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Tissue Glucose Fluctuations in Preterm Infants in the post-intensive care period

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Table of contents

I. List of Abbreviations

AAP	American Academy of Pediatrics
AGA	Adequate for Gestational Age
aEEG	Amplitude-Integrated Electroencephalography
BMI	Body Mass Index
BPD	Bronchopulmonary Dysplasia
CGM	Continuous Glucose Monitoring
CGMS	Continuous Glucose Monitoring System
CPAP	Continues Positive Airway Pressure
CRIB-score	Clinical Risk Index for Babies
DOL	Days of life
EEG	Electroencephalogram
ELBW	Extreme Low birth weight
FFA	Free Fatty Acid
GLUT	Glucose Transporter
HC	Head Circumference
HFNC	High Flow Nasal Cannula
ICH	Intracranial Hemorrhage
IVH	Intraventricular Hemorrhage
LBW	Low birth weight
LMU	Ludwig-Maximilian-University
MRI	Magnetic Resonance Imaging
NICU	Neonatal Intensive Care Unit
PVL	Periventricular Leucomalacia
SD	Standard Deviation
SGA	Small for Gestational Age
TG	Tissue Glucose

II. List of illustrations

1. Introduction

1.1. Abstract

Background: We previously observed hypoglycemic episodes in preterm infants after achieving full enteral feeds and during a stable postnatal period. The purpose of this study was to prospectively determine subcutaneous glucose levels in this population.

Methods: Twenty preterm infants <32wks gestational age were enrolled for continuous subcutaneous glucose monitoring for 72hrs in two cohorts G1: <1000g BW (n=14) and G2: 1000–1500g BW (n=6). All infants were fed according to a standard feeding protocol where full feeds are provided at 150-180ml/kg/d of fortified EBM or premature formula at 110–135kcal/kg/d. Primary outcome was the frequency and quality of low tissue glucose episodes within 72 hours, defined as tissue glucose ≤45mg/dL.

Results: 93% of the infants in G1 and 34% in G2 suffered glucose fluctuations during monitoring. Low tissue glucose episodes occurred in 21% in G1, compared to 17% in G2. In G1 7% of infants in G1 reached glucose values <30mg/dL. We also observed high tissue glucose episodes (>150mg/dl) after feeds (G1: 50%, G2: 17%), followed by rapid drops in both cohorts. Cumulatively, all low- and high tissue glucose episodes lasted >60min (14%), 35-60min (21%), 10-30min (62%) and <5min (3%) per patient. The main risk factors for glucose instability were gestational age at birth and weight at trial.

Conclusions: Otherwise stable, well developing former very low birth weight preterm infants are at risk for glucose instability, generally considered as unfavourable. It remains unclear whether this instability is likely to influence long-term outcome and whether continuous feeds are preventive.

1.2. Abstrakt

Hintergrund: In einer vorangegangenen retrospektiven Studie wurden bei vollständig enteral ernährten Frühgeborenen mit adäquater Gewichtszunahme, eine erhöhte Inzidenz niedriger interstitieller Glukose-Wert Episoden festgestellt. In dieser prospektiven Studie wurden in der stabilen postnatalen Periode, kontinuierliche interstitielle Glucose Werte gemessen.

Methoden: 20 Frühgeborene <32 SSW wurden rekrutiert und nach Geburtsgewicht in zwei Kohorten eingeteilt, G1: 500-999g GG (n=14) und G2: 1000-1500g GG (n=6). Die kontinuierliche interstitielle Glukose Messung erfolgte über 72 Stunden. Alle Kinder wurden nach einem Standard Bolus-Ernährungsprotokoll mit 150-180 ml/Kg verstärkter Muttermilch oder Frühgeborenen-Nahrung mit einer Energiezufuhr von 110-135 kcal/Kg ernährt. Alle Kinder waren während der Studie klinisch stabil. Ziel dieser Studie war die asymptomatischen niedrigen- ($\leq$ 45mg/dL) sowie erhöhte (>150 mg/dL) interstitielle Glukose-Wert Episoden während 72 Stunden zu registrieren.

Ergebnisse: 93% der Frühgeborenen in G1 und 34% in G2 hatten Fluktuationen der Glucose-Werte während der kontinuierlichen Messung. Niedrige und erhöhte interstitielle Glukose-Wert Episoden waren häufiger und schwerwiegender in G1 (21% vs. 17% und 50% vs. 17%). 7% in G1 erreichten Glucose Werte <30mg/dL, 57% Werte >200mg/dL. Auf postprandiale hyperglykämische Peaks folgten rasche Glucose-Wert-Absenkungen. Die wichtigsten Risikofaktoren für die Glucose-Instabilität waren Gestationsalter bei Geburt und Gewicht bei Studieneintritt.

Diskussion: Klinisch stabile, adäquat wachsende Frühgeborene zeigen nach den ersten Lebenswochen ausgeprägte Schwankungen der Gewebe-Glukose-Werte. Diese Fluktuationen werden im Allgemeinen als ungünstig interpretiert. Es ist noch unklar ob diese metabolische Instabilität Konsequenzen für die spätere Entwicklung der Kinder hat und ob eine kontinuierliche Ernährung die Schwankungen verhindern könnte.

1.3. Background Information

Glucose imbalances resulting in hypo- and hyperglycemic events during the neonatal period are an important problem as survival of the most fragile infants continues to improve. The estimated incidence of symptomatic hypoglycemia in full-term newborns is 1-3/1000 live births; the asymptomatic events are an unknown number [1, 2]. Regarding preterm infants, this incidence is even three times higher [3]. When it comes to hyperglycemia the incidence in term newborns is 20/1000 live births and 450/1000 for preterm infants [4].

Neonatal hypo- and hyperglycemia correlate with brain damage and subsequent neurodevelopmental impairment. This has been well established by several research groups and studies [4-9]. Despite the evident vital importance of these clinical entities, it has not yet been possible to establish universally accepted definitions (either) for hypoglycemia or for hyperglycemia [10]. Each Neonatal Intensive Care Unit (NICU) has its own threshold to determine the accepted and tolerated glycemia levels in their patients. There is no general agreement regarding the definition of a safe limit for newborns. Each clinical situation has its own clinical management guidelines thus; the diagnosis and treatment of asymptomatic neonatal hypo- and hyperglycemic events require an individual assessment of each patient and situation.

The objective of this prospective cohort study was to determine the incidence of silent hypoglycemias in fully enteral fed, clinically stable, former preterm infants. A Continuous Glucose Monitoring System (CGMS) – providing real time interstitial glucose values was used. Furthermore, the question was addressed as to whether tissue glucose fluctuations are related to a hypercaloric, preterm standard formula, or to the presence of other potential risk factors.

So far no published reports have addressed this subject in preterm infants older than ≥ 4 postnatal weeks [11]. There is one study by Staffler et al. in 98 infants with the same demographic characteristics as in this prospective study, showing an incidence of hypoglycemia of 44% in preterm newborns with a birth weight <1000g and 23% in those born with 1000-1500g [12].

The fact that preterm newborns are metabolically unstable is well known. Therefore these children are closely monitored and their glucose metabolism thoroughly controlled to prevent the relatively frequent hyper- and hypoglycemic derails at this age. But after achieving full enteral feeds in apparently metabolic stable baby at a NICU on either supplemented breast milk or preterm formula blood glucose controls

are scarce. It is generally assumed that at this stage, without current risk factors apart from the former prematurity and low birth weight, the glucose concentration pattern does not fluctuate and is maintained within normal ranges.
This study demonstrates for the first time that metabolic fluctuations between hyper- and hypoglycemic values since birth continue beyond at least the $32^{nd} \pm 6$ DOL and might have potential consequences for long time morbidity in these infants.

1.4. Definition of Hypo- and Hyperglycemia:

Hypoglycemia and Hyperglycemia represent an imbalance between glucose supply and utilization. These imbalances may result from a multitude of disturbed regulatory mechanisms, and mostly occur when the normal metabolic adaptation mechanism after birth fails [4, 13, 14]. There is no consensus in the medical community regarding the definition of either neonatal hypo- or hyperglycemia. Kalhan et al. refers to this controversy as “the inability to reach a consensus is because there is no absolute correlation between plasma glucose values, clinical symptoms and long-term sequels” [15]. In neonates there is not always a correlation between blood glucose concentration and the classical clinical manifestations of these entities. Thus clinical symptoms are not a reliable parameter to estimate blood glucose to be at an optimal level for maintaining e.g. brain metabolism. Additionally, if clinical symptoms of hypoglycemia are obvious in an newborn infant, the risk of neurological damage increases dramatically [16]. In 1988 Koh and colleagues addressed the controversial subject regarding the definition of pathological glucose levels in the newborn. Their results showed that among textbooks, guidelines and NICUs, the lower limit of normality for circulating blood glucose concentrations ranged from <18 mg/dL to <72mg/dL [14]. For hyperglycemia the values ranged from >120 -150mg/dL throughout >200mg/dL [4]. The thresholds depend upon the studied population, type (plasma or serum) and source (venous, capillary or arterial) of the blood samples, the methods of assay, feeding schedules of the measured infants and the type of nutrition they are receiving (breast milk or formula). This increased diversity of the variables only adds confusion to the subject [17]. Some authors propose a symptomatic definition of hypoglycemia, which depends on the presence of autonomic and /or neurological symptoms [10]. Other studies take into account an

additional definition correlating frequent previous hypoglycemic episodes and adverse long-term neurodevelopmental sequel and outcomes [11].

The definition of hyperglycemia remains equally unsettled and a symptomatic definition is almost impossible to make due to the lack of specific symptoms in newborn infants. At this point in time, there is no absolute safe lower margin for blood glucose level in newborns, because individual susceptibility as well as several different variables like duration, severity, cerebral blood flow, rates of glucose uptake and availability of alternative substrates during the event could individually determine poor neurodevelopmental outcome [18, 19]. In healthy term neonates blood glucose concentrations of 30mg/dL are common 1 to 2 hours after birth and are considered physiological and are well tolerated. These events are transient and asymptomatic and do not require treatment at all [19]. But this premise cannot be transferred to all newborns, because infants with risk factors do not tolerate the same glucose concentrations as well as healthy term newborns [11, 20]. Premature neonates have an immature control of glucose production and/or diminished substrate availability. This may be related to their prematurity, low birth weight- (LBW) or extreme low birth weight- (ELBW) or the fact of being small for gestational age (SGA). Other risk factors for hypoglycemia, which have a high prevalence in this specific group, are perinatal asphyxia and cerebral ischemia, hypothermia and infections [3, 15, 21]. Risk factors for term newborns are the administration of intrapartum glucose infusion to the mother and being offspring of mothers with diabetes mellitus or gestational diabetes [22].

Therefore, there is as yet no generally established and accepted clinical situation and glucose concentration that could be defined as the cut-off value for when to screen and eventually treat an asymptomatic episode. It depends on the patient, individual risk factors and the current clinical situation.

After the first hours of life, the neonatal glucose concentration tends to rise to higher and stable values, in general >45 mg/dL by 12 hours after birth [19, 23]. But if the glucose concentration rises above normal parameters, into hyperglycemia, the neonatal risk increases yet again. Extremes, hypo- and hyperglycemic ranges are equally dangerous [6, 8, 9, 11, 24].

1.5. Significance and Sequelae of Hypo- and Hyperglycemia

Glucose is the brains primary essential energy substrate and it accounts for up to 90% of the glucose-uptake in neonates [24-26]. Infants born during the critical rapid brain growth period between the 20th and 32nd gestational week postmenstrual age have most certainly a disturbed glucose homeostasis and therefore the highest risk for brain damage among the paediatric population [8]. Despite the confusion about the definition of hypo- and hyperglycemia it should be noted that there is no controversy regarding the deleterious effects on the brain due to a recurrent or persistent glucose imbalance during the neonatal and perinatal period [15, 24].

1.5.1. Consequences of Neonatal Hypoglycemia

There is a correlation between glucose production and glucose consumption by the brain and the estimated brain weight at all ages. The brain uses about 20 times more glucose than muscle and fat and due to the infants' large brain to body weight ratio (12% vs. 2% in adults) it results in a higher glucose turnover rate than in adults. Children with previous repetitive hypoglycemic episodes have a suboptimal head circumference (HC) and head growth, because if needed, cerebral structural substrates may become degraded to energy usable intermediates, resulting in decreased brain mass. These substrates may be lactate, pyruvate, alanin, glycerol and ketone bodies. They support brain metabolism, but at the expense of brain growth [5, 6, 24, 26, 27].

Lactate is, at this time, the best characterized alternative fuel. The cerebral lactate utilization rate correlates directly with the arterial plasma lactate concentration. Also ketone bodies (hydroxybutiric acid and acetoacetic acid) increase their concentration when plasma glucose concentration is low [21, 28]. Alternative substrates may be used by a variety of body tissues, decreasing their glucose demands and sparing it for the nervous system. Several studies have shown that the immature brain has a relatively greater tolerance to low glucose levels then a mature one and has the ability to use lactate and ketone bodies, the only alternative fuel able to cross the blood-brain barrier, partially decreasing the need for glucose. This is a protective mechanism against the deleterious effects of hypoglycemia on the central nervous system [24].

Several study groups have postulated other possible neuroprotective mechanisms like glycogen storage in astrocytes and increased cerebral blood flow during

hypoglycemic events, to ensure the glucose delivery which occurs via blood supply [6, 29-31]. It has also been demonstrated in several clinical studies that a clinically relevant hypoglycemia or a long lasting one has a direct proportional impact on neurological outcome [32, 33].

The pathogenesis and pathophysiology of the hypoglycemic damage is the correlation of low glucose concentrations with changes in cerebral blood flow, glucose uptake, oxygen consumption, intracellular energy and electrolyte changes. Hypoglycemia disturbs the cellular energy state, causing an energy failure, leading to necrosis by excitotoxicity. Glutamate accumulates and stimulates its receptors excessively, which triggers a calcium influx and potassium efflux causing the acceleration of proteolytic and lipolytic reactions culminating in apoptosis or necrosis. Reactive oxygen species (ROS) are also involved in causing neurological damage as a result of hypoglycemia, because this leads to an increased brain blood flow, causing a loss of autoregulation of the brain vessels, culminating in an oxidative stress, radical injury and cellular apoptosis [3, 18, 31].

Pryds et al. [30] recommend that blood glucose concentrations be maintained at more than 40-45 mg/dL to avoid cerebral hyperperfusion and epinephrine secretion. The epinephrine secretion indicates the activation of one of the brain protective mechanism to ensure its glucose supply. Among the neurological abnormalities seen on MRI (magnetic resonance imaging) of children who had symptomatic and/or asymptomatic neonatal hypoglycemic episodes are white matter alterations, white matter hemorrhages and different cortical injury patterns. The most frequent neuroimaging alteration involves the cortex and white matter of the parietal and occipital lobes. A large cerebral bleeding, regardless of its cause (hypo-hyperglycemia, prematurity or other), disturbs brain metabolism [25, 31].

Sixty-five percent of the infants in a study by Burns et al. [5] demonstrated neurological impairment at 18 months of age related to white matter injuries due to repetitive hypoglycemic events. The cognitive impairment of the infants was greater than the motoric one, showing low IQ-scores (< 86) at 5 to 7 years of age.

Lucas et al. [11] reported reductions in Bayley motor and mental developmental scores at 18 months of age associated with repetitive episodes of neonatal hypoglycemia. The neurological deficits were not as striking at 8 years of age as they were at 18 months; but some impairment remained (in arithmetic and motor tests).

The permanent neurological impact of a low plasma glucose concentration in a particular infant is dependent on multiple factors: brain maturity, presence of ischemia and hypoxia, glycogen stores, presence of alternative fuels and genetically determined neural plasticity response [13, 16]. Some authors state that even a hypoglycemic episode of <47 mg/dL can have neurodevelopmental consequences like non febrile seizures, abnormalities in brain-stem auditory or somatosensory evoked potentials, isoelectric electroencephalogram (EEG) and even death [6, 7, 11, 34, 35].

Apparently for some authors the decisive variable in the cause of brain injury is not the length and severity of a hypoglycemic event but rather the mere existence of one [5, 32]. The question that still arises is which glucose concentration causes brain damage and how long the event needs to be. Unfortunately at this point in time there is neither a correct nor a unanimous answer to it.

1.5.2. Consequences of Neonatal Hyperglycemia

In preterm infants, stress-reactive hormones such as epinephrine and norepinephrine increase in response to delivery, thermal instability, hypovolemia, low blood pressure and sepsis. These hormones and the often infused counterparts dobutamin and dopamine-inhibit insulin secretion and action, promoting instead glycogen breakdown which could lead to hyperglycemia [33].

Several studies have demonstrated that hyperglycemia is an independent risk factor for neonatal morbidity and mortality. There is also a connection between high glucose concentrations, abnormal brain MRI scans and an increased incidence of intracranial hemorrhages. The elevated blood glucose concentrations cause a rise in serum osmolarity (every 18 mg/dL rise in glucose concentration results in an osmolarity rise of 1 mOsm/L)[1, 36], rapidly shifting water from the intracellular to the extracellular compartment, which may result in brain cell dehydration and capillary dilatation increasing the already elevated risk of cerebral hemorrhage in preterm infants. A further hazard is also the subsequent osmotic diuresis and polyuria with a possible consequential systemic dehydration. If cerebral bleeding occurs, the brain metabolism gets disturbed and a reduction in glucose consumption by the affected area follows. This again leads to hyperglycemia. Therefore, a hemorrhage could be the cause or the consequence of hyperglycemia. As for the MRI scans, a reduction in white matter has been described, as well as an increased prevalence of

retinopathy in the preterm newborn with glucose values >155mg/dL, a higher incidence of sepsis and increased length in hospital stay. Experimental studies suggest that the pathophysiology in hyperglycemic events is an increase in oxidative factors, like in the hypoglycemic events. The excitotoxic injury of the brain cells produces brain damage [4, 8, 24, 25, 37].

1.6. Glucose Homeostasis in the Term, Preterm and SGA Newborn

1.6.1. Metabolism in the Term Newborn

Until the umbilical cord is clamped, there is no need for fetal glucose production during pregnancy [15, 24]. The glucose supply depends exclusively on the transplacentally glucose transport. The fetal glycemia is about 80% of the maternal glucose concentration with values oscillating between 70-90 mg/dL during a normal pregnancy. This ratio fetal – maternal glucose must be maintained at approximately 2:3, because it provides the necessary gradient for the transplacentally glucose transport. Glucose crosses the placental membranes through facilitated diffusion, mediated by a carrier protein, GLUT3, which has the highest glucose affinity of all GLU- transporters. GLUTs are a family of glucose transporters, which, are located across the plasma membranes of cells and are structurally similar. Their expression is tissue-specific and the ontogeny of these transporters explains some of the metabolic differences between the neonate and the adult [28].

The transporters GLUT-1 and GLUT-3 are primarily expressed in the central nervous system and are responsible for the basal glucose uptake across the blood-brain barrier and into the neurons. GLUT-2 is predominantly expressed in hepatocytes and pancreatic β cells, has a low affinity to glucose, regulates the hepatic glucose uptake and release and is part of the β cell glucose sensor together with the enzyme glukokinase. In the placenta the predominant transporter is GLUT3. All these transporters are for the most part insulin independent. This means that the glucose uptake in those tissues is not affected by plasma insulin concentrations. In contrast, the GLUT-4 transporter, which is located predominantly in muscle, fat tissue and cardiac muscle, is an insulin-sensitive and dependant transporter [3, 4].

