haemolysis (102).

Another aspect of chronic foetal hypoxia is described by Teramo in 2010 (103). As the stillbirth- rate is 4-6 times higher in IDM than in non diabetic pregnancies and neonatal 2.4 times higher, he postulated a correlation of infant death with chronic hypoxia. The rate of foetal deaths significantly increased after 35 weeks of gestation. The risk for late gestation stillbirths is lowest within a weight of 2750g to 4000g in diabetic pregnancies, but even in non- diabetic pregnancies the risk for stillbirth or neonatal death is increased starting at 4000g. Teramo described a correlation of bad maternal glycaemic control and stillbirths. Not only the higher demand due to increased foetal metabolism is thought to be causal, but also a shift in maternal oxyhaemoglobin dissociation curve. Increased glycosylation of haemoglobin leads to a decrease of oxygen supply from the mother to the infant caused by reduced oxygen release of red blood cells. This aspect could also be an explanation of stillbirths in infants missing macrosomia. Foetal hyperinsulinaemia is also a risk factor for impaired placental- umbilical blood flow, which worsens the prediction additionally. He claimed that the number of infant deaths, stillborn or death in neonatal period did not change dramatically in the years from 1990 to 2010, despite progress in foetal and neonatal monitoring. Maternal glycaemic control remains to be the main factor to prevent adverse pregnancy outcome.

In 1996 Weintrob et al. (95) postulated a higher incidence of chronic intrauterine hypoxia in infants of diabetic mothers. Consequently, they have an increase in risk for polycythaemia up to 20% compared to infants of non diabetic mothers, which have a risk of about 3% to 5%. The chronic hypoxia in IDM

stimulates the erythropoietin excretion, which as a consequence leads to increased production of erythrocytes. Weintrob et al. based their conclusions on the results of studies performed on rhesus foetuses and lambs.

3.6.2. Changes in neonatal period

Another condition often seen in IDM is hyperbilirubinaemia in the newborn. In 1988 Weiss et al. (104) compared clinical data of 228 women with gestational diabetes mellitus, of which 33 had an elevation of insulin levels in amniotic fluid. He observed a significantly higher increase in prevalence for hyperbilirubinaemia in those infants with increased levels of insulin in the amniotic fluid. In the group with normal insulin levels, consistent of 195 participants, only 7.7% of the infants presented with hyperbilirubinaemia, whereas within the group of increased amniotic insulin levels 36.4% showed elevated bilirubin levels. Or more precisely, hyperbilirubinaemia was observed in 57% of children born to women with gestational diabetes mellitus, who had increased insulin levels in the amniotic and who had dietary therapy only. But only 21% in those treated with insulin and dietary measurements.

The mechanism hereof is not clearly understood yet. It is thought to be due to elevated erythropoietic metabolism, with immature haematopoiesis, or prematurity of the erythrocytes (73,95).

3.7. Polycythaemia and hyperviscosity syndrome

Infantile haematocrit and blood viscosity values are affected by many different factors like site of sampling or age of the infant. Polycythaemia in infants is defined as a haematocrit level ≥65% in samples drawn from a peripheral vein. Whereas viscosity is influenced by a variance of factors, most importantly red and white blood cells as well as the amount of platelets. Additionally, blood pH, fibrinogen and proteins contribute to blood viscosity (105).

The resulting polycythaemia of above described increased erythropoesis needs to be treated immediately, as it can lead to hyperviscosity syndrome in the

neonate with renal vein thrombosis, necrotising enterocolitis or aggravation of respiratory distress (95).

3.7.1. Changes in foetal period

One main factor for foetal polycythaemia is chronic intrauterine hypoxia, known to be present in infants of diabetic mothers. But as mentioned above foetal increase of red blood cells has a variation of possible causes, connected to foetal hyperinsulinism. The consequences of chronic oxygen deficiency in foetal period and subsequently the consequences of polycythaemia are mainly present in neonatal period.

3.7.2. Changes in neonatal period

Clinical presentation of the neonates is similar to the symptoms of hyperinsulinaemic hypoglycaemia described previously. Symptoms like lethargy, irritability, tachypnea, jaundice, vomiting and poor feeding are described frequently (105).

Polycythaemia and hyperviscosity is thought to have most impact on the tissue supplied by small vessels, as presented in a study including 11 infants diagnosed with polycythaemia and hyperviscosity. Polycythaemia was defined as a haematocrit of 63% or more. Hyperviscosity was diagnosed independently from polycythaemia. For control parameters seven healthy infants were examined. All infants were measured twice within an interval of 6 hours, affected infants underwent partial exchange transfusion after the initial measurement. Cerebral arterial blood flow was then obtained by Doppler measurements of the anterior cerebral artery via the anterior fontanel. Individuals suffering of polycythaemia not only showed a decreased cerebral blood flow, with improvement after exchange transfusion. But also heart rate of the infants changed after transfusion. Increase of heart rate after transfusion might be a compensatory mechanism to the decreased RBC and therefore less oxygen supply of peripheral tissues. But also decrease of hyperviscosity is discussed as a cause. Whether the decrease in cerebral blood flow has a negative impact on cerebral function or not is not clear (106).

Polycythaemia is also thought to affect kidney function with impairment of filtration and metabolism. Renal vein thrombosis (95), which would lead to loss of function of the subsequent tissue, as well as a decrease in glomerular filtration rate (105) are described. Additionally, hyperviscosity is thought to disturb vitamin D metabolism at the renal level (107).

Another frequently described complication of polycythaemia is necrotising enterocolitis, which on the other hand often comes along with different comorbidities, therefore a straight correlation could not be assured (105,108).

3.8. Hepatic changes

The liver is known to play a main role in the individual's metabolism. Especially for carbohydrate metabolism with breakdown of glucose molecules and storage of glycogen it is essential and therefore vulnerable for disturbances.

3.8.1. Changes in foetal period

Roberts and colleagues (87) showed changes in foetal liver length. They included 104 pregnant women, divided into group 1 consisting of 26 women with type 1 diabetes, group 2 of 54 women with type 2 DM and group 3 including 24 obese women as a control group. In group 3 present DM was excluded at 28 weeks of gestation. Additionally, they used reference values of a standard group and original liver length of unselected patients delivered at the National Women's Hospital in Auckland. Foetal measurements were obtained at 18, 28 and 36 weeks of pregnancy. Liver length of the control group was only obtained once at 36 weeks of gestation.

For presentation of the results they combined group 1 and group 2 as there was no difference found, neither in liver length nor in other parameters measured by ultrasound. A significant increase of femur length, abdominal circumference and liver length of diabetic patients was found compared to reference values. Liver length showed an even greater increase than AC or FL and showed a progress in excessive growth throughout pregnancy. At 18 weeks mean values of overgrowth was measured to be 12% in the diabetic group, at 36 weeks of GA infants showed

liver lengths about 19.3% greater than reference values. Similar results were shown in the obese group, in which length was increased about 9.4% in mean compared to reference parameters.

Matching results were found in 38 infants of diabetic mothers and LGA. Compared to non LGA infants, those 38 foetuses showed an accelerated growth rate of the liver, amongst others. Whereas in the control group the growth rate was in mean 1.8 mm per week, it was 2.7 mm per week in LGA infants. Together with fat mass the liver was the only organ showing a significant difference in growth rate, compared to the control subjects. Nevertheless, a total increase of 12% in lean body mass was described in infants born LGA (84).

3.8.2. Changes in neonatal period

Naeye (80) compared the autopsy results of 25 infants of diabetic mothers, who were stillborn or died within the first two weeks of life, to the data of 14 infants born to non diabetic mothers. Additionally, to the general body weight increase, Naeye raised results for six different body organs, all of them with an increase in weight compared to a control group of infants born to non diabetic mothers. As described already before, he found liver weights increased up to 179% of the values of the control group. As the median diameter of cell nuclei was idem in the control group and the overweight infants, he postulated that overweight infants had a higher number of hepatic cells in general. Furthermore, he described an increase of cytoplasm by 63% and an increased level of erythropoietic precursor cells. In synopsis all those factors might cause the increased liver weight.

Also the results of Kehl et al. (84) are consistent with the results of several other studies. In their study in IDM the liver showed the greatest increase in growth regarding lean body mass, which is allegeable by the fact of liver tissue being highly sensitive to insulin, which might lead to increased glycogen stores in the liver. Whereas the kidneys and bones are relatively insensitive

3.9. Placental changes

The placenta, with its function as connecting organ of maternal and foetal metabolism, is exposed to influencing factors of both organisms. Likewise, it has a great impact on foetal development. Furthermore, placental changes are very likely to appear due to strong interaction of maternal and foetal metabolism.

