Glucose Derangements and Brain Function in Neonatal Encephalopathy

by

Elana Pinchefsky

A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

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Master of Science
Institute of Medical Science
University of Toronto
2018

Abstract

Hypoglycemia and hyperglycemia are common in neonates with encephalopathy and may be independently associated with unfavourable outcomes. This thesis describes how glucose derangements detected with continuous interstitial glucose monitoring commenced soon after birth are associated with brain function on amplitude-integrated electroencephalography (aEEG) and continuous EEG (cEEG) in the context of neonatal encephalopathy. We hypothesize that hypoglycemia, hyperglycemia and glucose variability in neonatal encephalopathy are associated with abnormal brain background activity and increased electrographic seizures on aEEG and cEEG. In neonates with encephalopathy, epochs of hyperglycemia, but not hypoglycemia, were common and temporally associated with worse aEEG background scores, less sleep-wake cycling, and more electrographic seizures, including after adjusting for clinical markers of hypoxia-ischemia. Epochs of hyperglycemia also were associated with worse cEEG background scores, including after adjusting for hypoxia-ischemia severity. Our data support the hypothesis that proactive avoidance of hyperglycemia may be a neuroprotective strategy in infants with neonatal encephalopathy.
Acknowledgments

This thesis could not have been produced without the help and support of many amazing people. First and foremost, I would like to express my sincere gratitude to my supervisors, Drs. Emily Tam and Cecil Hahn for their mentorship, time and support that have allowed me to develop my research and clinical skills. I have felt continuously supported, challenged and encouraged throughout the past 2 years. Your patience, insight and guidance with my research and future career development have been invaluable.

Secondly, thank you to Dr. Vann Chau for all of your extremely valuable advice, mentorship and continued friendship that have helped me through my training. And thank you to Dr. Steven Miller for all your mentorship, words of wisdom, and research and clinical pearls that you have shared. I have learnt so much during my time at SickKids Hospital and the University of Toronto and I am very grateful for the kind and supportive work environment.

Importantly, I would also like to express my sincere gratitude to my program advisory committee members, Drs. Carter Snead and Mark Palmert for their time, expertise and investment in my research career. Your extremely helpful constructive feedback helped me shape and improve the work within this thesis.

I am also grateful to Dr. Sampsa Vanhatalo and his research group from Helsinki, including Nathan Stevenson, Karoliina Tapani and Minna Kauppila for such an exciting collaboration. Your input and expertise in quantitative EEG analysis have been vital and I look forward to working further with your group.

Furthermore, thank you to Daphne Kamino, our clinical research project manager, who keeps the NOGIN study organized and answers my many questions, at all times of the day. I am so grateful for your support and friendship. I would also like to thank all the members of the Tam and Miller labs for your support and for providing such a wonderful and inspirational work environment. I would especially like to thank Diane Wilson, Claire Watt, Ashley LeBlanc, Torin Glass, Lara Leijser, Julienne Schneider, Amr Al-Shahed, Jojy Varghese, Marie Metrailler, Isabel Benavente-Fernandez, Dalit Cayam-Rand, and Asma Al- Mazroei. Thank you for your friendship and support throughout my fellowship. You have all been great team members and
have taught me so much. In addition, this project would not have been possible without Dr. Ayako Ochi, Dr. Tina Go, Dr. Aideen Moore, the wonderful neurophysiology technologists, and Matthew Keyzers and the Acute Care Transport Services team.

A special thank you to my family and friends for their continued love, support and encouragement, and especially my fiancé, Andrei Isac for being unfailingly supportive during my two years away. You have always pushed me to be the best that I can be.

Lastly, I would like to extend the largest thank you, and my sincerest appreciation to all the NOGIN study subjects and their families, without whom this study could not have happened.
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AAP</td>
<td>American Academy of Pediatrics</td>
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<tr>
<td>ACNS</td>
<td>American Clinical Neurophysiology Society</td>
</tr>
<tr>
<td>aEEG</td>
<td>Amplitude-integrated EEG</td>
</tr>
<tr>
<td>AGA</td>
<td>Appropriate for gestational age</td>
</tr>
<tr>
<td>BS</td>
<td>Burst suppression</td>
</tr>
<tr>
<td>BSID</td>
<td>Bayley Scales of Infant Development</td>
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<td>cEEG</td>
<td>Continuous EEG</td>
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<td>CGM</td>
<td>Continuous glucose monitor</td>
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<tr>
<td>CHD</td>
<td>Congenital heart disease</td>
</tr>
<tr>
<td>CLV</td>
<td>Continuous low voltage</td>
</tr>
<tr>
<td>CNV</td>
<td>Continuous normal voltage</td>
</tr>
<tr>
<td>CONGA</td>
<td>Continuous overlapping net glycemic action</td>
</tr>
<tr>
<td>CP</td>
<td>Cerebral palsy</td>
</tr>
<tr>
<td>CPS</td>
<td>Canadian Pediatric Society</td>
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<tr>
<td>DFA</td>
<td>Detrended fluctuation analysis</td>
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<tr>
<td>DNV</td>
<td>Discontinuous normal voltage</td>
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<tr>
<td>DWI</td>
<td>Diffusion weighted imaging</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<td>ELBW</td>
<td>Extremely low birth weight neonates</td>
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<tr>
<td>ES</td>
<td>Electrographic seizures</td>
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<td>ESE</td>
<td>Electrographic status epilepticus</td>
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<tr>
<td>FSIQ</td>
<td>Full-scale Intelligence Quotient</td>
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<tr>
<td>FT</td>
<td>Flat tracing</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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<tr>
<td>GA</td>
<td>Gestational age</td>
</tr>
<tr>
<td>GMFCS</td>
<td>Gross Motor Function Classification System</td>
</tr>
<tr>
<td>GOS-E Peds</td>
<td>Glasgow Outcome Scale - Extended, Pediatric</td>
</tr>
<tr>
<td>HI</td>
<td>Hypoxic-ischemia</td>
</tr>
<tr>
<td>HIE</td>
<td>Hypoxic-ischemic encephalopathy</td>
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<tr>
<td>IBI</td>
<td>Interburst interval</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive care unit</td>
</tr>
<tr>
<td>IDM</td>
<td>Infants of diabetic mother</td>
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<tr>
<td>IQ</td>
<td>Intelligence quotient</td>
</tr>
<tr>
<td>KOSCHI</td>
<td>King’s Outcome Scale for Childhood Head Injury</td>
</tr>
<tr>
<td>LGA</td>
<td>Large-for-gestational age</td>
</tr>
<tr>
<td>MAG</td>
<td>Mean absolute glucose change</td>
</tr>
<tr>
<td>MAGE</td>
<td>Mean amplitude of glucose excursions</td>
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<tr>
<td>MF-DFA</td>
<td>Multifractal DFA</td>
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<tr>
<td>MODD</td>
<td>Mean of daily differences</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NICHD</td>
<td>National Institute of Child Health and Human Development</td>
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<tr>
<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<tr>
<td>PCPC</td>
<td>Paediatric Cerebral Performance Category</td>
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<tr>
<td>PES</td>
<td>Pediatric Endocrine Society</td>
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<tr>
<td>PICU</td>
<td>Pediatric intensive care unit</td>
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<tr>
<td>PMA</td>
<td>Postmenstrual age</td>
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<tr>
<td>pp</td>
<td>Peak to peak</td>
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<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
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<tr>
<td>SB</td>
<td>Seizure burden</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SGA</td>
<td>Small-for-gestational-age</td>
</tr>
<tr>
<td>SWC</td>
<td>Sleep-wake cycling</td>
</tr>
<tr>
<td>TH</td>
<td>Therapeutic hypothermia</td>
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<tr>
<td>VLBW</td>
<td>Very low birth weight</td>
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Chapter 1

1 Literature Review

1.1 Introduction

Neonatal encephalopathy is defined as a clinical syndrome of disturbed neurological function with many etiologies. Hypoxic–ischemic encephalopathy (HIE) after perinatal asphyxia is the most common cause of neonatal encephalopathy and is an important cause of neonatal mortality and morbidity (Volpe, 2012). Although therapeutic hypothermia (TH) has reduced mortality and major neurodevelopmental disability at 18 months of age (Azzopardi, Strohm, et al., 2014; Jacobs et al., 2013), mortality rates in moderate to severe HIE remain at approximately 25%. Similarly, morbidity with major neurodevelopmental disability occurs in approximately 20% of survivors of HIE (Jacobs et al., 2013). Thus, current neuroprotective strategies require further optimization.

Both hypoglycemia and hyperglycemia are common in neonates with HIE (Basu et al., 2016; Tam et al., 2012; Wong et al., 2013), and are potentially modifiable risk factors. In neonates with HIE, hypoglycemia has been detected in 34% (Basu et al., 2016; Tam et al., 2012; Wong et al., 2013) and hyperglycemia in almost 50% of neonates using intermittent glucose testing (Basu et al., 2016). Although studies suggest that hypoglycemia and hyperglycemia may be independently associated with worse outcomes in these neonates (Basu et al., 2016; Chouthai et al., 2015; Salhab et al., 2004; Spies et al., 2014; Tam et al., 2012), available evidence is conflicting and putative mechanisms remain poorly understood (Callahan et al., 1990; Nadeem et al., 2011; Park et al., 2001; Sheldon et al., 1992; Tottman et al., 2017; Vannucci et al., 1987). Furthermore, glucose instability and rapid correction of hypoglycemia may also be associated with worse outcomes (Al Shafouri et al., 2015; McKinlay et al., 2015; McKinlay, Alsweiler, et al., 2017). Despite the above observations, there is limited evidence available to guide effective hypoglycemia management to prevent associated brain injury and neurodevelopmental sequelae (Harding et al., 2017). The Pediatric Endocrine Society advise that a ‘safe target’ during the first 48 hours should be close to the mean for healthy newborns and above the threshold for neuroglycopenic symptoms (2.8 mmol/L) (Thornton et al., 2015). Moreover, reference ranges in
healthy term newborns may not be appropriate in infants at risk for impaired metabolic adaptation as individual susceptibility to brain injury can vary depending on comorbid conditions and an infant’s ability to produce and use alternative fuels (Harris et al., 2015).

Electroencephalography (EEG) is an important tool for assessing brain activity since EEG background abnormalities and recovery are important early prognostic indicators of long-term neurodevelopmental outcome in neonates with HIE (Azzopardi & Toby study group, 2014; Chandrasekaran et al., 2017; Dunne et al., 2017; Skranes et al., 2017; Spitzmiller et al., 2007; Thoresen et al., 2010; van Laerhoven et al., 2013).

We hypothesize that hypoglycemia, hyperglycemia and glucose variability in neonates with encephalopathy are associated with abnormal brain background activity and increased electrographic seizures on amplitude-integrated EEG (aEEG) and continuous EEG (cEEG). The objective of the research presented in this thesis is to inform potential neuroprotective glucose management strategies. We sought to investigate how abnormalities in glucose homeostasis correlate with brain function as measured by aEEG and cEEG in the context of neonatal encephalopathy.

1.2 Neonatal Encephalopathy

1.2.1 Overview

Neonatal encephalopathy is defined as a clinical syndrome of disturbed neurological function, manifested by abnormal level of consciousness or seizures, and may be associated with other features such as difficulty initiating and maintaining respiration and depression of tone and reflexes (D'Alton et al., 2014). There are many etiologies of encephalopathy in newborns (such as congenital infections, genetic or neurometabolic disorders). The term HIE applies when peripartum or intrapartum hypoxia-ischemia is identified as the likely pathogenesis. The incidence varies due to methodological and population differences between studies. Nonetheless, based on population data, the incidence of neonatal encephalopathy is 3 per 1000 live births.
(95% CI 2.7 to 3.3) and the incidence of HIE is 1.5 per 1000 live births (95% CI 1.3 to 1.7) (Kurinczuk et al., 2010).

Guidelines have been developed by the American College of Obstetricians and Gynecologists’ Task Force on Neonatal Encephalopathy for retrospective definition of an intrapartum event sufficient to cause cerebral palsy (CP). Neonatal encephalopathy is likely due to acute hypoxia-ischemia when one or more of the following elements are present:

(1) Neonatal signs consistent with an acute peripartum or intrapartum event:

   a. Apgar Score of less than 5 at 5 minutes and 10 minutes,
   b. Fetal umbilical artery acidemia (fetal umbilical artery pH <7.0, or base deficit ≥12 mmol/L, or both),
   c. Neuroimaging evidence of acute brain injury seen on brain magnetic resonance imaging (MRI) or Magnetic Resonance Spectroscopy consistent with hypoxia–ischemia,
   d. Presence of multisystem organ failure consistent with HIE.

(2) Type and timing of contributing factors that are consistent with an acute peripartum or intrapartum event:

   a. Sentinel hypoxic or ischemic event occurring immediately before or during labor and delivery (e.g. uterine rupture, abruption placenta, umbilical cord prolapse),
   b. Fetal heart rate monitor patterns consistent with an acute peripartum or intrapartum event,
   c. Timing and type of brain injury patterns based on imaging studies consistent with an etiology of an acute peripartum or intrapartum event (including deep nuclear gray matter or watershed cortical injury),
   d. No evidence of other proximal or distal factors that could be contributing factors.

(3) Developmental outcome is spastic quadriplegia or dyskinetic cerebral palsy:

   a. Other subtypes of cerebral palsy are less likely to be associated with acute intrapartum hypoxic-ischemic events,
   b. Other developmental abnormalities may occur, but they are not specific to acute intrapartum HIE and may arise from a variety of other causes (D'Alton et al., 2014).
Eleven randomized controlled trials (RCTs) have shown that therapeutic hypothermia started within 6 hours after birth for term infants with moderate to severe HIE reduces mortality or major neurodevelopmental disability at 18 months of age (Azzopardi, Strohm, et al., 2014; Jacobs et al., 2013). Longer-term neurodevelopmental outcomes up to 6 to 8 years old support these 18-month findings (Azzopardi, Strohm, et al., 2014; Guillet et al., 2012). Therapeutic hypothermia has thus become the standard of care for neonates that meet the criteria that were used in the published trials, which were designed to select neonates with presumed HIE within the 6-hour time window (Azzopardi et al., 2009; Gluckman et al., 2005; Jacobs et al., 2011; Shankaran et al., 2005; Simbruner et al., 2010; Zhou et al., 2010). The fundamental understanding that was key to development of therapeutic hypothermia was that of delayed secondary injury. Following the initial primary injury during the acute hypoxic-ischemic (HI) event, there is a “latent” phase of initial partial recovery lasting approximately 6 hours. In moderate to severe injury, this period is followed by “secondary” injury characterized by progressive failure of cerebral mitochondrial oxidative metabolism, cytotoxic edema and seizures (Lorek et al., 1994; Wassink et al., 2014). Still, the number needed to treat with therapeutic hypothermia for an additional beneficial outcome is seven (Jacobs et al., 2013), thus there is still a proportion of neonates for whom this therapy is ineffective.

Mortality rates in moderate to severe HIE with therapeutic hypothermia treatment remain at approximately 25% and with major neurodevelopmental disability occurring in approximately 20% of survivors of HIE (Jacobs et al., 2013). Major disabilities include cerebral palsy, intellectual disability, sensorineural hearing loss and visual impairment. CP develops in 12.5-32% of children (Jacobs et al., 2013) and intellectual disability in approximately 25% (Pappas et al., 2015). In a secondary analysis of the National Institute of Child Health and Human Development (NICHD) whole-body hypothermia trial, intellectual impairment occurred in 96% of children with CP at 6 to 7 years old (intelligence quotient (IQ) <70). In children without CP, 9% had intellectual impairment and 31% had IQ scores of 70 to 84 (Pappas et al., 2015). A variety of other deficits are reported; including specific memory and language problems, deficits in visual-motor integration, delays in school-related activities (such as reading, spelling, and arithmetic), lower performance and full-scale IQ scores compared with population norms, difficulties in executive function, and motor deficits (Lindstrom et al., 2006; Perez et al., 2013; Robertson et al., 1988; Robertson et al., 1989).
There is also growing evidence that mild HIE is not a benign syndrome with observational studies showing both short- and long-term complications. Short-term consequences in neonates classified clinically as having mild encephalopathy have included EEG documented seizures, abnormal aEEG and EEG background (including excessive discontinuity and burst suppression patterns), MRI abnormalities in up to half of neonates (including deep gray matter injury, watershed injury, white matter lesions and strokes), feeding difficulties and abnormal neurological exam on discharge (DuPont et al., 2013; Massaro, Murthy, et al., 2015; Prempunpong et al., 2018; Walsh et al., 2017). Long-term outcome studies have demonstrated behaviour problems (van Handel et al., 2010) and lower full-scale IQ, verbal IQ and performance IQ (Murray et al., 2016).

Several ongoing trials are looking at adjunctive therapies to therapeutic hypothermia. Promising agents indicated by preclinical and clinical trial data include erythropoietin (Wu et al., 2012; Y. W. Wu et al., 2016) with a phase III trial underway (NCT03079167), melatonin (Aly et al., 2015; Robertson et al., 2013), stem cells (Bennet et al., 2012; Cotten et al., 2014), topiramate (Filippi et al., 2018), argon (Broad et al., 2016; Gunes et al., 2007), and allopurinol (Peeters-Scholte et al., 2003) with the ALBINO study (NCT03162653) to start recruitment.

Despite advances in neuroprotective therapies, good resuscitation and supportive care are fundamental for management in HIE to avoid additional brain injury. Supportive management is directed at possible hypoxic damage to organs other than the brain; such as fluid restriction in the setting of oliguria, monitoring for coagulopathy, closely monitoring adequacy of ventilation, volume replacement and inotropic medications as needed to maintain blood pressure and appropriate cerebral perfusion, and control of seizures. As well, it is important that both hypoglycemia and hyperglycemia be avoided (Basu et al., 2016; Giesinger et al., 2017; Lingappan et al., 2016; Martinello et al., 2017; Yager et al., 2009).

1.2.2 Prevalence of glucose derangements

Neonatal hypoglycemia (<2.6 mmol/L) is common and occurs in up to 19% of healthy newborns (Kaiser et al., 2015). However, neonatal hypoglycemia occurs in approximately half of at-risk neonates. This category includes infants of diabetic mothers, late-preterm (35 to <37...
weeks’ gestation), small (<10th percentile or <2500 g) or large (>90th percentile or >4500 g) weight at birth, or other reasons identified clinically such as poor feeding (Harris et al., 2012). A first episode of hypoglycemia may occur after 3 normal blood glucose measurements and over 24 hours after birth (Harris et al., 2012). Additional risk factors for neonatal hypoglycemia include intrauterine growth restriction, intrapartum fever, temperature instability in the newborn, sepsis, public insurance compared with those privately insured, and perinatal asphyxia (DePuy et al., 2009; Knobloch et al., 1967; VanHaltren et al., 2013; Wackernagel et al., 2016; Zhou et al., 2015). There are also several genetic predispositions which place neonates at high risk of hypoglycemia (Thompson-Branch et al., 2017) such as congenital hyperinsulinism, growth hormone or cortisol deficiency, and inborn errors of metabolism.

Studies in neonates with encephalopathy have detected hypoglycemia in 13-34% using intermittent glucose testing (Basu et al., 2016; Tam et al., 2012; Wong et al., 2013). Early hyperglycemia was found in 48% of a cohort of neonates with HIE (Basu et al., 2016) (Figure 1.1). Hyperglycemia is also frequently encountered in preterm neonates, especially those with very low birth weight (VLBW; birth weight <1500 g). Hays et al. reported a cohort of extremely low birth weight neonates (ELBW; birth weight <1000 g) where hyperglycemia (>8.3 mmol/L) was seen in 57% and glucose values above 13.9 mmol/L were seen in 32% of neonates (Hays et al., 2006). Furthermore, clinically stable well-developing preterm infants on full enteral feeds who are beyond their initial period of intensive care can continue to have glucose fluctuations, with frequent hypoglycemia (<2.6 mmol/L) and hyperglycemia (>8.3 mmol/L) detected by continuous glucose monitoring (CGM) in approximately 40 and 70% of neonates respectively (Mola-Schenzle et al., 2015).
Figure 1.1 Newborns with encephalopathy have a high incidence of hypoglycemia and hyperglycemia, which may conspire to worsen brain injury in this population already at high risk of adverse neurodevelopmental outcomes. Hypoxic-ischemic encephalopathy is a common cause of neonatal encephalopathy.

1.3 Glucose physiology in neonates
1.3.1 Normal transitional period and challenges of defining neonatal hypoglycemia

Many physiological, metabolic and endocrine changes must occur in order to maintain glucose homeostasis as a neonate transitions from the fetal environment to the extra-uterine world (Figure 1.2). In utero, fetal glucose is supplied continuously from the mother (Kalhan et al., 1979) and fetal insulin functions primarily to regulate intrauterine growth (Hattersley et al., 1998). Once the umbilical cord is cut, the blood glucose concentration declines during the first 2 to 4 hours of life (Srinivasan et al., 1986). This fall in glucose levels leads to decreased insulin secretion and increase in levels of counter-regulatory hormones (e.g. glucagon, catecholamines and glucocorticoids) (Mitanchez, 2007; Sperling et al., 1984). This then initiates endogenous glucose production via glycogenolysis and gluconeogenesis in order to stabilize the blood glucose concentrations (Fowden, 1980). Blood glucose concentrations in healthy term neonates
reach levels comparable to fasting levels in children and adults by approximately 72 hours of life (Hawdon et al., 1992). Brief periods of hypoglycemia commonly seen in normal newborns during the transition from fetal to extra-uterine life are referred to as transitional neonatal hypoglycemia (Stanley et al., 2015).