After birth, the residual fetal insulin precipitates a decline in plasma glucose concentration in the first neonatal hours. Not till then do the counter-regulatory hormones (epinephrine, glucagon, corticosteroids and growth hormone) suppress the

insulin dominance, favouring the induction of gluconeogenesis and allowing the blood glucose levels to rise by 3-4 hours of age [15, 28, 38]. This environment is a prerequisite to the induction of the hepatic synthesis of neural fuels: glucose (at a rate of 4-6 mg/kg/min) and ketone bodies [15]. The presence of ketone bodies in the blood is a good indicator of neonatal adaptation and that the insulin activity is being restrained or suppressed [39]. During this transition and adaptation to extra-uterine life, the newborn's circulating glucose concentration may decrease to 30-40 mg/dL, because of the limited hepatic glycogen content, but the hepatic gluconeogenesis and ß-oxidation of free fatty acids (FFAs) via ketogenesis are activated within the first 6-8 hours of life. These processes are essential for maintaining euglycemia. The gluconeogenic pathway is activated in the immediate neonatal period regardless the gestational age of the neonate, indicating that it is the birth process itself that activates the key gluconeogenic enzymes [3, 24, 40].

Metabolic adjustment and glucose stabilization in healthy full-term neonates can be expected after the first 4-72 hours of life [2, 7, 24, 38]. By that time in life an adaptation to the intermittent feeding with milk and a stabilization of the blood glucose levels may assumed.

Even if enteral feeds are withheld, the hypoglycemic events in the first hours of life in an adequate for gestational age (AGA) term infant are self limiting and blood glucose concentrations rise rapidly to the metabolic demands of extrauterine life because of the counter regulatory metabolic response [7, 13, 24]. It has been shown that healthy term newborns have optimal metabolic management and maintain euglycemia during the alternating periods of enteral milk feeding and fasting [41].

1.6.2. Metabolism in the Preterm Newborn

Metabolic adaptation differs for term, preterm and term/preterm SGA newborns. Immature neonates (24-29 weeks gestational age and birth weight between 600-1200g) can produce glucose via glycogenolysis and gluconeogenesis at rates comparable to term newborns during their first DOL, but a certain functional immaturity of the enzymatic systems increases the propensity to hypoglycemic episodes [22, 24]. During the first postnatal week the relation between glucose and other metabolic fuels diverge among both groups because preterm infants have small body fuel stores and cannot sustain gluconeogenesis at the same rate as term newborns. The accumulation of glycogen, fat and protein occurs in the last 8 weeks

of pregnancy, and since premature infants are born before or during that time, they are not able to tolerate long periods of fasting [24, 28]. Blood glucose values show an increased variability in preterm infants. On one hand, the immaturity of the glucose sensor in the pancreatic β cell leads to an inability to down regulate insulin secretion during hypoglycemia: on the other hand, the hepatocytes do not respond accurately to the circulating insulin concentrations during hyperglycemia, instead continuing with their glucose production [4].

Preterm infants also have lower ketone body concentrations, even at lower glucose values, than term infants [42]. Glucose regulated insulin secretion (first detected at the 11^{th} week of gestation, but not released until the 20^{th} week) by the pancreatic β cell is still immature in preterm newborns, resulting in unregulated and inadequate insulin production and secretion. The plasma insulin concentration and the insulin/glucose ratio increase exponentially with gestation, indicating the maturation of the pancreas [22]. The relative lack of adipose tissue (<2% of total body weight) and glycogen stores in this group of neonates results in an incapability to produce alternative fuels to maintain euglycemia via glycogenolysis and gluconeogenesis for long periods of fast, causing a limitation for protective metabolic counterregulation during hypoglycaemia [28]. In preterm infants the endocrine and enzyme control of intermediate metabolism is immature and not fully developed, leading to a failed homeostasis. They are less able than term infants to adapt to cessation of intrauterine nutrition and they depend partly on total parental nutrition (TPN) during the first weeks of life [3, 4, 24, 42].

1.6.3. Metabolism in the SGA Newborn

Small for gestational age infants (SGA - neonates who fall below the 10th percentile for birth weight) have a higher risk for suffering transient hypoglycemia, have a higher mean blood lactate concentration and a lower mean ketone body concentration than AGA infants. This is also true for preterm SGA vs. preterm AGA babies, who have the lowest blood glucose and ketone body concentration among the 4 described categories of neonates most frequently present in a neonatal unit. Their disturbed homeostasis leads to a depletion of metabolic fuels predisposing to hypoglycaemia [20].

If extreme clinical conditions (e.g. sepsis, perinatal asphyxia and cold stress) occur concomitantly and demand more energy than available metabolic reserves, the overall risk for hypoglycemia increases even further [3]. Lubchenco et al [23] determined in 1971 the incidence of hypoglycemia in the first few hours after birth in newborn infants in a general obstetric service (Fig. 1.6.3.-1). The SGA group shows the highest incidence of hypoglycemia, a result that many other authors have confirmed since and is an indicator for the inappropriate adaptation to the extrauterine life [20, 23, 24].

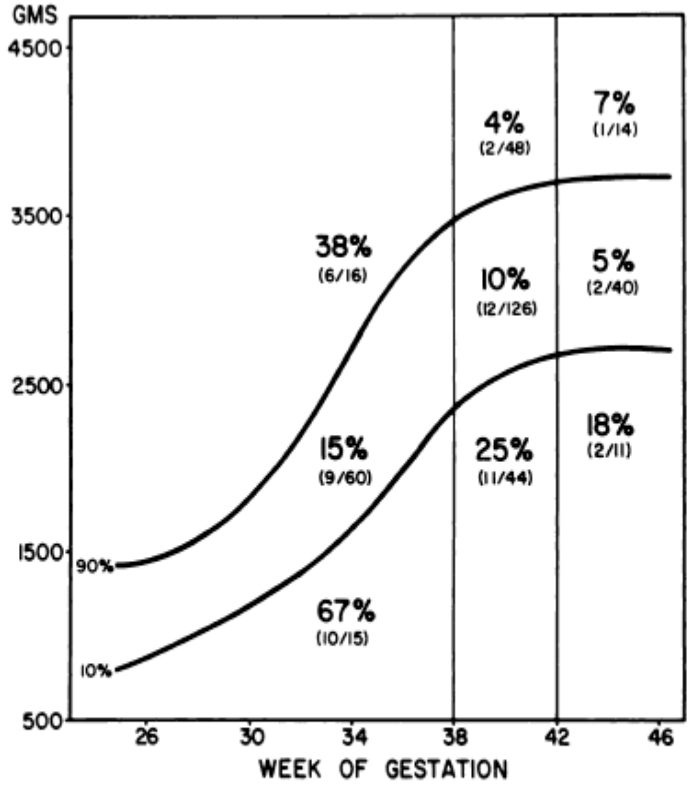

Fig. 1.6.3.-1: Incidence of Neonatal Hypoglycemia by birthweight, gestational age and intrauterine growth. Reproduced with permission from Pediatrics, Vol.47 (5), Pages 831-8, Copyright © 1971 by the AAP

1.6.4. Enzyme maturation and glucose homeostasis

Maturation of neonatal homeostasis is influenced by the integrity of specific pathways in the intermediary metabolism, which are more disordered with decreasing maturity. The activity of important enzymes participating in the gluconeogenesis, which don't

increase their activity until the perinatal period [24], can make the first hours and days of life a difficult period for maintaining euglycemia. The insulin release at the 20th week postmenstrual age, in response to elevated glucose concentrations may not be fully developed at birth and a relative unresponsiveness to insulin action may continue even in later development [43]. The hyperglycemic episodes are also more common in preterm infants due to the immaturity of the GLUT-system present in the hepatocyte and the pancreatic β cell [17]. The GLUT- 4 progressively increases its expression during gestation, coincident with the growth of insulin-sensitive tissues (skeletal muscle and adipose tissue) and the concomitant production and secretion of insulin into the blood stream. The effect of insulin causes the translocation of the transporter to the cell membrane and enhances the cellular glucose uptake, decreasing its blood concentration. If the transporter expression is low and the presence of insulin-dependent tissues, which express these transporters, is spare, it contributes to a peripheral insulin resistance with a diminished glucose uptake.

The expression, function and/or sensitivity of GLUT-2 is decreased during gestation, therefore the insulin secretion in response to hyperglycemia in the perinatal period is diminished. There is also a persistent production of hepatic glucose despite elevated glucose values and significant elevations in plasma insulin. This is in part caused by the immaturity of the glucose sensor and in part by a decreased activity in other metabolic linkages in the β cell [1, 28]. It seems that the transporters are developmentally regulated and except for GLUT-1, which is abundant in fetal life and decreases after birth, the others appear to increase in number and sensitivity after birth, reaching adult levels later on in life [28].

During the neonatal and perinatal period, low insulin levels caused by an absolute or relative insulin insufficiency, a peripheral insulin resistance and/or inadequate responsiveness may lead to hyperglycemia. Preterm infants have an incomplete suppression of hepatic glucose production regardless of the circulating insulin concentration and concomitantly reduced peripheral glucose utilization [24, 44]. This decrease in the utilisation of peripheral glucose is partly caused by free fatty acids that alter the enzymatic activity, which preferentially leads to fatty acid carbon oxidation rather than glucose oxidation, promoting high glucose concentrations. Fatty acids also inhibit the effect of insulin on the suppression of hepatic glucose production. The insulin secretion is stimulated, but its action in the periphery is inhibited. The result is a peripheral glucose intolerance and central (hepatic) insulin

resistance, increasing the blood glucose concentrations. Other causes for hyperglycemic episodes are exogenous glucose infusions (which do not inhibit gluconeogenesis), the lipid component of the parenteral and enteral nutrition, physiologic stress in neonates caused by painful procedures, irritation during ventilatory treatment and catecholamine infusion, sepsis and some medications (e.g. theopylline, glucocorticoids, epinephrine, glucagon and fentanyl). To summarize the mechanism leading to hyperglycemia: there is a decreased sensitivity to insulin and an excessive or inappropriate insulin secretion rate and plasma glucose production [4, 25, 37, 40, 45].

A decreased sensitivity to insulin has also been described in AGA or SGA preterm infants with a relatively normal rate of insulation secretion, even in those who have sufficient adipose tissue stores. Persistent hepatic glucose production at this age increases the risk of having insulin resistance, glucose intolerance and Diabetes Mellitus manifesting itself at adolescence and young adulthood [45-49].

1.7. Clinical Manifestations in Infancy

A rapid decline in blood glucose concentration activates an autonomic response and causes neuroglucopenia, which may lead to neurological dysfunction.

Table 1 - Clinical Signs and Symptoms of Hypoglycemia in Childhood[2]

Features Associated with Cerebral Glucopenia	Features Associated with Activation of the Autonomic Nervous System and Epinephrine Release
Headache, Mental confusion	Anxiety
Visual disturbances	Perspiration
Organic personality changes	Tachycardia, Palpitation
Inability to concentrate	Tachypnea
Dysarthria, Ataxia, Aphasia	Pallor
Staring	Tremulousness
Paresthesias	Weakness
Dizziness	Hunger
Amnesia, Somnolence, Lethargy	Nausea, Emesis
Seizures, Stroke	
Hemiplegia	
Decerebrate or decorticate posture	
Coma, Death	

The relation between low blood glucose values and clinical symptoms is relatively easy to observe in older children and in adults; but in newborns the signs are more subtle and too often asymptomatic [15]. The symptoms in newborns and infants include cyanosis, pallor, apneic episodes, tachypnea, hypothermia, hypotonia (floppiness), poor feeding, abnormal cry (weak or high-pitched), irritability, diaphoresis, tremors, jitteriness, exaggerated Moro reflex, lethargy and seizures. Some of these symptoms may be so mild that they are missed. It is of utmost importance to identify an event even in the absence of a correlation between low blood glucose concentrations and the presence of abnormal clinical signs [2, 7, 16, 19, 21, 27, 28, 31, 50].

Most of the enumerated signs are not specific for hypoglycemia and can also be manifestations of other neonatal disorders (e.g. septicaemia, congenital heart disease, ventricular hemorrhage and respiratory distress syndrome), the common factor being poor peripheral circulation and tissue perfusion associated with hypoxemia and lactic acidosis [28]. To diagnose an event the index of suspicion for hypoglycemia must be high and other etiologies have to be excluded [4, 16, 19, 21, 24].

Neonatal hyperglycemia is also mostly asymptomatic and possible signs are also indicative of other pathological processes. The symptoms include dehydration due to osmotic diuresis, weight loss, failure to thrive, fever, glucosuria, ketosis and metabolic acidosis [4].

1.8. Diagnosis of Hypo- and Hyperglycemia

Routine monitoring is usually only performed in infants with risk factors during their first weeks of life at an NICU. Healthy full-term infants, born after entirely normal pregnancies and deliveries, which have no symptoms whatsoever, do not require blood glucose monitoring [35].

The presence of (1) clinical manifestations, (2) a reliable significantly low blood glucose value and (3) a prompt response to adequate therapy fulfill the requirements of Whipple´s Triad, the basis for making the diagnosis of significant hypoglycemia at any age. The problem arising with this “clinical diagnosis” is that most hypoglycemic episodes in the newborn patient are silent. Therefore, the latest guidelines from the American Academy of Pediatrics (AAP) [19] state that if either hypo- or

hyperglycemia are suspected, the plasma or blood glucose concentration must be determined immediately by using one of the laboratory enzymatic methods (glucose oxidase, hexokinase or dehydrogenase method). Plasma values tend to be 10-18% higher than whole blood values because of the plasma's higher water content. If bedside methods are used (they allow a prompt diagnosis and initiation of therapy), the blood or plasma glucose concentration must be confirmed by laboratory methods. Although laboratory methods are the gold standard and the most reliable ones, their results may not be immediately available, relegating them to the confirmation of events [7, 19, 21].

In Neonatology, the incidence of hypo- and hyperglycemia depends on the criteria of diagnosis, the population surveyed, as well as the method for blood collection and glucose analysis [16, 31].

1.9. Management of Neonatal Hypo- and Hyperglycemia

It is important to ensure in preterm and SGA infants an early provision of exogenous calories. If possible it should be enteral milk feedings, which enhance metabolic adaptation and gut maturation. In this specific demographic group the sucking reflex is not yet fully established: therefore, at first, feeding must be a continuous intra-gastric infusion, if bolus feeds are not tolerated. If enteral feeding is not tolerated at all, an intravenous glucose infusion is indicated [51].

In term newborns, breast-feeding is encouraged and it should be on demand and unrestricted in duration and frequency. These babies are more likely to have low glucose levels but higher blood ketone body concentrations than artificially fed ones. If supplementation is needed, formula can be used, but it does not substitute breast-feeding in any way [7, 13, 41, 42, 52].

1.9.1. Hypoglycemic Event Therapy

If hypoglycemia is diagnosed in a symptomatic infant, regardless of the glucose value, a prompt intervention with an intravenous glucose infusion is required ("minibolus" of 200mg of glucose per kg, 2 mL/kg dextrose in 10% water). It rapidly corrects the hypoglycemic episode, without resulting in hyperglycemia. On the other hand, if the episode develops asymptomatic and it was an incidental diagnosis, with blood glucose concentrations of ≤45 mg/dL at the NICU Perinatal Center

Großhadern, the feeding frequency should be increased. If the episode is asymptomatic, but the glucose concentration is <36 mg/dL and the glycemia does not increase after a feed, or the infant develops abnormal clinical signs; an intravenous glucose infusion is needed. In either case, follow-up glucose concentrations and clinical evaluation must always be obtained for monitoring and control [7, 19].
The reasonable safe lower limit target value should be maintained at >45 mg/dL [21].

1.9.2. Hyperglycemic Event Therapy

Hyperglycemic episodes need to be treated based on the infant's diagnosis and suspected etiology. Potential dehydration, osmotic diuresis and serum electrolytes should be determined to calculate fluid therapy replacement. In the range 125-350 mg/dL the gradual reduction of exogenous glucose administration should be sufficient to bring the glucose level back to normal. If severe hyperglycemia persists (>300-400 mg/dL), exogenous insulin administration may be needed, in the form of a continuous infusion beginning at 0.02 to 0.05 IU/kg/hour. With the addition of potassium the risk of hypokalemia can be minimized. Plasma glucose and serum potassium monitoring is advised (every 1 to 2 hours or whenever signs of hypokalemia and/or hypoglycemia develop), until achieving normoglycemia. The insulin-treatment is highly controversial among guidelines, experts and NICUs [4].

2. Methods

This doctoral thesis is based on a prospective observational cohort study with a total n=20 infants. Infants were enrolled at the Perinatal Center Grosshadern, Ludwig-Maximilian University, Munich.

We measured the interstitial glucose concentration continuously for three days in stable preterm infants who receive enteral high caloric nutrition according to guidelines [51]. The objective of this study was to diagnose the incidence of silent but clinically- relevant hypoglycemias and their correlation with high caloric feedings and other possible risk variables at about 30 DOL.

Despite the fact that the protocol of our study has as its focus on the measurements with the CGMS and the results regarding incidence of hypoglycemic episodes and the possible variables affecting the metabolism of these infants, we decided retrospectively to look also at the charts of the included infants and to gather their blood glucose values obtained by capillary samples with the ABL-blood gas analyzer during their first ten days of life.

Ethical approval was granted by the Ethics Committee of the LMU (2008.06.18) and informed consent was obtained from the parents for the inclusion of each infant in the study. The first infant was recruited and measured in April 2011 and the 20th in February 2012.

2.1. Study Population

All infants enrolled were born at the Perinatal Center, Grosshadern in the year 2011.

The **inclusion criteria** were (1) birth weight < 1500g and (2) gestational age < 32 weeks postmenstrual age. All infants were on full enteral feeds for a median of 26 days and achieved a stable growth rate.

The **exclusion criteria** were (1) a pathological outcome in the neonatal screening test on the 3rd day of life, (2) a positive family anamnesis for metabolic diseases besides Diabetes Mellitus and (3) acute congenital malformations.

Between April 2011 and February 2012, of the total number of neonates born at the Perinatal Center, 213 infants were admitted in the NICU. Thirty-one percent of them were potential candidates for the study, but due to transfer to other facilities, ongoing

infections, received medication or the non-consent of the parents, only 30% of the potential candidates could be included and measured.

2.1.1. Cohort distribution and characteristics

Infants were classified into two cohorts. The first group (G1, n=14) had a birth weight <1000g, infants in the second group (G2 n=6) had a birth weight between 1000 - 1500g. The total number of included newborns for this thesis was n=20.

Three infants were SGA-infants and were included in their respective weight-cohort. They all belonged to G1. Infants selected for this study were considered to be without major medical problems by the neonatologist in charge at the time of the measurements.

2.1.2. Demographic Characteristics

The following biomedical and perinatal information was collected: gestational age, gender, anthropometric values at birth, type of delivery, Apgar score at 1, 2, 5 and 10 minutes, venous cord blood sample at birth, maternal obstetric history including complications and diseases (e.g. Diabetes Mellitus or Gestational Diabetes), reception of antenatal steroids, and neonatal problems and complications.