A frequently discussed aspect is the transport of nutrition by the placenta. The placenta is connected to maternal and foetal circulation and therefore susceptible for altered states of both. With advancing pregnancy foetal impact is increasing (Fig.8). Not only is insulin accountable for altered expression of genes related to signal transduction, metabolism as well as transport. But also may foetal insulin lead to increased glycogen synthesis in endothelial cells (109).

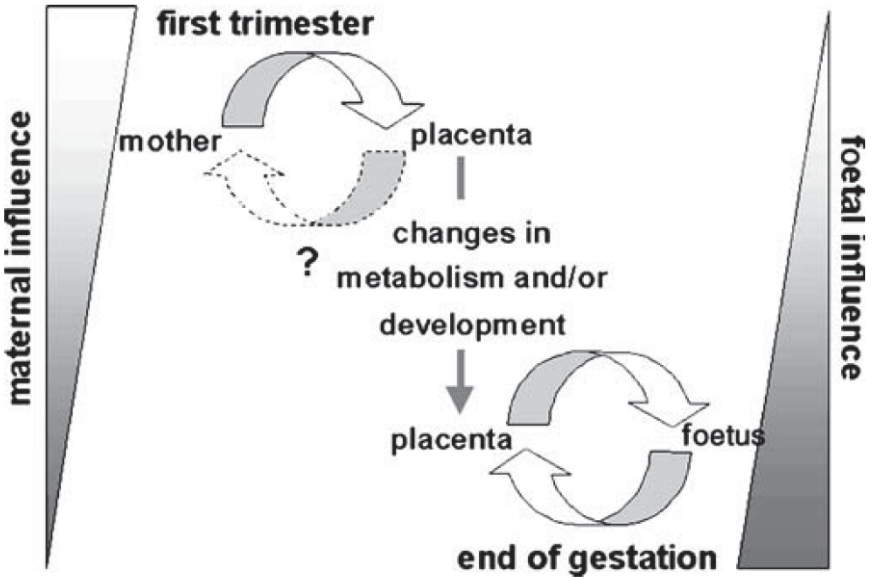

Figure 8: Altered maternal and foetal influence on the placenta troughout pregnancy (111)

The study group of M. Rodríguez-Morán (110) investigated the correlation of infantile growth, hyperinsulinism and placental maturity. They included 272 term born babies were included, mothers with possible confounding factors, like (gestational) diabetes mellitus, diagnosed DM in previous pregnancies, high blood pressure, intrauterine infections, preterm labour, malnutrition or dextrose use prior to birth, were excluded from the study. Placental maturity was evaluated after birth at microscopy and insulin levels were measured in cord blood serum. They found a

strong correlation of infants born SGA and hyperinsulinism, which stays in contrast to above described findings of macrosomia. Possible explanations for infants born SGA despite high levels of insulin are placental immaturity or an early in- utero insulin resistance, leading to a limited effect of insulin in the foetal organism. Rodríguez-Morán et al. found a correlation of smoking, young maternal age, family history of DM and high insulin levels at birth. Therefore, they are suggesting further investigations in this regard. It is to be identified whether hyperinsulinaemia is cause or consequence of intrauterine growth restriction and placental immaturity.

Another frequently discussed effect of insulin acting on the placenta is via increased angiogenesis leading to hypervascularisation in placental tissue. For this purpose Lassance and colleagues (111) used third trimester foeto- placental endothelial cells of human placentas. They showed in vitro pro- angiogenetic effects of insulin. Insulin did not show a direct proliferative effect on the cell cycle, but stimulated angiogenesis on different signalling pathways. These results reassure the hypothesis of insulin as one responsible factor for the frequently observed hypervascularization on the placenta in pregnancies complicated by GDM.

Additional to increased angiogenesis other morphological changes were observed. Increased placental weight and immature terminal villi are frequently described. Both are seen in pregnancies with pregestational and gestational DM (112).

3.10. Residual organ systems

Little human data was found regarding the remaining organ systems, like endocrinological or digestive system. Some case reports describe alternate findings, but no studies including a greater population.

Within his analysis, Naeye (80) also discovered a 27% increase in the weight of the spleen in IDM. On further examinations on the splenic tissue, he again saw augmented numbers of erythrocytes with an increased haematopoiesis, whereas lymphoid cell mass was diminished. The weight of the thymus glands was in average increased about 37% and the adrenal glands about 58%. Only minimal

weight gain (5%) was described for the kidneys in overweight infants. Whereas kidneys and thymus glands showed an equal increase of cortical mass and medulla, adrenal glands primarily had an augmented cortical foetal zone. Matching histological findings of a hyplerplastic cortical zone, there were some signs of hyperadrenocorticism such as hypokalaemia.

Consistent findings are described by Farquhar in 1962 (113). Where increased levels of corticosteroids were described in amniotic fluid, urine and blood samples.

Also the weight of the kidneys was found to be 105% of control values. For all organs the cytoplasm of the cells was increased, whereas the nuclei were in general not affected. Nevertheless, in some organs a greater mass of nuclei was found, indicating an increase of singular cells (80). Nephromegaly is also describe in two cases of macrosomic infants weighing 5.6 kg and 5.7 kg. As confounding factors like Beck- Wiedeman- syndrome were excluded, THI is thought to be causal (114).

The same infants showed signs of intestinal dysmotility, which is a frequently described finding in hyperinsulinaemic individuals. Commonly presenting with persistent vomiting (bile stained) and intolerance to enteral feeding (114,115). Investigations in adults showed a decrease of gastric and small intestine motility at a hyperinsulinaemic condition (116). Similar results are described in a study including five infants diagnosed with severe PHHI and in a case report with two more infants suffering PHHI. In both resources, EEG was performed to investigate the origin of vomiting. Additionally, imaging studies of the two individuals showed a half-time of gastric emptying of 115 minutes, normal ranges are usually between 35 and 55 minutes for gastric emptying. The underlying cause is thought to be a disturbance of myoelectrical activity (115,117).

An in vitro study showed the effect of insulin on human skin fibroblasts. Cells were isolated and grown, to induce collagen synthesis to a maximum ascorbic acid was used. Subsequently non- essential amino acids, glucose in different concentrations and/or insulin were added to the processed cells. Thereby, cells supplied by a high glucose medium secreted a higher amount of collagen than cells in a physiologic glucose condition. Additional insulin, however, did not further increase the collagen synthesis, but accumulation of non- collagen protein was

stimulated. This effect is thought to be due to the growth stimulating effect of insulin (118).

Although the occurrence of congenital hyperinsulinism with consequently (severe) hypoglycaemic episodes in neonatal period is known for many years now, it is still a severe condition. Mainly due to the fact that it remains unrecognized or symptoms are misinterpreted.

3.11. Case Reports

The case report of Chinoy et al. (119) published 2018 showed the consequences of unrecognized hyperinsulinism. In this case the newborn's condition was mistaken as sepsis twice. The infant was born slightly premature (34+4 weeks) and spent the first ten days at the neonatal unit, treated with antibiotics for a strong suspicion of sepsis despite negative blood cultures. Simultaneously, he was treated with phototherapy for hyperbilirubinaemia. Several times severe hypoglycaemic blood levels were measured and treated with frequent feedings. The cause of hypoglycaemia was not the point of interest at this moment and thought to be due to inadequate uptake of nutrients in line with prematurity. At the following days his situation stabilised and the newborn was discharged in a good condition. At the age of four months the parents sought for help at the hospital again, he was treated with antibiotics due to suspected balanitis. The following days his situations worsened, he started vomiting and got diarrhoea, to the point of unresponsiveness. In hospital he presented pale, hypothermic and with circulatory failure missing visible signs of severe blood loss. Blood samples detected pancytopenia, severe hypoglycaemia (<1mmol/L) and acidosis. The baby died within 4 hours after presentation at the hospital, despite extended resuscitation actions. At the following days an autopsy was performed. Only at this point they detected focal hyperinsulinism, no other organ malformations or signs for known syndromes as Beckwith- Wiedemann were found. Distinctive left shift of bone marrow with an increased number of precursor cells was detected at histology examinations. Also at this point no signs for an infection were present. In this case hyperinsulinaemic hypoglycaemia is thought to be the cause of sudden infant death.