**Figure 1.2** The metabolic, endocrine and physiological changes which occur at the time of birth to allow a normal term newborn to adapt to the change from the fetal environment to the extra-uterine world. Figure originally published by Guemes et al. (2016) in *Arch Dis Child*; reproduced with permission from BMJ Publishing Group Ltd.

During the initial transitional phase in the hours after birth, glucose values show marked variability between neonates and range between 1.4 mmol/L and 6.2 mmol/L in healthy, appropriate for gestational age (AGA) neonates (Acharya et al., 1965; Diwakar et al., 2002; Hawdon et al., 1992; Heck et al., 1987; Hoseth et al., 2000; Srinivasan et al., 1986) with a mean
initial glucose between 3.1 to 3.6 mmol/L (Stanley et al., 2015) (Figure 1.3). It has also been shown that blood glucose concentrations in healthy neonates are lower in those being breast fed than bottle fed, yet ketone body concentrations are higher with breastfeeding (Hawdon et al., 1992). Glucose concentrations are also relatively stable and unaffected by the timing of the initial feed (Sweet et al., 1999; Zhou et al., 2017).

**Figure 1.3** Range of plasma glucose concentrations during the first days after birth measured in (A) 223 full-term appropriate weight for gestational age breastfed neonates (B) 200 term appropriate weight for gestational age and exclusively breastfed neonates at 3 h, 6 h, 24 h and 72 h of age. A) Reproduced from Hoseth et al. (2000) Arch Dis Child Fetal Neonatal Ed with permission from BMJ Publishing Group Ltd. B) Figure was adapted from Diwakar et al. (2000) and originally published by Guemes et al. (2016) in Arch Dis Child; reproduced with permission from BMJ Publishing Group Ltd.

In older children, ketone synthesis and gluconeogenesis is noted in response to low glucose levels. However, mechanisms associated with fasting adaptations are not fully developed at the time of birth. Early studies have shown that during hypoglycemia in term AGA neonates, ketone levels were low despite elevated levels of fatty acid precursors and higher levels of the glucose precursors lactate and alanine (Hawdon et al., 1992; Stanley et al., 1979). This suppression of ketones seen in term neonates is similar to that seen in hyperinsulinemic hypoglycemia (Stanley et al., 1976). It has also been shown that plasma insulin concentrations
are not completely suppressed at the low glucose concentrations seen during transitional neonatal hypoglycemia in normal newborns (Adam et al., 1968; Hawdon, Aynsley-Green, Alberti, et al., 1993). Furthermore, transitional neonatal hypoglycemia is also associated with an inappropriately large glycemic response to glucagon or epinephrine suggestive of inappropriate retention of liver glycogen stores (Cornblath et al., 1958). This constellation of metabolic and hormonal features (inefficient ketone production, incomplete suppression of insulin, and inappropriate retention of glycogen stores) supports the idea that transitional neonatal hypoglycemia in normal AGA newborns resembles hyperinsulinemic hypoglycemia due to congenital hyperinsulinism in which the set point for suppression of insulin secretion is reduced (Stanley et al., 2015).

Controversy remains as to whether the newborn brain is more or less vulnerable to low glucose levels (Kim et al., 2005; Vannucci et al., 2001). A number of putative physiologic mechanisms may protect the brain from such low glucose levels. These include a compensatory increase in cerebral blood flow (Ichord et al., 1994; Mujisce et al., 1989; Pryds et al., 1988; Skov et al., 1992), enhanced glucose extraction from blood into the brain and low cerebral energy demands (Mujisce et al., 1989), and the availability and utilization of alternative cerebral fuels (Hellmann et al., 1982; Vannucci et al., 1981). However, there is no evidence that the glucose concentration which impairs brain function and leads to neuroglycopenic symptoms is different in neonates than in older children and adults (Thornton et al., 2015). Neonates also have a proportionally larger brain relative to their body size and almost all of the daily total body glucose utilization can be accounted for by the brain (Bier et al., 1977). Although glucose is the primary substrate metabolized by the brain, there are alternate fuels which can be utilized including pyruvate, lactate, and ketones (Cremer et al., 1979; Hellmann et al., 1982; Wyss et al., 2011; Zhang et al., 2013). In neonates, ketone use by the brain is directly proportional to the plasma concentration of ketones (Bougueré et al., 1986) and lactate may also be used as fuel by the brain in the neonatal period (Costello et al., 2000; Vannucci et al., 1981; Vannucci et al., 2000). Yet, in high-risk neonates there may be inefficient ketone production and plasma lactate levels may not be sufficiently elevated, making them particularly vulnerable to hypoglycemia-induced brain injury (Harris et al., 2015; Hawdon et al., 1992; Stanley et al., 1979).

Failure of neonatal metabolic adaptations can lead to hyperglycemia as well. Etiologies for hyperglycemia in neonates may be partially explained by several mechanisms. These include
an apparent inefficiency of glucose homeostasis, with studies demonstrating that with intravenous glucose or insulin infusions, there is variability and lack of suppression of gluconeogenesis in neonates and in particular preterm neonates (Chacko et al., 2011; Cowett et al., 1983; Sunehag et al., 1994). Animal studies have also shown newborn pups are less efficient at handling excess glucose due to decreased hepatic and peripheral sensitivity to insulin (Varma et al., 1973). Treatments such as epinephrine can increase glucose levels (Cornblath, 1955; Desmond et al., 1950) and hypothermia may delay the return of glucose concentrations back to normal levels (Baum et al., 1968; Desmond et al., 1950). A study of 28 neonates undergoing investigations for hypo- or hyperglycemia showed that blood glucose concentrations are predominantly influenced by the rate of glucose administration, as there are incomplete internal control mechanisms. Similar to hypoglycemia, there was a variable insulin response to hyperglycemia seen, and glucoregulatory hormones (insulin and glucagon) were not adequately controlling hepatic glucose production (Hawdon, Aynsley-Green, Bartlett, et al., 1993). How these mechanisms may be altered by conditions such as HIE remains unclear.

1.3.2 Glucose physiology in neonatal encephalopathy

Intrapartum asphyxia disturbs the typical physiological changes described above and increases the likelihood of hypoglycemia through several mechanisms. Intrapartum asphyxia leads to increased glucose metabolism in all tissues and is possibly associated with decreased placental glucose transport to the fetus. Prolonged anaerobic metabolism leads to increased lactate, a fall in pH and a decrease in high energy phosphates. The increased glucose consumption leads to depletion of endogenous glucose stores, especially in the liver, and consequent hypoglycemia once the neonate is delivered (Randall, 1979; Vannucci et al., 2001; Volpe, 2008). Reduced perfusion and impairment of liver function may also have direct effect on substrate supply. Furthermore, enteral feeding with breast milk which promotes ketogenesis is often delayed (Hawdon et al., 1992).

Additionally, neonates with HIE may behave as neonates with hyperinsulinemic hypoglycemia or transitional neonatal hypoglycemia, during which ketogenesis is suppressed (Collins et al., 1984; Hoe et al., 2006). Therefore, as mentioned above, it cannot be assumed that
ketones are available as an alternative fuel to support brain metabolism during hypoglycemia, leaving them particularly vulnerable to hypoglycemia-induced brain injury (Harris et al., 2015; Harris et al., 2011; Stanley et al., 2015). Lactate concentrations may be high enough to potentially be a source of alternative fuel (Harris et al., 2015), however levels may also not be sufficiently elevated to compensate for low glucose in these vulnerable neonates (Harris et al., 2015; Harris et al., 2011).

Animal studies demonstrate that hypoglycemia during hypoxic-ischemic (HI) injury can be detrimental (Chang et al., 1999; Kim et al., 1994). Studies by Vannucci et al. in newborn rats have demonstrated the detrimental effect of hypoglycemia on survival following anoxia with 100% nitrogen. Hypoglycemic animals survived only one-tenth as long as controls with normal glucose following anoxia and the vulnerability was dependent on the amount of endogenous cerebral glucose reserves at the time of insult. Increased vulnerability correlated with accelerated depletion of endogenous high energy phosphate levels. Based on their findings they concluded that glucose levels that are sufficient to meet energy demands in well neonates may be inadequate during periods of reduced systemic or cerebral oxygenation (Vannucci et al., 1978). Similar findings were shown in newborn dogs when insulin-induced hypoglycemia was combined with asphyxia, there was a more rapid exhaustion of high-energy phosphate reserves. Furthermore, blood glucose levels were not related to cerebral perfusion after asphyxia, and rather correlated with systemic blood pressure regardless of blood glucose levels (Vannucci et al., 1980). Thus, although compensatory increase in cerebral blood flow may be a protective mechanism during hypoglycemia alone (Ichord et al., 1994; Mujsc et al., 1989; Pryds et al., 1988; Skov et al., 1992) cerebral vascular autoregulation can be lost during hypoxia or asphyxia and cerebral blood flow becomes pressure passive (Freeman et al., 1968; Massaro, Govindan, et al., 2015). Hypoglycemia may also further impair cerebrovascular autoregulation, as demonstrated in rats and newborn dogs, which may further increase vulnerability of the watershed regions to ischemia in HIE (Anwar et al., 1988; Siesjo et al., 1983). It is important to note that animal studies use insulin-induced rather than calorie-restricted hypoglycemia, which is non-physiological and may lead to distinctly different uses of alternative fuels. Studies have also demonstrated high levels of ketone bodies available as metabolic fuel to the brain in suckling rats (Cremer et al., 1979; Hawkins et al., 1971).
Hyperglycemia after hypoxia-ischemia may occur due to reduced net metabolism of severely damaged tissues (Jensen et al., 2006) or prolonged elevation of stress hormones after asphyxia, which may be further prolonged by TH (Davidson et al., 2008). However, regarding effects of hyperglycemia in this context, animal studies in neonatal models for HIE have been contradictory with early studies in neonatal rat models suggesting hyperglycemia prior, during, or after HI did not lead to accentuated damage and was perhaps neuroprotective (Callahan et al., 1990; Hattori et al., 1990; Vannucci, Brucklacher, et al., 1996; Voorhies et al., 1986). There were also beneficial effects seen with pretreatment with glucose in newborn lamb and rhesus monkeys prior to asphyxia (Dawes, Jacobson, et al., 1963; Dawes, Mott, et al., 1963; Rosenberg et al., 1990). Plus, hyperglycemia was shown to reduce energy depletion during HI in piglets (Laptook et al., 1992). Conversely, a study examining hyperglycemia after HI injury in newborn rats demonstrated more severe neuronal injury (Sheldon et al., 1992). Hyperglycemia during hypothermic circulatory arrest in newborn dogs increased brain injury in specific regions, especially the caudate, amygdala and brainstem (Vannucci, Rossini, et al., 1996). Hyperglycemia prior to ischemia in fetal sheep (Petersson et al., 2004) and during HI injury in newborn piglets also worsened brain injury (Chang et al., 1998; LeBlanc et al., 1993), whereas a study with hyperglycemia after HI injury did not (Leblanc et al., 1994). Further studies with newborn piglet models of HI also suggest that hyperglycemia after HI interferes with recovery of brain cell membrane function and energy metabolism (Park et al., 2001). There may be methodological and species differences. It is argued that the newborn piglet is a superior animal model of neonatal HIE as the piglet brain is comparable in glucose metabolism and maturity to human neonates at birth (Dobbing et al., 1979), whereas rat pups have limited ability for brain glucose metabolism (Booth et al., 1980; Fuglsang et al., 1986; Nehlig et al., 1988). Additionally, understanding the effects of blood glucose after HI is more clinically practical as it may not be possible to modify glucose levels during the primary asphyxia insult in neonates.

Hyperglycemia is also common in preterm neonates thus animal studies have examined hyperglycemia in this context and demonstrated a detrimental effect of hyperglycemia. A study in preterm Sprague-Dawley rats demonstrated that increasing severity of hyperglycemia was associated with greater severity of brain injury and apoptosis, particularly in the hippocampus. Severity of hyperglycemia was also associated with increased incidence of mortality and decreased brain density and weight gain in survivors (Tayman et al., 2014). A study by Lear et
al. in preterm sheep (equivalent to 28 to 32 weeks gestational age [GA]) compared fetuses that received glucose infusions prior to asphyxia to maternal injection of dexamethasone and controls who received saline infusions, in order to examine the hypothesis that dexamethasone treatment increased asphyxial brain injury at least in part by producing hyperglycemia. Both maternal dexamethasone treatment and glucose infusions prior to asphyxia were associated with severe cystic brain injury in multiple brain regions (compared to diffuse injury in control animals). They were also both associated with improved neurophysiological adaptation to asphyxia but worsened neurophysiological recovery, and higher glucose levels were significantly correlated with greater numbers of seizures (Lear et al., 2017).

1.3.3 Glucose management guidelines

1.3.3.1 Hypoglycemia management

There is controversy and variable definitions of both neonatal hypoglycemia and hyperglycemia in the various available clinical guidelines for glucose management in neonates (Cornblath et al., 2000; Harding et al., 2017; Tin, 2014). The National Institutes of Child Health and Human Development workshop reviewed the evidence and identified many gaps in knowledge regarding neonatal hypoglycemia and the lack of an evidence based definition in neonates, as well as the need to determine the effects of comorbidities (e.g. HI, sepsis) and their contribution to adverse outcomes with low plasma glucose (Hay et al., 2009).

On the basis of neurophysiological and neurodevelopmental outcome studies, a commonly used definition of neonatal hypoglycemia is less than 2.6 mmol/L (Koh et al., 1988; Lucas et al., 1988). However, the level and duration of hypoglycemia associated with brain injury and neurologic sequelae has not been established and the individual neonates’ response to inadequate delivery of glucose to the target organ (e.g. the brain) depends on their physiological and hormonal responses, as well as concurrent pathology. Consequently, it is difficult to define a single blood glucose concentration cutoff for hypoglycemia. The blood glucose concentration must be interpreted within the clinical context, whether there was recent antecedent hypoglycemia, and considering the presence of alternative fuels and intermediate metabolites (Guemes et al., 2016; Thornton et al., 2015). Additionally, reference ranges in healthy term
newborns may not be appropriate in infants at risk for impaired metabolic adaptation and at risk for adverse neurodevelopment, such as with perinatal asphyxia (Harding et al., 2017).

In 2000, Cornblath suggested “operational thresholds” with different cutoffs proposed if the neonate was ‘symptomatic’ or not (Cornblath et al., 2000). Clinical hypoglycemia refers to when low blood glucose concentrations are associated with symptoms and signs attributable to glucose deficiency in the target tissues and which are improved by the administration of glucose (Vannucci et al., 2001). Neurogenic symptoms occur due to sympathetic nervous system activation and include tachycardia, vomiting, sweating, pallor, tremulousness, temperature instability, and irritability. Neurogenic signs and symptoms occur at higher glucose levels than neuroglycopenic symptoms. Neuroglycopenic symptoms occur when there is impaired brain energy metabolism. Symptoms are nonspecific and include lethargy, poor feeding, irritability, hypotonia, apnea, seizures and coma (Mitrakou et al., 1991; Pildes et al., 1967). The glucose level at which symptoms occur may depend on the occurrence of previous episodes of hypoglycemia and the presence of alternative fuels, such as ketones and lactate (Guemes et al., 2016). Although children and adults can communicate when they have symptoms of hypoglycemia, neonates cannot. Furthermore, these symptoms are nonspecific and may occur due to many possible etiologies in sick neonates. In the study by Lucas et al., 222 of the 433 neonates with plasma glucose concentrations below 2.6 mmol/L had symptoms of hypoglycemia (e.g. recurrent apnea, vomiting, jitteriness and seizures), however these symptoms were equally prevalent in the 228 neonates without hypoglycemia (Lucas et al., 1988). Also, a recent prospective study by Harris et al. found that almost 80% of neonates that became hypoglycemic showed no clinical signs (Harris et al., 2012).

The American Academy of Pediatrics (AAP) and Canadian Pediatric Society (CPS) provide advice for screening and management of transitional hypoglycemia in the first 24 to 36 hours after birth. The AAP guidelines for management of newborns at risk born at ≥ 34 weeks’ GA advises thresholds which are dependent on postnatal age; ranging from 1.4 to 2.2 mmol/L in the first hours of life, 1.9 to 2.5 mmol/L from 4 to 24 hours, and 2.5 mmol/L for babies over 24 hours old. In babies with clinical signs of hypoglycemia, the advised threshold for intervention is a blood glucose concentration of ≤ 2.2 mmol/L (Adamkin et al., 2011). The CPS advises that at-risk babies require intervention if they have a blood glucose concentration <1.8 mmol/L at 2 hours of age or <2.0 mmol/L after subsequent feeding or if blood glucose concentration are
repeatedly below 2.6 mmol/L despite subsequent feeding. Though symptomatic neonates should be treated immediately for a blood glucose concentration <2.6 mmol/L (Aziz K et al., 2004).

Whereas the AAP and CPS guidelines use the lower ranges of glucose concentrations found in asymptomatic infant, the Pediatric Endocrine Society (PES) approach uses mean glucose values of healthy newborns and focuses on identification of persistent hypoglycemia when hypoglycemia persists beyond 48 hours of life. They advise that a ‘safe target’ during the first 48 hours should be close to the mean for a healthy newborn and above the threshold for neuroglycopenic symptoms (2.8 mmol/L). This target should be increased after 48 hours to 3.3 mmol/L to be above the threshold for neurogenic symptoms and close to the target for older infants and children (Thornton et al., 2015).

The PES guidelines take into consideration that neurogenic symptoms are perceived in older children and adults at plasma glucose concentrations <3.0 mmol/L and cognitive function is impaired <2.8 mmol/L (Boyle et al., 1988; Schwartz et al., 1987). They also take into account that although attempts to establish thresholds for neuroglycopenic symptoms in response to hypoglycemia in neonates have not been successful, there is no reason to assume lower threshold for intervention in neonates than children and adults, particularly considering they have a proportionally larger demand for glucose by the brain (Harding et al., 2017; Stanley et al., 2015; Thornton et al., 2015). The PES also considers that in hypoketotic conditions such as hyperinsulinism, ketones and lactate are not available in sufficient concentrations to serve as an alternative fuel for the brain and thus there is greater risk of brain energy failure and hypoglycemia-induced brain damage (Thornton et al., 2015).

At-risk categories for screening in the CPS and AAP guidelines include small-for-gestational-age (SGA), large-for-gestational age (LGA), infants of diabetic mothers (IDM) or preterm neonates (Adamkin et al., 2011; Aziz K et al., 2004). The PES expands the at-risk categories that should be screened to also include: perinatal stress (birth asphyxia, ischemia, cesarean section for fetal distress, maternal preeclampsia/eclampsia or hypertension, meconium aspiration syndrome, erythroblastosis fetalis, polycythemia, hypothermia), premature or postmature delivery, family history of genetic forms of hypoglycemia, congenital syndromes (e.g., Beckwith Wiedemann) and neonates with abnormal physical findings (e.g., midline facial malformations, microphallus).
Depending on glucose level and symptoms, management of hypoglycemia includes feeding or IV dextrose (Aziz K et al., 2004; Lilien et al., 1980). Another option is oral dextrose gel which has been shown to be effective to improve glucose concentrations in late preterm and term neonates, and reduce admission to the neonatal intensive care unit (NICU) and formula-feeding at 2 weeks of age (Harris et al., 2013) and appeared safe at 2-year follow-up (Harris et al., 2016). For more severe hypoglycemia, treatment options may also include glucagon (Miralles et al., 2002), diazoxide (Drash et al., 1968) and octreotide (Thornton et al., 1993).

Experts suggest that a balance between the different approaches using the lower threshold recommended by the AAP and CPS, while maintaining awareness of the need to exclude persistent hypoglycemia when low glucose concentrations persist beyond 48 hours, may be considered to reduce concerns of unnecessary screening and potentially unnecessary treatments, while still identifying persistent hypoglycemia (Harding et al., 2017; Tin, 2014).

1.3.3.2 Hyperglycemia management

Hyperglycemia may be considered as part of the physiological response to stress (Davidson et al., 2008; Dungan et al., 2009), however there is accumulating evidence that hyperglycemia is associated with greater mortality and morbidity in preterm neonates (Blanco et al., 2006; Hall et al., 2004; Hays et al., 2006; Heimann et al., 2007; Kao et al., 2006; Zarif et al., 1976) and with unfavorable outcomes in term neonates with HIE (Basu et al., 2016; Chouthai et al., 2015; Spies et al., 2014).