Weight (g), length and head circumference (cm) and their corresponding percentiles were recorded again at trial entry, before, during and after measurements. For each infant the CRIB-Score (Clinical Risk Index for Babies, range from 0 – 23, higher score indicating more severe illness) [37] and Body Mass Index (BMI) [27] at birth were also calculated.

On the Continuous Glucose Monitoring (CGM) – days, information about the energy intake and detailed data on nutrition and medication were collected, as well as on the 7 days before and after the measurements. Other parameters and data like breathing support, occurrence of seizures, Bronchopulmonary Dysplasia (BPD), Intraventricular Haemorrhage (IVH) and Periventricular Leucomalacia (PVL) were also gathered. The diagnosis of grade I to IV IVH and PVL was determined through cranial ultrasonography (Logiq E9 Ultrasonography – General Electric's Healthcare) by an attending neonatologist.

Weight gain was calculated (g/kg/d) 7 days before and after measurement and the overall weight gain during hospital stay.

Table 2 - Baseline Characteristics of Infants at Recruitment

Characteristics (Median + SD)	<1000g	1000 -1500g	P-Value *
Total n: 20	14	6	
Gestational age at birth (weeks)	25.1 ± 1.9	28.9 ± 1.4	.002
Birth weight (g)	640.0± 165.8	1135.0 ± 65.9	.000
Gestational age at trial (weeks)	31.5 ± 1.8	33.4 ± 0.9	.207
DOL at trial	48.0 ± 14.7	32.5 ± 6.0	.009
Head circumference at trial (cm)	26.4 ± 1.8	28.5 ± 1.1	.020
Length at trial(cm)	39.0 ± 2.9	41.0 ± 1.8	.051
Weight at trial(g)	1362.5 ± 209.4	1642.5 ± 147.7	.012
Sex n (%) male 13 (65%)	8 (57%)	5 (83%)	
CRIB score	7 ± 3	1 ± 1	.003
BMI at birth	6,9 ± 0.8	7,4 ± 1.2	.009
Arterial Cord blood pH at birth	7,36	7,38	.444
Antenatal glucocorticoids (%)	86%	100%	.659
Mode of Delivery Vaginal	2 (14%)	1 (17%)	.968
Cesarian	12 (86%)	5 (83%)	
Total length of hospital stay (days)	69.0 ± 17.3	61.0 ± 14.2	.033

** P < 0.05*

Regarding respiratory support (Table 3), 85% were receiving Continuous Positive Airway Pressure (CPAP), High Flow Nasal Cannula (HFNC) or alternated between both systems. 15% did not need any oxygen or ventilatory support during the CGM-days.

Table 3 - Details of Respiratory Support among Cohorts during the CGM-Days

Cohort	<1000g	1000-1500g
n=20	14	6
No Support (%)	7	33
HFNC (%)	36	67
CPAP (%)	21	-
HFNC + CPAP (%)	36	-

All infants received oral medications like vitamins and iron, 18 infants still received coffein base for the treatment of apnea and bradycardia episodes, 10 received fluconazol, 8 inhaled budesonid, 5 morphine, 4 phenobarbital, 3 teicoplanin, salbutamol, formoterol or ceftazidim and 2 were receiving erythromycin during the CGM-days.

2.1.3. Nutrition at birth and during the CGM-Days

All children included in this study started enteral and partial parenteral nutrition on their first DOL. Initial enteral nutrition consisted of 8-12 meals of max 30ml/kg/day according to feeding tolerance. The energy intake was advanced daily, as recommended by guidelines, 110-135 kcal/kg/day. Nutritional composition was 10-15% proteins, 40-45% carbohydrates and 30-35% lipids and essential fatty acids plus minerals (e.g. Iron) and vitamins (A, D, E and K) [53].

The goal for each preterm infant <1500g was to feed with breast milk fortified with human milk-fortifier FM85 (Nestlé®, Vevey, Switzerland) to meet nutritional requirements. If breast milk was not available or contraindicated, infants were fed with preterm formula (Beba FGN, Nestlé®, Vevey, Switzerland) plus LC PUFA (milk fortifier with long chain poly unsaturated fatty acids). Parenteral nutrition was increased daily to reach the nutritional recommendations of a total intake of 3.5 – 4g/kg/day of proteins and 2.5 – 3g/kg/day of lipids, with the average energy intake of 120 kcal/kg/day (European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) and the American Association of Paediatrics (AAP)) [51]. The detailed nutrition characteristics are listed in Table 4.

Table 4 - Feeding Characteristics of Infants on the CGM-Days

Nutritional Intake (Median ± SD)	<1000g	1000-1500g
Feeding volume (ml/kg/day)	151.2 ± 13.6	153.2 ± 8.2
Energy intake (kcal/kg/day)	126.6 ± 9.1	127.7 ± 6.9
Carbohydrate intake (g/kg/day)	15.7 ± 1.8	15.5 ± 1.5
Protein intake (g/kg/day)	3.1 ± 0.3	3.1 ± 0.3
Lipid intake (g/kg/day)	6.0 ± 0.4	6.1 ± 0.3

In G1 the median amount of days fully enteral fed at study entry was 25.0 days ± 16.7 and in G2 it was 20.5 ± 5.3 days. During the 3-day measurement period, the received nutritional intake for all infants was as described in Table 4. One patient in G1 and two patients in G2 received sunflower oil (Mazola-Oil) as supplement. That nutrition enrichment is taken into account in Table 4.

2.2. Data Collection and Test Methods

2.2.1. Blood Glucose Sample Collection

Intermittent capillary blood sampling by heelstick was routinely taken preprandial at least twice a week until the 35th week postmenstrual age. The samples were analyzed with an automated `Point of care blood gas analyzer (ABL 700´ Radiometer, Copenhagen, Denmark). The POC analyzer determines glucose value by an amperometric method. All blood glucose values taken during the selected infants´ hospital stay were recorded and analyzed. Blood glucose values within the first 10 DOL and those during the CGM-days were used to determine the presence of early fluctuations and to calculate the correlation between glucose values obtained from the ABL-analyzer and the continuous glucose monitoring system, respectively. It is important to remember that the whole blood glucose concentration is usually 10-15% lower than the corresponding plasma glucose value [22].

2.2.2. CGMS Equipment Description

Every enrolled child, once it was adequately full enteral fed, clinically stable and had reached at least its 30th gestational week postmenstrual age, was measured continuously for 72 hours.

The device used for the CGM (Guardian® REAL-Time Continuous Glucose monitoring System, CGMS, Medtronic®, Northridge CA, USA) consists of a sensor, a transmitter and a receiver. The sensor (SOF-SENSOR, Medtronic®, Northridge CA, USA) is minimally invasive, soft, flexible, disposable and is placed subcutaneously.

Fig. 2.2.2-1: Minilink Transmitter and Sof-Sensor.

This device is about 1mm in width, 10 mm in length and is mounted through a hollow needle. It has a platinum electrode which catalyses interstitial glucose oxidation, generating an electrical current every 10 seconds.

The sensor is connected to a wireless transmitter (MiniLink, Medtronic®, Northridge CA, USA), which provides power for the glucose sensor, collects data and transmits those to the receiver [54].

The receiver (Guardian Monitor, Guardian® Real-Time Continuous Glucose Monitoring System – Medtronic, Düsseldorf, Germany) records mean values every 5 minutes, 24 hours a day, providing 288 interstitial glucose values per day. It displays the measured interstitial glucose level in real time on a screen together with graphic representation of the actual glucose pattern. Arrows indicate the direction and rate of change in the actual interstitial glucose levels. The device in this study was configured to alarm for low glucose levels (≤45 mg/dL) and displays the actual trends [55].

Fig. 2.2.2-2: Transmitter shown with a 50 cent Euro coin (24.25 mm diameter) for size comparison.

Fig. 2.2.2-3: Transmitter with charging device and receiver

2.2.3. Sensor Collocation and Removal

After parental consent, the sensor was introduced subcutaneous into the lateral aspect of the infant's thigh by the attending neonatologist under sterile conditions. The skin in the chosen area had to be intact.

Before insertion, the chosen skin area was treated with a local anaesthetic patch, containing lidocaine and prilocaine (Emla® AstraZeneca GmbH, Wedel, Germany).

In one of 20 infants a small amount of blood at the insertion site was observed and the sensor replaced. In all infants insertion site was inspected continuously by the nurse in charge. The whole procedure was well tolerated by all infants.

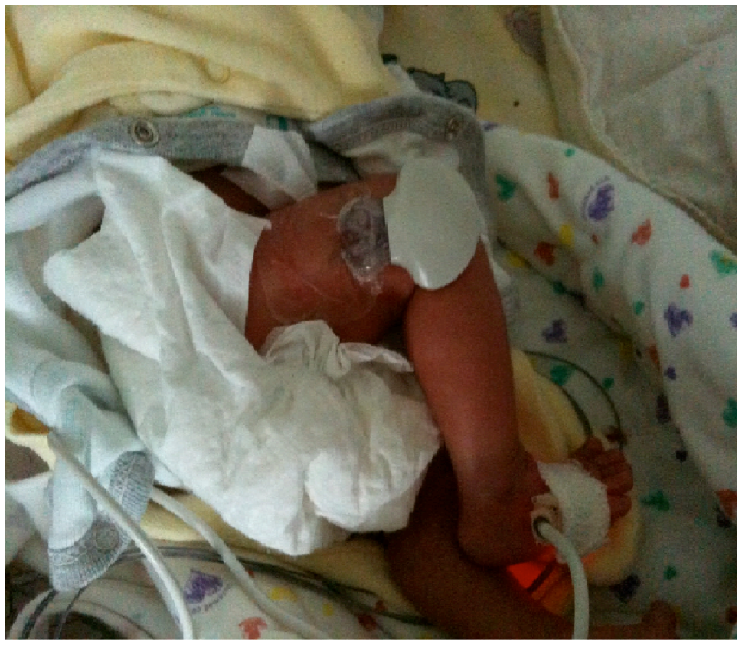

Fig. 2.2.3-1: Sensor and transmitter inserted into the subcutaneous tissue

There were no signs of irritation or inflammation of the skin in any infant throughout the study. The interstitial sensor takes two hours to stabilize after insertion before data recording starts.

After the completion of the 3 days of CGM the attending neonatologist removed the sensor. Within a day after removal the puncture site at the thigh healed without residues in all infants [54]. In the weeks following the removal of the sensor, no infant showed any signs of infection or complications, which could be related to the CGMS.

2.2.4. CGMS Calibration

The CGMS system needs to be calibrated against POC blood glucose values two to three times a day to maintain its accuracy. Blood glucose values measured with a reflectance meter were discarded.

If a hypoglycemic episode was measured (display showing an interstitial glucose value $\leq$ 45mg/dL), a control whole blood sample of 50µL for glucose and 170µL for insulin were collected and sent on ice (+4°C) to the laboratory. The samples were

analyzed at the hospital clinical laboratory using the hexokinase method and an immunological method, respectively.

2.3. Definitions, Statistical Methods and Analysis

2.3.1. Operative Definition of low- and high Tissue Glucose Value Episodes

Low tissue glucose value episodes were defined as an interstitial sensor glucose value ≤ 45 mg/dL (operational threshold at the NICU Perinatal Center Grosshadern). In order to determine the severity of these events, interstitial glucose values were ranked as described in Table 5.

Table 5 – Tissue Glucose (TG) Value Classification

Normal TG	> 45 mg/dl
Moderate low TG	41 - 45 mg/dL
Severe low TG	31 - 40 mg/dL
Extreme low TG	< 30 mg/dL

To define high tissue glucose value episodes, two values have been selected: >150mg/dL and >200mg/dL. These are the glucose cut-offs most frequently used in most publications, being >150mg/dL consistent with the renal glucose threshold and the observation of glucosuria in the preterm neonate and the commonly used value to define hyperglycemia in older children and adults and >200mg/dL the operational threshold at our NICU [1, 4, 8, 9, 25, 28, 36].

2.3.2. Statistical Analysis

Data was analysed using the Statistical Package for Social Sciences 18.0 (IBM, SPSS Inc, Chicago, IL, USA). The level of significance was specified at $P < .05$.

Since many of the measurement variables were not distributed normally, the median and standard deviations (SD) for each cohort were calculated and are the main measures reported. The statistical analysis was carried out with chi-squared tests to rule out any effect of confounding factors. To calculate the agreement between the paired samples obtained by the CGMS and those obtained by blood sample, a Related Samples Wilcoxon Signed Ranks Test was used and assessed by

Pearson´s correlation coefficient and a Bland-Altman method. The 95% confidence interval was determined with a two-sample T-Test.

For the calculation of significance levels across cohorts the nonparametric Mann-Whitney U Test or Kruskal Wallis Test were used. The positive and negative relationship between the different variables and the hypo- and hyperglycemic events are described with the Spearman's rank correlation coefficient.

3. Results

3.1. Correlation of capillary blood glucose and interstitial glucose concentration

We calculated the correlation between paired glucose readings from capillary blood samples measured with the ABL-analyzer and the corresponding CGMS- interstitial glucose values. A total of 133-paired values were included in the analysis. The results were r=0.80, r^2=0.68, p< .001 and the median difference between paired values equalled 0 mg/dL. The mean glucose difference between both monitoring systems was 0.48 mg/dL (95% confidence interval -2.80 to 3.66). The correlation remained excellent for lower glucose values (<150 mg/dL, r=0.81 and p = .000), with a mean glucose difference of -1.12 mg/dL between both methods, but the correlation decreased for higher ranges of values (>150 mg/dL).

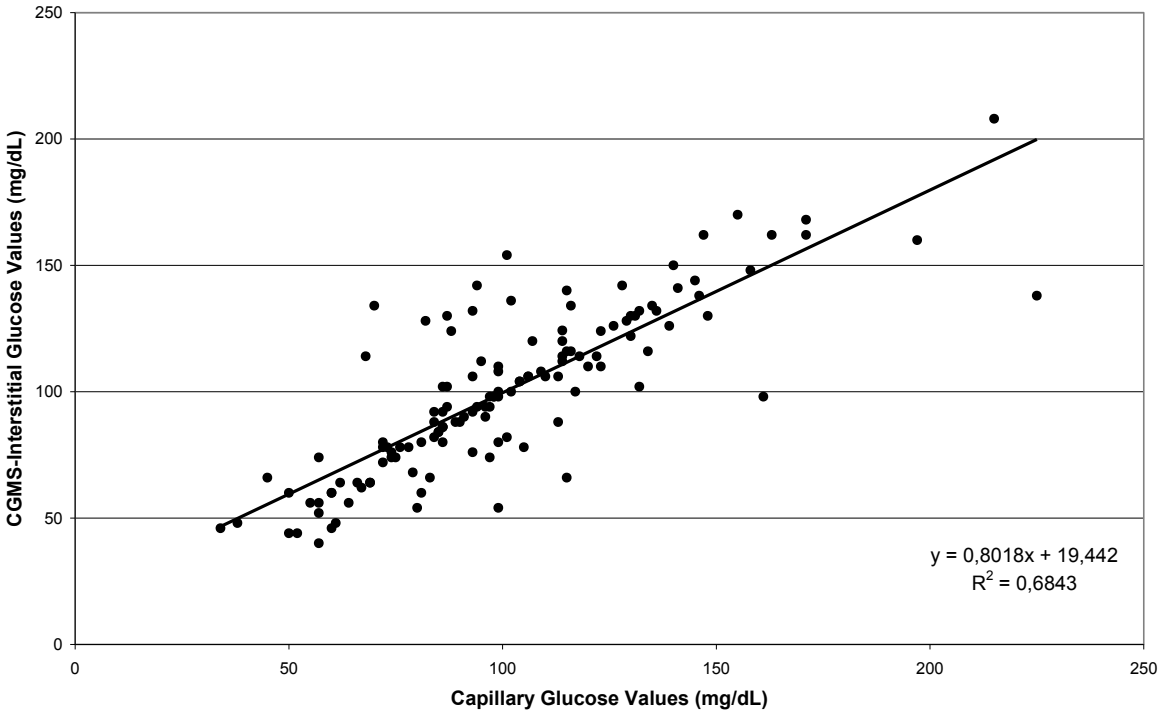

Figure 3.1-1*:* Accuracy of agreement between capillary glucose and CGMS-interstitial glucose values.

A Bland-Altman plot (difference plot) was made to illustrate the correlation and agreement between the two glucose-monitoring methods (Figure 3.1-2).
The limit of agreement is set at ± 2 SD (average difference ± 2 standard deviations of the difference)

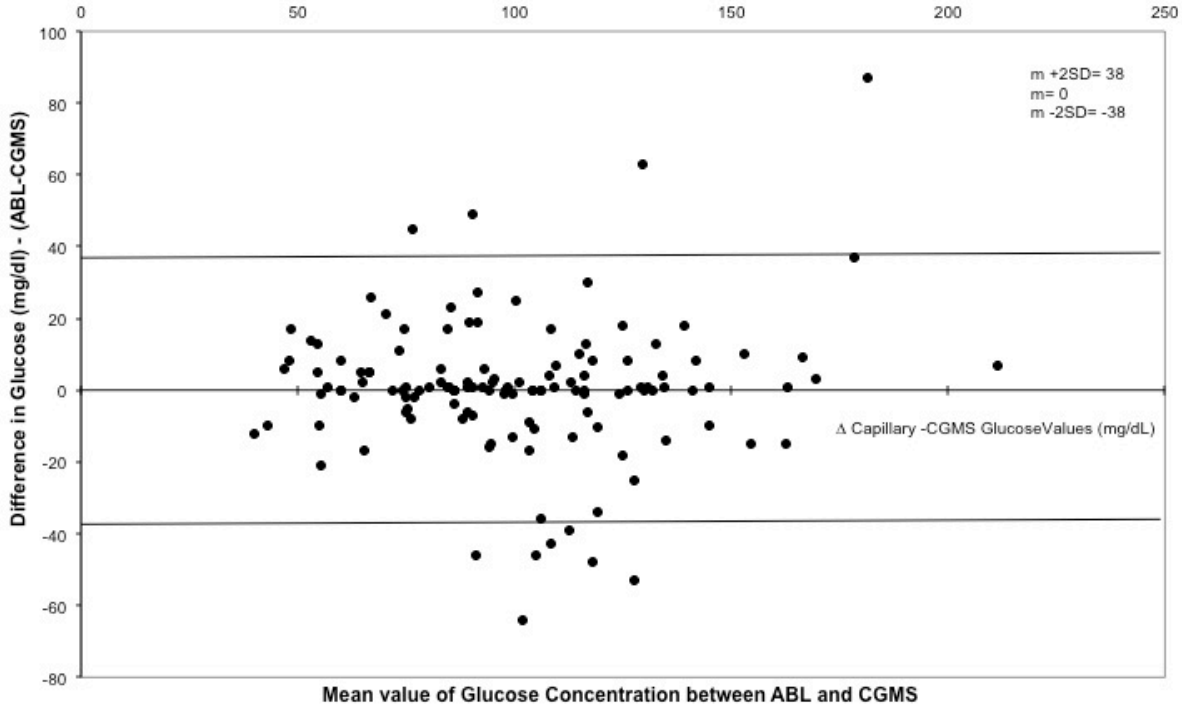

Figure 3.1-2: Accuracy of agreement between the two methods (CGMS and capillary Glucose). Bland Altman graphical representation. M, mean

The capillary glucose values used for calibration of the CGM-device were compared to the interstitial glucose values registered with the device. We took the ABL-value as "time 0" and compared it to the value registered by the CGM-device at 0, plus 5, 10, 15, 20, 25 and 30 minutes. The best correlation coefficients were at 15 (0,488) - and 20 minutes (0,489) after the blood sample was obtained.