Another case of hypoketotic hyperinsulinaemic hypoglycaemia in siblings is reported. The siblings are born two years apart, both presented with persistent hypoglycaemias in the first days of life. In both patients an ABCC8 compound mutation was detected later on. The first infant was male and an early term with 4220 g birth weight. The mother was diagnosed with gestational diabetes at 20th week of gestation, the diabetes was dietary controlled. The infant presented with impaired consciousness, bradycardia and hypotonia due to severe and persistent hypoglycaemic episodes 36 hours after the delivery. Abdominal sonography and computed tomography were performed, but no abdominal abnormalities were described. Laboratory findings revealed increased levels of ketone bodies and free fatty acids, with low glucose levels and high levels of insulin. After high glucose infusion rates, the infant was treated medically with diazoxide and later on hydrochlorothiazide. At the age of 3 months the patient underwent a partial pancreatectomy (>90%), due to persistent neurological events like seizures. Postoperatively no improvement was observed, enteral feeding and subcutaneous octreotide was initiated. At the age of 5 years the boy's developmental status was evaluated, he presented with psychomotoric delay, hemiparesis, spontaneous involuntary movements of the left arm and dysesthesias. The MRI showed cerebral atrophy and hyperintensity in the medial part of the right temporal lobe, indicating periodic hypoglycaemic episodes. During puberty the glycaemic situation improved, by the age of 18 years the medical therapy for blood glucose controll could be reduced and switched to lanreotide 60 mg every 30 days. He remained with slight neurological deficits, but without epileptic activity under carbamazepine therapy and his body composition (BMI 22kg/m^2) consistently improved (120).

Two years later the woman gave birth to a term born girl with 3 600 grams. Again at 20th weeks of gestation the woman was diagnosed with GDM, treated with dietary measures. Only one hour after birth the infant showed sever hypoglycaemia with 20mg/dL. Initial therapy with diazoxide and hydrochlorothiazide at the age of 1 month, showed no improvement regarding the blood glucose levels. Equal to the brother's course, she was treated with octreotide and enteral feeding. During puberty the number of hypoglycaemic episodes declined and episodes were less severe. Again the therapy was switched to lanreotide 60 mg every 28 days. Obese body weight was detected up to the age of 6 years, continuously decreasing. At the

age of 18 years her BMI was 22.6 kg/m^2, she has no neuropsychological impairment or seizures, no signs of long term damage due to hypoglycaemia (120).

A case report of Mehta A. and Hussain K. described two infants born with severe transient hyperinsulinism. One mother had a history of mild gestational diabetes, whereas the second mother had no signs of metabolic disturbances during or after the pregnancy. In both cases macrosomia, with 5.6 kg and 5.7 kg, hepatomegaly, nephromegaly and HOCM was detected at ultrasound examination (114).

Shortly after the delivery patient one fell to a nadir of 27 mg/dl with his blood glucose level. After high glucose infusion rates and later on continuous octreotide and glucagon infusions, as well as high amount of volume replacements the patient remained stable and could be taken off ventilatory support. Glucose infusion rates were reduced and enteral feeding initiated, which led to recurrent vomiting and retching. Subsequently, an electrogastrogram (EGG) was derived. The clinical presentation in synopsis with the EGG results confirmed the suspicion of intestinal dysmotility. At the age of 3 weeks full enteral feeding was established and the patient was slowly tapered of medical therapy. At the discharge he was able to retain normal blood glucose levels without any therapy in this regard. The infant obtained oral propranolol therapy for the HOCM, which showed itself regressive at the sonography at 6 weeks of age (114).

Patient two was cyanotic and hypotonic, with need for resuscitation and ventilatory support right after birth. During the course this patient also presented itself with frequent vomiting after enteral feedings. The EGG again revealed a profound intestinal dysmotility requiring parenteral nutrition. At the age of 4 weeks octreotide, glucagon and parenteral feedings could be stopped without recurrence of hypoglycaemic episodes. The follow up at 3 moths of age showed complete regression of the cardiomyopathy.

Both cases showed typical features of HI like hyperfattyacidaemia, hyperketonaemia, hyperinsulinism and hypoglycaemia. Primary suspected BWS was excluded in both cases, consequently THI was diagnosed (114).

In another case report the authors described the rare case of a newborn dying due to heart failure as a consequence of cardiomegaly. As described above

cardiomegaly usually resolves itself within weeks with supportive actions only. In this case the infant was detected to be macrosomic (5500g) with an estimated weight above the 97th percentile. As in this case the mother suffered from obesity, had a history of GDM and also pathological glucose testing, a hyperinsulinaemic condition in the infant is highly likely. On the autopsy record not only a macrosomia and cardiomegaly was described, but also weight increase in the liver, kidneys, spleen and thymus. All of these organ's weights were above 99th percentile for gestational age. Moreover, the infant was cyanotic on initial appearance and at the autopsy they found petechiae at the alveolar, pleural and pericardial site, which additionally indicates intrauterine hypoxia. The results of this case report are consistent with the results of Naeye in 1965, who also found an overall increase in the different organ weights (73).

4. Discussion

The research on infants of diabetic mothers started many years ago. First descriptions of IDM are reported back in 1826 by Bennewitz, who described the abnormal appearance of an infant born to a diabetic mother. Pedersen in 1954 was then the first one to link the changes in neonates to foetal hyperinsulinism. He set the hypothesis of maternal diabetes mellitus potentially causing excessive supply of nutrients, including glucose, to the infant leading to foetal hyperinsulinaemia. The following years this hypothesis was affirmed and supported by many studies.

The aim of this thesis is to present overall changes in foetal and neonatal period linked to hyperinsulinaemia. Human studies were included only, which gives restricted access to recent results. Main data about changes of the organ systems in IDM was extracted from papers published in the time period from 1970 to 2000.

Limitations in this study field are given by the nature of the subject. Especially in utero examinations are difficult and only few methods are available. The most common one is sonography, which is low in risk and cost effective. But the results obtained by sonography are highly dependent on the examiners routine. Measurements differ depending on the examiner and his or her experience. Rarely any studies mentioned if the examination was performed by one or multiple

examiners. Additionally, foetal movements are complicating the situation. Therefore, multiple measurements by different examiners and mean values of this data would give a more objective result for comparison. This approach was used by Kehl et al. (84), where ultrasound examinations were performed by two different examiners. Results showed a coefficient of variance of 5% in seven of nine parameters, and a highest of 8% for subcutaneous thigh fat measurements.

Further confounding factors are the stricter ethical guidelines established nowadays. Consequently, study designs are frequently retrospective or of small group size. Additionally, the increase of preventive measurements in terms of pregnancies complicated by pre-existent and gestational DM leads to less severe consequences of hyperinsulinism in this population.

Another aspect, which needs to be considered for reviewing, are the inconsistent definitions of hyperinsulinaemia and the variable thresholds of tolerance in the individuals.

Different animal studies give a more detailed information about the impact hyperinsulinism in the foetus.

Susa and Schwarzt (121) investigated the effects of increased insulin levels in primate foetuses. Concordant with the case reports and studies mentioned previously they described elevated weights of liver, spleen and heart. Susa and Schwarzt collected data from a control group, a group with low- dose insulin injections and a group with high dose insulin injections to the foetus. Whereas increase in weight of the heart was observed in both insulin- injected groups, enlargement of the liver and spleen was only seen in the high dosage group. Furthermore, a higher mortality was observed in the group with higher dosages of insulin with a stillborn rate of 40% compared to 20% in the low- dose injection group.

Glucagon as one of various hormones to increase blood glucose levels is one of the major antagonists of insulin and vice versa. This suggests an impact of excessive insulin levels on glucagon synthesis and function. In this regard no studies with human data were found. The condition of hypoglycaemic episodes in neonates is allegeable by the fact of insulin itself lowering the blood glucose level and as an additive component suppressing antagonists like glucagon, which would,

in a physiologically functioning organism, elevate blood glucose levels by utilization of glycogen stores. Glucagon suppression does not occur in neonates only, but also in utero (121). However, a decrease of glucagon producing cells is not described.