There are no clear guidelines for management of hyperglycemia in neonates, and in particular there is a lack of studies to guide management decisions of hyperglycemia in neonates with HIE. As such, there are wide variations in the definition used for hyperglycemia in neonates as well as large variations in the criteria used in NICUs for starting insulin in neonates with hyperglycemia.

There are a few studies that have assessed management of hyperglycemia in preterm neonates, but still, treatment approaches differ widely between centers. A survey in Australasia found that criteria for commencing insulin in preterm neonates varied in the different neonatal
units between glucose levels of 8 to 15 mmol/L (Alsweiler et al., 2007). Similarly, studies looking at outcomes in preterm neonates with hyperglycemia have used varying threshold; including >8.3 mmol/L (Blanco et al., 2006; Hays et al., 2006; Heimann et al., 2007), >8.5 mmol/L (Ertl et al., 2006; Slidsborg et al., 2018), >10 mmol/L (Beardsall et al., 2008; Kao et al., 2006), or >12 mmol/L (Manzoni et al., 2006).

Two small randomized trials of continuous insulin infusion for management of hyperglycemia in ELBW infants found that it appears safe and helps to maintain normal caloric intake and weight gain (Collins et al., 1991; Meetze et al., 1998). However, only short-term outcomes were measured in those trials and a 2011 Cochrane review found insufficient evidence to determine the effects of insulin treatment on death or major morbidities (Bottino et al., 2011). A randomized, controlled non-blinded trial compared tight glycemic control to standard practice in neonates that became hyperglycemic (>8.5 mmol/L) found that tight glycemic control with insulin was associated with increased risk of hypoglycemia as well as lower rates of linear growth despite greater head circumference and growth and weight gain (Alsweiler et al., 2012). The American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) clinical guidelines for hyperglycemia in neonates receiving parenteral nutrition suggest that excess energy and dextrose delivery be avoided and fat emulsion be added to the parenteral nutrition infusion. They make a strong recommendation against the use of early insulin therapy to prevent hyperglycemia (Arsenault et al., 2012).

“Early” continuous insulin therapy has been investigated in preterm neonates, in which insulin is started within 24 hours of birth and supported by 20% dextrose in order to prevent hyperglycemia. The NIRTURE study was an international randomized, controlled trial in VLBW infants comparing “early” continuous insulin to standard neonatal care. The efficacy of glucose control was assessed by continuous glucose monitoring. The authors found that early insulin therapy may lead to a significant improvement in glucose control and an increase in energy intake during the first week of life, but with an increased risk of hypoglycemia. There were no differences between the two study groups with regard to the primary outcome of mortality at the expected date of delivery or to morbidity outcomes. The study was discontinued early because of concerns about futility with regard to the primary outcome (Beardsall et al., 2008). The pilot study had shown that treatment with early insulin therapy improved glucose
control and increased weight gain and leg length growth, with no difference in hypoglycemia between the two groups (Beardsall et al., 2007).

Similarly, potential benefits of insulin therapy in critically ill pediatric and adult patients also remains unclear. Several studies have looked at intensive insulin therapy in the pediatric ICU (PICU) as hyperglycemia has also been associated with adverse outcome in critically ill children (Falcao et al., 2008; Faustino et al., 2005; Srinivasan et al., 2004; Wintergerst et al., 2006; Y. Wu et al., 2016). A prospective randomized controlled study of 700 critically ill pediatric patients compared intensive insulin therapy (targeted to age-adjusted normoglycemia) to conventional therapy (continuous insulin therapy started after 2 blood glucose concentrations >11.9 mmol/L). The cohort included 317 infants less than 1 year of age (45% of cohort) and three-quarters of patients were admitted for cardiac surgery for congenital heart defects. Intensive insulin therapy was shown to improve mortality and morbidity (protected the cardiovascular system, prevented secondary infections and attenuated the inflammatory response, and reduced the duration of PICU stay) but increased hypoglycemia (Vlasselaers et al., 2009). Long-term follow-up was obtained in 569 of the patients and demonstrated that tight glucose control did not affect the full-scale IQ (FSIQ) score compared to usual care, although tight glucose controlled improved motor coordination and cognitive flexibility. Episodes of hypoglycemia secondary to tight glucose control were not associated with worse neurocognitive outcomes (Mesotten et al., 2012).

The finding in a randomized trial that correction of hyperglycemia improves morbidity and mortality may be suggestive of a causal relationship between hyperglycemia and worse outcomes. However, other trials have found contradictory results. Two multicenter trials of hyperglycemia control after cardiac surgery or admission to the PICU in critically ill neonates and children (the Control of Hyperglycaemia in Paediatric Intensive Care [CHiP] trial and the Safe Pediatric Euglycemia in Cardiac Surgery [SPECS]) trial found that tight glycemic control in critically ill children had no significant effect on major clinical outcomes (Agus et al., 2012; Macrae et al., 2014) and potentially a higher incidence of hypoglycemia with tight glucose control (Macrae et al., 2014). Prospective follow-up was performed at 9-18 months in children in the SPECS trial using the Bayley scale of infant development (BSID), Adaptive Behavior Assessment System, Ages and Stages Questionnaire, and Brief Infant Toddler Social-Emotional Assessment. No significant differences were seen in neurodevelopment outcome scores with
tight glycemic control compared to standard care. However, moderate to severe hypoglycemia (<2.8 mmol/L) was associated with worse functioning in the cognitive, language, and motor domains, including after adjusting for potential confounders, such as complexity of cardiac physiology and duration of cardiac ICU stay (Sadhwani et al., 2016). Additionally, the recent Heart and Lung Failure–Pediatric Insulin Titration (HALF-PINT) trial investigated tight glycemic control in children with cardiovascular or respiratory failure excluding patients who had undergone cardiac surgery. They also found no significant between-group difference in ICU-free days or in any secondary outcomes, and the study was stopped early due to a low likelihood of benefit and evidence of the possibility of harm (nonsignificant higher mortality, health care-associated infection profile, and risk of severe hypoglycemia) in the lower glucose target group (Agus et al., 2017).

Equally, in adults with brain injury, hyperglycemia has repeatedly been shown to be associated with worse clinical outcomes (Bruno et al., 2002; Lam et al., 1991; Passero et al., 2003; Wartenberg et al., 2006), yet controversy exists regarding glycemic management. Randomized studies in critically ill adults in intensive care units had demonstrated that tighter glucose control may improve clinical outcomes (Van den Berghe et al., 2006; van den Berghe et al., 2001). However, not all studies showed a benefit. An international randomized trial showed intensive glucose control increased mortality among adults in the ICU (Finfer et al., 2009). A network meta-analysis did not find any benefit of tight glycemic control for mortality (Yamada et al., 2017). Furthermore, studies in brain injured adults using cerebral microdialysis (which allow in vivo detection of neurochemical changes in interstitial tissue of the brain) are concerning because the data indicate that excessive insulin-induced decrease in glucose in order to achieve tight glycemic control may compromise brain energy metabolism and worsen neuronal injury (Oddo et al., 2008; Vespa et al., 2006).

In summary, the literature suggests that while tight glycemic control shows no clear benefit, the appropriate threshold for treatment of hyperglycemia remains unclear. As well, there is variability between centers in treatment approaches to hyperglycemia. Further understanding of the long-term effects of hyperglycemia and different management approaches are crucial to inform treatment decisions in critically ill neonates at high risk for neurodevelopmental impairments.
1.4 Neurodevelopmental outcomes following neonatal glucose derangements

1.4.1 Overview

There is agreement in the literature that recurrent and severe hypoglycemia can cause brain injury. Neuroimaging abnormalities are seen in 18-39% of neonates after severe and recurrent hypoglycemia (Kinnala et al., 1999; Tam et al., 2008). Severe and recurrent hypoglycemia in term neonates leads to a specific neuroimaging pattern which includes parieto-occipital white matter abnormalities, abnormal signal in the thalamus and basal ganglia (Alkalay et al., 2005; Barkovich et al., 1998; Gataullina et al., 2013; Kinnala et al., 1999; Murakami et al., 1999; Spar et al., 1994; Traill et al., 1998) and restricted diffusion in the parieto-occipital lobes, corpus collosum and optic radiations on diffusion-weighted imaging (DWI) (Filan et al., 2006; Kim et al., 2006; Tam et al., 2008) (Figure 1.4). However, Burns et al. reported a retrospective cohort of 35 infants with neonatal hypoglycemia and more diffuse injury, including cortical watershed parasagittal lesions, focal infarction and hemorrhage. Still, 29% of the cases had a predominantly posterior pattern of brain injury. Their cohort may represent a mixture of etiologies; as 40% had IUGR, and 11% had endocrine disorders. As well, delivery by emergency cesarean section, need for resuscitation in the delivery room, and/or cord pH 7.0-7.1 was more frequent in cases than controls (Burns et al., 2008). Of note, neuroimaging findings following hypoglycemia may not be persistent and the children may have no apparent neurodevelopmental sequelae at follow-up (Kinnala et al., 1999; Tam et al., 2008).
Figure 1.4 Neuroimaging findings after neonatal hypoglycemia in a 5-day old term neonate demonstrating bilateral parieto-occipital diffusion restriction which is extending to the left posterior temporal lobe and pulvinar nuclei of the thalami bilaterally but more marked on the left, and into the posterior limb of the internal capsule. (A) DWI and (B) ADC maps

Long-term neurodevelopmental outcomes of neonatal hypoglycemia include epilepsy, microcephaly, global developmental delay, cerebral palsy and intellectual disability (Koivisto et al., 1972; Per et al., 2008; Udani et al., 2009; Yalnizoglu et al., 2007) and visual outcomes including cortical visual deficits (e.g. cortical blindness, homonymous hemianopsia) (Karimzadeh et al., 2011; Tam et al., 2008; Yalnizoglu et al., 2007) have been described. Symptomatic epilepsy secondary to hypoglycemia is often focal (most frequently occipital lobe epilepsy) and usually has a good prognosis. However, occasionally neonatal hypoglycemia can lead to refractory seizures and epileptic encephalopathy (including epileptic spasms, atonic and tonic seizures). There has not been a clear relationship identified between seizure outcome and the severity, duration or cause of neonatal hypoglycemia, or the presence of neonatal seizures (Alrifai et al., 2014; Arhan et al., 2017; Caraballo et al., 2004; Fong et al., 2014; Kumaran et al., 2010; Montassir et al., 2010; Yang et al., 2016). Occurrence of acute symptomatic seizures in the
neonatal period may be associated with worse long-term neurodevelopmental outcomes (Koivisto et al., 1972; Pildes et al., 1974).

Duration, frequency, and severity of hypoglycemia seem to play a role, although the specific level and duration of hypoglycemia that leads to brain injury or neurodevelopmental sequelae is not established. Severe and recurrent episodes of hypoglycemia are more predictable of long term sequelae than single hypoglycemic episodes (Duvanel et al., 1999; McKinlay, Alsweiler, et al., 2017). Tam et al. found that hypoglycemia measured on 2 or more days was associated with cortical visual deficits but none of the neonates with hypoglycemia measured on only 1 day had cortical visual deficits in long-term follow-up (Tam et al., 2008). Longer duration of hypoglycemia has also been directly related to neurodevelopmental outcome (Montassir et al., 2009; Singh et al., 1991).

Regarding hyperglycemia, studies in neonates have focused predominantly on outcomes in preterm neonates and term neonates with HIE, and will be discussed further below.

1.4.2 Short term outcomes

It was recognized almost 60 years ago that low blood glucose levels in SGA and preterm neonates were associated with seizures (Cornblath et al., 1959). Further studies have also reported symptomatic seizures in close temporal relationship to documented hypoglycemia (Filan et al., 2006; Kim et al., 2006; Tam et al., 2008; Traill et al., 1998).

Short-term effects also include neurophysiologic abnormalities detected using evoked potentials. Abnormal evoked potentials during hypoglycemia were reported by Koh et al. and supported the common clinical use of 2.6 mmol/L as a cutoff for hypoglycemia. They performed evoked potentials (sensory evoked potentials and brainstem auditory evoked potentials) during spontaneous and induced hypoglycemia in a cohort of 5 neonates and 12 children. Abnormalities in the evoked potentials occurred over a range of glucose concentrations between 0.7 to 2.5 mmol/L, suggesting individuals have varying levels of susceptibility. No subjects whose blood glucose concentration was maintained at 2.6 mmol/L or greater had abnormalities noted in their evoked potentials (Koh et al., 1988). A subsequent study by Cowett et al. of 50 neonates (33-40 weeks GA) with glucose levels between 1.38 to 6.83 mmol/L found no correlation with
brainstem auditory evoked potentials. Yet the blood glucose concentrations were predominantly within the normal range and the single glucose concentration of 1.38 mmol/L was associated with a prolonged wave V latency in the term neonate (Cowett et al., 1997). In a cohort of term neonates admitted to NICU with a variety of perinatal conditions (excluding HIE), hypoglycemia was the main risk factor for reduction in amplitude of wave I on brainstem auditory evoked responses. The main conditions in the cohort included hypotension, hypoglycemia, meconium aspiration syndrome, sepsis, metabolic acidosis, hemolytic or non-hemolytic hyperbilirubinemia, and pneumonia (Jiang et al., 2013). Occipital restricted diffusion after neonatal hypoglycemia has also been associated with abnormal visual evoked potentials within 1 week after birth (Tam et al., 2008).

Several studies have shown hyperglycemia to be associated with worse short-term outcomes in preterm neonates than hypoglycemia. Hyperglycemia in preterm neonates is associated with an increased risk of mortality (Alexandrou et al., 2010; Hays et al., 2006; Heimann et al., 2007; Kao et al., 2006), grade 3 or 4 intraventricular hemorrhage (Bermick et al., 2016; Hays et al., 2006), sepsis (Kao et al., 2006), necrotizing enterocolitis (Hall et al., 2004; Kao et al., 2006), retinopathy of prematurity (Blanco et al., 2006; Ertl et al., 2006; Mohamed et al., 2013; Mohsen et al., 2014; Slidsborg et al., 2018), and greater length of hospital stay (Hays et al., 2006). Data are lacking regarding short-term outcomes of hyperglycemia in term neonates.

1.4.3 Long term outcomes

1.4.3.1 Well babies

As discussed, severe and persistent hypoglycemia can cause acute seizures and brain injury in neonates, however, the long-term significance of early asymptomatic or transitional hypoglycemia remains controversial. Neurodevelopmental difficulties may emerge as children get older, but may not be evident at initial follow-up at younger ages. Therefore, long-term follow-up studies are essential. A systematic review in 2006 (Boluyt et al., 2006) identified 18 studies spanning over 40 years, of which only 2 studies were of sufficient methodological quality for quantitative analysis, one study in term (Brand et al., 2005) and the other in preterm neonates (Lucas et al., 1988).
Some but not all studies have found an association between neonatal hypoglycemia with long-term outcome. Still, presence of hypoglycemia in the neonatal period has been associated with worse long-term neurodevelopmental outcome in several studies. A study in 28 children of diabetic mothers assessed long-term outcome at 8 years of age. Thirteen of the children had hypoglycemia (<1.5 mmol/L) in the neonatal period, and most were asymptomatic. The children with neonatal hypoglycemia exhibited significantly more difficulties on a screening test for attention deficit disorder and were more frequently reported to be hyperactive, impulsive, and easily distracted. On psychological assessment, they had a lower total developmental score than the normoglycemic children born to diabetic mothers as well as control age-matched healthy children (Stenninger et al., 1998). In a recent prospective study, 72 hypoglycemic (plasma glucose <2.8mmol/L) and 70 weight and gestational age matched control neonates (GA >32 weeks) were enrolled from the NICU in the first week of life. There were significantly lower levels of blood glucose in the symptomatic than asymptomatic hypoglycemia group. The mean motor and mental developmental quotients were significantly lower in neonates with both symptomatic and asymptomatic hypoglycemia than euglycemic infants. A cutoff of 2.2 mmol/L was associated with lower scores. Neurodevelopment was assessed at 6 and 12 months old with the Developmental Assessment Score for Indian Infants using the DASII scoring system which is an Indian adaptation of the BSID, and which was administered by the un-blinded principal investigator. In this study, the proportion of neonates with comorbidities (respiratory distress, polycythemia and sepsis) was significantly higher in the hypoglycemic group (Mahajan et al., 2017).

Conversely, the study in term neonates by Brand et al. retrospectively identified a cohort of 117 LGA healthy neonates with transient hypoglycemia on the first day of life (<2.2 mmol/L 1 hour after birth or <2.5 mmol/L afterwards) and obtained neurodevelopmental testing in 75 of the children at 4 years old (64% of original cohort). These authors found no significant difference between neonates with and without hypoglycemia on the Denver Developmental Scale scores, Child Behavior Checklist scores or total IQ scores. Although one IQ subscale (reasoning) was significantly lower in hypoglycemic neonates, there was no correlation between lowest plasma glucose level and the reasoning-IQ. These investigators also noted that the Denver Developmental Scale score may not have been sensitive enough to detect neurodevelopmental
sequelae of hypoglycemia because the scores were found to be normal in almost all children tested (Brand et al., 2005).

To further investigate the relationship between the duration, frequency, and severity of low glucose concentrations in the neonatal period and later neurodevelopment, the Children with Hypoglycaemia and Their Later Development (CHYLD) study followed a large prospective cohort of term and late-preterm neonates born at risk for hypoglycemia (IDM, preterm birth, SGA or LGA). The study included 614 well babies born at $\geq 32$ weeks GA at risk for hypoglycemia. Glucose concentrations were tested by intermittent blood glucose measurements and by masked continuous interstitial glucose monitoring. Hypoglycemia was defined as whole blood glucose $<2.6$ mmol/L and interstitial episodes were defined as interstitial glucose concentrations $<2.6$ mmol/L for $\geq 10$ minutes. At 2 years of age, they assessed 404 children born $\geq 35$ weeks GA with a comprehensive neuropsychological assessment. Hypoglycemia occurred in 53% of the neonates. The investigators found that hypoglycemia, when treated to maintain blood glucose concentration $\geq 2.6$ mmol/L, was not associated with increased risk of neurosensory impairment and processing difficulty compared to at-risk children that did not have hypoglycemia. Neonates with severe, multiple or prolonged hypoglycemia episodes also did not have worse outcomes. Further, the risks were not increased among children with clinically unrecognized hypoglycemia. Interestingly, the factor in the first 48 hours that was most predictive of an adverse 2-year outcome was glucose instability, which was defined as the time spent outside the central range of 3 to 4 mmol/L. Both higher glucose levels and less glucose stability in the first 48 hours were associated with neurosensory impairment, especially cognitive delay. Of note, the higher glucose concentrations were predominantly within the normal range as only 3 neonates had clinical hyperglycemia ($>8$ mmol/L). A more rapid increase in glucose concentration following dextrose treatment for hypoglycemia in the first 12 hours after birth was also associated with neurosensory impairments at 2 years of age (McKinlay et al., 2015).

It has been proposed that increased glycemic variability could generate more reactive oxygen species due to hyperglycemia-induced oxidative stress (Hirsch et al., 2005). Preclinical studies using cell culture and rodent models have shown that neuronal death was triggered by glucose reperfusion rather than hypoglycemia itself, and that hyperglycemia following hypoglycemia can worsen neuronal injury. Superoxide production may be influenced by blood glucose concentrations achieved during the period immediately after hypoglycemia correction;
with correction to higher glucose levels during the first hour after hypoglycemia being associated with greater superoxide production and neuronal death (Ennis et al., 2015; Suh et al., 2007). These findings suggest that rate of correction of hypoglycemia as well as prevention of rebound hyperglycemia may be important and should be investigated further.

The CHYLD study recently published 4.5 year outcomes, when emerging neurocognitive functions could be further assessed. In this follow-up study, 477 children born at ≥ 32 weeks GA were assessed. Neonatal hypoglycemia occurred in 59% of children and although it was still not associated with an increased risk of neurosensory impairments, hypoglycemia was associated with an increased risk of low executive function and low visual motor function. Risk was highest in children that had severe, recurrent, or clinically undetected hypoglycemia (interstitial episodes only). This was the first study to show that clinically undetected low glucose concentrations can be associated with an adverse outcome. Furthermore, children who developed neurosensory impairment between 2 and 4.5 years had a steeper rise in interstitial glucose concentrations after hypoglycemia, whereas children with stable neurosensory status had similar interstitial glucose concentrations in the first 48 hours (McKinlay, Alsweiler, et al., 2017). Impaired executive function and visual motor function may be a risk factor for later school difficulties.

In accordance with these findings, early transient newborn hypoglycemia was associated with lower achievement test scores in literacy and mathematics at 10 years old in a retrospective population-based cohort where the investigators controlled for multiple perinatal factors, including maternal educational and socioeconomic status. Newborns underwent universal early glucose screening and medical data was matched to their 4th grade student achievement test scores in 1395 newborn-student pairs. Although there are several limitations of the retrospective design, the associations were large and potentially clinically significant (Kaiser et al., 2015).