3.2. CGMS Data characteristics and results

At study-entry, the median gestational age was 31.5 ± 1.8 for G1 and 33.4 ± 0.9 weeks for G2. At that time 16 infants (80%) were fed with human milk + FM85 fortifier and 4 (20%) with preterm formula.

A total number of 16874 subcutaneous glucose readings were recorded, which correlates with 1429 hours and 14 minutes of data. This means a number of measurements and record time per child of 844 ± 78 and 71:27 hours ± 6:49.

The highest and lowest median interstitial glucose values for n=20 were 190 mg/dL and 57 mg/dL.

The median daily weight gain (g/kg/day) one week before the measurements was 19.0 ± 15.2 (G1) and 36.8 ± 17,5 (G2) and one week after the completion of the CGM-days was 28.6 ± 8.9 (G1) and 41.2 ± 18.2 (G2). For these two variables there were no statistically relevant differences among cohorts. The overall median weight gain for each cohort during the entire hospital stay was 20.2 ± 4.3 (G1) vs. 25.4 ± 5.2 (G2) g/kg/day (p = .044).

Table 6 - Characteristics of Infants at CGM-Days

Value (Median ± SD)	< 1000g	1000-1500g	P-Value*
Gestational age at trial (weeks)	31.5 ± 1.8	33.4 ± 0.9	.207
DOL at trial	48.0 ± 14.7	32.5 ± 6.0	.009
Weight at trial (g)	1362.5 ± 209.4	1642.5 ± 147.7	.012
Measured Time (hours)	71.5 ± 8.2	70.67 ± 1.25	.680
Nr. of measurments/infant	849.0 ± 94.0	829.0 ± 15.0	.650
Risk factors for hypo- hyperglycemia n (%)			
Prematurity	14 (100%)	6 (100%)	
Infant of mother with Diabetes Mellitus / Gestational Diabetes	1 (7%)	1 (17%)	.476
SGA		3 (21%)	.156
Interstitial Glucose values (Median + SD and Range)			
Highest interstitial glucose concentration (mg/dL)	210 ± 64 (396-146)	136 ± 26 (190-120)	.004
Lowest interstitial glucose concentration (mg/dL)	54 ± 15 (29-74)	57 ± 11 (40-68)	.648
Nutrition at CGM-Days (nr. of infants and %)			
Fortified Human Milk (+ FM85)	13 (93%)	3 (50%)	-
Preterm Formula (Beba + LC PUFA)	1 (7%)	3 (50%)	-
Weight gain (g/kg/day)			
Daily weight gain 7 days before measurement	19.0 ± 15.2	36.8 ± 17.9	.284
Daily weight gain 7 days after measurement	28.6 ± 8.9	41.2 ± 18.2	.219
Overall weight gain during hospital stay	20.2 ± 4.3	25.4 ± 5.2	.044

**P < 0.05*

3.3. Prevalence and characteristics of Low- and High tissue glucose value events

3.3.1. Prevalence and severity of Low- and High tissue glucose value events

During the CGM-days, low tissue glucose value episodes (tissue glucose ≤ 45 mg/dL) were documented in 43% of the infants in G1 and in 17% in G2. All three SGA infants (G1) presented tissue glucose values within normal parameters during the 72 hours of measurement.

The prevalence of high tissue glucose values overall (episodes with interstitial glucose values >150mg/dL) was 71% in G1 and 17% G2 (p= .028). Considering the threshold >200mg/dL, 57% of the infants (in G1) had severe high tissue glucose value events. In contrast, no infant in G2 had high tissue glucose value incidents with interstitial glucose values >200mg/dL (p= .020, table 7).

Interstitial glucose values of some infants fluctuated between normal, high- and/or low ranges during the 72 hours of measurement.

Table 7 - Metabolic Fluctuations among Cohorts during the CGM-Days

Cohort	<1000g	1000-1500g	P-Value*
n	14	6	
Normal TG Value (%)	7	66	.006
Low TG Value Events (≤45mg/dL) (%)	21	17	.273
High TG Value Events (>150mg/dL) (%)	50	17	.028
Low - and High TG Value Events (%)	22	-	.010

** P < .0.05*

In total 93% of the infants in G1 and 34% in G2 had abnormal interstitial glucose values during the CGM-days (p= .010, figure 3.3.3-1.). The following graph illustrates the data in Table 7.

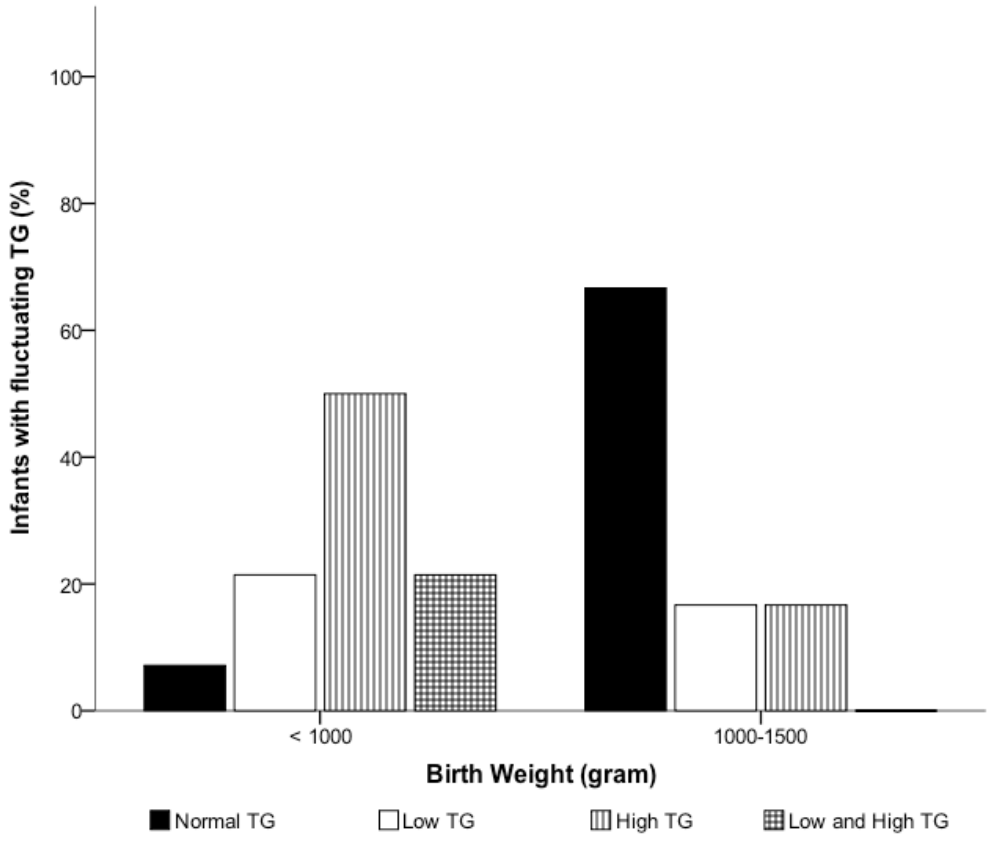

Figure 3.3.1-1: Tissue Glucose fluctuation within weight cohorts during CGM-days.

Figure 3.3.1-2 and 3.3.1-3 illustrate the relation between birth weight and the incidence of low interstitial glucose values. The pattern repeats itself for low birth weight and high tissue glucose values. The median highest interstitial glucose value was 210 in G1 and 136 mg/dl. The median lowest interstitial glucose value were 54 in G1 and 57 in G2.

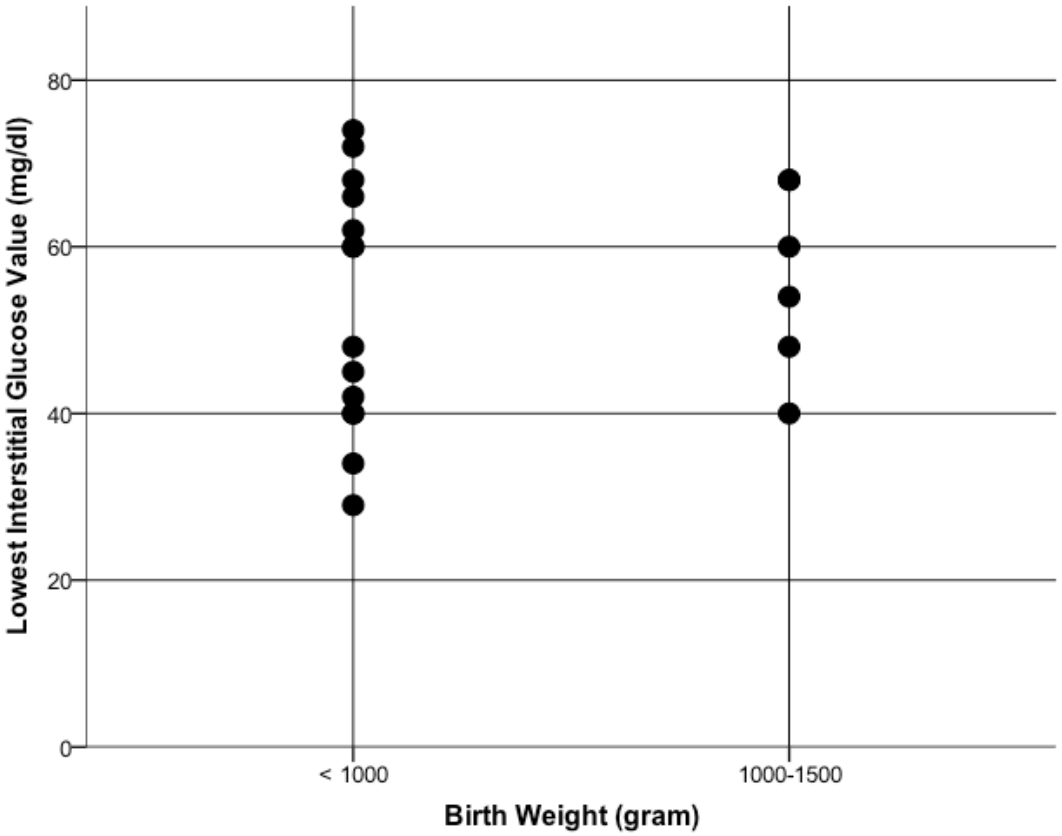

Figure 3.3.1-2: Infants with lower birth weight experienced lower TG values.

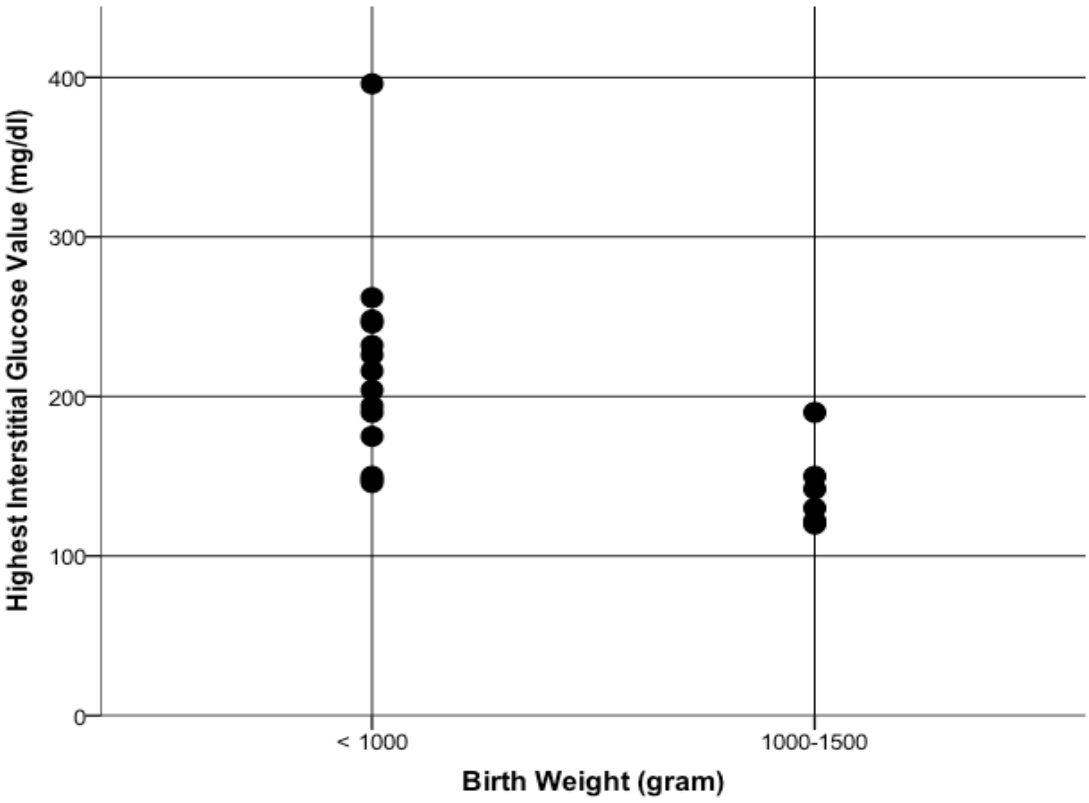

Figure 3.3.1-3: Infants with lower birth weight experienced higher TG values.

Seven percent of the infants in G1 had tissue glucose measurements below 30 mg/dL, 22% between 30 and 40 mg/dL, 14% had moderate low tissue glucose events of 41-45 mg/dL and 57% had interstitial glucose values >45 mg/dL. 17% in G2 presented moderate low tissue glucose episodes with glucose values ranging between 41-45 mg/dL and 83% maintained normal tissue glucose values (>45 mg/dL) as depicted in Figure 3.3.1-4.

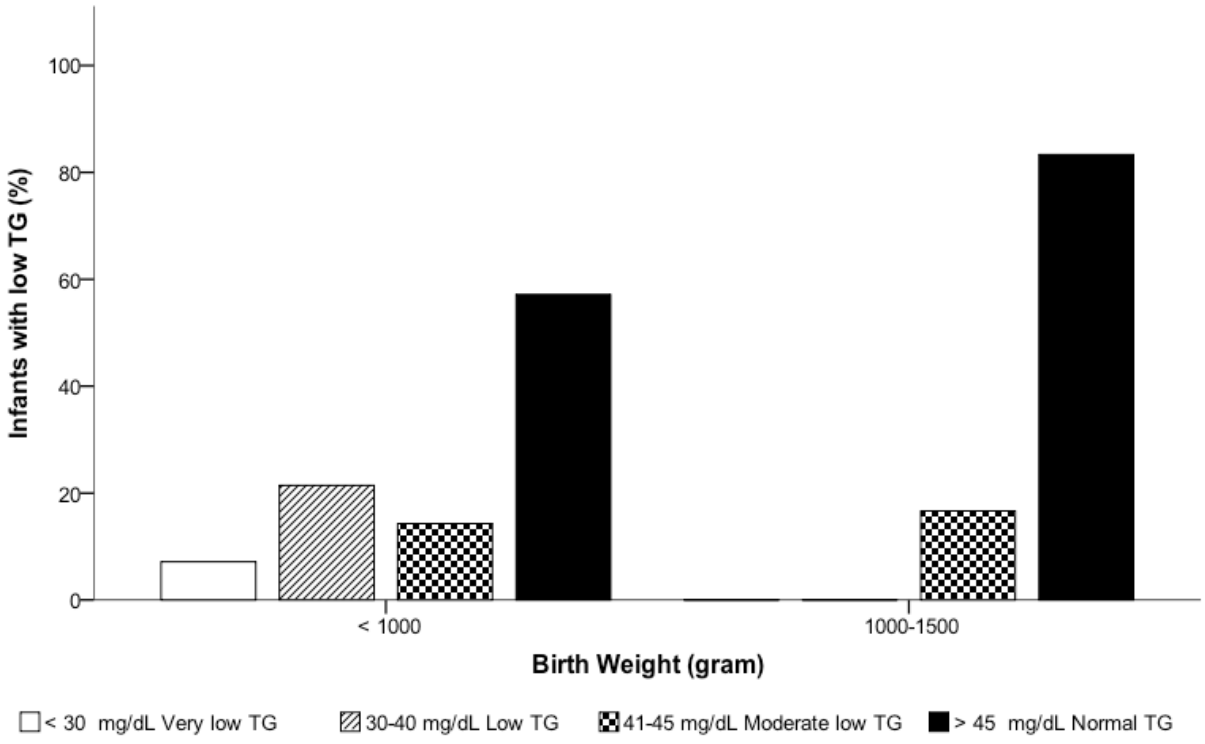

Figure 3.3.1-4: Incidence and severity of low tissue glucose events in each cohort

In G1 the overall incidence of high tissue glucose episodes (>150 mg/dL) was 71%. Only 29% of these infants were able to maintain glucose values below 150 mg/dL. In 14% of infants from G1 we observed interstitial glucose values ranging between 150-200 mg/dL, 57% had events with values >200 mg/dL. In contrast, only 17% of infants in G2 had moderate high tissue glucose episodes with interstitial glucose values between 150-200 mg/dL as shown in Figure 3.3.1-5.