Another often discussed topic is the influence of hyperinsulinism on glucocorticoid metabolism. Cortisol as a known antagonist of insulin and important factor for foetal lung maturation is thought to be highly impaired by hyperinsulinaemia. Whereas in former times hypercortisolism was assumed to be present in foetuses and neonates with hyperinsulinaemia, insulin is now thought do suppress the release of corticosteroids. In animal studies as well as in vitro models not only a decrease of surfactant synthesis, but also an insufficient response to a cortisol bolus in hyperinsulinaemic foetal lambs was observed. In summary, due to the infants hyperinsulinaemia physiological lung maturation is impaired and facilities of external support by corticosteroids are insufficient (112,122). Furthermore, an augmented lung volume in lungs of hyperinsulinaemic foetal rhesus monkeys was described by Beck et al. in 1981 (123), thought do be caused by increased connective tissue.

Based on animal studies different references postulate an indirect effect of insulin on surfactant synthesis by supressing glucocorticoids. Glucocorticoids play a major role in lung maturation in terms of surfactant production, especially in late gestation. Impairing the lecithin synthesis and therefore an adequate surfactant production, elevated insulin level may lead to surfactant deficiency and consequently neonatal respiratory distress syndrome (93,124,125).

These findings could give a causal explanation of the increased risk for IDM in humans to suffer from RDS. The condition of impaired surfactant production and elevated amount of connective tissue may be contributing to the infants' condition.

In contrast to above mentioned findings of decreased cortisol levels, Susa and Schwarzt described no changes in cortisol level at birth in primate foetuses. They observed neither in the group treated with high doses of insulin nor in the group treated with low doses a significant change compared to the control group (121).

Whereas human data to muscular changes in hyperinsulinaemic infants are not significant, a study published in 2016 described changes in muscle fiber of foetal sheep. The sheep received continuous insulin infusion and simultaneously dextrose

infusions to maintain a euglycaemic state and were compared to a control group. Tissue samples of the biceps femoris, tibialis anterior and soleus muscle were examined. In the hyperinsulinaemic group the authors found a 1.3- fold increase in myoblast proliferation and a decrease in differentiation of cells (126).

Insulin impairing the development of different tissues has been frequently reported. In 1999 A. Plagemann et al. (127), focused on a different aspect by investigating the impact of hyperinsulinaemia on hypothalamic nuclei in rats. The aim of this study was to gain detailed information about hypothalamic changes due to a hyperinsulinaemic condition, knowing that hypothalamic tissue plays a main role in metabolism, food intake and insulin secretion. Pregnant rats received a single streptozotocin injection to obtain impaired glucose tolerance. The investigations showed structural disorganisation and reduced cytoplasm in hypothalamic nuclei of hyperinsulinaemic offsprings. This morphological changes together with changes in quantity of hormones and neurotransmitters, like leptine or catecholamine, may lead to altered hypothalamic regulation of metabolism and body weight, which might persist throughout life.

Due to the wide spread effects of hyperinsulinism, early recognition and therapy of foetal and neonatal hyperinsulinism is important to prevent long term consequences for infants. Some conditions in adulthood, i.e. obesity, might have its origin very early in human development, therefore besides early therapy interventions preventive measures are most important. Long term changes were not mentioned specifically prior in this thesis as they are exceeding foetal and neonatal period.

One of the main life time consequence for IDM is an increased risk for obesity throughout puberty and adulthood in contrast to infants of non diabetic mothers. Consistent with increased risk for obesity, IDM are found to have a higher risk to develop Type II DM, which comes along with various comorbidities such as, microangiopathy leading to visus problems and kidney failure at end- stage. The risk for Type I DM does not seem to be markedly increased, unless the patients underwent therapeutic pancreatectomy (95).

For other aspects, like neurodevelopment, it seems like neurodevelopmental delay is very rare in later life, presupposed euglycaemic levels are obtained as early as possible. Most infants were at a normal developmental stage at the latest age of 6 years and were able to receive regular education. But, it is reported that some patients have learning difficulties in a cohort treated medically only (95,128). It is to mention that infants treated only medically usually suffer a lighter course of HI and therefore complications are likely to be less severe. Also Menni et al. (64) described a more severe outcome if hyperinsulinism was of early onset, late recognition or if surgery was required. In general the outcome is less severe with the awareness of hyperinsulinism as a possible cause for hypoglycaemia.

However, vision and auditory difficulties are frequently described hyperinsulinaemic infants, which might persist throughout life. Also, structural abnormalities, like previously described occipital atrophy persist for several years and are not expected to be rebuild to the full extent.

For all the described findings a difference in abnormalities was not found to be connected to focal or diffuse type of HI. But the aetiology seems to have an indirect impact on the outcome. Hyperinsulinaemia due to genetic cause is described to be more difficult to treat in some cases and therefore infants suffer a more severe course. Especially mutations of K_{ATP}- channel are mentioned to be unresponsive to diazoxide or octreotide (43,129).

Additionally, genetic mutations are rather described to be causal for persistent hyperinsulinism than transient hyperinsulinism. Maternal diabetes, in contrast, often results in transient HI of the foetus and neonate. They are known to be at risk to develop HI and are detected early. Transient hyperinsulinism in general is more responsive to medical therapy and therefore consequences are usually of weaker characteristics (130). Regarding the short term outcome, a difference between gestational DM and pre-existing Types of DM was not found. Neither showed the infants any difference in clinical presentation, nor is a difference in number of affected infants described. However, differences can be suspected due to the fact that pregestational DM is known before pregnancy and therapeutic measures can be adapted from the beginning. In contrast to this is gestational DM commonly tested around 24- 28 weeks of gestation, where hyperinsulinaemia might be already

present for several weeks. Therefore, not a difference in symptoms but in the number of affected offsprings might be present.

Globally, different approaches regarding the screening recommendations for GDM are present. A general screening of all pregnant women is put in contrast to a screening only of those at risk. Whereas testing of all women is thought to stress healthy pregnancies unnecessarily, a screening of at risk patients only leads to undetected cases. According to the German Diabetes Association a screening only for women at risk is not recommended as up to 40% of cases with DM remain unrecognised and therefore do not receive an adequate treatment (30).

An OGTT is routinely recommended for all pregnant women to be performed in the second trimenon (24-28 weeks of gestation) in Austria since 2010 and in Germany since 2012 (29,30). This preventive measures lead to an early recognition of (gestational) DM and subsequently a decrease of risk for the infant.

The guidelines are based on the data of the HAPO study, which included 25000 pregnant women all over the world. This study offers data for the correlation of maternal hyperglycaemia and adverse perinatal outcome, with emphasised interest for parameters like foetal size, neonatal morbidity, mode of delivery and foetal insulin levels (131).

Another aspect is that OGTT results are repeatedly at the borderline to be pathologic, but within normal values. Depending on the doctor in charge further investigations are then performed or not. In practise, repeatedly pregnancies with indices for GDM are seen but the diagnosis has not been confirmed by previous tests.

Also shown in a study with 29 macrosomic infants born to healthy mothers with normal OGTT test (132). This suggests that current guidelines might need to be revised to increase sensitivity and specificity.

A possible approach would be to perform a glucose testing earlier in pregnancy than it is currently performed. Due to the fact that current guidelines recommend an OGTT earliest at 24 weeks of gestation and foetal insulin secretion starts at around 10^{th} week of gestation, there might be increased insulin levels present for 14 weeks without being noticed. Another alternative approach would be measuring at two different points of time during pregnancy to increase sensitivity.

For the interpretation of OGTT results, the so called ‘foetal glucose steal phenomenon’ is to be considered. Foetal organism is provided with glucose by facilitated diffusion from maternal to foetal compartment. The diffusion rate adapts, depending on the maternal- to- foetal gradient of glucose concentration. In this situation foetal circulation is thought to draw increased amounts of glucose from the maternal metabolism and consequently mask maternal hyperglycaemia (133).

In 2016 Desoye and Nolan (133) stated an aggravation of this phenomenon in pregnancies with a hyperinsulinaemic foetus. In terms of hyperinsulinaemia, glucose transportation to sensitive cells is augmented and foetal blood glucose levels are constantly lower than maternal blood glucose. Therefore, increased levels of foetal insulin are thought to lead to an augmented glucose flux from maternal to foetal circulation, which again is transported to and uptaken by the cells and foetal blood glucose level is lowered, forming a vicious cycle.

The management of diabetic women to improve the pregnancy outcome was subject of several studies. Weiss et al. (104) compared diabetic pregnancies with dietary therapy only to dietary measures in combination with insulin therapy. In this study, with 228 diabetic pregnancies, a significant increase in perinatal mortality in the subgroup of dietary therapy only was observed. Moreover, comorbidities of infants with hyperinsulinaemia, like respiratory distress or hyperbilirubinaemia, were found to be significantly decreased if insulin therapy was added.