In summary, the presence of hypoglycemia in the neonatal period has been associated with worse long-term neurodevelopmental outcome in several studies. Studies in well babies suggest that long-term follow-up is essential as the potential long-term neurodevelopmental consequences of transient hypoglycemia on higher-order cognitive functions may emerge at later ages. These studies also highlight the potential negative consequences of higher glucose levels as well as rapid increase in glucose levels with treatment.
1.4.3.2 Premature infants

Several studies have assessed the association of glucose derangements in preterm neonates and long-term outcomes, however the results have been mixed. An influential study by Lucas et al. examining neonatal hypoglycemia in a cohort of 661 premature neonates demonstrated an association between repeated episodes of hypoglycemia and reduced mental and motor developmental scores on the BSID at 18 months corrected age. Bayley scores were regressed on days of hypoglycemia using varying blood glucose concentration cutoffs between 0.5 to 4 mmol/L and found a significant association with glucose levels below 2.5 mmol/L. They therefore chose 2.6 mmol/L as a cutoff and showed that the presence of hypoglycemia on 5 or more days was related to reduced BSID scores (Lucas et al., 1988). Follow-up at 7.5-8 years old showed persistent impairment in arithmetic and motor function (Lucas et al., 1999).

The findings of an association between hypoglycemia with long term outcome was supported by Duvanel et al. in a cohort of 85 SGA preterm neonates (27 to 34 weeks GA). They showed that repeated episodes of hypoglycemia were associated with smaller head circumferences and lower psychometric scores (perceptive and motricity scales) at 3.5 years, although a significant difference could not be detected at 5 years (Duvanel et al., 1999). An association has also been noted in moderately preterm neonates (born 32 to 36 weeks GA) in the Longitudinal Preterm Outcome Project. Parents of children born moderately preterm completed the Ages and Stages Questionnaire at 4 years old. The investigators looked at many risk factors and morbidities from the neonatal period and in multivariate analysis, the only factor associated with developmental delay at 4 years was hypoglycemia, defined as ≥1 glucose level <1.7 mmol/L in the first 72 h of life (Kerstjens et al., 2012).

However, a study by Tin et al. attempted to reproduce the findings of Lucas et al. in premature neonates (<32 weeks GA) and they were not able to confirm the association between recurrent hypoglycemia and neurodevelopmental outcome when measured at 2 and 15 years of age. Of note, cognitive function at 15 years was relatively low in both the exposed and matched control group (full scale IQ 80.7 vs 81.2) (Tin et al., 2012; Tin et al., 1998). Moreover, the study by Lucas et al. adjusted for social class and mother’s educational level whereas the study by Tin et al. did not. Socioeconomic status or maternal education level have been shown to be associated with neurodevelopmental outcomes, including in the study by Lucas et al. (Ansell et
al., 2017; Dollaghan et al., 1999; Fernald et al., 2013; Lewis et al., 1989; Lucas et al., 1988; Vohr et al., 2000).

Likewise, another retrospective observational cohort of 443 very preterm neonates (GA <30 weeks or weight <1500g) examined several measures of neonatal glycemia, including hypoglycemia, hyperglycemia, unstable glucose (≥1 blood glucose concentration ≤2.5 mmol/L and ≥1 blood glucose concentration ≥8.6 mmol/L), and glucose variability (defined as the standard deviation around the mean after log transformation of all blood glucose concentrations). After correction for gestational age, birth weight z-score and socioeconomic status, measures of neonatal glycemia were not independent predictors of neonatal illness or outcomes at 2 years old (Tottman et al., 2017). Although Tottman et al. did not find an association with hyperglycemia and long-term outcomes, hyperglycemia on the first day of life in extremely preterm neonates (<27 weeks GA) has been associated with white matter reduction on term equivalent MRI scan (Alexandrou et al., 2010). And another study of hyperglycemia in very preterm neonates (≤32 weeks GA) showed an association of hyperglycemia with neurological and behavior problems at 2 years of age (van der Lugt et al., 2010).

Hence, several studies have demonstrated that both early life hypo- and hyperglycemia in premature neonates are associated with worse-long term outcomes, although not all studies have supported these conclusions.

1.4.4 Outcomes following glucose derangements in neonatal encephalopathy

As discussed, there is no universally accepted “safe” blood glucose level for all newborns since individual susceptibility to brain injury varies, such as in the presence of comorbid conditions. In HIE, the neonates’ ability to produce and use alternative fuels may not be sufficient to compensate for low glucose levels, making these neonates particularly vulnerable to hypoglycemia induced brain injury. Several studies in neonates with HIE have shown that glucose derangements on intermittent glucose testing may be an important factor associated with brain injury and worse neurodevelopmental outcomes.
The posterior predominant MRI pattern of hypoglycemic brain injury can be distinguished from patterns of brain injury in HIE which include watershed, basal ganglia, total or focal-multifocal white matter injury (Figure 1.5). In a prospective cohort of 160 term neonates with HIE, selective posterior white matter and pulvinar edema were most predictive of clinical hypoglycemia and could be superimposed on the predominant pattern of HIE (Wong et al., 2013). Hypoglycemia may also be associated with a more severe MRI pattern of perinatal hypoxia-ischemia. In a cohort of 94 term neonates with encephalopathy, occurrence of hypoglycemia was associated with increased odds of corticospinal tract injury (Tam et al., 2012).

Figure 1.5 Neuroimaging features of basal ganglia pattern of brain injury in a 3-day old term neonate with HIE. (A) DWI shows restricted diffusion involving the bilateral basal ganglia and thalami (B) corresponding ADC map

Higher rates of hypoglycemia have been associated with more severe clinical signs of neonatal encephalopathy (Basu et al., 2009). A retrospective chart review of 185 depressed term neonates with severe fetal acidemia showed that hypoglycemia was associated with adverse short-term outcomes (death or evidence of moderate to severe encephalopathy with or without
seizures) including in multivariate analysis (Salhab et al., 2004). Subsequently, studies looking at long-term outcomes have shown neonatal hypoglycemia to also be associated with worse neurodevelopmental outcomes. In the study discussed above by Tam et al. there were 1-year outcomes available for 73 children of the cohort of 94 term neonates with encephalopathy. They demonstrated that hypoglycemia in neonatal encephalopathy was associated with worse motor outcomes as well as worse cognitive and language score on the BSID (Tam et al., 2012). Conversely, in a retrospective cohort of 52 term neonates’ with HIE, although early hypoglycemia (<6 hours of life) was associated with neurodevelopmental outcome at 24 months old on univariate analysis, it was not a significant predictor after adjusting for severity of clinical neonatal encephalopathy (Sarnat score at 24 hours of life). Nadeem and colleagues thus concluded that early hypoglycemia was associated with severe HIE. Occurrence of hyperglycemia also was not associated with adverse outcome (Nadeem et al., 2011). However, these authors adjusted for clinical severity of encephalopathy which not only may be related to severity of HIE, but could be worsened due to hypoglycemia or other comorbidities. Of note, none of the neonates in the above studies received TH except for 11 of 94 neonates in the study by Tam et al (2012).

In a post-hoc analysis of the CoolCap study, it was demonstrated that not only hypoglycemia (≤2.2 mmol/L) but also hyperglycemia (>8.3 mmol/L) were very common in neonates with HIE and that both were associated with unfavorable neurodevelopmental outcome (death and/or severe neurodevelopmental disability) at 18 months corrected, independent of severity of HIE and TH (Basu et al., 2016). The CoolCap Study included 234 neonates ≥36 weeks’ GA with moderate to severe HIE and neonates who were randomized to previous standard care or head cooling for 72 hours within 6 hours after birth. Plasma glucose concentrations were collected at specific time points after randomization (0, 4, 8 and 12 hours after randomization). They further showed that neonates with hyperglycemia within 12 hours after randomization appeared to benefit most from TH (Basu, Salemi, et al., 2017).

Some noteworthy differences have been reported between neonates with HIE who experience hypo- or hyperglycemia. Neonates with HIE and hyperglycemia had higher rates of reported sentinel events (e.g. prolapsed cord, umbilical cord tear, placental abruption and ruptured uterus) and emergency caesarian delivery compared to normoglycemic and hypoglycemic neonates (Basu, Salemi, et al., 2017). Consequently Basu et al. hypothesize that
hyperglycemic neonates were more likely to have a temporally acute and relatively intense insult and that the evolution of brain injury in some hyperglycemic neonates may have been more likely to be within the therapeutic window for TH to be beneficial. These neonates had milder multiorgan injury and may have intact gluconeogenesis and stress hormone responses with decreased glucose utilization due to brain injury from perinatal asphyxia (Basu, Salemi, et al., 2017; Shi et al., 2012; Thorngren-Jerneck et al., 2001). In support of this idea, these authors presented an abstract at the Pediatric Academic Society Meeting recently describing higher odds of predominant basal ganglia or global patterns of injury in hyperglycemic neonates (Basu, Ottolini, et al., 2017). Conversely, neonates with HIE and hypoglycemia have been reported to more commonly have a watershed pattern of hypoxic-ischemic injury (Basu, Ottolini, et al., 2017; Wong et al., 2013) which has been associated with prolonged partial asphyxia (Barkovich et al., 1995). Further, neonates without a history of a sentinel event but rather with a history of decreased fetal movements, have a watershed predominant injury and less clear response to therapeutic hypothermia (Bonifacio et al., 2011).

Hyperglycemia has also been shown to adversely affect long-term outcome in neonates with HIE in two small retrospective studies, in which 24 to 36% of babies underwent TH in each. In a chart review of 41 neonates with HIE, the authors found that hyperglycemia in the first 12 hours of life was associated with poor gross motor outcomes (Gross Motor Function Classification System [GMFCS] ≥ 1 or death [in NICU or within 6 months with severe motor deficits]) in univariate analysis (Spies et al., 2014). The other retrospective study identified 56 neonates with HIE of which outcome data was available for 41 children and they likewise found an association of hyperglycemia in neonates undergoing TH with poor outcome (death, microcephaly, and/or moderate to severe CP documented between 18 to 36 months) (Chouthai et al., 2015).

Lastly, looking at glycemic variability, there was one small retrospective cohort of 23 term neonates with HIE treated with TH which showed that glucose variability was associated with severe neurodevelopmental disability (GMFCS 3 to 5, Bayley III Motor Standard Score <70, Bayley III Language Score <70 and Bayley III Cognitive Standard Score <70). As a measure of glucose variability, they used mean absolute glucose (MAG) change which was calculated as the sum of differences between successive arterial glucose values divided by 24 hours (Al Shafouri et al., 2015). Although their cohort was small, these findings are consistent
with the CHYLD study which showed greater glycemic variability was associated with neurosensory impairment at 2 years in well babies. Similarly, a relationship between glucose variability (as measured by standard deviation [SD]) with mortality was demonstrated in VLBW preterm neonates (Fendler et al., 2012). Also, it has been shown that hypoglycemia, hyperglycemia and glucose variability (SD or glucose variability index) have been associated with mortality, nosocomial infections and increased length of hospital stay in term neonates and children admitted to the PICU (Faustino et al., 2005; Hirshberg et al., 2008; Rake et al., 2010; Wintergerst et al., 2006). As well, a longer duration of hyperglycemia and higher peak glucose levels have been associated with increased mortality in the PICU (Srinivasan et al., 2004). In adults, glucose variability also has been associated with increased mortality in the ICU (Ali et al., 2008; Bagshaw et al., 2009; Dossett et al., 2008; Egi et al., 2006; Hermanides et al., 2010; Krinsley, 2008; Pidcoke et al., 2009; Waeschle et al., 2008). Measures of glycemic variability used in these studies have included SD, coefficient of variability, and presence of both an episode of hypo- and hyperglycemia within 24 hours of ICU admission.

The data regarding the effects of tight glucose control in critically ill adults on outcome is conflicting. Van den Berghe et al. showed a reduction in mortality with tight glucose control (van den Berghe et al., 2001) whereas the NICE-SUGAR study found an increase in mortality with intensive glucose control (Finfer et al., 2009). The study by van den Berghe et al. showed a markedly lower SD in the intensively treated group (SD of morning blood glucose, 1.1 mmol/L in the intense treatment group vs. 1.8 mmol/L in the conventional treatment group) compared to the NICE-SUGAR study where SD of morning glucose was equal in both groups (Siegelaar et al., 2010). One hypothesis for these conflicting results is the differential effect of glucose variability in the studies (Siegelaar et al., 2010).

In summary, in neonates with HIE, not only hypo- and hyperglycemia but also glycemic variability may be important factors associated with brain injury and poor neurodevelopmental outcomes. It is essential to identify whether hypoglycemia, hyperglycemia and glucose variability merely reflect the severity of the underlying illness, or whether these factors contribute further to brain injury and poorer long-term outcomes.
1.5 Continuous interstitial glucose monitoring

1.5.1 Continuous interstitial glucose monitors overview

Continuous interstitial glucose monitors (CGMs) were developed for use in management of diabetes mellitus and have been found to improve glucose control (Battelino et al., 2012; Cemeroglu et al., 2010) and were shown to be safe and potentially useful in the ICU setting as well (Allen et al., 2008; Bridges et al., 2010; Piper et al., 2006). CGM devices and insulin pumps can also now be combined to form a closed loop system, also known as artificial pancreas systems. They utilize a complex set of algorithms that interpret CGM glucose levels and adjust insulin delivery via an insulin pump. Closed-loop systems have been shown to be beneficial to improve glycemic control without increases in hypoglycemia in children and adults (Anderson et al., 2016; Chernavsky et al., 2016; DeBoer et al., 2017; Leelarathna et al., 2014; Tauschmann et al., 2016).

CGMs contain a disposable glucose oxidase-based platinum electrode sensor inserted subcutaneously which catalyzes interstitial glucose oxidation generating an electrical current every 10 seconds. Glucose concentrations are estimated from this signal using proprietary algorithms which require regular calibration with blood glucose measurements. Although the sensor measures the concentration of interstitial glucose every 10 seconds, the monitor records averaged glucose values every 5 minutes. Some systems display the output in real-time, whereas for others, the data cannot be viewed in real time and are therefore a useful research tool, as it does not impact on clinical care. The CGM data are downloaded after completion. The software does not display data when glucose concentrations fall below 2.2 mmol/L or above 22 mmol/L (Beardsall et al., 2005; Beardsall et al., 2013; McKinlay, Chase, et al., 2017). Retrospective analysis, filtering and recalibration of the raw signal may be possible (Signal et al., 2012). Of note, the interstitial glucose sensor can also take as long as 2 hours to stabilize after insertion (Harris et al., 2010). The sensor is well tolerated, including in small premature infants, with no evident local edema, inflammation or infection at the sensor site or problems of skin debridement with removal (Beardsall et al., 2005; Beardsall et al., 2013). However, there is limited evidence for the benefits of treating newborns based on CGM monitoring.

Errors in CGM measures may occur. There can be random error of the sensor due to sensor technology and its interstitial location (referred to as zero-mean error). Plus, conversion of
the raw signal to a glucose concentrations requires the regular input of calibration measures (i.e. blood or plasma glucose concentrations) and thus the resulting output will reflect the reliability of the calibration sample used. Ideally, CGMs should be calibrated to plasma equivalent whole-blood glucose concentrations rather than point-of-care glucometers (McKinlay, Chase, et al., 2017; Thomas et al., 2014). There is the potential for sensor drift where there is shifts in sensor output between calibration points. The CGMs rely on a continuous shifting internal algorithm to generate glucose concentrations from the raw sensor signal. The regular calibrations use the ‘true’ glucose concentrations which are entered into the device. The drift between calibrations measurements may impact accuracy, which could result in apparently stable CGM values when the blood glucose concentrations are falling (McKinlay, Chase, et al., 2017; Signal et al., 2012).

Also, studies have reported that interstitial glucose concentrations can have a variable “lag” behind the blood glucose concentrations by as long as 20 minutes due to delays in diffusion of glucose from the vascular into the interstitial space, as well as patient-specific and instrumental factors. Variability in this time lag has been documented and there may be increasing positive error as the blood glucose concentrations decrease and increasing negative error when the glucose concentrations are rising (Boyne et al., 2003; Caplin et al., 2003; D. L. Harris et al., 2009; Kovatchev et al., 2009; Rebrin et al., 1999; Schmelzeisen-Redeker et al., 2015; Shah et al., 2018). A study in term and near-term neonates was not able to find a significant time lag but found a late increase in accuracy, with the highest accuracy for subcutaneous glucose concentrations observed after a 15 to 19-minute delay following blood glucose sampling (Wackernagel et al., 2016).

The two main CGM brands that have been used in neonates are Medtronic Minimed (Northridge, CA, United States) (Figure 1.6) and DexCom (San Diego, CA, United States), both of which manufacture retrospective and real-time devices. Of note, none of these devices have been approved for clinical use in neonates. The clinical significance of glucose disturbances detected on CGM is not clear, and in the absence of well-established clinical guidelines, there is a risk that CGM could lead to unnecessary or even harmful interventions (McKinlay, Chase, et al., 2017).
The sensor is inserted subcutaneously into the lateral aspect of the neonate’s thigh. The sensor catalyzes interstitial glucose oxidation, which is converted to an average glucose value every 5 minutes.

1.5.2 Continuous interstitial glucose monitor use in newborns

CGMs have been used in studies in term and preterm neonates. They have been validated in small preterm neonates and have been used over a 7-day period without any deterioration in accuracy over time. It was shown that the CGM data correlates well with point of care devices, with minimal bias. Analysis of CGMS System Gold (Medtronic, Minneapolis, Minnesota, USA), in 188 VLBW preterm neonates showed that overall the CGM marginally under reads but there was essentially no bias overall. At low glucose levels, there was a tendency to over read and at higher glucose levels there was a slight bias to under read. Newer CGM systems with modified sensors and monitors are now thought to have better accuracy, particularly at lower glucose levels (Beardsall et al., 2013).

The CHYLD study used blinded CGMs and a clinical management protocol aimed at maintaining blood glucose concentrations ≥2.6 mmol/L. CGM use detected low glucose concentrations in almost one quarter of neonate which were not detected clinically based on intermittent blood glucose monitoring (McKinlay et al., 2015). A recent RCT in VLBW preterm neonates also detected many more episodes of hypoglycemia using CGM than routine intermittent blood glucose testing (Uettwiller et al., 2015).

A recent randomized trial used CGM to guide glucose infusion rate in a cohort of 50 neonates ≤ 32 weeks’ GA or with birth weight ≤ 1500 g. Neonates were randomly assigned to receive computer-guided glucose infusion rate adjustments driven by unblinded CGM or by standard of care on the basis of blood glucose determinations. Neonates in the unblinded CGM group had a significantly greater percentage of time spent in the euglycemic range and decreased

Figure 1.6 The Medtronic iPro2 continuous glucose monitor. The sensor is inserted subcutaneously into the lateral aspect of the neonate’s thigh. The sensor catalyzes interstitial glucose oxidation, which is converted to an average glucose value every 5 minutes.
time spent in the mild (2.6 – 3.9 mmol/L) and severe (<2.6mmol/L) hypoglycemia range compared with the blinded CGM group. Use of CGM also decreased glycemic variability (measured by SD and coefficient of variation [percent value of SD divided by mean glucose]). With the ability to rapidly change glucose infusion rates, they were able to prevent both hypoglycemia and hyperglycemia while maintaining glucose intake and weight gain goals. Further, they showed that linking a CGM to a control algorithm guiding glucose titration can successfully achieve glucose control in this population without need for insulin (Galderisi et al., 2017).

CGMs have the potential to improve the detection of hypoglycemia and hyperglycemia episodes that would otherwise go undetected with intermittent blood sampling, while also providing information on duration and severity of the episodes. As well, CGMs have the potential to be a valuable tool to improve management in these neonates, although further research is needed.

1.5.3 Measures of glucose variability and complexity

Several methods of measuring glucose variability have been described in the literature, each with advantages and disadvantages. SD is the most commonly used parameter. It represents an index of dispersion of the glucose values, however it is important to keep in mind that glucose does not have a Gaussian or ‘normal’ distribution in the statistical sense (Eslami et al., 2011).

Many of the studies discussed above looking at glycemic variability have used SD, but various other measures were used as well. For example, the study by Wintergerst et al. in the PICU population calculated a glucose variability index by dividing the absolute difference of sequential glucose values by the difference in collection time (in hours + 0.01). The mean of the ratios for each subject formed was the variability index (Wintergerst et al., 2006). Egi et al. calculated the coefficient of variability \( (\text{Glu}^{\text{CV}} = \frac{\text{Glu}^{\text{SD}}}{100/\text{Glu}^{\text{Ave}}}) \) (Egi et al., 2006) and Ali et al. calculated a glycemic lability index. (Ali et al., 2008). A glycemic lability index can be calculated as \( \Sigma (\text{Glu}_n - \text{Glu}_{n+1} \text{ (mmol/L)})^2(\text{h}_n + 1 - \text{h}_n)^{-1})(\text{number of readings})^{-1} \). A potential benefit is that the differences are weighted individually, which gives more importance to the greatest differences, which are likely to be more detrimental (Ali et al., 2008; Le Floch et al.,
CGM provides the opportunity to measure glucose variability more precisely. An analysis of the blood glucose rate of change can be useful to evaluate the dynamics of glucose fluctuations on the time scale of minutes. The glucose rate of change at a certain time ($t_i$) is computed as the ratio \([\text{Glucose}(t_i) - \text{Glucose}(t_i-1)]/(t_i-t_i-1)\), where ($t_i$) and ($t_i-1$) are consecutive CGM readings taken at times $t_i$ and $t_i-1$, respectively. A larger (by absolute value) glucose rate of change indicates more rapid and pronounced glucose fluctuations. One could also use the average (over a certain time window, such as 1 h) absolute value of glucose rate of change as a measure of local glucose variability (Kovatchev et al., 2005).