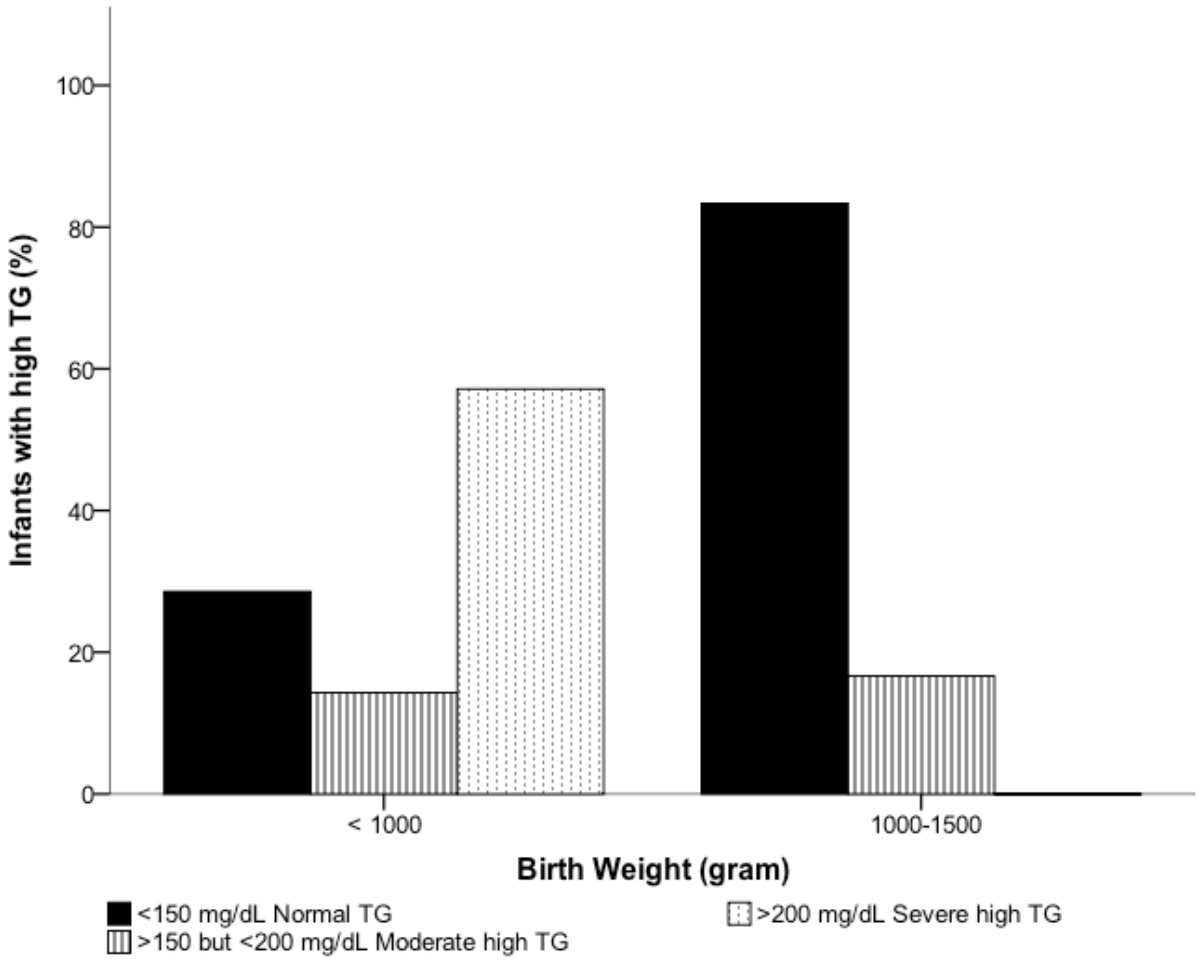

Figure 3.3.1-5: Incidence and severity of high tissue glucose events in each cohort

3.3.2. Length of high- and low tissue glucose episodes

Of the 20 infants included in this study, only 5 (25% of the total) maintained their tissue glucose at all times. The rest suffered different long episodes of glucose value fluctuations. Figure 3.3.2.-1 illustrates the duration and severity of the total 241 episodes in 20 babies recorded during their CGM-days. Twenty percent had at least ≥ 1 episode of low tissue glucose (≤ 45 mg/dL), 40% had at least ≥1 episode of high tissue glucose and 15% suffered high- and low tissue glucose episodes during the CGM-days. Overall there were 14 low tissue glucose episodes among all infants. Six episodes lasted between 10-30 minutes and four lasted between 35-60 minutes. During the CGM-days there were 43 high tissue glucose events with glucose values >200 mg/dL, and they were only present in G1. Twenty-seven episodes lasted 5 minutes and the rest of the episodes had a length of either 10-30 or 35-60 minutes. The high tissue glucose episodes with values >150 mg/dL were 184, of which 116 lasted between 10-30 minutes, 39 lasted 35-60 minutes and 25 lasted more than 60 minutes. The remaining episodes lasted a maximum of 5 minutes.

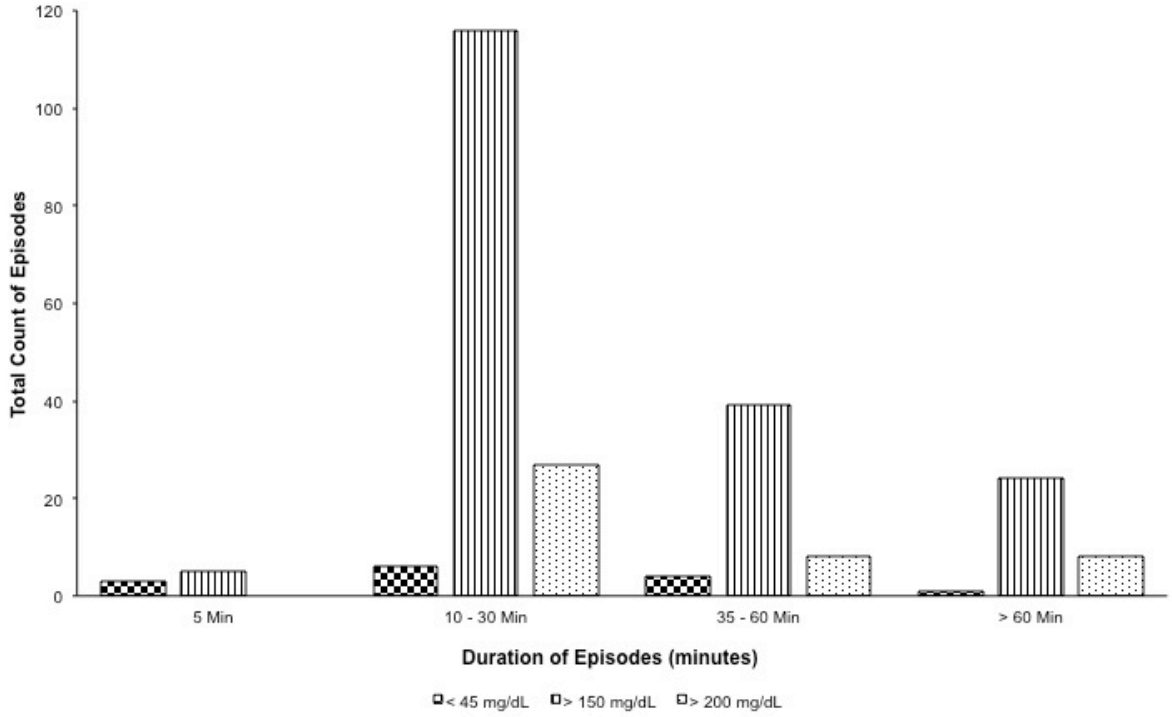

Figure 3.3.2-1: Number and total duration of high and low tissue glucose episodes

3.3.3. Risk factors at birth and during CGM-Days for glucose homeostasis fluctuations

Due to the currently small number of included infants in this study and their irregular distribution among cohorts (14 and 6), the significance of possible risk factors can only be preliminarily established. Based on the binary logistic regression analysis performed by Staffler et al [12], we included the same variables and added several more. The variables were: “gestational age at birth and at trial”, “carbohydrate, protein, lipid and energy intake at trial”, “length of parenteral nutrition”, SGA, BPD, BMI (Body Mass Index: birth weight / height²), CRIB-score and intracranial bleeding. At this point, none of the above mentioned variables showed a significant influence on the development of hypoglycemic events, neither for G1 nor for G2.

On the other hand, there appeared to be an association between gestational age at birth ($p= .020$) and at trial ($p= .033$) with an elevated incidence of high tissue glucose value events. High tissue glucose value events are significantly more frequent in more premature infants (G1, $p= .028$). There is also a negative correlation between high tissue glucose value events and a smaller feeding volume in the group with high tissue glucose values ($p = .033$), lower protein and lipid intake (both in g/kg/day, Spearman’s-Rho $\rho=-.526$, $p= .017$ and $\rho=-.589$ and $p= .006$, respectively). But this correlation was only significant on the first ($p= .014$ and $p= .024$) and second day ($p= .017$ and $p= .047$) of monitoring. There was no significant correlation of protein and lipid intake and a higher incidence of high tissue glucose values on the third day of measuring. A positive correlation was established between high tissue glucose value events during CGM-days and the total length of the received parenteral nutrition ($\rho= .639$ and $p= .003$). The variables ICH/PVL also showed a positive correlation between them and repetitive high tissue glucose value events ($\rho= .452$ and $p= .045$). But when the U-Test was performed, the significance value was >.05, indicating that the intracranial bleeding incidence among infants who suffered repetitive high tissue glucose value episodes and those who did not was actually the same.

SGA, BPD, low BMI and high CRIB score values did not seem to be predictable factors associated either with low- or high tissue glucose value events. We compared the BMI at birth with the metabolic patterns during the CGM-days and saw that infants who developed low- and high tissue glucose value events during the CGM-days had a smaller BMI at birth (6.9 kg/m²) than those who only suffered high- (7.2 kg/m²) or low tissue glucose value events (7.4 kg/m²), or those who remained with

their tissue glucose values within normal ranges (BMI of 7.7 kg/m²) during the CGM-days. (Figure 3.3.3 -1)

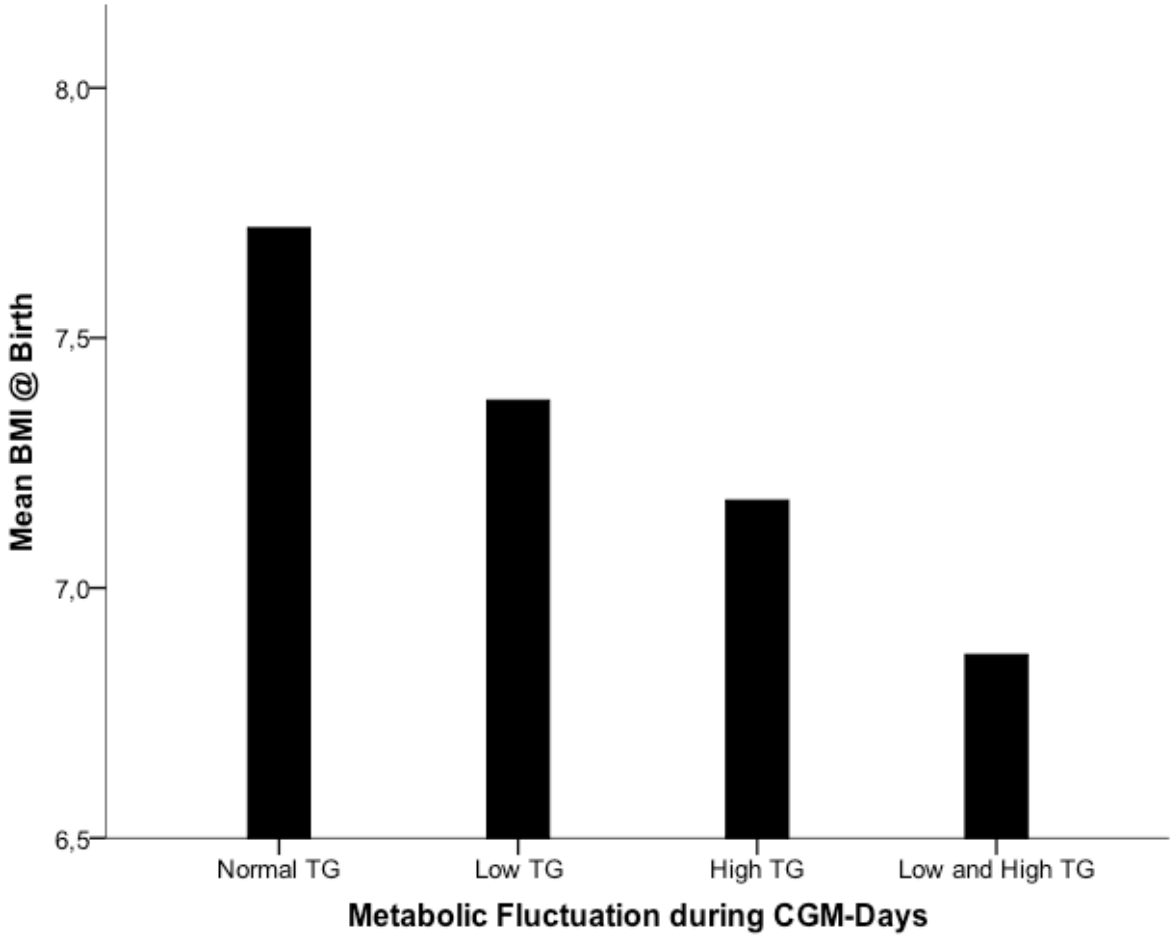

Figure 3.3.3 -1: BMI at birth vs. the metabolic fluctuations experienced by the infants during CGM-Days

Regarding the CRIB score (graph 3.3.3 -2), the infants with the highest scores (mean value of 7.67) are those with abnormal tissue glucose values, which fluctuate between low- and high tissue glucose value episodes (Kruskal-Wallis-Test p= .049 and Spearman Rho ρ= .496, p= .026), during the CGM-days. Infants with repeated low tissue glucose value episodes had the second highest CRIB scores with a mean value of 7.00. In third place, infants with high tissue glucose value episodes had a mean score of 6.25. Stable infants had scores below 2 points (mean score of 1.20).
The different scores among infants who fluctuated during CGM-days can also be seen if we analyze the fluctuations during the first 10 DOL and again during CGM-days with a significance of p= .016.

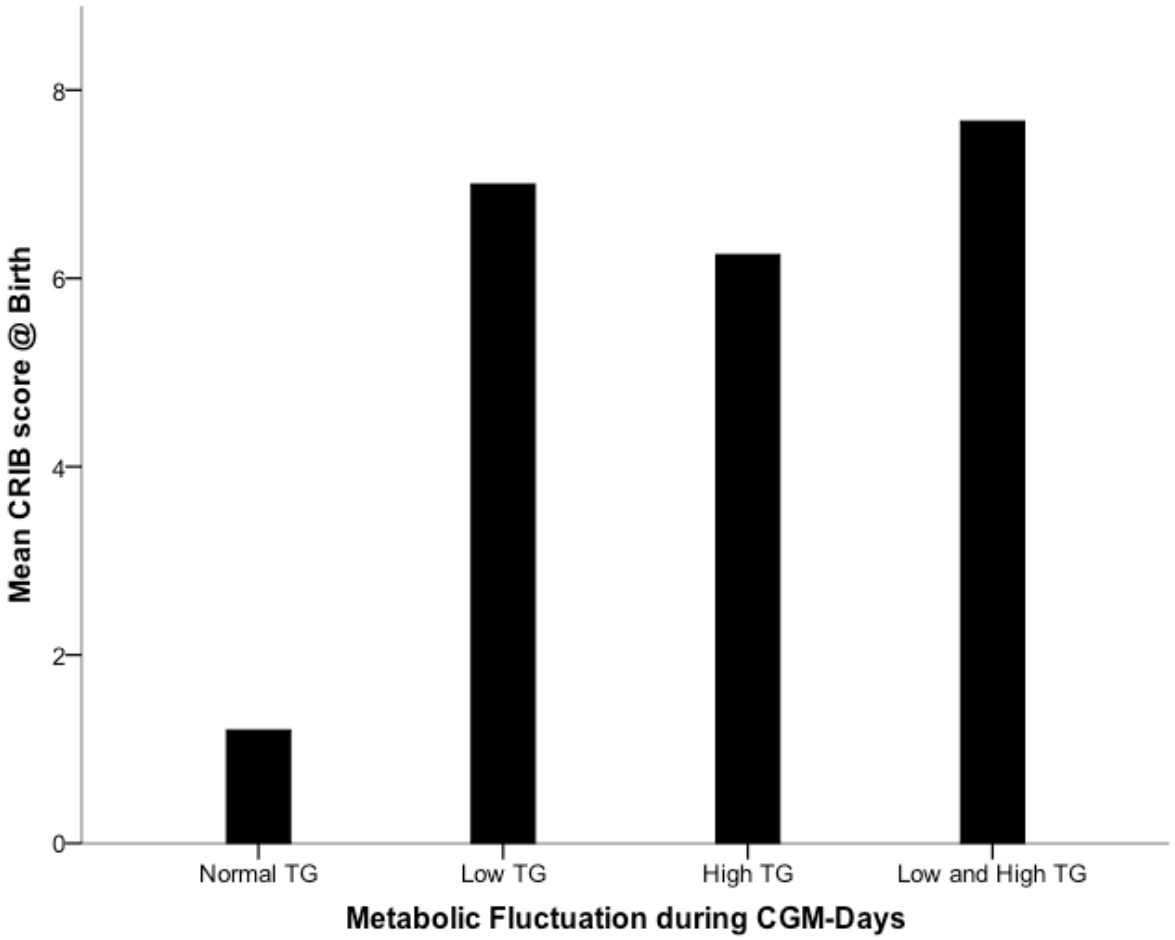

Figure 3.3.3 -2: CRIB score at birth vs. metabolic fluctuations during CGM-Days

We could not detect any significant difference when considering the growth rates for weight (g/kg/day) seven days before and after the CGM-days, either among cohorts or when trying to relate them to low- and/or high glucose tissue value events. There is one significant difference among cohorts regarding the overall weight gain during the hospital stay, where G2 had a significantly higher weight gain than G1 (p= .044).

We compared the characteristic birth weight, birth length and HC at birth with the incidence of low- and high glucose tissue values during the CGM-days and obtained one significant correlation (Figures 3.3.3-3, 3.3.3-4, 3.3.3-5). The HC at birth was significantly smaller in infants who afterwards, during the CGM-days, suffered repeated low tissue glucose value events (p= .002). When conducting the same calculations with the HC at trial, during the CGM-days, we could not determine any correlation.

If we compare the anthropometric parameters at birth of the infants, which remained with tissue glucose values within normal ranges to those infants who fluctuated

during the CGM-days, we see significant differences for all three of them (birth weight p= .049, birth length p= .023 and HC p= .004).

Weight and HC at trial vs. birth weight and HC at birth remain significantly different among cohorts. This was not the case for length at trial, but none of these parameters showed any correlation to a higher incidence of metabolic fluctuations during the CGM-days.