Also Coustan et al. (83) recommends a therapy, which targets values strictly within the recommended ranges. With a therapy controlled more strictly, they observed a better outcome in infants, with less severe consequences. They compared the outcome of prophylactic treatment with insulin to dietary controlled treatment and no treatment. Within the infants of women treated with insulin only 7% appeared to be macrosomic in contrast to 18.5% and 17.6% in the dietary and no- treatment groups, respectively. Also, the incidence of operative delivery methods, including i.e. vacuum extraction and forceps, were significantly increased in the latter groups. Moreover, women are often found to be at a borderline level for GDM, pre-diabetic or diagnosed with GDM requiring no treatment and with advanced pregnancy common complications of diabetic pregnancies appear.

Coustan's results show that prophylactic insulin treatment might have a great positive effect on the pregnancy outcome in those specific groups.

Consistent with Coustan and Weiss, the findings of White et al. showed that already mild forms of diabetes caused significant changes of foetal organs. That suggests an augmented sensitivity of foetal organs to metabolic changes. Mainly foetal organ weights were increased and changes were found to be significant in liver, lung, heart and intestine. Furthermore, an upregulation of receptors for the insulin/IGF system was observed in most of the foetal organs (134).

Preventive measures have been increasing over the years, but still pregnancies with typical characteristics of a diabetic pregnancy are observed repeatedly. Recommendations for management of HI vary greatly and also depend on the caretaking hospital. Clear guidelines for all infants are difficult to develop, as so far no clear definition for hypoglycaemia and hyperinsulinism in newborns could be set. Also, the study protocols about hyperinsulinaemia and hypoglycaemia are using different thresholds in the including criteria, which again has an impact on the population size, outcome and therapy.

Cornblath et al. (135) recommend to use a combination of the infant's blood glucose level and organ specific symptoms to define the threshold requiring interventions after birth. The possible complicating factor in this approach is the asymptomatic hypoglycaemia, which can be present and remains unnoticed to the point of first symptoms in some individuals. It is reported that repeated episodes of severe hypoglycaemia in a developing brain can cause neurodevelopmental delay. As the brain is very sensitive and dependent on glucose supply for adequate function, it is also very vulnerable to lack of fuel. Cerebral dysfunction is the most obvious symptom of a deficient metabolic state. Considering this, long term hypoglycaemia might have a greater impact on the development of specific organs, additional to the brain.

A case report mentioned above shows a benefit in siblings with CH for the younger one. Both suffered from congenital hyperinsulinism due to a genetic mutation in the ABBCC8 gene. Whereas the first born had deficits in neurological

development, the second one had a complete normal development. The knowledge gained during treatment of the first born contributed to a better course in the second one. Consequently, leading to a better neurodevelopmental outcome despite the two of them diagnosed with the same cause of hyperinsulinaemia. Which again indicates that, early recognition and treatment can affect the course and outcome of the disease significantly (120).

This again reassures the essentiality of early recognition and therapy, in best case even prevention of foetal hyperinsulinism. Due to the increasing incidence of gestational and non gestational DM and high frequently new gained research results a permanent revision of guidelines is crucial.

5. Conclusion

Most significant changes were present in foetal period, with enlargement of the liver and heart and augmentation of fat mass. Changes in foetal period are often the origin for neonatal consequences, i.e. lung maturation is impaired in foetal period and consequently neonates are at a higher risk to develop RDS. Different changes in different organ systems may interact with each other and increase the risk for more severe consequences. Polycythaemia and hyperviscosity together with a reduced mobility of the heart, increase the risk for thrombi.

However, consequences were described in every organ system of foetuses and neonates, except reproductive organs. No human data for changes in the reproductive system were found.

A high mortality rate of IDM in former times is reported frequently, most likely due to severe episodes of unrecognized hypoglycaemia. Although the risk of infant's death could be decreased remarkable over the years, there is still a higher risk in hyperinsulinaemic infants for late gestational or early infant death.

Besides changes in organ systems and a higher mortality rate due to hyperinsulinism, is commonly described, but also are infants with HI at a higher risk for birth injuries, need for birth assistance with i.e. forceps and C- section birth.

With this thesis an overview about existing knowledge regarding the morphological and non morphological consequences of foetal and neonatal

hyperinsulinism is given. Changes were described in foetuses, neonates and partly in early childhood. Hormonal changes and long term consequences lasting throughout adulthood still need to be investigated on a more detailed level to understand the full extent of foetal and neonatal hyperinsulinism.

Bibliography

1. Güemes M, Rahman SA, Hussain K. What is a normal blood glucose? Arch Dis Child. 2016 Jun;101(6):569–74.
2. Bozzetti P, Ferrari MM, Marconi AM, Ferrazzi E, Pardi G, Makowski EL, et al. The relationship of maternal and fetal glucose concentrations in the human from midgestation until term. Metabolism. 1988 Apr;37(4):358–63.
3. Hoseth E, Joergensen A, Ebbesen F, Moeller M. Blood glucose levels in a population of healthy, breast fed, term infants of appropriate size for gestational age. Arch Dis Child Fetal Neonatal Ed. 2000 Sep;83(2):F117-9.
4. Stanley CA, Rozance PJ, Thornton PS, De Leon DD, Harris D, Haymond MW, et al. Re-evaluating "transitional neonatal hypoglycemia": mechanism and implications for management. J Pediatr. 2015 Jun;166(6):1520–5.e1.
5. Baynes JW, Dominiczak MH. Glucose Homeostasis and Fuel Metabolism: Diabetes Mellitus. In: Medical Biochemistry. Sanders Elsevier; 2014.
6. Heinrich PC, Müller M, Graeve L. Insulin - Das wichtigste anabole Hormon. In: Löffler / Petrides Biochemie und Pathobiochemie. Springer-Lehrbuch; 2014. p. 1073.
7. Weiss M, Steiner DF, Philipson LH. Insulin Biosynthesis, Secretion, Structure, and Structure-Activity Relationships. Endotext. MDText.com, Inc.; 2014.
8. Lord K, De León DD. Hyperinsulinism in the Neonate. Clin Perinatol. 2018 Mar;45(1):61–74.
9. Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Curr Diabetes Rev. 2013 Jan 1;9(1):25–53.
10. Blackburn ST. Maternal, fetal, & neonatal physiology: a clinical perspective. Saunders Elsevier; 2007. 777 p.
11. Thorsson A V., Hintz RL. Insulin Receptors in the Newborn. N Engl J Med. 1977 Oct 27;297(17):908–12.

12. Devaskar S, McMenamy K, Holtzclaw L, Sadiq F. Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors. Am J Obstet Gynecol. 1990 Oct;163(4 Pt 1):1350–6.

13. Neufeld ND, Scott M, Kaplan SA. Ontogeny of the mammalian insulin receptor: Studies of human and rat fetal liver plasma membranes. Dev Biol. 1980 Jul 1;78(1):151–60.

14. Piper K, Brickwood S, Turnpenny LW, Cameron IT, Ball SG, Wilson DI, et al. Beta cell differentiation during early human pancreas development. J Endocrinol. 2004 Apr;181(1):11–23.

15. Navale AM, Paranjape AN. Glucose transporters: physiological and pathological roles. Biophys Rev. 2016 Mar;8(1):5–9.

16. Marshall WJ, Lapsley M, Day AP, Ayling RM. Clinical biochemistry : metabolic and clinical aspects. 932 p.

17. Wu X, Freeze HH. GLUT14, a Duplicon of GLUT3, Is Specifically Expressed in Testis as Alternative Splice Forms. Genomics. 2002 Dec 1;80(6):553–7.

18. Rao PNS, Shashidhar A, Ashok C. In utero fuel homeostasis: Lessons for a clinician. Indian J Endocrinol Metab. 2013 Jan;17(1):60–8.

19. Pamela C. Champe, Richard A. Harvey, Denise R. Ferrier. Metabolic Effects of Insulin and Glucagon. In: Biochemistry . 3rd Edition. 2005.

20. Gerich JE, Charles MA, Grodsky GM. Regulation of Pancreatic Insulin and Glucagon Secretion. Annu Rev Physiol. 1976 Mar 28;38(1):353–88.

21. Sadler TW. Medizinische Embryologie. 10. Auflag. Stuttgart: Georg Thieme Verlag KG; 2008.

22. Rodeck B, Zimmer K-P. Physiologie und Embryologie des Pankreas. In: Rodeck B, Zimmer K-P, editors. Pädiatrische Gastroenterologie, Hepatologie und Ernährung. 2. Auflage. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013.