Many other terminologies and measures have been used to assess variability, some of the common measures include the mean absolute glucose change per patient per hour (MAG), mean amplitude of glucose excursions (MAGE; average amplitude of upstrokes or downstrokes with magnitude greater than 1 SD), mean of daily differences (MODD; mean difference between glucose values obtained at the same time of day on 2 consecutive days under standardized conditions, and \(\text{MODD}_d\), which allows calculation of the MODD for a continuous time series involving many days), and the continuous overlapping net glycemic action (CONGa; the SD of the difference between values obtained exactly n hours apart) which was developed specifically for CGM (Rodbard, 2009a; Siegelaar et al., 2010). Limitations of each these measures exist, for instance, MAGE arbitrarily ignores excursions of less than 1 SD, yet the importance of these smaller excursions is not clear (Siegelaar et al., 2010). A systematic review of the literature identified 12 studies using 13 different indicators to measure glucose variability, with SD and the presence of both hypo- and hyperglycemia being the most common. In each study, at least one of the measures appeared to be associated with mortality. They conclude that due to methodological limitations, heterogeneity of studies, and the possibility of reporting bias, it is still not clear whether and which measure of glucose variability is independent of other confounders (Eslami et al., 2011).

Many of these indices have been shown to be highly correlated with each other and SD and thus convey largely the same information (Rodbard, 2009a; Saisho et al., 2015). As SD correlates highly with other variability measures, it is often used, even though glucose is not normally distributed (the mathematical condition for SD) and it doesn’t take into account
glycemic swings (Siegelaar et al., 2010). Conversely it has also been shown that the different indices and ratios may provide some complementary information (Le Floch et al., 2016; Rodbard, 2009b). One study in diabetic patients showed that the most reliable markers of glucose variability were the pairs of MAG and MAG/m (MAG to mean ratio) as well as two new markers; the glucose fluctuation index (GFI), and its ratio to the mean of glucose values, the glucose coefficient of fluctuation (GCF) (Le Floch et al., 2016). Consequently, no universal consensus exists on how to measure glycemic variability.

In addition to glucose variability, which looks at the magnitude of glucose fluctuations over time, one could also consider glucose complexity. Glucose complexity is a dynamic measure of glucose time series. Glycemic variability depends on both exogenous and endogenous factors, whereas glucose complexity describes the endogenous glucose regulation that is independent of exogenous factors (Brunner et al., 2012). It is hypothesized that the regulatory system should normally make frequent corrections of glucose levels and result in a ‘complex’ profile. Whereas critically ill patients will have a ‘low complexity’ glucose profile due to inability to correct glucose fluctuations frequently and quickly. In critically ill adult patients, loss of glucose complexity has been shown to be associated with mortality (Brunner et al., 2012; Lundelin et al., 2010).

Glucose complexity can be calculated using detrended fluctuation analysis (DFA). DFA is a unitless measure which estimates the degree of long-range correlations within a signal. It analyzes how the time series and its linear regression diverge as the ‘time window’ increases. Lower DFA values represents higher complexities (Brunner et al., 2012; Lundelin et al., 2010). Loss of complexity has been associated with greater glycemic variability (Garcia Maset et al., 2016). Two modes of DFA can be used depending on the properties of the time series. Monofractal DFA is used when the scaling properties of the time series can be quantified by a single power law exponent (independent of time and space). As real-world physiological signals often do not exhibit monofractal scaling behavior over the entire time period, multiple scaling exponent are required to fully characterize the correlation properties of the signal, and multifractal DFA should be employed. Assessment of sensor glucose data from CGM devices suggests the scaling properties of the time series are multifractal and that multifractal DFA appears to be a more appropriate analysis technique (Signal et al., 2013).
1.6 Neurophysiology

1.6.1 Continuous electroencephalography (cEEG)

EEG is a fundamental tool for assessing brain activity. Traditionally EEG is interpreted by visual inspection, using a set of qualitative rules developed through clinical experience by experts in the field. EEG measures electrical fields generated by neuronal activity by recording the amplified potential differences between electrodes placed on the scalp. The synchronous electrical activity of more than $10^8$ neurons in a cortical area is required to record visible scalp EEG potentials. The major contributors to the EEG signal are postsynaptic potentials produced by the cortical neurons (both excitatory postsynaptic potentials and inhibitory postsynaptic potentials). The scalp EEG signal is modified by electrical conductive properties of the tissues between the electrical source and the recording electrode on the scalp, conductive properties of the electrodes, and the orientation of the cortical generator (Current Practice of Clinical Electroencephalography, 2014; Olejniczak, 2006).

Scalp electrodes are traditionally placed according to the International 10-20 system. Specific measurements are made between bony landmarks, and then from these landmarks, specific measurements are made and 10% and 20% of a specified distance is used at the inter-electrode distance. This provides uniform inter-electrode distances in all laboratories and symmetric placement of electrodes over the left and right hemispheres. The orderly arrangement of multiple channels is termed a montage. The American Clinical Neurophysiology Society (ACNS) has recommended using a minimum of 21 electrodes for the International 10-20 system. Each electrode is named by a letter/number combination. The letter designates the approximate anatomical location (e.g. “C” for central). Odd-numbers designate electrodes over the left hemisphere and even-numbered electrodes over the right (Current Practice of Clinical Electroencephalography, 2014). In neonates, EEG electrodes are also applied using the international 10-20 system, but often modified with fewer electrodes because of the smaller neonatal head circumference (Figure 1.7). Simultaneous video recording with EEG is strongly recommended to provide clinical correlate for seizures and to assist in detecting artifacts. Recordings should include relevant polygraphic data (electrocardiogram and respiration). Other extracerebral channels including extraocular movements, oxygenation, and electromyogram are useful, but not universally required (McCoy et al., 2013; Shellhaas et al., 2011).
There is no universally accepted EEG classification for neonatal HIE and published studies have used different classification schemes (Andre et al., 2010; Birca et al., 2016; Glass et al., 2014; Holmes et al., 1993; Leijser et al., 2007; Nash et al., 2011; Obeid et al., 2017; Tsuchida, 2013; Walsh et al., 2011). In 2013 the ACNS published an expert consensus of standardized neonatal EEG nomenclature with the goal of improving consistency and facilitating collaborative research. The consensus focuses on normal state changes, background features, graphoelements (or named neonatal EEG features), seizures, and rhythmic or periodic patterns. Several normal behavior states of term infants are described. During wakefulness, the EEG background contains continuous, low to medium amplitude mixed frequency activity (predominance of theta and delta with overriding beta activity), often referred to as activité moyenne. Sleep is classified as active, quiet, transitional and indeterminate. The EEG during active sleep consists of activité moyenne, indistinguishable from normal wakefulness. Quiet sleep near term consists of tracé alternant, which is an alternating trace of higher voltage bursts (50–150 mcV peak to peak [pp]) lasting 4 to 10 seconds alternating with briefer, lower voltage (25–50 mcV pp) interburst intervals (IBI) composed mostly of mixed theta and delta frequencies. Tracé alternant is first seen at 34 to 36 weeks postmenstrual age (PMA). There is a progressive decrease in normal IBI durations with increasing PMA. At 34-36 weeks PMA, the maximum normal IBI is 10 seconds and at 37 to 40 weeks PMA the maximum normal IBI is 6 seconds. Sleep-wake cycling (SWC) is the normal pattern of alterations among behavioral states. In a term infant, a complete sleep and waking cycle has a typical duration of 3 to 4 hours. An isolated sleep-only cycle typically lasts 40 to 70 minutes (Tsuchida et al., 2013).

Excessive discontinuity refers to abnormally discontinuous background activity with bursts that contain some normal patterns and graphoelements separated by IBIs that are too prolonged or voltage too depressed for PMA. Tracé discontinu is the normal discontinuous pattern seen in healthy preterm babies. This EEG pattern is characterized by bursts of high voltage (50–300 mcV pp) activity that is interrupted by low voltage interburst periods (<25 mV pp). The duration of the low voltage interburst periods is dependent on PMA, and decreases with increasing PMA. Burst suppression pattern is characterized by a more severe disruption of the continuity of the EEG. Burst suppression consists of invariant, abnormally composed EEG bursts (no normal features within the bursts) separated by prolonged and abnormally low voltage IBIs periods (<5 mcV pp). The definition does allow for one electrode with sparse activity during the
IBI (up to 15 mcV pp) or <2 seconds with transient activity up to 15 mV pp, or >2:1 asymmetry in voltage in multiple electrodes (Tsuchida et al., 2013). A low voltage suppressed or low voltage undifferentiated background is also invariant and unreactive. It is composed of persistently low voltage activity without normal background features. The baseline voltage is <10 mcV, yet can be interspersed with higher voltage (≥10 mcV pp) transient activity for <2 seconds (Tsuchida et al., 2013).

Variability (lability) signifies noticeable spontaneous EEG responses to internal stimuli (e.g. those that occur during typical sleep–wake cycling). Of note, arousals from sleep can result in transient attenuation of EEG voltages, which should not be considered discontinuity. Reactivity of the EEG is demonstrated by a noticeable cerebral EEG response to external stimuli. These EEG responses to stimuli can consist of changes in any electrical domain: frequency, continuity or voltage (Tsuchida et al., 2013).

The ACNS classifies neonatal seizures as: clinical-only seizure, electroclinical seizure (clinical signs with simultaneous EEG correlate), or electrographic only (EEG seizure with no specific visible clinical signs). A clinical-only seizure consists of a sudden paroxysmal clinical change which does not correlate with a simultaneous EEG seizure. Clinical signs may include unnatural posturing, obligatory stereotyped movements, sudden behavioral arrest, or autonomic dysfunction (such as episodic tachycardia or hypertension, flushing, pallor, or salivation). An electrographic seizure is defined as a sudden, abnormal EEG event defined by a repetitive and evolving pattern, with a minimum voltage of 2 mcV pp and minimum duration of at least 10 seconds. “Evolving” is defined as an unequivocal evolution in frequency, voltage, morphology, or location. There is no minimum frequency required in the definition of neonatal seizures, in contrast to the definition of seizures in older children and adults. If brief rhythmic repetitive discharges last <10 seconds but with evolution, they would be considered brief rhythmic discharges and not seizures. There must be at least 10 seconds separating 2 distinct seizure events. Status epilepticus is present when the summed duration of seizures comprise ≥50% of an arbitrarily defined 1-hour epoch (Tsuchida et al., 2013). The use of 10 seconds as a minimum duration for seizures is largely arbitrary, and electrographic events with a clinical correlate are usually considered electroclinical seizures even if they last less than 10 seconds (Massey et al., 2018).
A study applying the ACNS standardized terminology and categorization found good or very good interrater agreement for identification of seizures and classification of EEG background. Interrater agreement was good in classifying EEG backgrounds on a 5-category scale (normal, excessively discontinuous, burst suppression, status epilepticus, or electrocerebral inactivity) (kappa = 0.70, p<0.001), with perfect agreement in 72% of records (43 of 60). Interrater agreement was very good for identification of seizures (kappa = 0.93, p<0.001), with perfect agreement in 95% of records (57 of 60). Although there was limited interrater agreement for other background features and abnormal sharp waves (Wusthoff et al., 2017). Another study had also shown that the inter-observer agreement for neonatal seizure detection on cEEG in a cohort of term neonates at risk of acute encephalopathy was high with temporal assessment resulting in a kappa of 0.827 (95% CI: 0.769 - 0.865; n = 70). The median agreement was 83.0% (IQR 76.6 - 89.5%; n = 33) for seizure and 99.7% (IQR 98.9 - 99.8%; n = 70) for nonseizure EEGs (Stevenson et al., 2015).

1.6.2 Amplitude-integrated electroencephalography (aEEG)

Continuous EEG is the gold standard for monitoring cerebral activity in neonates however there are several barriers to its widespread use. Interpretation of the EEG requires specialized training, as the EEG signals are low amplitude and easily contaminated by artifacts (e.g. electrocardiographic, respiration, pulsation, movement, electrodes, equipment). A neurophysiologist may not be available on an emergent basis throughout nights and weekends for interpretation. Furthermore, it is expensive and access to equipment and technologists to start a recording can be variable depending on time of day or day of the week.

aEEG is a simplified method for continuously monitoring cerebral activity that can be applied by bedside caregivers with minimal training and can be interpreted by neonatologists or other bedside caregivers (such as nurses or respiratory therapists) that do not have specialized neurophysiology backgrounds (Shah et al., 2015).

Due to the ease of application and interpretation of aEEG compared to cEEG, it has been used in NICUs in European centers for over 2 decades and increasingly used in North American centers in the recent years (Glass et al., 2013). EEG processing includes an asymmetric band
pass filter (to attenuate activity below 2 Hz and above 15 Hz), semi-logarithmic amplitude compression, rectifying, smoothing, and time compression. The amplitude display is linear between 0 to 10 mcV and logarithmic from 10 to 100 mcV. Detailed evaluation is lost, however long-term trends and evolution of the background over time become more evident with the compressed time scale (Hellström-Westas et al., 2006).

For aEEG, a minimum of 2 electrodes and a reference are placed for a single channel montage or 4 electrodes and a reference for a dual channel montage (provides one channel from each hemisphere) (Figure 1.7). Dual-channel aEEG recordings can provide information about the presence of unilateral brain injury and can improve seizure identification in these neonates (van Rooij, de Vries, et al., 2010). Originally, single-channel aEEG placed leads over the parietal region (P3 and P4) because it is over the cerebrovascular watershed, an area at high risk for acquired injury. However, the adjacent C3 and C4 channels probably provide similar data for single-channel aEEG. Most contemporary machines allow the display of dual-channel recordings (usually C3-P3 and C4-P4), along with the raw EEG from which the aEEG signals are derived. When reduced channel aEEG is obtained simultaneously to complement ongoing cEEG monitoring, P3 and P4 may be added to the conventional neonatal recording montage (Shellhaas et al., 2011).

Figure 1.7 Standard placement for EEG electrodes A) according to the 10-20 system modified for neonates. B) aEEG lead placement for dual channel aEEG recordings
Generally, there is good correlation between findings on EEG and aEEG (Hellström-Westas et al., 2006; Shellhaas et al., 2008). aEEG can demonstrate background abnormalities and sleep wake cycling. Hypothermia does not significantly affect aEEG background activity (Burnsed et al., 2011; Horan et al., 2007). There are 2 main background classification systems used. Al Naqeeb et al. created a background classification for term infants which includes 3 categories. The classification is based on aEEG amplitudes. aEEG background is classified as normal amplitude when the upper margin of aEEG activity is >10 mcV and the lower margin >5 mcV; as moderately abnormal amplitude when the upper margin of aEEG activity is >10 mcV and the lower margin <5 mcV; and as suppressed when the upper margin of aEEG activity is <10 mcV and lower margin <5 mcV (al Naqeeb et al., 1999).

An alternate classification proposed by Hellstrom-Westas et al. classifies aEEG background patterns using pattern recognition based on EEG terminology, including categorization of sleep-wake cycling, which could be used in all newborns. The 5 background categories were defined as: continuous with minimum (lower) amplitude around (5 to) 7 to 10 mcV and maximum (upper) amplitude of 10 to 25 (to 50) mcV; discontinuous with minimum amplitude variable, but below 5 mcV, and maximum amplitude above 10 mcV; burst-suppression as a discontinuous background with minimum amplitude without variability at 0 to 1 (2) mcV and bursts with amplitude >25 mcV; low voltage as a continuous background pattern of very low voltage (around or below 5 mcV); and inactive, flat describes a primarily inactive (isoelectric tracing) background below 5 mcV (Figure 1.8). A classification for SWC in the aEEG was also proposed. SWC is characterized by smooth sinusoidal variations of the bandwidth, mostly in the minimum amplitude. A wider bandwidth represents the discontinuous background activity during quiet sleep (tracé alternant EEG in term infants), and the narrower bandwidth corresponds to the more continuous activity seen during wakefulness and active sleep. SWC was classified in 3 categories: no SWC (where there is no cyclic variation of the aEEG background); imminent/immature SWC (when there is some, but not fully developed cyclic variation of the lower amplitude, and not developed as compared with normative gestational age representative data); and developed SWC (when there are clearly identifiable sinusoidal variations between discontinuous and more continuous background activity, with cycle duration >20 min) (Hellström-Westas et al., 2006).
Figure 1.8   Examples of aEEG background patterns.

Classifications similar to the pattern classification system had been previously used and described in cohorts of neonates with HIE and were shown to be correlated with prognosis. Neonates with continuous normal voltage (CNV) or discontinuous normal voltage (DNV) background patterns during the first 6 hours after birth were likely to survive without sequelae, whereas neonates with burst suppression (BS), continuous low voltage (CLV) or flat tracings (FT) had a high risk for death or neurodevelopmental disability (Hellstrom-Westas et al., 1995; ter Horst et al., 2004; Toet et al., 1999). aEEG monitoring can be a useful tool in neonates with HIE to monitor degree of encephalopathy and to detect seizures, as well as to assess recovery and predict outcome. Its role in outcome prediction will be discussed further below.

Seizures can be identified on aEEG as an abrupt, transient rise in the lower and upper margins and narrowing of the bandwidth. aEEG can detect approximately one third of neonatal seizures. Seizures that are infrequent, short (<30 seconds), and low amplitude are difficult to detect (Lawrence et al., 2009; Mastrangelo et al., 2013; Rakshasbhuvankar et al., 2015; Shellhaas, Soaita, et al., 2007). Seizure detection may be dependent on user experience (Frenkel et al., 2011) and on availability of the raw aEEG for confirmation, which improved the
sensitivity to 76% and specificity to 78% in one study (Shah et al., 2008). Still, aEEG is superior to clinical detection, as clinicians are poor at clinical recognition of seizure and nonseizure movements (Malone et al., 2009) and up to 85% of seizures in neonates are subclinical (electrographic only), meaning they would go undetected without EEG (Bye et al., 1995; Glass et al., 2016; Naim et al., 2015; Nash et al., 2011; Wietstock et al., 2016; Wusthoff et al., 2011). The majority of seizures occur in the central region (56%), occasionally in the temporal (25%) and occipital regions (14%), and rarely in the frontal regions (5%) (Shellhaas & Clancy, 2007). Thus, placement of electrodes over the central region is important for seizure detection.

1.7 Neurophysiology and neurodevelopmental outcomes

1.7.1 Background and neurodevelopmental outcomes

aEEG background abnormalities and recovery have been shown to be predictive of long-term neurodevelopmental outcome in term neonates with HIE (Awal et al., 2016; Azzopardi & Toby study group, 2014; Chandrasekaran et al., 2017; Dunne et al., 2017; Skranes et al., 2017; Spitzmiller et al., 2007; Thoresen et al., 2010; van Laerhoven et al., 2013). An early normal or mildly abnormal EEG is predictive of a favorable neurodevelopmental outcome (Awal et al., 2016; Murray et al., 2009; Pressler et al., 2001; Toet et al., 1999; Weeke et al., 2016). Whereas, EEG background abnormalities, in particular burst suppression, low voltage or a flat trace is associated with poor long-term neurodevelopmental outcomes (at 12 months of age or older) (Awal et al., 2016). However, even neonates with mild EEG abnormalities have reduced intact survival rates. A study that performed cognitive and motor assessments at 5 years of age showed that 25% of neonates with mild EEG abnormalities 6 hours after birth had lower cognitive scores that the healthy comparison group recruited at birth (Murray et al., 2016). Also, serial recordings improve the prognostic accuracy of EEGs, as progressive improvements in the background are associated with more favorable outcomes (Murray et al., 2009; Nash et al., 2011; Pressler et al., 2001), and the worsening of a background pattern or the development of an abnormal background is predictive of unfavorable outcomes (Khan et al., 2008). Thus, the evolution of aEEG background over time, using a classification system that defines specific patterns of
change in aEEG over the course of therapeutic hypothermia may also be useful for outcome prediction, rather than grading only at a discrete time interval (Sewell et al., 2018).