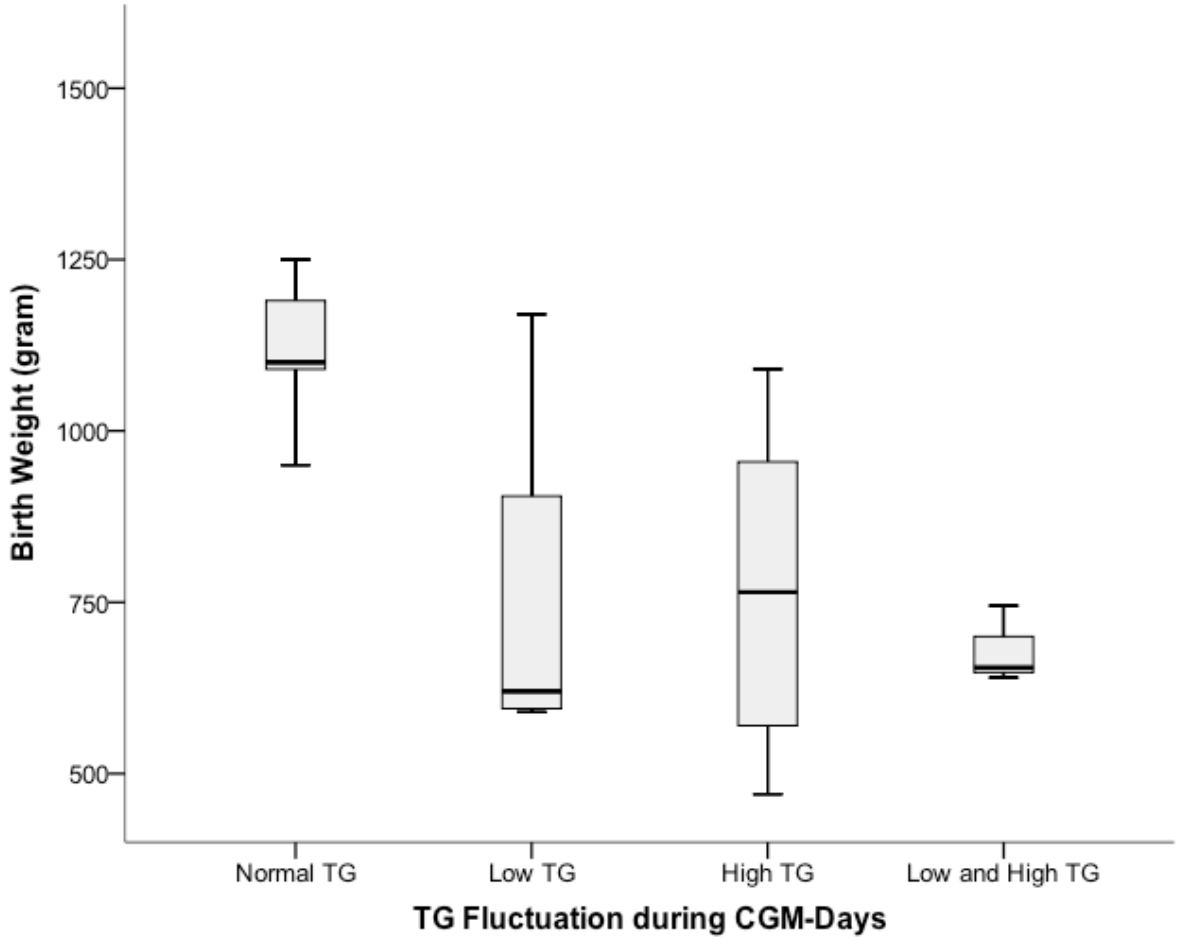

Figure 3.3.3-3: Birth weight and tissue glucose fluctuations during CGM-Days

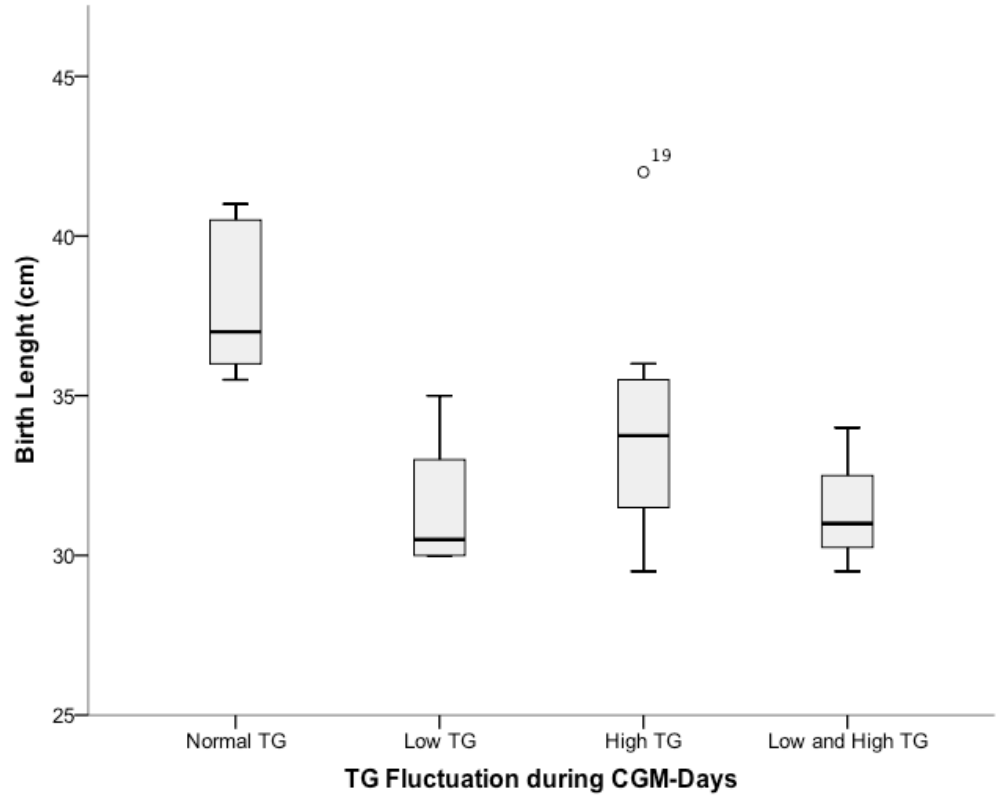

Figure 3.3.3-4: Length at birth and tissue glucose fluctuations during CGM-Days

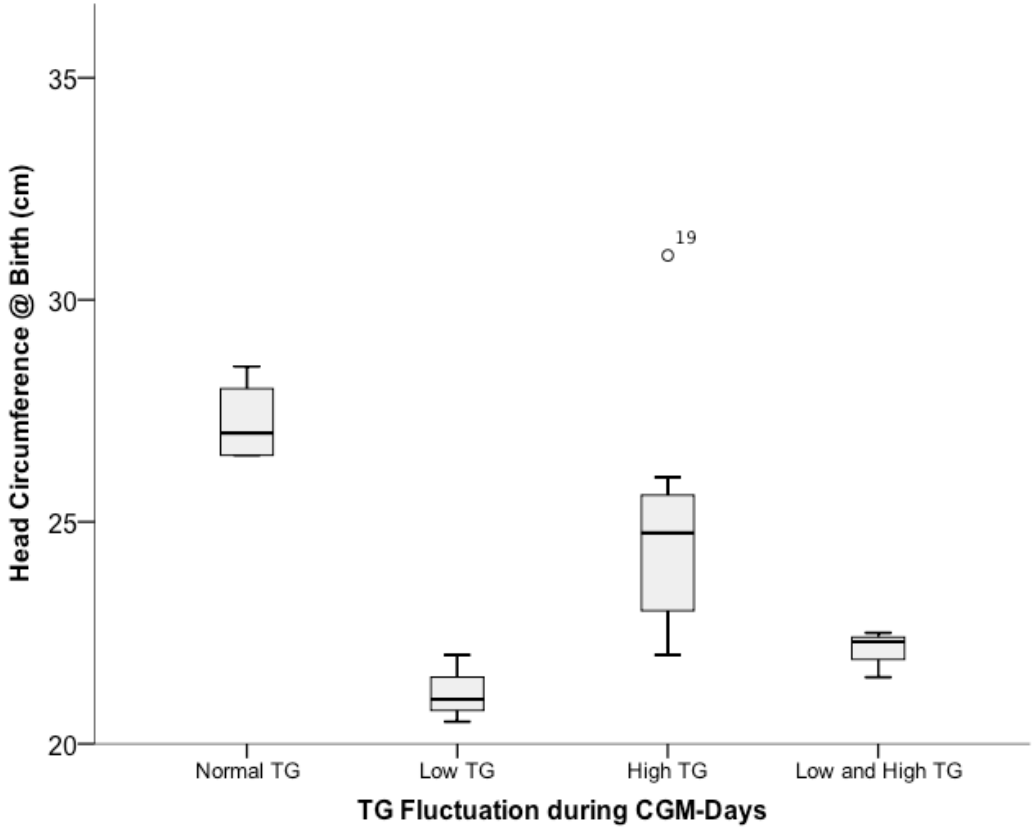

Figure 3.3.3-5: HC at birth and tissue glucose fluctuation during CGM-Days

3.4. Interstitial Glucose Pattern

The interstitial glucose values obtained with the CGMS showed cyclic tissue glucose fluctuation within 24 hours according to the infants feeding schedule. The figure 3.4-1 is derived from measurements in an infant who was receiving 8 bolus feeds per day.

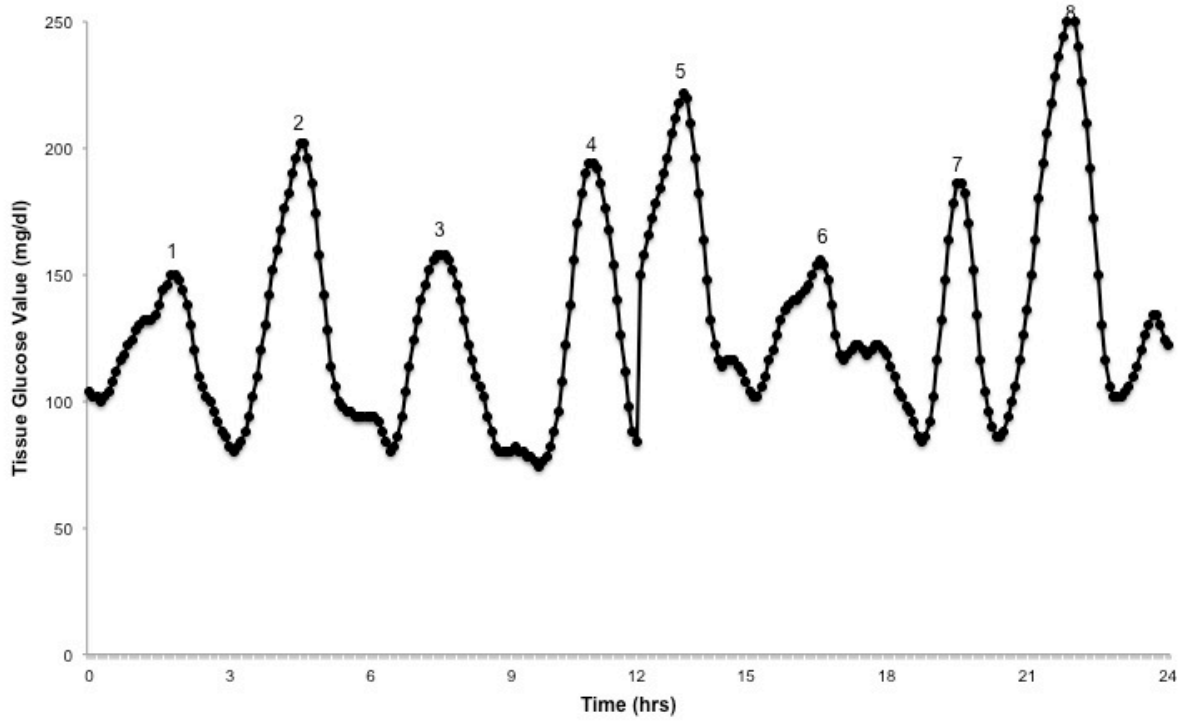

Figure 3.4-1: Glucose pattern of an infant with eight meals in 24 hours.

The second figure 3.4-2 is derived from measurements in an infant who was receiving 12 bolus feeds per day.

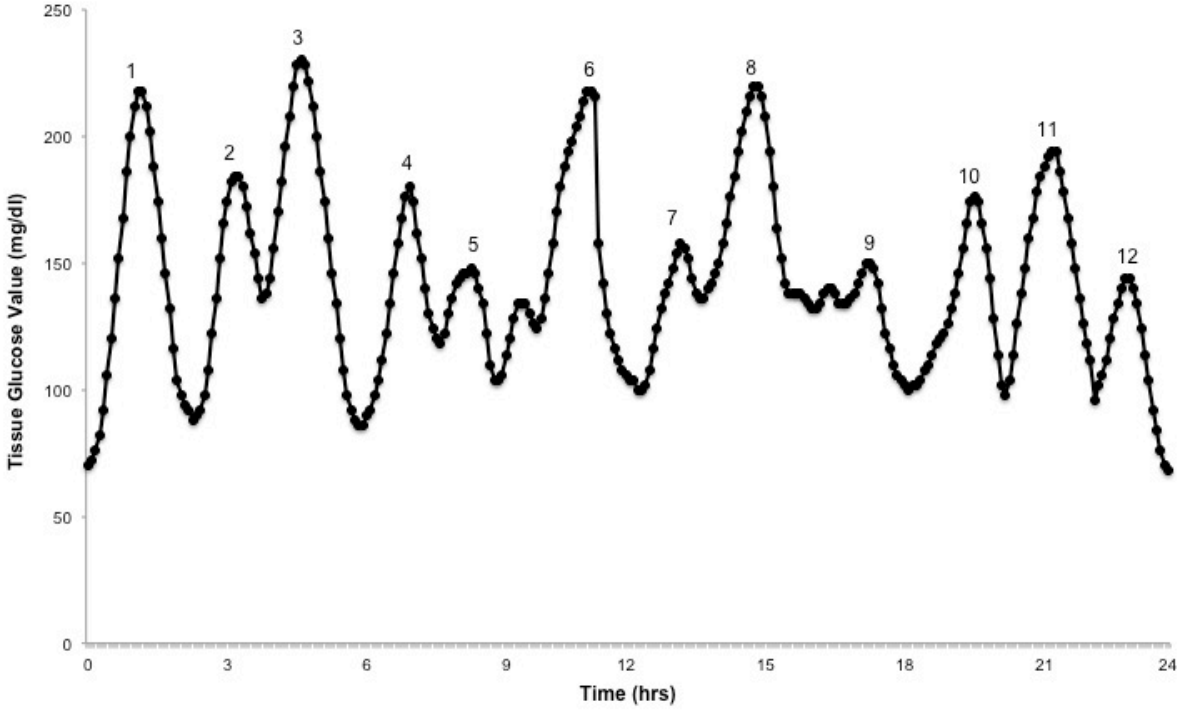

Figure 3.4-2: Tissue glucose pattern with 12 meals in 24 hours

Graph 3.4-3 shows an infant who received 6 meals per day and suffered repeated low tissue glucose values through to 40 mg/dl, in 24 hours. And graph 3.4-4 is derived from one of the 5 infants with tissue glucose values within the normal range, receiving 8 bolus feeds. In these five infants tissue glucose was independent of the number of meals they received per day (8, 10 or 12), and glucose stayed within normal limits in all infants (> 45 mg/dL and <150 mg/dL) at all times.

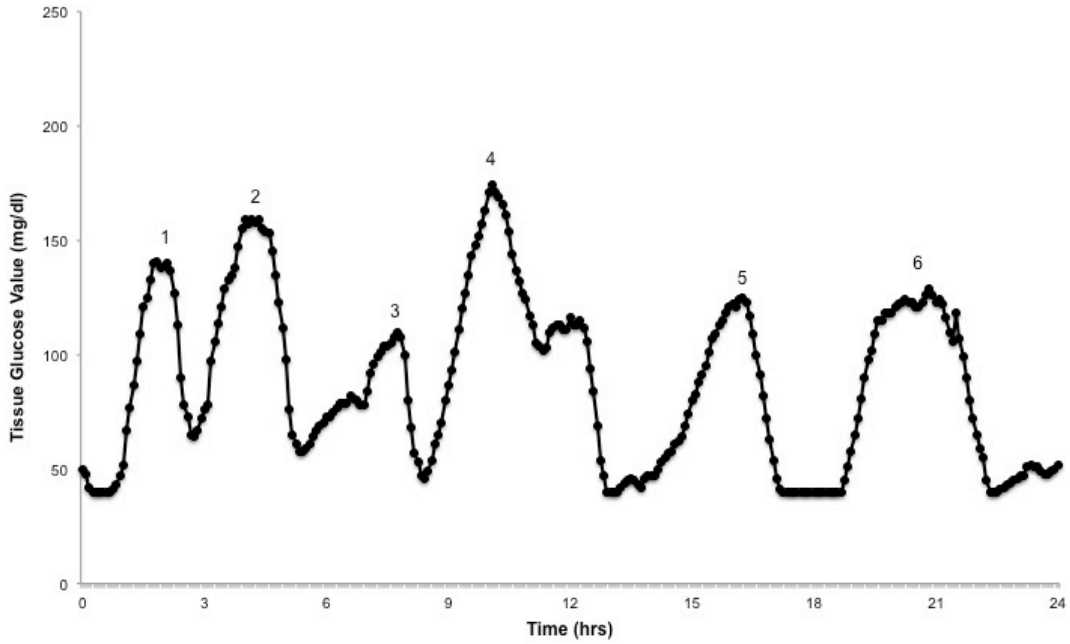

Figure 3.4-3: Repeated low tissue glucose values in infant with six meals in 24 hours.

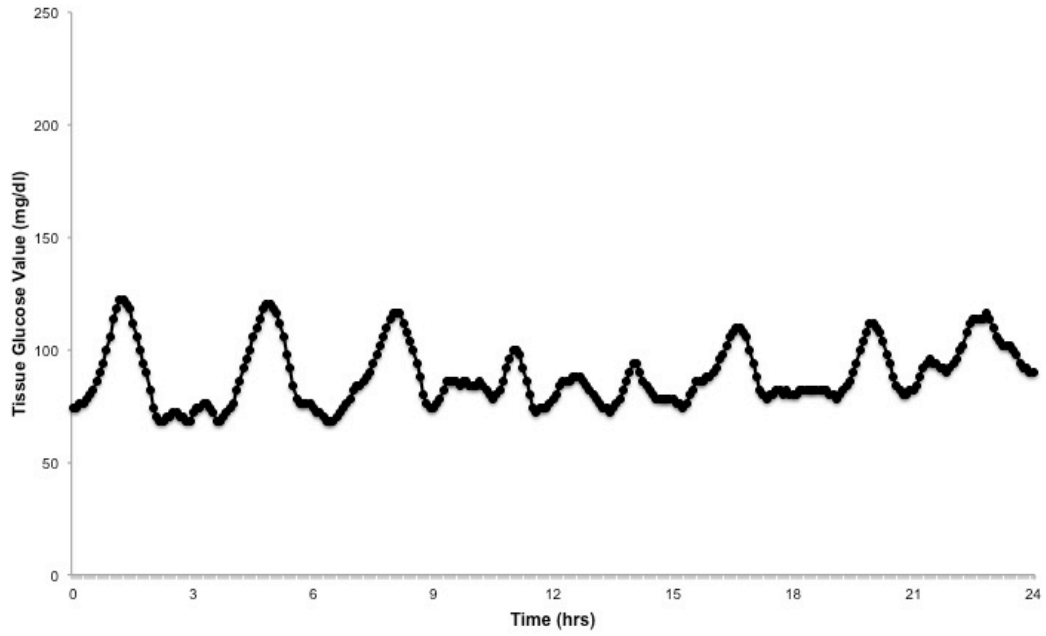

Figure 3.4-4: Glucose pattern of a stable infant

3.5. Metabolic instability during DOL 1-10th and CGM-Days

Many studies on neonatal hypo- and hyperglycemia describe these entities as a very common problem during the first 10 DOL in preterm infants [4, 9, 11, 23, 54, 56]. We recorded a total of 699 measurements, a median number of 35 ± 11 capillary blood samples per infant, performed over their first ten days of life. The median glucose value for G1 was 146 ± 23 mg/dL and for G2 110 ± 36 mg/dL. The maximum glucose values ranged from 279-136 mg/dL / 303-115 mg/dL in G1/G2 and the minimum values were 39-113 mg/dl / 59-87 mg/dL respectively. Only 21% of the infants in G1 presented with a hypoglycemic event, with no relevant statistical differences among cohorts.

In contrast, 100% of the infants in G1 and 50% of the infants in G2 suffered at some point during their first 10 DOL a hyperglycemic event of > 150 mg/dL. When comparing cohorts the difference reached significance (p= .004). And lastly, hyperglycemic events > 200 mg/dL were registered in 57% only in G1 (p= .017).

Only 10% of the total number of infants remained euglycemic from their first DOL throughout the CGM-days. These infants belonged exclusively to G2. The rest of the infants had a derail in their glycemia at some point in time.

The infants were classified into 4 categories. These are the infants who maintained normal tissue glucose throughout the measurement period (10%, belonging exclusively in G2), the ones fluctuating during the first days of life and again during CGM-days (70%) and finally those who only fluctuated whether during the first days of life (15%) or exclusively during the CGM-days (5%, also only in G2).