23. Laidlaw GF. Nesidioblastoma, the islet tumor of the pancreas. Am J Pathol. 1938 Mar;14(2):125–134.5.

24. West NJ, Thorpe M. Neonatal hyperinsulinism secondary to maternal intake of high-sugar drinks. BMJ Case Rep. 2011 Jul 15;2011.

25. Alaei MR, Akbaroghli S, Keramatipour M, Alaei A. A Case Series: Congenital Hyperinsulinism. Int J Endocrinol Metab. 2016 Oct;14(4):e37311.

26. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, et al. Recommendations from the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. J Pediatr. 2015 Aug;167(2):238–45.

27. Burlina S, Dalfrà MG, Lapolla A. Short- and long-term consequences for offspring exposed to maternal diabetes: a review. J Matern Neonatal Med. 2017 Oct 2;1–171.

28. Holemans K, Aerts L, Van Assche FA. Lifetime consequences of abnormal fetal pancreatic development. J Physiol. 2003 Feb 15;547(Pt 1):11–20.

29. Bancher-Todesca D, Worda C, Klein K, Kautzky-Willer A, Repa A. LEITLINIE GESTATIONSDIABETES [Internet]. 2010. Available from: https://frauenheilkunde-innsbruck.tirol-kliniken.at/data.cfm?vpath=diverse/oeggg_leitlinie_gdm_7_5_2010pdf

30. Kleinwechter H, Schäfer-Graf U, Bührer C, Hoesli I, Kainer F, Kautzky-Willer A, et al. Evidenzbasierte Leitlinie zu Diagnostik, Therapie u. Nachsorge der Deutschen Diabetes-Gesellschaft (DDG) und der Deutschen Gesellschaft für Gynäkologie und Geburtshilfe (DGGG) [Internet]. 2011. Available from: http://www.deutsche-diabetes-gesellschaft.de/fileadmin/Redakteur/Leitlinien/Evidenzbasierte_Leitlinien/Gestationsdiabetes_EbLL_Endfassung_2011_08_11_.pdf

31. Catalano PM, Shankar K. Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child. BMJ. 2017 Feb 8;356:j1.

32. Stothard KJ, Tennant PWG, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: A systematic review and meta-analysis. JAMA - J Am Med Assoc. 2009 Feb 11;301(6):636–50.

33. White P. Pregnancy complicating diabetes. Am J Med. 1949 Nov 1;7(5):609–16.

34. James C, Kapoor RR, Ismail D, Hussain K. The genetic basis of congenital hyperinsulinism. J Med Genet. 2009 May 1;46(5):289–99.

35. Avatapalle HB, Banerjee I, Shah S, Pryce M, Nicholson J, Rigby L, et al. Abnormal Neurodevelopmental Outcomes are Common in Children with Transient Congenital Hyperinsulinism. Front Endocrinol (Lausanne). 2013;4:60.

36. Zielinsky P, Piccoli AL. Myocardial hypertrophy and dysfunction in maternal diabetes. Early Hum Dev. 2012 May;88(5):273–8.

37. Pinar H, Pinar T, Singer DB. Beta-Cell Hyperplasia in Macrosomic Infants and Fetuses of Nondiabetic Mothers. Pediatr Dev Pathol. 3(1):48–52.

38. Eades CE, Cameron DM, Evans JMM. Prevalence of gestational diabetes mellitus in Europe: A meta-analysis. Diabetes Res Clin Pract. 2017 Jul;129:173–81.

39. American Diabetes Association. Gestational diabetes mellitus. Diabetes Care. 2003 Jan 1;26 Suppl 1(suppl 1):S103-5.

40. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and Trends in Obesity Among US Adults, 1999-2008. JAMA. 2010 Jan 20;303(3):235.

41. Mohamed Z, Arya VB, Hussain K. Hyperinsulinaemic hypoglycaemia:genetic mechanisms, diagnosis and management. J Clin Res Pediatr Endocrinol. 2012 Dec;4(4):169–81.

42. De León DD, Stanley CA. Mechanisms of Disease: advances in diagnosis and treatment of hyperinsulinism in neonates. Nat Clin Pract Endocrinol Metab. 2007 Jan;3(1):57–68.

43. Pinney SE, MacMullen C, Becker S, Lin Y-W, Hanna C, Thornton P, et al. Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. J Clin Invest. 2008 Aug 1;118(8):2877–86.

44. De Lonlay P, Giurgea I, Sempoux C, Touati G, Jaubert F, Rahier J, et al. Dominantly inherited hyperinsulinaemic hypoglycaemia. J Inherit Metab Dis. 2005 May;28(3):267–76.

45. Aynsley-Green A, Hussain K, Hall J, Saudubray JM, Nihoul-Fékété C, De Lonlay-Debeney P, et al. Practical management of hyperinsulinism in infancy. Arch Dis Child Fetal Neonatal Ed. 2000 Mar;82(2):F98–107.

46. Hardy OT, Hernandez-Pampaloni M, Saffer JR, Suchi M, Ruchelli E, Zhuang H, et al. Diagnosis and localization of focal congenital hyperinsulinism by 18F-fluorodopa PET scan. J Pediatr. 2007 Feb;150(2):140–5.

47. Laje P, States LJ, Zhuang H, Becker SA, Palladino AA, Stanley CA, et al. Accuracy of PET/CT Scan in the diagnosis of the focal form of congenital hyperinsulinism. J Pediatr Surg. 2013 Feb;48(2):388–93.

48. Güemes M, Hussain K. Hyperinsulinemic Hypoglycemia. Pediatr Clin North Am. 2015 Aug;62(4):1017–36.

49. Kizu R, Nishimura K, Sato R, Kosaki K, Tanaka T, Tanigawara Y, et al. Population Pharmacokinetics of Diazoxide in Children with Hyperinsulinemic Hypoglycemia. Horm Res Paediatr. 2017;88(5):316–23.

50. McMahon AW, Wharton GT, Thornton P, De Leon DD. Octreotide use and safety in infants with hyperinsulinism. Pharmacoepidemiol Drug Saf. 2017;26(1):26–31.

51. Laje P, Halaby L, Adzick NS, Stanley CA. Necrotizing enterocolitis in neonates receiving octreotide for the management of congenital hyperinsulinism. Pediatr Diabetes. 2010 Mar;11(2):142–7.

52. Senniappan S, Alexandrescu S, Tatevian N, Shah P, Arya V, Flanagan S, et al. Sirolimus Therapy in Infants with Severe Hyperinsulinemic Hypoglycemia. N Engl J Med. 2014 Mar 20;370(12):1131–7.

53. Adamkin DH. Neonatal hypoglycemia. Semin Fetal Neonatal Med. 2017 Feb;22(1):36–41.

54. Stomnaroska-Damcevski O, Petkovska E, Jancevska S, Danilovski D. Neonatal Hypoglycemia: A Continuing Debate in Definition and Management. PRILOZI. 2015 Jan 1;36(3):91–7.

55. Harris DL, Weston PJ, Harding JE. Incidence of Neonatal Hypoglycemia in Babies Identified as at Risk. J Pediatr. 2012 Nov;161(5):787–91.

56. McGowan JE. Neonatal Hypoglycemia. Pediatr Rev. 1999;

57. Pildes R, Forbes AE, O'Connor SM, Cornblath M. The incidence of neonatal hypoglycemia--a completed survey. J Pediatr. 1967 Jan 1;70(1):76–80.

58. Smith NC. Epidemiology of Spontaneous Abortion in Insulin-Dependent Diabetic Women. In: Carbohydrate Metabolism in Pregnancy and the Newborn · IV. London: Springer London; 1989. p. 75–81.

59. Fee BA, Weil WB. BODY COMPOSITION OF INFANTS OF DIABETIC MOTHERS BY DIRECT ANALYSIS*. Ann N Y Acad Sci. 1963 Dec 15;110(2):869–97.

60. Pildes RS. Infants of Diabetic Mothers. N Engl J Med. 1973 Oct 25;289(17):902–4.

61. Linder K, Schleger F, Kiefer-Schmidt I, Fritsche L, Kümmel S, Heni M, et al. Gestational Diabetes Impairs Human Fetal Postprandial Brain Activity. J Clin Endocrinol Metab. 2015 Nov 1;100(11):4029–36.