A meta-analysis of 8 studies prior to the introduction of therapeutic hypothermia showed a pooled sensitivity of 91% and specificity of 88% of early severe aEEG backgrounds to accurately predict poor neurodevelopmental outcome (Azzopardi & Toby study group, 2014; Spitzmiller et al., 2007). Suppressed aEEG background patterns within the first 24 hours of life were predictive of adverse outcome (al Naqeeb et al., 1999; Toet et al., 1999), however with therapeutic hypothermia, the predictive value of the aEEG is delayed until 24 to 48 hours of life. Still, a persistently abnormal aEEG background at 48 hours remains an important prognostic indicator (Chandrasekaran et al., 2017; Cseko et al., 2013; Massaro et al., 2012; Shellhaas et al., 2015; Thoresen et al., 2010). In a cohort of term neonates with HIE that received therapeutic hypothermia, the positive predictive value (PPV) of EEG background activity for abnormal outcome was 100% at 36 hours and 48 hours and the negative predictive value (NPV) was 75% at 36 hours and 69% at 48 hours (Weeke et al., 2016). Cumulative doses of morphine, phenobarbital or midazolam do not significantly delay aEEG recovery (Cseko et al., 2013).

Several EEG features have also been associated with unfavorable outcome including, background amplitude of <30 mcV, interburst interval of >60 seconds and electrographic seizures (Kontio et al., 2013; Menache et al., 2002; Murray et al., 2009; Obeid et al., 2017; Pressler et al., 2001; Shah et al., 2014b). Quantitative automated analysis of EEG discontinuity may improve the objectivity of EEG assessment in newborns. Dunne et al. (2017) calculated EEG discontinuity using a novel algorithm and showed that higher mean discontinuity at 24 hours and 48 hours were associated with severe cerebral tissue injury on MRI and unfavorable neurodevelopmental outcome at 24 months. Quantitative analysis of the total suppression length has also been associated with death or neurodevelopmental disability (Flisberg et al., 2011). More detailed EEG characteristics of discontinuous tracings relate to outcome and clinical features (Biagioni et al., 1999). Quantitative measures looking at novel features of bursts such as measures of burst area and duration and their interrelationships, may improve prognostic capabilities beyond the dichotomous classification of presence-versus-absence of a burst suppression pattern or length of IBIs (Iyer et al., 2014). An increase in EEG discontinuity may be seen with rewarming after therapeutic hypothermia, principally in neonates with severe HIE (Birca et al., 2016), although another study found that severity of HIE but not core body
temperature affected the EEG (Burnsed et al., 2011). Abnormal interhemispheric synchrony (defined as when the onset of bursts between hemispheres is separated by more than 1.5 seconds in discontinuous background) was also shown in a study to have good sensitivity and specificity for predicting survival without disability, especially when combined with EEG background analysis (Leroy-Terquem et al., 2017).

A support vector machine classifier using a multimodal combination of routine clinical markers, EEG and heart rate parameters was evaluated for neurodevelopmental outcome prediction at 24 months in newborn infants with HIE. They identified 12 multimodal features that provided promising prediction results, 9 of which were EEG features and 3 were clinical features. EEG achieved the highest performance as a predictor of clinical outcome (Temko et al., 2015). Accordingly, although therapeutic hypothermia has changed the time-frame in which tools such as aEEG and EEG are the most predictive of outcome, they are still the best objective tools to identify the highest-risk patients for poor outcome (Bonifacio et al., 2015).

In summary, EEG is a good marker of long-term neurodevelopmental outcome and is thus a useful surrogate marker in the acute setting in neonates with encephalopathy.

1.7.2 Sleep-wake-cycling and neurodevelopmental outcomes

Neonatal sleep involves endogenous driven brain activity and is fundamental for neuronal survival and guidance of brain networks (Dereymaeker et al., 2017; Scher et al., 2009). Long-term, neonatal sleep cycling has been shown to be a marker of brain function and enhance prediction of neurodevelopmental outcomes. The failure to develop SWC has been shown to strongly predict poor neurodevelopmental outcomes in neonates with HIE (Massaro et al., 2012; Murray et al., 2009; Osredkar et al., 2005; Takenouchi et al., 2011; Thoresen et al., 2010). A study in term neonates with HIE that received therapeutic hypothermia showed that failure to develop SWC by 45.5 hours of life was associated with adverse outcomes (Sewell et al., 2018). Another study in term HIE neonates treated with hypothermia further showed that the presence of SWC was a better predictor of a good outcome than the recovery of aEEG background pattern, while the absence of SWC was less likely to predict poor outcome then prolonged abnormal background pattern (Cseko et al., 2013). The amount of time spent in different awake
and sleep states can also be fragmented due to frequent handling and painful procedures in the NICU. Handling-related sleep deprivation may lead to an increase in quiet sleep (Axelin et al., 2013). Whereas in healthy term infants, SWC is present immediately after birth and is dominated by active sleep (Korotchikova et al., 2016).

Furthermore, a recent multimodality neuromonitoring study in a cohort of neonates at risk for cerebral dysfunction showed that inefficient neonatal sleep patterns were independent predictors of 18-month neurodevelopmental outcome evaluated with BSID-III. Eleven of 29 children in the cohort had HIE, of which 8 received therapeutic hypothermia. They used objective measures of sleep–wake physiology including conventional video EEG, cerebral near-infrared spectroscopy (NIRS), and a 12-hour full bedside polysomnography. Increased time spent in quiet sleep predicted worse cognitive and motor scores and lower 0.5-2Hz EEG power during quiet sleep predicted worse language and motor scores at 18-months (Shellhaas et al., 2017).

Thus, assessment of SWC on EEG enhances prediction of long-term neurodevelopmental outcomes with aEEG and cEEG.
1.7.3 Seizures and neurodevelopmental outcomes

This section is modified from work published in Current Opinion in Neurology:
Pinchefsky EF and Hahn CD. Outcomes following electrographic seizures and electrographic status epilepticus in the pediatric and neonatal ICUs.
Publication Date: 2017/04/01. DOI: 10.1097/WCO.0000000000000425

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A link to the published paper can be found at:
https://journals.lww.com/co-neurology/Abstract/2017/04000/Outcomes_following_electrographic_seizures_and.7.aspx

Abstract

Purpose of review:
Increasing recognition of electrographic seizures and electrographic status epilepticus in critically ill neonates and children has highlighted the importance of identifying their potential contributions to neurological outcomes in order to guide optimal management.

Recent findings:
Recent studies in children and neonates have found an independent association between increasing seizure burden and worse short-term and long-term outcomes, even after adjusting for other important contributors to outcome such as seizure etiology and illness severity. The risk of worse neurological outcome has been shown to increase above a seizure burden threshold of 12-13 minutes per hour, which is considerably lower than the conventional definition of status epilepticus of 30 minutes per hour. Randomized controlled trials in neonates have demonstrated that EEG-targeted therapy can successfully reduce seizure burden, but due to their small size these trials have not been able to demonstrate that more aggressive EEG-targeted treatment of both subclinical and clinical seizures results in improved outcome.

Summary:
Despite mounting evidence for an independent association between increasing seizure burden and worse outcome, further study is needed to determine whether early seizure identification and aggressive antiseizure treatment can improve neurodevelopmental outcomes.
Key points:

- Evidence has accumulated demonstrating an association between electrographic seizures, immediate pathophysiological changes and worse short-term and long-term neurodevelopmental outcomes in neonates and children, even after adjusting for potential confounders. However, a causal link between seizure burden and outcome remains to be proven.
- The observed association between seizure burden and outcome is potentially confounded by the contribution of the underlying etiology of the seizures to acute brain injury.
- Whether more timely electrographic seizure detection and more aggressive electrographic seizure management improves outcomes remains unknown.
- Further research is needed to delineate disease-specific seizure burden thresholds above which seizures have clinically relevant adverse effects that warrant aggressive antiseizure treatment.

INTRODUCTION

With increasing use of continuous electroencephalogram (cEEG) monitoring (Sanchez, Carpenter, et al., 2013), electrographic seizures and electrographic status epilepticus (ESE) are being increasingly recognized in critically ill neonates and children. Estimates of seizure prevalence in the pediatric ICU (PICU) populations have varied widely, with reports of electrographic seizures in 10-47% and ESE in 9-32% of critically ill children undergoing cEEG (Abend et al., 2013; Abend et al., 2011; Abend et al., 2009; Arndt et al., 2013; Gwer et al., 2012; Kirkham et al., 2012; Payne et al., 2014; Piantino et al., 2013; Sanchez, Arndt, et al., 2013; Schreiber et al., 2012; Shahwan et al., 2010; Topjian et al., 2013; Vaewpanich et al., 2016). This variability is likely due to variations in the PICU populations and variable indications for cEEG monitoring (Abend, 2015). In neonates at risk for seizures undergoing cEEG, the reported prevalence of electrographic seizures is 26-82%, and ESE is 16-25% (Glass et al., 2016; Laroia et al., 1998; McBride et al., 2000; Wietstock et al., 2016). Among specific neonatal subpopulations, ES have been observed in 30-65% of neonates with hypoxic ischemic encephalopathy (HIE) (Bashir et al., 2016; Glass et al., 2011; Glass et al., 2014; Kharoshankaya et al., 2016; Nash et al., 2011; Srinivasakumar et al., 2015; Wusthoff et al., 2011), 82% of neonates with stroke (Fox et al., 2016), and 8-13% of neonates and infants with congenital heart disease (CHD) in the perioperative period (Gaynor et al., 2013; Naim et al., 2015). Importantly,
up to 85% of seizures in neonates are subclinical (also known as electrographic only), meaning they would go undetected without EEG (Bye et al., 1995; Glass et al., 2016; Naim et al., 2015; Nash et al., 2011; Wietstock et al., 2016; Wusthoff et al., 2011). ‘Uncoupling’, a phenomenon by which ES persist after clinical seizures have ceased, is common in neonates, especially after administration of antiseizure medications (Glykys et al., 2009; Scher et al., 2003). Furthermore, it has been shown that clinical identification of seizures in neonates is unreliable, thus cEEG monitoring is vital for accurate detection (Malone et al., 2009).

There is growing evidence that electrographic seizures and ESE are associated with worse neurological outcomes. Greater awareness of the potential deleterious effects of seizures and the high seizure prevalence among at-risk patients has motivated recommendations for more widespread cEEG monitoring in the pediatric and neonatal ICU. However, the relationship between seizures, brain injury and outcomes is likely complex, and remains incompletely understood. This review will highlight recent evidence that has furthered our understanding of the potential contribution of electrographic seizures and ESE to brain injury and their relationship to short and long-term outcomes in neonates and children.

**POTENTIAL MECHANISMS FOR SEIZURE-INDUCED BRAIN INJURY**

Among critically ill children and neonates, electrographic seizures and ESE are recognized as an important biomarker of more severe brain injury. However, mounting evidence is revealing that seizures may independently contribute to brain injury through several plausible mechanisms. In critically-ill adults, seizures have been linked to glutamate-mediated worsening of cerebral edema (Vespa et al., 1998), long-term ipsilateral hippocampal atrophy (Vespa et al., 2010), transient increases in intracranial pressure (Vespa et al., 2007) and cortical spreading depression (Fabricius et al., 2008; Hartings et al., 2014). Pathological studies in both adults and children support the hypothesis of excitotoxic neuronal injury (Tsuchida et al., 2007). Recently, electrographic seizures in adults with severe traumatic brain injury were associated with a state of “metabolic crisis”, demonstrated by significant elevations in the lactate/pyruvate ratio measured by cerebral microdialysis (Vespa et al., 2016).

In neonates, there is also evidence that seizures are associated with impairments of energy metabolism and neuronal integrity. Clinical seizure severity has been associated with increased lactate/choline ratio and diminished N-acetylaspartate/choline ratio on magnetic
resonance spectroscopy (Miller et al., 2002). Neonates who experienced seizures during phosphorous-31 magnetic resonance spectroscopy demonstrated a 33% decrease in phosphocreatine during seizures, followed by a rapid normalization following administration of antiseizure medications (Younkin et al., 1986).

SEIZURES & OUTCOME – ASSOCIATION OR CAUSATION?
Several clinical studies have reported that electrographic seizures or ESE are associated with worse outcomes, even after adjusting for potential confounders such as diagnosis and illness severity (Abend et al., 2015; Arndt et al., 2013; Fox et al., 2017; Kirkham et al., 2012; O'Neill et al., 2015; Payne et al., 2014; Topjian et al., 2013; Wagenman et al., 2014). Although these observational studies support the hypothesis that seizures contribute to brain injury and worsen outcome, they cannot prove a causal link between higher seizure burden and worse outcome. Recently, this hypothesis has been tested by two noteworthy trials in neonates designed to evaluate whether more aggressive antiseizure treatment can decrease seizure burden, reduce secondary brain injury and improve neurodevelopmental outcomes. These trials employed amplitude-integrated EEG (aEEG) or cEEG monitoring to quantify seizure burden, and then randomized neonates at risk for seizures to either treatment of both clinical and subclinical (electrographic-only) seizures or treatment of clinical seizures alone.

The first Dutch-Flemish multicenter trial enrolled a total of 33 term neonates who developed seizures following moderate-to-severe HIE, and employed 1-channel aEEG monitoring to evaluate seizure burden. The primary outcome measure was an MRI injury score at 4-10 days after birth. Neonates treated for both clinical and subclinical seizures had a lower total seizure duration, however this was not significant, perhaps because only 8 of the 19 neonates in this group were appropriately promptly treated for seizures. Longer seizure duration was associated with worse MRI injury scores when analyzing the entire cohort, however there was no significant difference in MRI injury scores between the two treatment groups. The lack of difference may have been due to the small sample size, which resulted from difficulties with study recruitment (van Rooij, Toet, et al., 2010).

A second American single-center trial enrolled a total of 35 term neonates who developed seizures following moderate-to-severe HIE, and employed conventional cEEG monitoring to evaluate seizure burden. Outcome measures included MRI injury scoring and
neurodevelopmental outcome assessed at age 18-24 months using the Bayley Scales of Infant Development (BSID). Neonates treated for both clinical and subclinical seizures had a significantly lower seizure burden. Seizure treatment was initiated earlier in this group, but there was no difference in the number of antiseizure medications used or phenobarbital levels between the two groups. Higher seizure burden was correlated with worse brain injury and worse long-term neurodevelopmental outcome, but these findings were significant only in the combined cohort, and were not adjusted for HIE severity. When comparing the two treatment groups, there was no significant difference in MRI injury scores or neurodevelopmental outcome. Again, due to difficulties with recruitment, the study was likely underpowered to detect a difference between the treatment groups (Srinivasakumar et al., 2015).

In summary, there is now substantial evidence for an independent association between seizure burden and worse outcome, but these findings do not prove causation. Although randomized controlled trials in neonates have demonstrated that EEG-targeted therapy can successfully reduce seizure burden, due to their small size, these trials have not been able to demonstrate that more aggressive EEG-targeted treatment of both subclinical and clinical seizures results in improved outcome. Therefore, a causal link between electrographic seizures and outcome remains to be proven.

CRITICALLY ILL CHILDREN
Several observational studies in critically-ill children have examined short-term outcomes after electrographic seizures and ESE with a few recent studies examining longer-term outcomes. See Table 1.1 for a summary of the most important and recent studies.

Short-term outcomes
Several studies have found an association between ESE and an increased risk of mortality, even after adjusting for age, EEG background and neurological diagnosis (Abend et al., 2013; Lambrechtse n et al., 2008; Topjian et al., 2013). However, no such association has been found between increased electrographic seizures burden and mortality (Kirkham et al., 2012; Payne et al., 2014; Piantino et al., 2013; Sanchez Fernandez et al., 2014). ESE has also been associated with increased length of PICU stay (Abend et al., 2013).

In pediatric traumatic brain injury, the occurrence of seizures and ESE was associated with increased length of hospital stay (Arndt et al., 2013; O’Neill et al., 2015) and ICU stay
(O’Neill et al., 2015), lower King’s Outcome Scale for Childhood Head Injury (KOSCHI) scores at hospital discharge (Arndt et al., 2013), worse Glasgow Outcome Scale scores (GOS-E Peds), and worse neurocognitive and functional evaluations (Vaewpanich et al., 2016).

Higher seizure burden has also been associated with worse short-term outcomes in several studies. A prospective observational study of a combined cohort of 204 comatose neonates and children using 1-3 channel EEG showed that the presence of electrographic seizures was associated with an unfavorable neurological outcome (severe handicap or persistent vegetative state) at 1 month in a multivariate analysis adjusting for etiology, age, pediatric index of mortality, Adelaide coma score, and EEG background. Moreover, no children had favorable outcomes if they had greater than 139 seizures, their total duration of electrographic seizures was greater than 759 minutes, or any individual seizure lasted longer than 360 minutes (Kirkham et al., 2012). A prospective observational study of 200 children undergoing cEEG for acute encephalopathy found that ESE, but not electrographic seizures, was associated with higher mortality and worse short-term outcome, measured as Paediatric Cerebral Performance Category (PCPC) score at ICU discharge, even after adjusting for age, acute neurologic disorder, prior neurodevelopmental status and EEG background category (Topjian et al., 2013). Conversely, a study of 84 children with non-traumatic coma showed that both seizures and status epilepticus were associated with a similarly increased risk of poor outcome (death or gross motor deficits at discharge) (Gwer et al., 2012).

To further investigate the relationship between seizure burden and outcome, a prospective observational single-center study of 259 critically ill neonates and children quantified the maximum hourly seizure burden on cEEG and correlated this with the PCPC score at hospital discharge, adjusting for diagnosis and illness severity. On multivariable analysis, for every 1% increase in maximum hourly seizure burden the odds of neurological decline increased by 1.13. Furthermore, above a maximum hourly seizure burden threshold of 12 minutes, there was a marked increase in both the probability and magnitude of neurological decline. These observations of a seizure burden “dose effect” strengthen the case for an association between seizures and outcome, and identify an hourly seizure burden threshold of 12 minutes as a potential therapeutic target (Payne et al., 2014). This study also observed that the magnitude of the relationship between seizure burden and outcome appears to depend on the underlying cause of seizures. For example, seizures in the context of anoxic brain injury appear to have less
influence on outcome, whereas seizures in the context of stroke or meningitis may have a greater influence on outcome (Figure 1.9). Further work is required to more precisely define these varying relationships between seizure burden and outcome, in order to inform patient-specific decisions on how aggressively to treat electrographic seizures (Hahn et al., 2013).

**Figure 1.9** Schematic illustration of potential relationship between seizure burden and outcome. The potential deleterious effects of seizures in the context of acute brain injury are likely to depend on the underlying cause. The probability of poor outcome might increase linearly or exponentially with increasing seizure burden, or a threshold might exist, above which seizures are harmful. Previously published by Hahn and Jette (Hahn et al., 2013).