Table 8 - Metabolic Fluctuations during the first 10 DOL and on the CGM-Days

Cohort	<1000g	1000-1500g	P-Value*
n	14	6	
Euglycemic (%)	-	33	.026
Fluctuation during first 10- and CGM-Days (%)	93	17	.026
Fluctuation only during first 10 DOL (%)	7	33	>.05
Fluctuation only during CGM-Days (%)	-	17	>.05

** P < 0.05*

The fluctuation (Table 8) was present in 93% of the infants in G1 during their first ten days of life and again on the CGM-days. This was also corroborated for 17% of the infants in G2. The rest of the infants in both groups had glycemia fluctuations only during their first 10 DOL or only during the CGM-days.

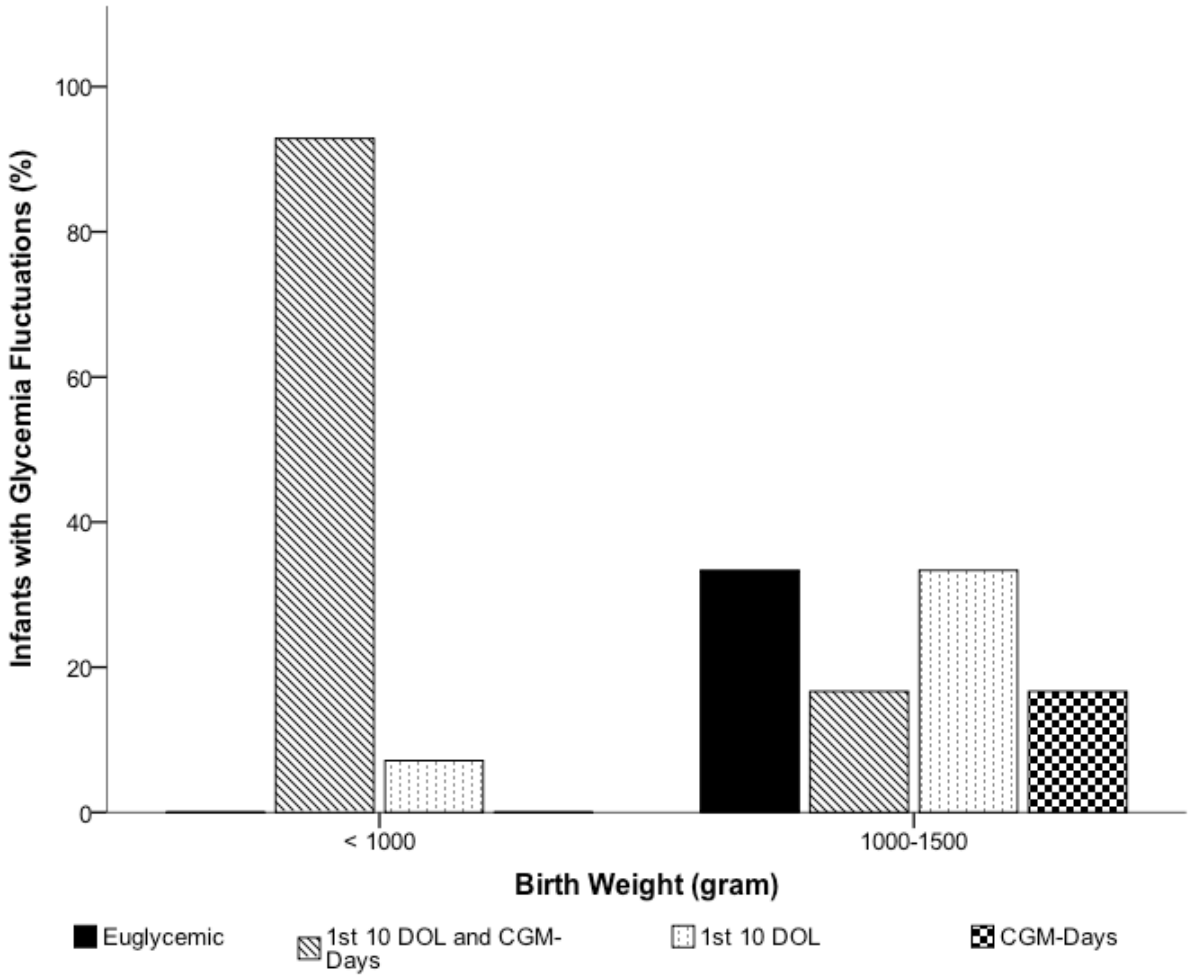

Figure 3.5.-1: Incidence of metabolic fluctuations during the first 10 DOL and during CGM-Days

We compared birthweigth, length and HC at birth of the infants who reimained euglycemic during the entire hospital stay, with the values of the fluctuating infants. We obtained significant differences among the groups for all three variables (birthweight p= .022, birth length p= .028 and HC p= .044). The results are illustrated in figures 3.5.-2, 3.5.-3 and 3.5.-4. At trial entry, the weight, length and HC showed no relevant differences, nor any correlation with metabolic fluctuations. The overall weight gain during the hospital stay was significantly lower for the infants who suffered glycemia fluctuations during their first days of life and again during the CGM-days (p= .032). The length of the hospital stay itself was the longest for the group who fluctuated at the beginning of their life and again during the period of continous interstitial glucose measurements (p= .030).

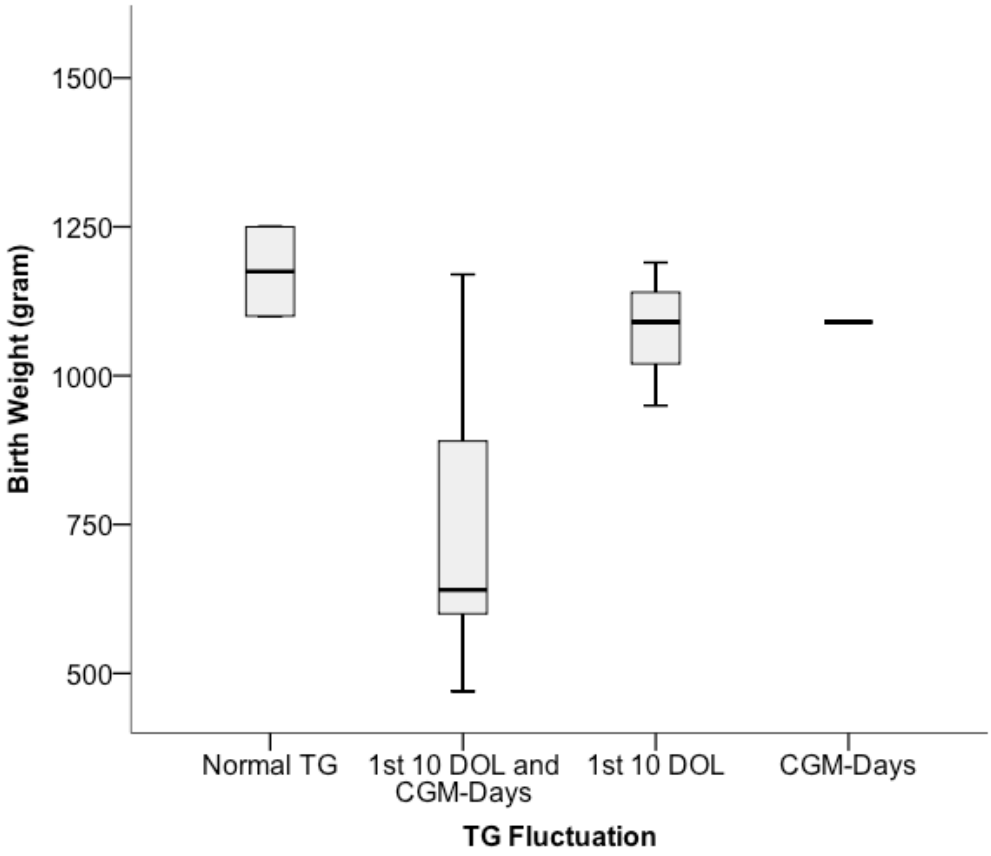

Figure 3.5-2: Birth weight and metabolic fluctuations during the perinatal period

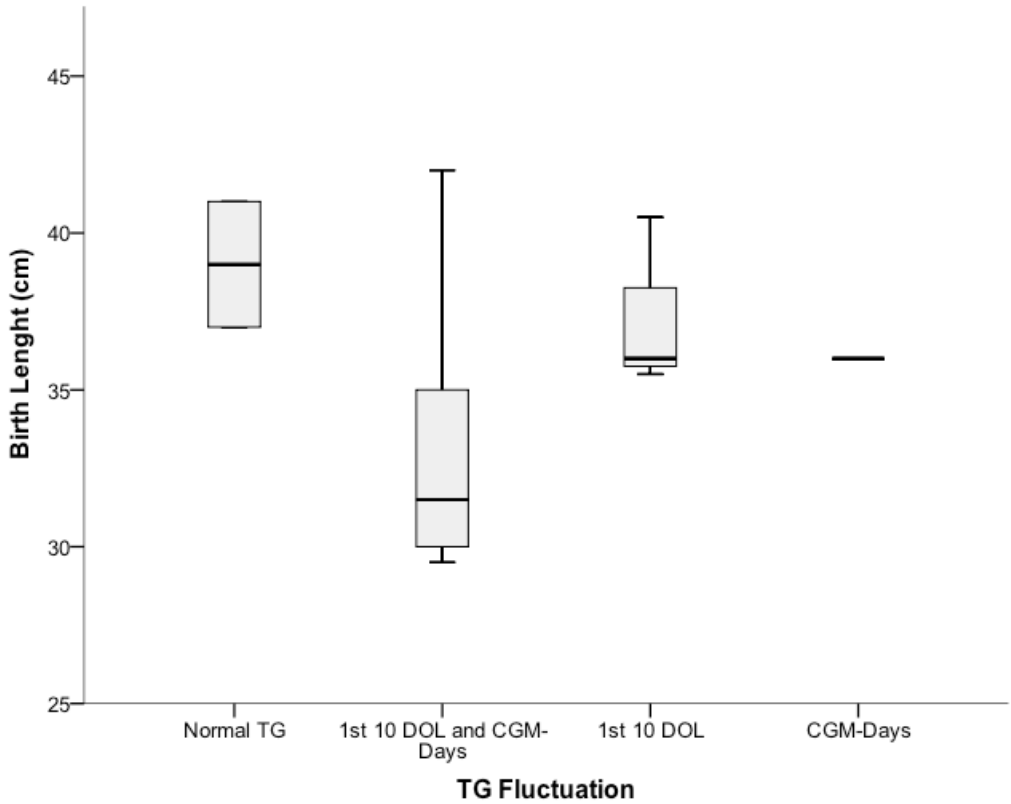

Figure 3.5-3: Lenght at birth and metabolic fluctuations during the perinatal period

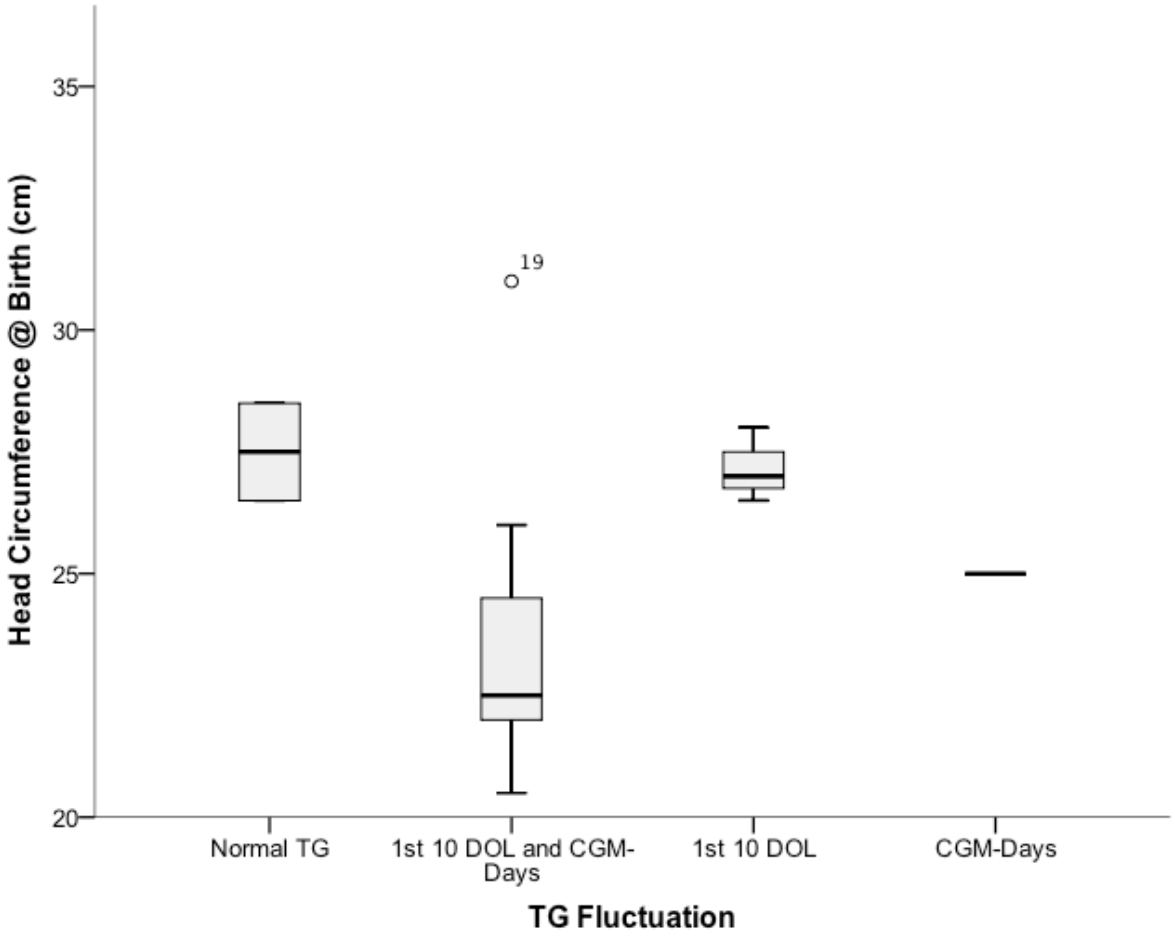

Figure 3.5-4: HC at birth and metabolic fluctuations during the perinatal period

4. Discussion

In this study we determined for the first time that 85% of the infants in this study, with a median age of 48.0 days in G1 and 32.0 days in G2, were actually unable to maintain tissue glucose values within the normal range during 72 hours of CGM measurement. These glucose fluctuations during monitoring had a pattern of hyperglycemic episodes (>150mg/dl) after feeds, followed by rapid glucose drops in both cohorts. The main risk factors for glucose instability were prematurity, gestational age at birth and low weight at trial.

We employed CGMS to measure tissue glucose values. This technique was developed for diabetic patients to maintain tissue glucose within the normal limits during insulin therapy. However, CGMS have already been applied in preterm infants before. Comparing our correlation analysis results between the CGMS and the ABL-Radiometer (r^2=0.68, r = .827, p< .001) with the results obtained at this same NICU back in the year 2008, we can confirm the accuracy of the sensor measurements. The results in 2008 were r^2=0.69 with p<0.05. Harris et al [57] reported a mean difference of 0.0 mg/dL, 95% CI, -20 to 20, Beardsell et al. [54] a r^2=0.87, p<.001, mean difference -11mg/dL, 95% CI -53 to 51, Garg et al. [3] a r^2= 0.87, p<.001 and Iglesias et al [58] r=0.96, p<.001) between different measurement methods using a CGMS in their studies. Their results are similar to ours. Therefore we can agree on an overall good correlation between the interstitial glucose values obtained with the CGMS and the corresponding glucose values obtained by capillary blood sample in this specific paediatric population [3, 37, 54, 57, 59].

When analyzing the time that shows the optimal accuracy between the blood glucose value and the interstitial glucose value, we determined the best concordance between 15 and 20 minutes after the blood sample was taken. This means that the CGM-device has a delay of 15-20 minutes in the representation of the blood glucose value. This is explained by the fact that the tissue has a buffer-capacity and it takes some minutes until the blood glucose has reached the interstitial space. When the sensor gives the alarm for increasing or decreasing tissue glucose values at a rapid rate, it means that the changes in the glycemia are happening or already happened. Our results agree with those obtained by Wackernagel et al. [60].

We compared glucose homeostasis of the same infants during their first days and after a few weeks of life, paying special attention to their glucose values as a

reflection of their metabolic stability and progressive maturation. In the early neonatal period, preterm infants are in a catabolic state, because their caloric intake is insufficient to satisfy their demands. After the conclusion of the neonatal period (per definition the first 28 days after birth), when all infants were medically stable, receiving an adequate full enteral caloric intake, and being ca. 48 (G1) and 32 (G2) days old, they should be in an anabolic state. We calculated the weight gain rates and obtained positive results for both cohorts. None of the infants lost weight, neither before nor after the completion of the continuous measurements, indicating the presence of anabolic processes (weight gain).

When comparing both cohorts, the distribution of infants who received fortified breast milk (16) or preterm formula (4) was statistically the same and there were no significant differences between feeding volumes, energy intake and amount of carbohydrates, proteins and lipids received during their hospital stay. All infants were fed according to the ESPGAN guidelines [51]. All infants were partially parenteral fed initially. Each infant received an individualized feeding plan, based on gestational age, birth weight, clinical condition and mothers' breast milk availability. The goal of the alimentation plan is to achieve a postnatal growth rate approximating intrauterine growth at the same postconceptional age [51, 61, 62]. Infants in G1 received more days of parenteral nutrition than the more mature infants (G2).They also took longer to reach full enteral feeds and they were hospitalized longer. Due to the overall immaturity of preterm neonates, the coordination of sucking, swallowing and respiration appears later in life (32-34 weeks of gestation). Until these reflexes appear, the nutrition must be partially parenteral to ensure an adequate energy supply and to prevent aspirations [61, 63]. During the first 10 days of life, the possible causes for the high incidence of low and/or high glucose events are well known, e.g. the overall physiological immaturity that is achieved later in postnatal life and the lipid, protein and glucose intake in the parenteral nutrition [17]. But the causes for the still metabolic instability at the time of the study, being fully enterally fed for a median of 25.0 ± 16.7 (G1) and 20.5 ± 5.3 (G2) days is a difficult to explain. At the time of measurements the nutritional goal to reach a total energy intake of ca. 110-130 kcal/kg/day was achieved by all infants. None of the infants were overfed nor underfed, and all showed growth rates (weight, length and HC) according to their percentiles.

Despite the fact that there are significant differences between fortified breast milk and preterm formula (Table 4) regarding energy intake, carbohydrates, proteins and lipids content, this had no influence on the incidence of metabolic fluctuations during CGM-days. There was no correlation of any of these parameters with the development of low tissue glucose events. When analyzing them for hyperglycemic events, there were three significant correlations. The high tissue glucose group had received a lower feeding volume and a lower protein and lipid intake on their first day of CGM, compared to infants who did not develop high tissue glucose values. When we analyzed the nutrition of the second day of CGM, there were no longer three significant correlations but only two (the lower protein p= .017 and lipid intake p= .047). And on the third day of CGM, the nutritional characteristics were the same across both groups (high glucose vs. normal glucose during CGM-days). The data about protein and lipid intake is very difficult to interpret, because of the impact of other possible cofounding variables in the ELGAN and VLBW infant [64].