62. Schleger F, Linder K, Fritsche A, Preissl H. Maternal Insulin Sensitivity and Fetal Brain Activity. In: Diet, Nutrition, and Fetal Programming. Cham: Springer International Publishing; 2017. p. 67–78.

63. Filan PM, Inder TE, Cameron FJ, Kean MJ, Hunt RW. Neonatal hypoglycemia and occipital cerebral injury. J Pediatr. 2006 Apr;148(4):552–5.

64. Menni F, de Lonlay P, Sevin C, Touati G, Peigne C, Barbier V, et al. Neurologic Outcomes of 90 Neonates and Infants With Persistent Hyperinsulinemic Hypoglycemia. Pediatrics. 2001 Mar 1;107(3):476–9.

65. Meissner T, Wendel U, Burgard P, Schaetzle S, Mayatepek E. Long-term follow-up of 114 patients with congenital hyperinsulinism. Eur J Endocrinol. 2003 Jul 1;149(1):43–51.

66. Burns CM, Rutherford MA, Boardman JP, Cowan FM, Satodia P, Moore AM. Patterns of Cerebral Injury and Neurodevelopmental Outcomes After Symptomatic Neonatal Hypoglycemia. Pediatrics. 2008 Jul 1;122(1):65–74.

67. GATAULLINA S, LONLAY P DE, DELLATOLAS G, VALAYANNAPOULOS V, NAPURI S, DAMAJ L, et al. Topography of brain damage in metabolic hypoglycaemia is determined by age at which hypoglycaemia occurred. Dev Med Child Neurol. 2013 Feb;55(2):162–6.

68. Begum-Hasan J. Congenital Hyperinsulinemia With Grade 4 Intraventricular Hemorrhage. Adv Neonatal Care. 2014 Dec;14(6):392–7.

69. Jarjour IT, Ryan CM, Becker DJ. Regional cerebral blood flow during hypoglycaemia in children with IDDM. Diabetologia. 1995 Sep;38(9):1090–5.

70. Anderson JM, Milner RD, Strich SJ. Effects of neonatal hypoglycaemia on the nervous system: a pathological study. J Neurol Neurosurg Psychiatry. 1967 Aug;30(4):295–310.

71. Dervisoglu P, Kosecik M, Kumbasar S. Effects of gestational and pregestational diabetes mellitus on the foetal heart: a cross-sectional study. J Obstet Gynaecol (Lahore). 2018 Jan 21;1–5.

72. Gandhi JA, Zhang XY, Maidman JE. Fetal cardiac hypertrophy and cardiac function in diabetic pregnancies. Am J Obstet Gynecol. 1995 Oct;173(4):1132–6.

73. Sardesai MG, Gray AA, McGrath MM, Ford SE. Fatal hypertrophic cardiomyopathy in the fetus of a woman with diabetes. Obstet Gynecol. 2001 Nov;98(5 Pt 2):925–7.

74. Bulbul A, Bolat F, Comert S, Demirin H, Tanik C, Bulbul L, et al. PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA WITH LEFT VENTRICULAR HYPERTROPHY AND DYSRHYTHMIA: A Case Report. Fetal Pediatr Pathol. 2010 May 7;29(3):165–71.

75. Poissonnet CM, Burdi AR, Garn SM. The chronology of adipose tissue appearance and distribution in the human fetus. Early Hum Dev. 1984 Sep 1;10(1–2):1–11.

76. Rosati P, Exacoustós C, Caruso A, Mancuso S. Ultrasound diagnosis of fetal macrosomia. Ultrasound Obstet Gynecol. 1992 Jan 1;2(1):23–9.

77. Larciprete G, Valensise H, Vasapollo B, Novelli GP, Parretti E, Altomare F, et al. Fetal subcutaneous tissue thickness (SCTT) in healthy and gestational diabetic pregnancies. Ultrasound Obstet Gynecol. 2003 Dec 1;22(6):591–7.

78. Carpenter MW, Canick JA, Hogan JW, Shellum C, Somers M, Star JA. Amniotic fluid insulin at 14-20 weeks' gestation: association with later maternal glucose intolerance and birth macrosomia. Diabetes Care. 2001 Jul 1;24(7):1259–63.

79. PEDERSEN J. Weight and length at birth of infants of diabetic mothers. Acta Endocrinol (Copenh). 1954 Aug;16(4):330–42.

80. Naeye RL. Infants of Diabetic Mothers: a Quantitative, Morphologic Study. Pediatrics. 1965 Jun;35:980–8.

81. SCHULZ DM, GIORDANO DA, SCHULZ DH. Weights of organs of fetuses and infants. Arch Pathol. 1962 Sep;74:244–50.

82. Hoegsberg B, Gruppuso PA, Coustan DR. Hyperinsulinemia in macrosomic infants of nondiabetic mothers. Diabetes Care. 1993 Jan 1;16(1):32–6.

83. Coustan DR, Imarah J. Prophylactic insulin treatment of gestational diabetes reduces the incidence of macrosomia, operative delivery, and birth trauma. Am J Obstet Gynecol. 1984 Dec 1;150(7):836–42.

84. Kehl RJ, Krew MA, Thomas A, Catalano PA. Fetal Growth and Body Composition in Infants of Women with Diabetes Mellitus During Pregnancy. J Matern Neonatal Med. 1996 Jan;5(5):273–80.

85. Widness JA, Teramo KA, Clemons GK, Voutilainen P, Stenman U-H, McKinlay SM, et al. Direct relationship of antepartum glucose control and fetal erythropoietin in human Type 1 (insulin-dependent) diabetic pregnancy. Diabetologia. 1990 Jun;33(6):378–83.

86. Mimouni F, Miodovnik M, Siddiqi TA, Butler JB, Holroyde J, Tsang RC. Neonatal polycythemia in infants of insulin-dependent diabetic mothers. Obstet Gynecol. 1986 Sep;68(3):370–2.

87. Roberts AB, Mitchell J, Murphy C, Koya H, Cundy T. Fetal liver length in diabetic pregnancy. Am J Obstet Gynecol. 1994 May 1;170(5):1308–12.

88. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of Obese Mothers Develop Insulin Resistance in Utero. Diabetes Care. 2009 Jun 1;32(6):1076–80.

89. Susa JB, McCormick KL, Widness JA, Singer DB, Oh W, Adamsons K, et al. Chronic hyperinsulinemia in the fetal rhesus monkey: effects on fetal growth and composition. Diabetes. 1979 Dec 1;28(12):1058–63.

90. BALLARD PL. Hormonal Regulation of Pulmonary Surfactant. Endocr Rev. 1989 May 1;10(2):165–81.

91. Piper JM, Langer O. Does maternal diabetes delay fetal pulmonary maturity? Am J Obstet Gynecol. 1993 Mar 1;168(3):783–6.

92. Bourbon JR, Farrell PM. Fetal lung development in the diabetic pregnancy. Pediatr Res. 1985 Mar;19(3):253–67.

93. Robert MF, Neff RK, Hubbell JP, Taeusch HW, Avery ME. Association between Maternal Diabetes and the Respiratory-Distress Syndrome in the Newborn. N Engl J Med. 1976 Feb 12;294(7):357–60.

94. Allen RD, Palumbo PJ. Respiratory distress and neonatal mortality in infants of diabetic and prediabetic mothers. Acta Diabetol Lat. 1981 Apr;18(2):101–6.

95. Weintrob N, Karp M, Hod M. Short- and long-range complications in offspring of diabetic mothers. J Diabetes Complications. 1996;10(5):294–301.

96. Salvesen DR, Michael Brudenell J, Snijders RJM, Ireland RM, Nicolaides KH. Fetal plasma erythropoietin in pregnancies complicated by maternal diabetes mellitus. Am J Obstet Gynecol. 1993 Jan 1;168(1):88–94.

97. Green DW, Khoury J, Mimouni F. Neonatal hematocrit and maternal glycemic control in insulin-dependent diabetes. J Pediatr. 1992 Feb 1;120(2 Pt 1):302–5.

98. Mackintosh TF, Walker CH. Blood viscosity in the newborn. Arch Dis Child. 1973 Jul 1;48(7):547–53.

99. McCarty MF. Hyperinsulinemia may boost both hematocrit and iron absorption by up-regulating activity of hypoxia-inducible factor-1α. Med Hypotheses. 2003 Nov 1;61(5–6):567–73.

100. Petry CD, Wobken JD, McKay H, Eaton MA, Seybold VS, Johnson DE, et al. Placental transferrin receptor in diabetic pregnancies with increased fetal iron demand. Am J Physiol. 1994 Oct;267(4 Pt 1):E507-14.