**Long-term outcomes**

One study has assessed the impact of seizures on long-term outcomes in critically ill children. Long-term follow-up was obtained on 60/137 children who were previously neurodevelopmentally normal, from an original cohort of 300 critically ill children who underwent clinically-indicated cEEG monitoring (Abend et al., 2015; Wagenman et al., 2014). Among these 60 children, those with ESE but not electrographic seizures demonstrated worse long-term functional outcome scores (GOS-E Peds), a lower health-related quality of life, and were more likely to develop epilepsy, even after controlling for age, acute neurological diagnosis and EEG background category. A favorable long-term functional outcome was seen in 64% of critically ill children without seizures, but in only 13% of those with ESE (Wagenman et al., 2014). Furthermore, both electrographic seizures and ESE were associated with worse adaptive functioning scores on multivariable analysis (Abend et al., 2015).
### Table 1.1 Recent studies of interest on outcomes following electrographic seizures and electrographic status epilepticus in children and neonates

<table>
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<th>References</th>
<th>Population and comorbidities</th>
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<tr>
<td>Vaewpanich et al. 2016</td>
<td>16 patients with TBI</td>
<td>cEEG</td>
<td>SE = ongoing seizure &gt;30 min</td>
<td>Short-term (at discharge and 4-6 wks post-discharge)</td>
<td>- All patients with seizures had poor outcome on the GOS-Epeds, and speech pathology neurocognitive/functional evaluations (SPNFE)</td>
</tr>
<tr>
<td>Abend et al. 2015</td>
<td>60 patients with normal neurodevelopment prior to PICU admission for AMS</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or SB &gt;50% in any 1-h epoch</td>
<td>Long-term (median 2.6 years)</td>
<td>- ES and ESE were associated with worse adaptive functioning scores on multivariate analysis - Non-significant trend to worse scores on behavioral-emotional &amp; executive function scales after ESE</td>
</tr>
<tr>
<td>O’Neill et al. 2015</td>
<td>144 patients with TBI</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or &gt;50% of EEG recording</td>
<td>Short-term (at discharge)</td>
<td>- Presence of seizures did not correlate with discharge disposition - Seizures associated with longer hospital and ICU stay (no difference between ESE compared to ES)</td>
</tr>
<tr>
<td>Payne et al. 2014</td>
<td>259 paediatric and neonatal patients admitted to ICU with clinically ordered cEEG</td>
<td>cEEG</td>
<td>Maximum hourly SB &gt;20%/h</td>
<td>Short-term (at discharge)</td>
<td>- No association with mortality - ↑ probability and magnitude of neurologic decline with SB &gt; 20% (12 minutes) - On multivariate analysis, every 1% ↑ in maximum hourly SB was associated with a 1.13 odds of neurological decline</td>
</tr>
<tr>
<td>Sanchez Fernandez et al. 2014</td>
<td>98 patients with CSE who underwent cEEG monitoring</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or &gt;50% SB in any 1-h epoch</td>
<td>Short-term (at discharge)</td>
<td>- No association with mortality - Longer PICU stay if ES and significantly longer if ESE</td>
</tr>
<tr>
<td>Wagenman et al. 2014</td>
<td>60 patients with normal neurodevelopment prior to PICU admission for AMS</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or SB &gt;50% in any 1-h epoch</td>
<td>Long-term (median 2.7 years)</td>
<td>- ESE, but not ES, associated with worse long term functional outcome scores and lower health-related quality of life - ESE associated with ↑ risk of subsequent diagnosis of epilepsy</td>
</tr>
<tr>
<td>Abend et al. 2013</td>
<td>550 consecutive PICU patients who underwent cEEG monitoring</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or SB &gt;50% in any 1-h epoch</td>
<td>Short-term (at discharge)</td>
<td>- ESE, but not ES, associated with greater odds of in-hospital death - ↑ PICU LOS if ESE compared to no ES, and compared to ES without ESE</td>
</tr>
<tr>
<td>Arndt et al. 2013</td>
<td>87 patients with TBI</td>
<td>cEEG</td>
<td>SE = seizure &gt;15 min or &gt;3 seizures/h</td>
<td>Short-term (at discharge)</td>
<td>- ESE associated with ↑ hospital LOS (no difference in PICU LOS) - ES and ESE associated with ↓ discharge KOSCHI score</td>
</tr>
<tr>
<td>Study</td>
<td>Patients/Population Description</td>
<td>Methodology</td>
<td>EEG Criteria</td>
<td>Outcome of EEG Associations</td>
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<tr>
<td>Piantino et al. 2013</td>
<td>19 patients on ECMO</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or SB &gt;50% in any 1-h epoch</td>
<td>Short-term (at discharge) - ES were associated with structural abnormality on MRI - ES not associated with ↑ mortality - Trend for those with ES having more prolonged ECMO course - No difference in rate of complications</td>
<td></td>
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<tr>
<td>Topjian et al. 2013</td>
<td>200 patients with acute encephalopathy</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or SB &gt;50% in any 1-h epoch</td>
<td>Short-term (at discharge) - ESE, but not ES, were associated with ↑ risk mortality - No difference in PICU or hospital LOS - ESE, but not ES, were associated with PCPC worsening</td>
<td></td>
</tr>
<tr>
<td>Gwer et al. 2012</td>
<td>82 patients with acute nontraumatic coma</td>
<td>cEEG</td>
<td>ES = &gt;6 sec; ESE = seizure &gt;30 min or ≥3 seizures/hr</td>
<td>Short-term (at discharge) - ES and SE associated with greater risk of poor outcome (death or gross motor deficits at discharge) - SE had even greater risk of poor outcome</td>
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<tr>
<td>Kirkham et al. 2012</td>
<td>204 comatose pediatric and neonatal patients</td>
<td>1-3 channel cEEG/ aEEG</td>
<td>Number, total duration, and longest ES</td>
<td>Short-term and long-term (for 84 of the survivors) - ES associated with unfavourable short-term outcome (severe handicap or vegetative state) - No association with mortality</td>
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<td>Studies in Critically Ill Neonates</td>
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<tr>
<td>Glass et al. 2016</td>
<td>426 neonates with clinically suspected +/- EEG seizures</td>
<td>cEEG</td>
<td>High SB=≥7 ES; ESE=seizures &gt;50% of at least 1-h of recording</td>
<td>Short-term (at discharge) - High SB was a risk factor for mortality, ↑ hospital LOS, and abnormal neurological exam at discharge</td>
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</tr>
<tr>
<td>Kharoshankaya et al. 2016</td>
<td>47 neonates with HIE</td>
<td>cEEG</td>
<td>Total SB &gt;40 min. Maximum hourly SB &gt; 13 min</td>
<td>Long-term (24-48 months) - Presence of seizures alone was not associated with abnormal outcome - A total SB of &gt;40 min and a maximum hourly SB of &gt;13 min/hr were associated with ↑ risk of abnormal development</td>
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<tr>
<td>Srinivasakumar et al. 2015</td>
<td>69 neonates with HIE</td>
<td>cEEG</td>
<td>SB recorded in seconds</td>
<td>Short-term and long-term (18-24 months) - ↑ SB associated with higher brain injury score - ↑ SB in combined cohort associated with lower performance on all 3 domains of BSID (cognitive, motor, language)</td>
<td></td>
</tr>
<tr>
<td>Shah et al. 2014</td>
<td>85 neonates with HIE</td>
<td>aEEG</td>
<td>High SB = SB &gt;15 min per 1-h epoch ESE = SB &gt;30 min per 1-h epoch</td>
<td>Short-term (MRI brain at median 9 days of life) - High SB independently associated with greater injury on MRI (adjusted for 10 min Apgar and abnormal aEEG background)</td>
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<tr>
<td>Study</td>
<td>Participants</td>
<td>EEG Type</td>
<td>Criteria</td>
<td>Outcome</td>
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<tr>
<td>Glass et al. 2011</td>
<td>56 neonates</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or SB &gt;50% of 1–3 hrs of recording time</td>
<td>Short-term (MRI brain at median 5 days of life) – Moderate to severe MRI injury more common in newborns with seizures, and present in all with SE – Moderate to severe MRI injury associated with seizures that were multifocal, later onset, and ongoing seizures following 20 mg/kg phenobarbital</td>
<td></td>
</tr>
<tr>
<td>Nash et al. 2011</td>
<td>41 neonates</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or SB &gt;50% of 1–3 hrs of recording time</td>
<td>Short-term (MRI brain at median 5 days of life) – Isolated or recurrent seizures were more frequent in patients with moderate to severe MRI injury compared with those with no or mild injury – SE was only seen in newborns with moderate to severe MRI injury</td>
<td></td>
</tr>
<tr>
<td>van Rooij et al. 2010</td>
<td>42 neonates</td>
<td>aEEG</td>
<td>Single seizure, ≥3 seizures per 30 min, or SE = seizure &gt;30 min</td>
<td>Short-term (MRI brain at mean 5.5 days of life) – Severity of brain injury on MRI associated with a longer duration of seizure patterns in the blinded group and the whole cohort</td>
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</tbody>
</table>

### Studies in Neonates and Infants with CHD Undergoing Cardiac Surgery

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>EEG Type</th>
<th>Criteria</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naim et al. 2015</td>
<td>161 neonates</td>
<td>cEEG</td>
<td>SE = seizure &gt;30 min or SB &gt;30 min in any 1-h epoch</td>
<td>Short-term (at discharge) – ↑ Mortality among neonates with seizures – No difference in cardiac ICU or hospital LOS</td>
</tr>
<tr>
<td>Gaynor et al. 2013</td>
<td>132 neonates</td>
<td>cEEG</td>
<td>ES &gt;10 sec</td>
<td>Long-term (4 years) – ES were associated with worse executive function and impaired social interactions/restricted behaviour – ES were not associated with worse cognitive, language, or motor skills</td>
</tr>
<tr>
<td>Bellinger et al. 2011</td>
<td>139 infants</td>
<td>cEEG</td>
<td>ES &gt;6 sec</td>
<td>Long-term (16 years old) – ES in the postoperative period was the medical variable most consistently related to worse outcomes (academic achievement, memory, executive function, visual-spatial skills, and social cognition)</td>
</tr>
</tbody>
</table>

aEEG, amplitude integrated electroencephalogram; AMS, altered mental status; BSID, Bayley Scales of Infant Development; cEEG, continuous electroencephalogram; CHD, congenital heart disease; CSE, convulsive status epilepticus; ECMO, extracorporeal membrane oxygenation; ES, electrographic seizures; GOS-E Peds, Glasgow Outcome Scale scores; HIE, hypoxic-ischemic encephalopathy; KOSCHI, King’s Outcome Scale for Childhood Head Injury; LOS, length of stay; PCPC, Paediatric Cerebral Performance Category; SB, seizure burden; SE, status epilepticus; TGA, transposition of the great arteries.
CRITICALLY ILL NEONATES

Several studies have now shown that neonatal seizures and status epilepticus are associated with worse outcomes even after adjusting for potential confounding variables, and support the hypothesis that seizures worsen existing brain injury (see Table 1.1). However, increasing seizure burden is often accompanied by greater use of antiseizure medications such as phenobarbital and phenytoin, which have been shown to have neurotoxic effects in rodent models (Bittigau et al., 2002; Bittigau et al., 2003). Disentangling the potential deleterious effects of seizures from the potential toxicity due to their treatment remains an important challenge.

**Short-term and surrogate outcomes**

Studies in neonates undergoing therapeutic hypothermia for HIE have shown a relationship between higher seizure burden and worse MRI injury scores in univariate analysis (Glass et al., 2011; Nash et al., 2011; Srinivasakumar et al., 2015; van Rooij, Toet, et al., 2010). In two of these studies, all neonates with status epilepticus had moderate to severe injury on imaging (Glass et al., 2011; Nash et al., 2011). In multivariable analysis, a higher seizure burden on aEEG (more than 15 minutes in any 1-h epoch) was associated with greater injury on MRI, independent of aEEG background and Apgar score at 10 min (Shah et al., 2014a).

A multicenter cohort study described 426 term and preterm neonates with various diagnoses who underwent cEEG to characterize clinically suspected seizures or screen for subclinical seizures. Electrographic seizures were found in 82% of neonates. In a multivariable analysis, high seizure burden (defined as ≥7 electrographic seizures in total) was associated with mortality, longer hospital stay, and abnormal neurological examination at discharge (defined as abnormalities in consciousness, tone, and/or reflexes) (Glass et al., 2016).

**Long-term outcomes**

Seizures have also been linked to long-term neonatal outcomes. In a study of 47 neonates with HIE who underwent cEEG monitoring, outcomes were assessed at 24 to 48 months using either the Griffiths Mental Development Scales or BSID, as well as clinical diagnoses of cerebral palsy or epilepsy. The presence of seizures *per se* was not associated with abnormal long-term outcomes, but high total seizure burden and maximum hourly seizure burden were associated with abnormal outcome. For every 1-min increase in total seizure burden the odds of an
abnormal outcome increased by 2.2%, and for every 1-min increase in maximum hourly seizure burden the odds of an abnormal outcome increased by 16%. The seizure burden thresholds used to predict greater odds of abnormal outcome were a total seizure burden more than 40 min or a maximum hourly seizure burden more than 13 min, and these findings were independent of HIE severity or the use of therapeutic hypothermia (Kharoshankaya et al., 2016).

Further support for clinically relevant electrographic seizure burden thresholds include observations that a total neonatal seizure count more than 75 was associated with microcephaly, severe CP, and failure to thrive (McBride et al., 2000), an ‘ictal fraction’ more than 10 min was associated with an unfavorable neurodevelopmental outcome at 18 months (Pisani et al., 2008), and that status epilepticus was a stronger risk factor than recurrent seizures for poor neurodevelopmental outcome and postneonatal epilepsy at 24 months of age (Pisani et al., 2007).

**NEONATES AND INFANTS WITH CONGENITAL HEART DISEASE**

Post-operative seizures in children with CHD have been associated with worse outcome. A study that systematically utilized cEEG for 48 hours in 161 neonates upon return to the ICU following neonatal cardiac surgery found electrographic seizures in 8%, of whom 62% had ESE. Seizures were subclinical in 85% of neonates. The presence of electrographic seizures was associated with higher mortality (Naim et al., 2015).

Studies examining long-term outcomes after cardiac surgery have demonstrated that later neurodevelopmental difficulties can emerge that may not have been evident at younger ages. The greater academic and psychosocial demands placed on children as they grow older can reveal neurodevelopment abnormalities potentially associated with increased seizure burden in the developing brain. From an initial cohort of 178 neonates and infants with cardiac surgery requiring cardiopulmonary bypass who underwent 48 h of cEEG monitoring in the postoperative period, neurodevelopmental outcomes assessed at age 1 year in 114 children showed that postoperative electrographic seizures were not associated with worse outcomes on the BSID, except in the subset of children with frontal-onset seizures (Gaynor et al., 2006). When 132 children of the original cohort were subsequently assessed at age 4 years, postoperative electrographic seizures were associated with worse executive function and impaired social interactions or restricted behaviours, but were not associated with cognitive, language, or motor outcomes (Gaynor et al., 2013).
The Boston Circulatory Arrest Study reported on successive long-term follow-up at age 1, 4, 8, and 16 years in a cohort of 171 infants with transposition of the great arteries that underwent cardiac surgery requiring cardiopulmonary bypass during infancy. cEEG was performed for at least 2 h pre-operatively, during surgery and for 48 h following surgery. However, cEEGs were not interpreted clinically; therefore, electrographic seizures remained unrecognized and untreated. Electrographic seizures were found in 20% and clinical seizures in 6% (Helmers et al., 1997). At age 4 years, increased seizures in the postoperative period were associated with lower mean intelligent quotient scores and an increased risk of abnormalities on neurological exam (Bellinger et al., 1999). At age 8 years, postoperative seizures were associated with social and attention problems (Bellinger et al., 2009). At age 16 years, seizures in the postoperative period was the medical variable most consistently related to worse outcomes, specifically academic achievement, memory, executive function, visual-spatial skills, and social cognition (Bellinger et al., 2011).

CONCLUSIONS
Seizures are a cardinal sign of underlying brain injury. There is mounting evidence that higher seizure burden during critical illness in neonates and children is associated with worse neurodevelopmental outcomes, even after adjusting for potential confounding variables such as age, acute encephalopathy etiology, critical illness severity, MRI injury severity, EEG background, Apgar scores or use of therapeutic hypothermia. Both animal and human studies have identified plausible mechanisms by which seizures may cause secondary brain injury. To date, however, clinical trials have been unable to demonstrate that lowering seizure burden through more aggressive treatment improves MRI brain injury or short-term outcome. Therefore, a causal link between increased seizure burden and worse outcomes remains unproven. Larger randomized controlled trials will be required to distinguish the impact of seizure burden from other non-modifiable factors such as age, genetic background, seizure etiology and severity of brain injury, as well as the potential adverse effects of antiseizure medications.
1.8 Glucose disturbances and EEG

1.8.1 Hypoglycemia and EEG changes

Few studies have examined acute EEG changes associated with hypoglycemia. In newborns, studies using aEEG have not been able to detect clear changes during hypoglycemia except for one case report. A study of 12 neonates of diabetic mothers used subcutaneous microdialysis catheters for glucose measurements and visual interpretation of one-channel aEEG monitoring (performed calculation of the baseline level, the amplitude and the frequency distribution). Segments of aEEG during hypoglycemia (<2.2 mmol/L) were compared to recordings during normoglycemia (>2.5 mmol/L) and no significant changes on aEEG during episodes of hypoglycemia were found (Stenninger et al., 2001). Harris et al. used continuous interstitial glucose monitoring and concurrent 3-channel aEEG (C3-P3, C4-P4, O1-O2) recordings in a cohort of 101 well babies (≥32 weeks GA) at risk for hypoglycaemia. They added an occipital channel to the standard aEEG because the occipital regions may be most sensitive to hypoglycemic damage. Twenty-four of the neonates had low interstitial glucose concentrations (<2.6 mmol/L) during the period with matching aEEG data, and a total of 103 hypoglycemic episodes were recorded. Total EEG intensity (µV²), spectral edge frequency, amplitude and continuity measures were assessed, and manual review was performed by a pediatric neurologist for seizures. No changes on aEEG recordings were identified between 20-minute periods of low glucose concentration and adjacent 20 minutes of normoglycemia from the same baby (Harris et al., 2011). The same group had similarly used a modified 3-channel aEEG (parietal and occipital montages) in newborn lambs during insulin-induced hypoglycemia and had also not detected changes in any of the measured aEEG parameters during hypoglycemia (D. L. Harris et al., 2009). Although most studies using aEEG failed to detect any changes during hypoglycemia, a case report of a term newborn with sepsis and necrotizing enterocolitis detected changes on single channel aEEG during periods of hypoglycemia. Seizures were suspected initially during hypoglycemia. This was followed by very little aEEG activity, but the aEEG activity returned to normal when blood glucose levels improved (Hellstrom-Westas et al., 1989).

In contradistinction to the clinical experience, animal models of insulin-induced hypoglycemia studies have shown progressive background slowing of the EEG as glucose levels fall. In newborn dogs, slowing was seen at or below glucose levels of 1.5 mmol/L and
progressed to paroxysmal discharges and convulsive seizures as glucose levels below 0.6 mmol/L. A burst suppression pattern was seen in 2 animals at glucose levels of 0.1 mmol/L and 0.3 mmol/L (Vannucci et al., 1981). Another study by Vannucci et al. using insulin-induced hypoglycemia in newborn dogs suggested that the EEG response to hypoglycemia may depend not only on the concentration of glucose in the brain but also on the presence of associated metabolic derangements. No change was seen on EEG in the newborn dogs with hypoglycemia alone, but the EEG became isoelectric following respiratory arrest (Vannucci et al., 1980). Studies in adult rats have also shown a slowing of the EEG pattern during insulin-induced hypoglycemia as well as convulsive polyspike activity and progression to burst suppression and an isoelectric EEG (Agardh et al., 1983; Auer et al., 1984; Bryan et al., 1994; Lewis, Ljunggren, Norberg, et al., 1974; Lewis, Ljunggren, Ratcheson, et al., 1974; Norberg et al., 1976). In adult rats no pathological brain damage was seen unless an isoelectric or burst suppression pattern was seen on the EEG during hypoglycemia (Auer et al., 1984).

As opposed to neonates, young children may manifest changes on the EEG during hypoglycemia. An interesting study in diabetic and nondiabetic children performed quantitative spectral analysis of the EEG during a gradual decline in glucose concentrations using insulin. At a plasma glucose concentration of 4 mmol/L, an increase in delta and theta amplitudes were seen in both groups. At 3 mmol/L, there was a further and more widespread increase in low-frequency EEG activity, with greater slowing and epileptiform discharges seen in the diabetic than nondiabetic children (Bjorgaas et al., 1998).

There are also several case reports of hypoglycemia in children with concomitant EEG monitoring. A case series of idiopathic spontaneous hypoglycemia describes a 3-year-old girl with recurrent hypoglycemia who had an EEG performed during the fasting state (blood glucose of 2.3 mmol/L) which demonstrated slow wave activity which improved after treatment and glucose improved to 4.1 mmol/L. In the same series, a 10-month-old girl’s EEG showed high voltage, widespread slow activity while her blood glucose was 1.9 mmol/L. Repeat EEG several days later when the blood glucose was 2.3 mmol/L continued to show slow activity, although it had improved from previous recordings. At that point the child was treated and 30 minutes later with a blood glucose of 4.2 mmol/L the EEG had normalized (Haworth et al., 1960). Another case reported in this series was that of an 11-month-old girl with hypoglycemia (0.3 mmol/L) and coma due to an accidental overdose of a sulfonylurea. The EEG recording commenced.
during treatment with IV dextrose, and demonstrated asymmetric irregular medium amplitude slow activity appearing in runs as well as runs of irregular fast activity, periodic suppression and frequent seizures. The EEG gradually improved but after 2 weeks there were still persistent abnormalities (excess irregular slow activity and small spikes or sharp waves). Long term the child developed epilepsy with an EEG that showed persistent abnormalities (Pavone et al., 1980).

Finally, one study in neonates demonstrated persisting EEG changes within one week following symptomatic neonatal hypoglycemia (plasma glucose levels <2.2 mmol/L). Conventional 50-minute EEG recordings were performed in a cohort of 20 newborns with hypoglycemia. The authors observed an increased density of frontal sharp transients in all stages of sleep and less bilateral synchrony compared to controls. However, the cohort of neonates with hypoglycemia had various associated pathologies (such as hydrocephalus, asphyxia, sepsis, toxoplasmosis) whereas controls were normal newborns with no evidence of CNS or other disorders (Lahorgue Nunes et al., 2000). Another study had examined a cohort of 28 children of diabetic mothers, of which 13 children had neonatal hypoglycemia (<1.5 mmol/L), for persistent EEG changes at 8 years of age. Quantitative frequency analysis of the EEGs found no significant difference between children with or without neonatal hypoglycemia in maximal power frequency or the relative power frequency distribution quotient. However, a lower relative delta power frequency distribution in the frontotemporal region and a higher relative alpha power frequency distribution in frontal and parietal regions was identified in children with neonatal hypoglycemia vs. those without (Stenninger et al., 1998).