Our study has obvious limitations regarding its statistical power to explore correlations due to the small sample size, but the preliminary results show partial relevant nutritional variables (protein and lipid intake during CGM-days) that might influence glucose homeostasis of these former preterm infants.

The CGM-device allows the detection of silent tissue glucose fluctuations in both directions that may go by undetected with the usual intermittent blood glucose controls every other day, routinely taken at the NICU, according to guidelines for clinically stable preterm infants [19]. This intermittent way of testing is recommended but it gives only a partial view of a constantly changing metabolic situation, which has been shown to be rather unstable in preterm infants [58]. The same guidelines also state that an infant should be able to maintain systemic glucose values within normal limits over several feed-fast cycles during the first ten DOL. It has been stated previously that later in postnatal life repeated glucose measurements are no longer needed [19, 65]. However, the glucose values we obtained during the CGM-days demonstrate that this presumed metabolic stability does not hold true for preterm infants under full enteral bolus feeds. In our cohort only 25% (5/20) of the infants, with a median age of 48.0 days in G1 and 32.0 days in G2, were actually able to maintain tissue glucose values within the normal range during 72 hours of CGM measurement. Of these stable infants only 1/5 was borne extremely preterm with a

birth weight below 1000g (G1). These findings imply that a major risk factor for metabolic instability is extreme prematurity.

Since low- and high tissue glucose events are episodic and asymptomatic, only random blood samples in the exact right moment or a continuous measurement can diagnose them. In previous studies the percentage of hyper- and hypoglycemic infants varies enormously, depending upon the chosen thresholds, the studied population characteristics, especially their age and the particular guidelines regarding nutrition and frequency in glucose controls in each NICU. The primary objectives of most previous studies were the diagnosis, treatment and evaluation of sequels of neonatal hypo- and hyperglycemia within the first ten DOL. A few groups extended measurements up to six weeks of life [11], or even 12 weeks of life [1]. In these studies however the sampling rate in clinically stable and unremarkable infants was low. Our data illustrate that especially in high risk preterm infants occasional blood glucose sampling will underestimated the overall occurrence of low- and high tissue glucose value fluctuations

Our findings regarding the incidence of hypoglycemic events in clinically stable, full enteral fed infants with a median age of ca. 48 (G1) and 32 (G2) DOL during CGM-days (43% in G1 and 17% in G2), confirm our previous results from a retrospective analysis, indicating a high prevalence of silent hypoglycemic events (44% in G1 and 23% in G2, $p < .042$) [12]. It is generally stated that glucose concentrations less than 45 mg/dL are accepted during the first hours of life, glucose concentrations less than 50-60 mg/dL afterwards are considered abnormal [3].

High tissue glucose value episodes during the neonatal period have not been as extensively researched as its counterpart, low tissue glucose value episodes. The publications on this subject state an increased morbidity and mortality for infants who suffer recurrent hyperglycemic episodes [4, 9, 25, 58, 66, 67]. Yet again, these studies refer mostly to the first days or weeks in the newborn's life. To our surprise, the incidence of high tissue glucose value episodes (>150 mg/dL) for the infants in our study was 71% in G1 and 17% in G2 ($p = .028$). With glucose values >200mg/dL it affected 57% of the infants, predominantly in G1 ($p = .020$).

These data suggests that ELGAN and VLBW infants are not able to adjust either insulin secretion or glucose utilization according to availability until reaching a specific gestational age. In previous studies it has been demonstrated that only at around 32 weeks postmenstrual age preterm infants are capable to adapt glucose

metabolism by secreting adequate amounts of insulin [43]. The infants in our study had reached a median age of 31.5 ± 1.8 weeks of gestational age in G1 and 33.4 ± 0.9 weeks gestational age in G2 at the time of measurement. This could explain the higher fluctuation rate in G1 compared to G2.

The presence of hypo- and hyperglycemic derails in the first hours, days and weeks of life is commonly associated with low birth weight, low gestational age, mother with gestational or pregestagional diabetes (requiring or not requiring Insulin) and being SGA among other variables. Wintergerst et al. [9] reported a hypoglycemia incidence of 18.6% (glucose values <65mg/dL) and a hyperglycemia incidence of 61% (>150 mg/dL) / 35.2% (>200 mg/dL) in preterm newborns during their first 14 DOL. Lucas et al. [11] for his part published an incidence of 39% of infants <1000g with recurrent hypoglycemic events (<47mg/dL) during their first 3-30th DOL . Ten percent of them maintained these hypoglycemic episodes for more than 4 weeks of life. Lubchenco et al. [23] gathered the glucose values of preterm infants during their first 4 DOL, obtaining an incidence of hypoglycemia (<30 mg/dL) of 10% for AGA infants. This percentage was higher for SGA infants (25%). Alexandrou et al. [8] reported hypoglycemia in 41% and hyperglycemia (>150mg/dL) in 81% of his studied preterm infants in their first week of life. The results vary among authors, but a similar trend can be noted. Taking into account that these research groups focused on values during the first weeks of life in preterm infants and our results belong to infants with an median age of 48 (G1) and 32 DOL (G2), we can affirm that metabolic fluctuations described during the first days of life) continue after more than 4 weeks of life, despite clinical stability and full enteral feedings according to guidelines.

In our cohort we were able to identify at least one risk factor (prematurity) for developing low tissue glucose values. However, no significant differences were detected with regard to perinatal risk factors comparing infants who developed tissue glucose fluctuations and those who remained within normal tissue glucose values. In G1 were the infants with lower birth weight (<1000g, p= .001), and concomitantly also with the lower gestational age (p= .004). These infants had low tissue glucose values more often in comparison to infants in G2.

In our study most of the recurrent low- and high tissue glucose value episodes lasted for 10-30 minutes. Studies addressing the effect of high or low glucose episode duration on long term outcome are conflicting. Several previous studies indicated that repeated episodes of blood glucose values below 47 mg/dL (documented on 5 or

more separate days), whether they are symptomatic or not may impair neurodevelopment [11, 27, 68]. Others state that only prolonged hypoglycemic episodes are associated with neurodevelopment impairment and again others affirm that there is no clear correlation between duration or severity of the episodes and an abnormal neural outcome [5, 32]. Our study was not designed to shed light on this issue because a to small number of infants enrolled, thus further studies are required.

Regarding the tolerance of the neonatal brain to extreme glucose values (whether they are hypo- or hyperglycemic), Salhab et al. reported that the immature brain is more resistant to the potential damages of low glucose values than the mature brain. He only takes into account glucose values during the first days of life and there was no follow-up, therefore the data is not conclusive [32]. With regard to high glucose values, Hays et al. and Sinclair et al. affirm that there is no reason to believe that ELBW infants should be more tolerant to high tissue glucose value than older children or adults [25, 69]. The dichotomy, why should be low tissue glucose levels better tolerated than high tissue glucose levels in preterm neonates or not and the involved risk of underestimating both extremes needs further clarification.

Regarding the risk factors, the literature alludes only to their relevance merely during the first 10 DOL and after that, if no infection, hypoxia or ischemia is present, the glucose value controls are relaxed. During the CGM-days, none of the infants had ongoing infections or other potential variables that may have knowingly affected their glucose homeostasis. Our data confirm previous observations that extremely low gestational age infants are at risk to develop a failure to thrive, still present when we conducted our CGMS measurement [1, 11, 58]. This observation might be related to the increased risk of these infants to develop a metabolic instability late during their NICU-stay without apparent clinical signs and symptoms. The incidence of low tissue glucose value episodes is inversely related to birth weight and gestational age at birth, (G1) maintaining a higher frequency of metabolic instability even after 48 DOL during CGM-days than the infants in G2. Our current findings could not demonstrate a statistically significant association between low tissue glucose value episodes during the CGM-days and any of its well-known risk factors. Otherwise when it comes to high tissue glucose value episodes, a significant inverse relationship between gestational age at birth and at trial and can be made ($p= .020$ and $p= .033$).

We also found a correlation between high tissue glucose value episodes and a higher percentage of intracranial bleedings (Spearman's Rho 0.452, p= .045), but without statistical significance when performing the Mann-Whitney U- Test (p>.05). These data are important and need further evaluation because despite being only a trend, it might prove valid in a larger cohort.

Regarding the possible relation between a low BMI at birth and an increased incidence of low and high tissue glucose value episodes we observed the same trend as Lucas et al. [11] and Duvanel et al. [27]. We also determined that infants with fluctuating glucose values during the CGM-days had smaller BMI's at birth than those who remained with their interstitial glucose values within normal ranges. Preterm newborns with recurrent episodes of glucose imbalance when compared to newborns with normal glucose values had a lower BMI. This finding is consistent with reduced glycogen and adipose tissue stores, an important factor that increases the propensity to metabolic instability in this specific demographic group [11, 27].

The Clinical Risk Index for Babies (CRIB score) assesses initial neonatal risk and compares performances among NICUs [70, 71]. We initially compared the mean CRIB scores among cohorts, and G1 showed significantly higher scores than G2. We also compared the risk index with the incidence of low and high tissue glucose value episodes and came to the conclusion that neither one had a significant correlation with the risk index. However, we did see that the infants with the highest scores were those who fluctuated between extreme tissue glucose values, not only during CGM-days but also during their first 10 DOL and then again during the CGM-days. Hays et al [25] demonstrated in their study a significant relation between high CRIB scores and hyperglycemic events (>150mg/dL), although they only analyzed values of preterm neonates during their first week of life. In our study, we saw that the relation between a high risk score at birth and an increased incidence of metabolic fluctuations is still significant even after 4 weeks of life.

It has been reported that infants with repeated hypoglycemic episodes have a smaller head circumference (HC) than euglycemic infants [27]. When we compared the following characteristics: birth weight, length and HC at birth with the incidence of high and low glucose episodes we observed a significant correlation between a small HC at birth and an increased incidence of low glucose episodes. If we compared the anthropometric values at birth of infants with normal glucose values to the values of the rest of the infants in the study we saw significant discrepancies. Infants who

suffered metabolic fluctuations during the CGM-days had the lowest weight, length and HC at birth. We could not determine any significance when comparing the same anthropometric values obtained at trial entry with a possible increased incidence of metabolic fluctuations. The stable infants, regardless of their cohort, always had the best values for weight, length and HC at birth in comparison to the anthropometric values of infants who suffered repetitive low- and/or high glucose tissue value events. These data indicate again that infants with extreme low birth weight, length and HC are at increased risk to develop metabolic fluctuations later during their NICU course.
Duvanel et al [27] described a significantly smaller HC than expected in the follow-up controls of their study-infants who had suffered repeated neonatal hypoglycemic events. Follow-up data for this study are yet pending.
The current feeding practice for neonates involves small frequent feedings (i.e. 8-12 meals per/day, meaning every 3-2 hours). With the CGMS we obtained different glucose patterns, related to the feeding schedule in metabolically stable or instable infants. Infants with normal tissue glucose show very small fluctuations in response to bolus feeds (disregarding the number of feedings they received). Their metabolism is stable enough to control the secretion of insulin and its counterregulatory hormones, maintaining tissue glucose values within normal ranges at all times.
The extreme glucose level variations may be caused by the immaturity of the metabolic pathways, not having reached optimal levels for hormone secretion despite the fact that these infants are already older than ca. 30 weeks gestational age, clinically stable and fully enterally fed.
The observed glucose fluctuations could not only be related to a metabolic immaturity but also to concomitant variables like rates of gastric emptying [64]. Hunt et al determined that gastric emptying is independent of the initial volume and is rather determined by the nutritive density of the meal [72]. In addition, increased caloric density results in a slower gastric emptying [73]. Breast milk empties twice as fast as formula milk in preterm infants, but the whole process shows a large interindividual variability [74]. Since for both cohorts, during CGM-days, the volume and meal characteristics were equal, neither volume nor milk composition can be responsible for the different glucose patterns among infants. If we take a closer look at the patterns of the glucose fluctuating, those are exactly related to the number of bolus feeds. Consequently, the question arises if it would be better to switch to a continuous gastric feed scheme instead of boluses of milk and if this could prevent

the extreme glucose fluctuation. A meta-analysis of the available data concluded that that there is currently not enough evidence to determine which one of the feeding methods for low birth weight premature infants is better and no general recommendation on continuous feeding can be made [63].

When we compared the incidence of hypo- and hyperglycemic events during the first 10 days of life to those obtained by other research groups we can see the same trend as those obtained by other authors. We only compared the data to those who had a similar demographic group, preterm neonates who are partially enteral / parenteral fed and without major clinical problems during their first days of life [3, 4, 8, 9, 11, 23, 25, 28, 54, 56, 58]. Due to variations in birth weight, length of the analyzed period of time, the degree of stress, the type of nutrition received by the infants, and above all, the different thresholds considered as abnormal values, the incidences show some variations. The fact that 93% of the infants in G1 showed low and high glucose values repetitively during their first 10 DOL and again during the CGM-days, and that only this cohort experienced glucose values above 200 mg/dL demonstrates an inverse relation of metabolic fluctuations to gestational age and birth weight [24, 58]. Only 2 infants (10% of the total n) had normal glucose values during their first 10 days of life and again during CGM-days and they had a birth weight above 1000g (G2). In concordance with Hays et al., infants who developed high tissue glucose had a considerable longer hospital stay than those who remained euglycemic [25]. Previous studies describe the difficulties a term newborn has to go through, to achieve a stable postnatal glucose homeostasis and emphasises the fact that this process is much more difficult for a preterm newborn who has to add the major disadvantage of the immaturity of his metabolic pathways to the process [28]. It is very difficult for preterm to maintain their systemic glucose within normal limits postnatal. Without a glucose infusion preterm infants are at risk to slide into a hypoglycemic state due to their lack of energy stores (adipose and muscle tissue) and with a glucose infusion the risk of developing hyperglycemia increases [3, 20, 24]. An additional difficulty of postnatal glucose control is the fact that in newborns the endogenous rate of glucose production is not suppressed by high concentrations of blood glucose values. The immature pancreas is also not able to secret adequate amounts of insulin to suppress gluconeogenesis, creating a relative peripheral insulin resistance [40]. In summary, the control of glucose production and suppression in the preterm infant is a complex process that is only partially controlled by insulin and

glucose concentrations. The overall degree of immaturity plays a central role in the pathophysiology of glucose instability in ELGAN- and VLBW infants [17, 24, 28, 75, 76].

The glucose values of 70% of the total n=20 fluctuated into hypo-and/or hyperglycemia during the first days of life and again after ca. 4 weeks of life, during the CGM-days. It is much more likely that the fluctuations never ceased and were present all along, but because of the intermittent blood sampling went by undetected, rather than being present only during the first days of life, then stabilizing, and then reappearing at an average age of 48 DOL (G1) and 32 DOL (G2). The results agree with the concept that the metabolic transition from the complete dependence of the fetus to the complete independence of the neonate is a process, which takes time and continues for several months after the end of the neonatal period, reaching during infancy the developmental maturity necessary to switch to an adult-like glucose homeostasis [7, 17, 28].

Every infant is in a catabolic state after birth, showing a mean 10% decrease of bodyweight postnatal. Most infants regain their weight to birth weight within the first 10 days of life. It is possible that some infants, due to their prematurity and to metabolic perturbations related to their immaturity need a few more days to regain their birth weight, like it happened to some of our infants in the study. Kalhan et al state that most of the growth parameters may remain subnormal after reaching a corrected gestational age of 40 weeks in high risk neonates [64]. This was not the case for our infants at the time of the study: with a corrected age of 31.5 ± 1.8 (G1) and 33.4 ± 0.9 (G2), they all remained within their percentile curves for weight, length and HC during the entire hospital stay, indicating that they received an adequate amount of calories and proteins. Since they had no intercurrent illnesses or stress at the time of the study, the best conditions were present for optimal growth. We do have to note that there were statistically significant differences regarding anthropometric characteristics when comparing metabolically instable infants, regardless if this was exclusively during CGM-days or during the first 10 days of life and again during CGM-days. Infants who presented an unstable metabolic state during the entire hospital stay had the worst anthropometric values (weight, length and HC at birth) of all infants. However, weight, length and HC at trial did not show any relevant correlation with the metabolic fluctuations. Regardless of the fact that the values remained within the normal percentiles for preterm newborns, the low birth

weight, length and HC were significant factors indicating an increased incidence of fluctuations during the first days of life and during CGM-days.

Recent studies have linked low birth weight, gestational age and neonatal hypo- and hyperglycemic events during the first weeks of life with subsequent β cell dysfunction and impaired glucose tolerance, which manifest later in life [17, 47, 48, 77, 78]. There seems to be epigenetic changes and programming of different organ systems during development and there is an association between low birth weight and obesity, cardiovascular risk factors and insulin resistance of later onset [28, 48, 62, 79]. Since we concentrated on interstitial glucose values in infants who were much older than most of the infants in the published studies, we hope that with the metabolic follow-up at 4 and 8 years of age, we can gain information about the possible relation between hypo- and hyperglycemic events diagnosed during the neonatal period and beyond at an median age of 48 (G1) and 32 (G2) DOL and the development of a metabolic syndrome (obesity, insulin resistance, diabetes and cardiovascular disease) in adulthood. The velocity of weight gain might also be very interesting because some publications link a rapid weight gain during the first 4 months of life (corrected age) and during infancy with obesity in childhood and young adulthood [49, 78].

All 241 episodes of low and high episodes registered with the CGMS were apparently asymptomatic and clinically undetectable by the experienced NICU nursing staff and medical team. Clinical signs of disturbed neural function in the neonate and young infant are often too subtle to be recognized, but Koh et al. described abnormal changes in neural function in infants with glucose values below 47 mg/dL [68]. On the other hand Harris et al. induced hypoglycemic events in lambs with a simultaneous aEEG running and could not detect any changes in the measured parameters [80].

We will see if abnormal brain activity like subclinical seizure activity or transient neuronal activity deterioration was recorded during the hypoglycemic events in our study [17, 81].

5. Conclusions

The diagnosis of silent low and high interstitial glucose values is a very important subject due to the potential neurological impairment and metabolic consequences in preterm infants. The use of a CGMS is a feasible way of studying the dynamic glucose level variations in real time, in ELBW and VLBW preterm newborns. It is a harmless and almost low-risk method, which gives the maximum yield of information possible for an uncomplicated method. Only with a CGMS we are able to document the exact duration of an event and simultaneously diagnose and treat it. We have to take into account that due to the difficulty of patient recruitment for an invasive study in this specific population of infants, and due to the need to study the smallest number of subjects adequate to evaluate the proposed hypothesis adequately, this method has proven to be accurate and feasible.

Up to date, an extensive number of studies have addressed the subject of hypo- and hyperglycemia and the long-term outcome in preterm neonates, but there is a lack of uniformity in the definition of hypo- and hyperglycemia, in the characteristics of the population studied and in the length of the follow-up controls, making it difficult to come to conclusive results.

We expect this prospective cohort study to show potential correlations between the duration, number and severity of the suffered events during the entire hospital stay at the NICU and possible gravitating consequences at the 24 months Bailey test and the endocrinal checkups at 4 and 8 years of age. We have to wait for the follow-up control results to determine the impact of the described metabolic fluctuations and their connection to the development of a metabolic syndrome, diabetes mellitus and the functional impact of these problems at school age.

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Kontinuierliche Glukose Messung in enteral ernährten Frühgeborenen

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Preterm infants are at risk for recurrent glucose fluctuations while already stable and on full enteral nutrition

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