101. Petry CD, Eaton MA, Wobken JD, Mills MM, Johnson DE, Georgieff MK. Iron deficiency of liver, heart, and brain in newborn infants of diabetic mothers. J Pediatr. 1992 Jul 1;121(1):109–14.

102. KC K, Shakya S, Zhang H. Gestational Diabetes Mellitus and Macrosomia: A Literature Review. Ann Nutr Metab. 2015;66(2):14–20.

103. Teramo KA. Obstetric problems in diabetic pregnancy – The role of fetal hypoxia. Best Pract Res Clin Endocrinol Metab. 2010 Aug 1;24(4):663–71.

104. Weiss PAM, Hofmann HMH, Kainer F, Haas JG. Fetal outcome in gestational diabetes with elevated amniotic fluid insulin levels: Dietary versus insulin treatment. Diabetes Res Clin Pract. 1988 May 19;5(1):1–7.

105. Sarkar S, Rosenkrantz TS. Neonatal polycythemia and hyperviscosity. Semin Fetal Neonatal Med. 2008 Aug 1;13(4):248–55.

106. Rosenkrantz TS, Oh W. Cerebral blood flow velocity in infants with polycythemia and hyperviscosity: Effects of partial exchange transfusion with Plasmanate. J Pediatr. 1982 Jul 1;101(1):94–8.

107. Alkalay A, Pomerance JJ, Prause J, Mogilner BM, Edelstein S. Cholecalciferol metabolites in polycythemic newborns. Isr J Med Sci. 1985 Feb;21(2):95–7.

108. Jeevasankar M, Agarwal R, Chawla D, Paul VK, Deorari AK. Polycythemia in the newborn. Indian J Pediatr. 2008 Jan 20;75(1):68–72.

109. Hiden U, Maier A, Bilban M, Ghaffari-Tabrizi N, Wadsack C, Lang I, et al. Insulin control of placental gene expression shifts from mother to foetus over the course of pregnancy. Diabetologia. 2006 Jan 13;49(1):123–31.

110. Rodríguez-Morán M, Levario-Carrillo M, González JL, Sánchez-Ramírez B, Martínez-Aguilar G, Guerrero-Romero F. Placental immaturity and hyperinsulinaemia in full-term newborns. Eur J Clin Invest. 2007 Jul;37(7):529–34.

111. Lassance L, Miedl H, Absenger M, Diaz-Perez F, Lang U, Desoye G, et al. Hyperinsulinemia Stimulates Angiogenesis of Human Fetoplacental Endothelial Cells: A Possible Role of Insulin in Placental Hypervascularization in Diabetes Mellitus. J Clin Endocrinol Metab. 2013 Sep;98(9):E1438–47.

112. Huynh J, Dawson D, Roberts D, Bentley-Lewis R. A systematic review of placental pathology in maternal diabetes mellitus. Placenta. 2015 Feb;36(2):101–14.

113. Farquhar JW. Maternal Hyperglycæmia and Fœtal Hyperinsulinism in Diabetic Pregnancy. Postgrad Med J. 1962 Nov;38(445):612.

114. Mehta A, Hussain K. Transient hyperinsulinism associated with macrosomia, hypertrophic obstructive cardiomyopathy, hepatomegaly, and nephromegaly. Arch Dis Child. 2003 Sep;88(9):822–4.

115. Cade A, Abel G, Stringer MD, Milla PJ, Puntis JWL. Foregut Dysmotility Complicating Persistent Hyperinsulinaemic Hypoglycaemia of Infancy. J Pediatr Gastroenterol Nutr. 1998;27(3):355–8.

116. Eliasson B, Björnsson E, Urbanavicius V, Andersson H, Fowelin J, Attvall S, et al. Hyperinsulinaemia impairs gastrointestinal motility and slows carbohydrate absorption. Diabetologia. 1995 Jan;38(1):79–85.

117. Knafelz D, St Louis D, Aynsley-Green A, Dunne M, Lindley K, Milla P. SURFACE ELECTROGASTROGRAPHIC EVIDENCE OF FOREGUT DYSMOTILITY IN PERSISTENT HYPERINSULINAEMIC HYPOGLYCAEMIA OF INFANCY (PHHI). J Pediatr Gastroenterol Nutr. 1997;24(4):459.

118. VILLEE DB, POWERS ML. Effect of glucose and insulin on collagen secretion by human skin fibroblasts in vitro. Nature. 1977 Jul;268(5616):156–8.

119. Chinoy A, Banerjee I, Flanagan SE, Ellard S, Han B, Mohamed Z, et al. Focal Congenital Hyperinsulinism as a Cause for Sudden Infant Death. Pediatr Dev Pathol. 2018 Mar 20;109352661876537.

120. Sousa-Santos F, Simões H, Castro-Feijóo L, Rodríguez PC, Fernández-Marmiesse A, Fiaño RS, et al. Congenital hyperinsulinism in two siblings with ABCC8 mutation: same genotype, different phenotypes. Arch Endocrinol Metab. 2018 Oct;62(5):560–5.

121. Susa JB, Schwartz R. Effects of Hyperinsulinemia in the Primate Fetus. Diabetes. 1985 Jun 1;34(Supplement_2):36–41.

122. Warburton D. Chronic hyperglycemia with secondary hyperinsulinemia inhibits the maturational response of fetal lamb lungs to cortisol. J Clin Invest. 1983 Aug 1;72(2):433–40.

123. Beck JC, Johnson JW, Mitzner W, Lee PA, London WT, Sly DL. Glucocorticoids, hyperinsulinemia, and fetal lung maturation. Am J Obstet Gynecol. 1981 Feb 15;139(4):465–70.

124. Smith BT, Giroud CJ, Robert M, Avery ME. Insulin antagonism of cortisol action on lechithin synthesis by cultured fetal lung cells. J Pediatr. 1975 Dec;87(6 Pt 1):953–5.

125. Kotas R V, Avery ME. Accelerated appearance of pulmonary surfactant in the fetal rabbit. https://doi.org/101152/jappl1971303358. 1971;

126. Brown LD, Wesolowski SR, Kailey J, Bourque S, Wilson A, Andrews SE, et al. Chronic Hyperinsulinemia Increases Myoblast Proliferation in Fetal Sheep Skeletal Muscle. Endocrinology. 2016 Jun;157(6):2447–60.

127. Plagemann A, Harder T, Janert U, Rake A, Rittel F, Rohde W, et al. Malformations of hypothalamic nuclei in hyperinsulinemic offspring of rats with gestational diabetes. Dev Neurosci. 1999;21(1):58–67.

128. K Mazor-Aronovitch, D Gillis, D Lobel, H J Hirsch, O Pinhas-Hamiel, D Modan-Moses, et al. Long-term neurodevelopmental outcome in conservatively treated congenital hyperinsulinism. Eur J Endocrinol. 2007;157(4):491–7.

129. de Lonlay-Debeney P, Poggi-Travert F, Fournet J-C, Sempoux C, Vici CD, Brunelle F, et al. Clinical Features of 52 Neonates with Hyperinsulinism. N Engl J Med. 1999 Apr 15;340(15):1169–75.

130. Stanley CA. HYPERINSULINISM IN INFANTS AND CHILDREN. Pediatr Clin North Am. 1997 Apr 1;44(2):363–74.

131. Metzger BE, HAPO Study Cooperative Research Group. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Int J Gynecol Obstet. 2002 Jul 1;78(1):69–77.

132. Akinbi HT, Gerdes JS. Macrosomic infants of nondiabetic mothers and elevated C-peptide levels in cord blood. J Pediatr. 1995 Sep 1;127(3):481–4.

133. Desoye G, Nolan CJ. The fetal glucose steal: an underappreciated phenomenon in diabetic pregnancy. Diabetologia. 2016 Jun 19;59(6):1089–94.

134. White V, Jawerbaum A, Mazzucco MB, Gauster M, Desoye G, Hiden U. Diabetes-associated changes in the fetal insulin/insulin-like growth factor system are organ specific in rats. Pediatr Res. 2015 Jan 30;77(1):48–55.

135. Cornblath M, Hawdon JM, Williams AF, Aynsley-Green A, Ward-Platt MP, Schwartz R, et al. Controversies Regarding Definition of Neonatal Hypoglycemia: Suggested Operational Thresholds. Pediatrics. 2000 May 1;105(5):1141–5.