### 1.8.2 Hyperglycemia and EEG changes

In preterm neonates, hyperglycemia has been shown to be associated with acute EEG and aEEG changes (Granot et al., 2012; Schumacher et al., 2014; Wikstrom et al., 2011). In extremely preterm neonates (22-27 weeks GA) Wikström et al. found a U-shaped relationship with plasma glucose levels, whereby a greater discontinuity was seen on aEEG at plasma glucose concentrations both above or below 4.0 mmol/L. Moderate hyperglycemia was significantly associated with greater discontinuity on aEEG. These authors measured IBIs using an automated algorithm and used the average IBI from 10-minute artifact-free epochs before each blood sample. In addition, it was observed that increasing \( \text{PaCO}_2 \) was also associated with longer IBIs.
and aEEG power was attenuated at both higher and lower PaCO$_2$ values (Wikstrom et al., 2011). Similarly, a prospective cohort study by Granot et al. of premature neonates (<28 weeks’ gestation) during the first 3 days of life found that higher levels of blood glucose were significantly associated with depressed cerebral activity on aEEG. aEEG patterns were assessed in the 30 minutes preceding blood sampling. Respiratory acidosis also was correlated with depressed cerebral activity (Granot et al., 2012). Using cEEG monitoring (8 EEG electrodes) Schumacher et al. showed that increased blood glucose concentrations were associated with decreasing total absolute band power during the first 3 days of life in premature infants (<30 weeks GA). They calculated the mean total absolute band power on each day and correlated it with mean glucose levels and highest glucose levels in each neonate. These authors also observed an effect of hypercapnia on EEG, with decreased total absolute band power (Schumacher et al., 2014).

A study in fetal sheep that examined the effects on EEG with glucose infusion prior to ischemic insult showed that the amplitude and power of the EEG during ischemia was significantly higher in animals given glucose infusions than saline alone. Further, higher blood glucose concentrations appeared to help maintain EEG activity during ischemia. In this study, ischemia caused cerebral oxygen consumption to decrease, glucose uptake to increase and a net efflux of lactate to occur. The authors hypothesized that elevated blood glucose helped maintain EEG activity during ischemia perhaps by fueling additional anaerobic energy production (Chao et al., 1989). However, Lear et al. demonstrated in fetal sheep that although higher glucose levels improve neurophysiologic adaptation to asphyxia, there is more marked secondary deterioration and impaired neurophysiologic recovery (Lear et al., 2017). Their findings were consistent with the so-called ‘‘glucose paradox’’ that is seen in adult animals, whereby hyperglycemia is associated with improved metabolic function during hypoxia-ischemia, but later development of greater brain injury (Lear et al., 2017; MacDougall et al., 2011). In preterm fetal sheep (equivalent to 28-32 weeks human fetuses) either dexamethasone, glucose infusion or saline were injected before asphyxia. The authors found a higher EEG power and attenuated rate of rise of cortical impedance in dexamethasone and glucose treatment groups during asphyxia. However, the dexamethasone and glucose treatment groups showed a marked secondary rise in cortical impedance and impaired recovery of spectral edge frequency. Dexamethasone and glucose treatment were also associated with increased numbers of post-asphyxial seizures. At the
end of the experiment, there were burst suppression patterns seen in the dexamethasone and glucose treatment groups which was not observed in the saline-asphyxia group (Lear et al., 2017).

A study in rats also showed that recovery of EEG activity following cerebral ischemia was severely impaired in hyperglycemic animals. Following cerebral ischemia, the cerebral activity reappeared later in the hyperglycemic group with persistent burst suppression pattern seen at 1 hour, whereas the EEG of normoglycemic group had normalized by that time (Siemkowicz et al., 1981).

Lastly, we could also consider studies in children with hyperglycemia. A study used CGM and simultaneous EEG monitoring for 40 hours in children with type 1 diabetes and found that asymptomatic hyperglycemia (>11 mmol/L and mainly >15.5 mmol/L) was associated with changes in the power spectra of different frequency bands. During wakefulness, hyperglycemia was associated with increased EEG power of low frequencies (theta and delta) and decreased EEG power of high frequencies (beta and gamma). During sleep, hyperglycemia was associated with increased EEG power of low frequencies in all areas and increased high frequencies in frontal and central regions (Rachmiel et al., 2016).

In summary, a number of animal and clinical studies suggest that hypoglycemia may be associated with progressive slowing on the EEG which may not be detected on aEEG monitoring except perhaps in the presence of critical illness or associated metabolic derangements. However, hyperglycemia is more consistently associated with increased slow activity, increased discontinuity and depressed cerebral activity. Changes with hyperglycemia have been detected on both aEEG and EEG monitoring.
1.9 Research Hypothesis and Aims

The primary objective of this study was to understand the relationship between glucose levels and brain activity in neonates with encephalopathy.

Hypoglycemia and hyperglycemia are common problems in neonates and may be associated with greater brain injury and worse neurodevelopmental outcomes in neonatal encephalopathy. However, the level and duration of hypo- and hyperglycemia that is harmful to a neonate’s developing brain is not known. There is some evidence that glucose derangements can cause acute neurophysiologic changes, but the specific effects of blood glucose disturbances on the EEG in neonatal encephalopathy remain poorly understood. EEG abnormalities and recovery are predictive of long-term neurodevelopmental outcome in neonates with HIE. Thus, these investigations aim to detect changes in global brain function associated with hypo- and hyperglycemia using aEEG and cEEG monitoring, and assess whether cEEG monitoring could be a suitable method to determine the degree and duration of hypo- and hyperglycemia that correlate with changes in global brain function to aid in determining clinically significant target glucose ranges in these neonates.

1.9.1 Aims

1. To determine whether abnormal glucose levels and glucose variability in neonates with encephalopathy are associated with immediate changes in brain background activity and electrographic seizures, as measured using aEEG.

2. To determine whether abnormal glucose levels in neonates with encephalopathy are associated with immediate changes in brain background activity and electrographic seizures, as measured using cEEG.
1.9.2 Hypothesis

In keeping with our primary aims, we will test the following hypotheses:

1. Hypoglycemia, hyperglycemia and glucose variability in neonates with encephalopathy are associated with worse brain background activity and increased electrographic seizures on aEEG.

2. Hypoglycemia and hyperglycemia in neonates with encephalopathy are associated with abnormal background activity and increased electrographic seizures on cEEG.
Chapter 2

2 Methods

2.1 Study population

In an ongoing prospective cohort study enrolling term neonates with encephalopathy transferred to The Hospital for Sick Children, subjects enrolled between August 2014 and March 2017 were considered for this analysis. This comprises a consecutive convenience sample of the Neurological Outcome of Glucose in Neonatal encephalopathy (NOGIN) study cohort. The institutional research ethics board approved the study protocol and the parents or guardians of all participating neonates provided written informed consent. Newborns with encephalopathy were eligible if they had abnormal consciousness, in addition to either neonatal seizures or abnormalities in tone or reflexes. Exclusion criteria included suspected or confirmed congenital malformations, inborn errors of metabolism, congenital infections, gestational age <36 weeks, weight <1500 grams or if it was expected that a CGM could not be attached within 6 hours of life.

Clinical data was collected from patient medical records. All point of care testing and laboratory glucose measurements obtained clinically were collected. Point of care testing performed with i-STAT® (Abbott Laboratories, Abbott Park, Illinois, USA) uses the gold standard glucose oxidase reaction, and was thus considered as laboratory values. Study data were stored and managed using research electronic data capture (REDCap) hosted at The Hospital for Sick Children (P. A. Harris et al., 2009).

Therapeutic hypothermia (TH) was initiated within six hours after birth if clinically indicated, with target temperature (33–34 °C core temperature) continued for 72 hours followed by gradual rewarming by 0.5 °C per hour over 6 hours. Low-dose morphine or fentanyl was provided during hypothermia as needed.
2.2 Amplitude-integrated electroencephalography (aEEG) monitoring and analysis

Amplitude-integrated electroencephalography was commenced as soon as possible after admission as part of routine clinical care and interpreted by the neonatology team. Subsequent research analysis of aEEG recordings was performed by a single neurologist (E.F.P.), blinded to clinical course, glucose levels, and neurological outcome. Only dual channel aEEG recordings (C3-P3, C4-P4) were included. Over 6-hour epochs, the aEEG background was graded on an ordinal scale: 0: continuous normal voltage (CNV, aEEG maximum >10 µV and minimum >5 µV); 1: discontinuous normal voltage (DNV, aEEG maximum >10 µV and minimum ≤5 µV); 2: burst suppression pattern (virtual absence of activity [<2 µV] between bursts of high voltage [>25 µV]); 3: continuous low-voltage (CLV, aEEG maximum ≤10 µV); and 4: flat trace (isoelectric activity, aEEG maximum ≤5 µV). The predominant, best and worst background score present in each epoch were graded. Sleep-wake cycling was graded as: 0: developed cycling; 1: immature cycling; and 2: no cycling. Seizures were graded as: 0: no seizures; 1: single seizure; 2: repeated seizures (2 or more); and 3: status epilepticus (‘saw-tooth pattern’), defined as continuous or recurrent seizures lasting at least 30 minutes, or for more than 50% of the recording time (Hellström-Westas et al., 2008; Hellström-Westas et al., 2006; Oh et al., 2013; Oh et al., 2015; Thoresen et al., 2010; Toet et al., 1999) (Figure 2.1).

2.3 Continuous electroencephalography (cEEG) monitoring and analysis

Continuous electroencephalography (cEEG) was arranged as soon as possible after admission, lasting 48 hours unless clinically indicated. They were acquired using portable Stellate Harmonie or Xltek Brain Monitor ICU video-EEG systems (Natus Neurology, Oakville, Ontario, Canada). Electrodes were applied by registered EEG technologists according to the international 10–20 system of electrode placement modified for neonates (Fp1, C3, T3, O1, A1, Fp2, C4, T4, O2, A2, Fz, Cz, and Pz). With the introduction of the Xltek Brain Monitor ICU video-EEG systems, P3 and P4 electrodes were added in order to display Persyst aEEG trend on the same device for the bedside caregivers. All neonates had simultaneous recording of deltoid electromyogram,
electrocardiogram and abdominal respiration. As per institutional protocol, a clinical cEEG was requested if there were definite or suspected clinical seizures, definite or suspected electrographic seizures on aEEG monitoring, or abnormal background activity on aEEG monitoring defined as discontinuous normal voltage, burst suppression or suppression. Before initiating clinical cEEG monitoring, all patients are assessed by our neurology consultation service. The duration of clinical cEEG monitoring is typically 24 hours, or longer when electrographic seizures are detected. Thus, the studies were prolonged to complete 48 hours of recording if no longer clinically indicated prior, or were continued as long as clinically indicated beyond 48 hours. Clinical and electrographic seizures were treated with medications according to institutional guidelines and at the discretion of the treating physician. If clinical cEEG monitoring was not indicated, research cEEGs were arranged and continued for 48 hours. If seizures were detected during research cEEG monitoring, neurology consultation service was advised and further treatment and cEEG monitoring was managed by the treating physicians as described above for clinical cEEG monitoring.

Subsequent research analysis of cEEG recordings was performed by a single neurologist (E.F.P.), blinded to clinical course, glucose levels and neurological outcome. The EEG recordings were visually inspected and 5-minute EEG epochs were classified according to the following categories according to accepted criteria (Tsuchida et al., 2013) as 0:Continuous or tracé alternant (IBI voltage ≥25µV with IBI duration ≤6 seconds); 1:tracé alternant with excessive discontinuity (IBI voltage ≥25µV with IBI duration >6 seconds); 2:tracé discontinu: (IBI voltage <25µV); 3:depressed and undifferentiated (with persistently low-voltage background activity with amplitude between 5 µV and 15 µV and without normal features) or burst suppression (abnormally composed EEG bursts separated by prolonged and abnormally low voltage IBI periods <5 µV pp); 4:very low voltage (amplitude <5 µV or with no discernible cerebral activity). Epochs were skipped if the background pattern was unable to be assessed due to seizure or excessive movement artifact (Figure 2.2).

Electrographic seizures were defined as a sudden, abnormal, repetitive and evolving pattern, with a minimum voltage of 2 mcV pp and minimum duration of at least 10 seconds. “Evolving” was defined as an unequivocal evolution in frequency, voltage, morphology, or location. Distinct seizure events were separated by at least 10 seconds. Status epilepticus was present when the summed duration of seizures comprised ≥50% of an arbitrarily defined 1-hour
epoch (Tsuchida et al., 2013). Seizure presence, duration and maximal extent over course of seizure was marked. Video recordings were reviewed and clinical correlate was classified as absent or present. Clinical correlates were further defined as 1: clonic; 2: tonic; 3: subtle (e.g. eye deviation, eye opening); 4: automatisms (e.g. bicycling, lip smacking); 5: autonomic only (e.g. heart rate changes); or unable to assess (e.g. caregiver in front of patient, patient not on video). Every 6 hours, the presence of reactivity, variability and state changes was graded as 0: present; 1: some/immature; or 2: none.

2.4 Continuous interstitial glucose monitoring

Medtronic iPro2™ professional continuous glucose monitors with Enlite™ sensors (Medtronic of Canada Ltd., Brampton, Ontario) were placed as early as possible after study enrolment, either during transport or upon admission to hospital. The sensors were inserted interstitially into the lateral aspect of the thigh, secured with clear adhesive dressing. In both term and preterm neonates, this device has been used safely over a 7-day period and has been shown to be well tolerated (Beardsall et al., 2013; Harris et al., 2010). The iPro2 monitor is a blinded device that records average interstitial glucose concentrations every 5 minutes. This data was not made available to the care team due to limited evidence for treating newborns based on these measures. Clinicians treated newborns for hypoglycemia or hyperglycemia based on current standard of care using intermittent glucose testing. As per institutional protocol, blood glucose concentrations less than 2.7 mmol/L were treated with intravenous dextrose or glucagon. Hyperglycemia was treated with insulin infusion as clinically indicated.

Continuous glucose monitoring was continued for 72 hours, after which the data was downloaded onto a Windows-based notebook computer running Medtronic software. Only laboratory blood glucose values were used to calibrate the CGM. Due to software cut-offs, interstitial glucose readings below 2.2 and above 22.2 mmol/L were recalculated manually from the raw data.
The definition of hypoglycemia remains controversial and there are variable thresholds used in different consensus guidelines and research studies. On the basis of neurophysiological and neurodevelopmental outcome studies, a commonly used definition of neonatal hypoglycemia is less than 2.6 mmol/L (Koh et al., 1988; Lucas et al., 1988). However, the PES advises that a ‘safe target’ during the first 48 hours should be close to the mean for a healthy newborn and above the threshold for neuroglycopenic symptoms (2.8 mmol/L) (Thornton et al., 2015). Additionally, reference ranges in healthy term newborns may not be appropriate in infants at risk for impaired metabolic adaptation and at risk for adverse neurodevelopment, such as with perinatal asphyxia (Harding et al., 2017). The PES considers that in hypoketotic conditions, ketones and lactate may not be available in sufficient concentrations to serve as an alternative fuel for the brain and thus there is greater risk of brain energy failure and hypoglycemia-induced brain damage (Thornton et al., 2015).

Accordingly, hypoglycemia was defined as blood or interstitial glucose ≤2.8 mmol/L and hyperglycemia as glucose >8.0 mmol/L. Episodes of interstitial glucose derangements were defined as two or more consecutive data points (≥10 minutes) outside the normal range. Episodes of glucose derangement were treated as contiguous if they were separated by brief periods of normoglycemia lasting ≤10 minutes.

For each subject, mean, minimum and maximum glucose levels were calculated based on the interstitial measurements obtained over each 6-hour epoch. The area under the curve was calculated as the area of interstitial glucose concentration over/under the normal glucose range (hours*mmol/L), reflecting the extent and duration of the episode of interstitial glucose derangement. Specifically, for hypoglycemia the area of interstitial glucose concentrations ≤2.8 mmol/L was calculated and for hyperglycemia the area of interstitial glucose concentrations >8.0 mmol/L was calculated. Glucose variability was quantified using the standard deviation and mean glucose rate of change per hour (mmol/L/hour).
2.5 Statistical analysis

All statistical analyses were computed using SPSS, version 20 (SPSS, Inc, Chicago, IL, USA). Demographic data were summarized by standard descriptive statistics, including median/interquartile range (IQR) or mean/standard deviation (SD) for continuous variables, or as number and percentages for categorical variables.

2.5.1 Statistical analysis of aEEG monitoring

The primary analyses compared aEEG scores (background, sleep-wake cycling and seizure scores) during 6-hour epochs containing episodes of hypoglycemia or hyperglycemia to normoglycemic epochs using generalized estimating equations for repeated measures with a linear scale response model. An independent correlation structure and robust variance estimators were used. Comparisons were made relative to normoglycemia because a prior study reported greater aEEG background discontinuity at plasma glucose concentrations both above or below 4.0 mmol/L (Wikstrom et al., 2011). Findings were adjusted for clinical markers of hypoxia-ischemia severity: Apgar scores, umbilical artery pH, and base deficit. Secondary analyses investigated the association of the aEEG scores with other glucose measures (mean, maximum or minimum glucose values, duration of glucose disturbance, and area under the curve) and measures of glucose variability (standard deviation and mean glucose rate of change per hour). Two-tailed tests with p values of <0.05 were considered statistically significant.
Figure 2.1 Summary of aEEG analysis methods. Analysis was performed on 6-h epochs of concomitant CGM and aEEG. CGM provides average interstitial glucose values every 5-min. Six-hour epochs of CGM were assessed for the presence or absence hypo- or hyperglycemia, as well as calculation of the mean, minimum, maximum, area under the curve, standard deviation and mean glucose rate of change in each 6-h epoch. aEEGs were scored every 6-h for background, sleep wake cycling (SWC) and seizures. Background was graded as: 0: continuous normal voltage (CNV); 1: discontinuous normal voltage (DNV); 2: burst suppression pattern (BS); 3: continuous low-voltage (CLV); and 4: flat trace (FT). SWC was graded as: 0: developed cycling; 1: immature cycling; and 2: no cycling. Seizures were graded as: 0: no seizures; 1: single seizure; 2: repeated seizures; and 3: status epilepticus.

2.5.2 Statistical analysis of cEEG monitoring

The primary analyses compared cEEG scores background scores and presence of seizures during 5-minute epochs with or without episodes of hypoglycemia or hyperglycemia to normoglycemic epochs using generalized estimating equations for repeated measures with a linear scale response model. An independent correlation structure and robust variance estimators were used. All comparisons were again made relative to normoglycemia. Findings were adjusted for clinical markers of hypoxia-ischemia severity: Apgar scores, umbilical artery pH, and base deficit. Secondary analyses included comparison of mean interstitial glucose concentration in each 5-
minute epoch with cEEG background scores and presence of seizures. A subgroup analysis was performed examining the association of hyperglycemia with cEEG background scores only in the 8 patients that had occurrences of hyperglycemia on CGM monitoring as it is hypothesized that neonates with hyperglycemia may have more severe HIE and thus worse brain activity. Subgroup analysis was also performed examining the association of hypoglycemia with cEEG background scores only in the 5 patients that had occurrences of hypoglycemia on CGM. Two-tailed tests with p values of <0.05 were considered statistically significant.

**Figure 2.2** Summary of cEEG analysis methods. Analysis was performed on 5-min epochs of concomitant CGM and cEEG. CGM provides average interstitial glucose values every 5-min. The presence or absence hypo- or hyperglycemia in each 5-min epoch as well as the mean interstitial glucose values every 5-min were assessed. cEEG background was visually graded every 5-min as: 0: Continuous or tracé alternant; 1: tracé alternant with excessive discontinuity; 2: tracé discontinu; 3: depressed and undifferentiated or burst suppression; 4: very low voltage. Seizures were marked as present or absent, as well as the duration of seizure in each 5-min epoch.
Chapter 3

3 Results

3.1 Prevalence of glucose derangements in cohort and treatments received

Eighty families were approached to participate in the study and 29 declined (enrollment rate 64%), accordingly 51 neonates were included for analysis. Overall, 41 of 51 neonates (80%) had abnormal glucose values in the first 3 days of life when including both interstitial values as well as blood glucose measurements obtained prior to insertion of the CGM. The mean age at the start of CGM measurements was 8.9 hours of life (SD 3.9 h). There were 13 neonates with hypoglycemia only (25%), 18 neonates with hyperglycemia only (35%) and 10 neonates with both hypo- and hyperglycemia (20%). Of the first 51 neonates recruited, 2 had no available interstitial glucose values. Twenty-five neonates had glucose derangements recorded on CGM (51%). There were 7 neonates with hypoglycemia only (14%), 15 with hyperglycemia only (31%) and 3 with both hypo-and hyperglycemia (6%).

During initial resuscitation after birth, 9 neonates required epinephrine, of which 6 developed hyperglycemia within 12 hours after birth (median 2.5 hours of life, IQR 1.94-8.9 h) and one developed hyperglycemia at 12.67 hours of life. Thirty-three neonates had hypotension requiring intervention (65%), of which 20 neonates (39%) had hypertension responsive to fluid boluses. The other 13 neonates (25.5%) required further treatment, including dopamine, dobutamine, epinephrine, norepinephrine, hydrocortisone or vasopressin. Four of these neonates developed hyperglycemia after receiving vasopressors.

In the 49 patients with CGM monitoring, there were 22 episodes of hypoglycemia (≤2.8 mmol/L) in 10 neonates, of which there were 16 episodes with a minimum glucose ≤2.6 mmol/L in 8 neonates. Only three of these episodes on CGM were detected and treated clinically with D10W IV boluses. Overall, 17 neonates received treatment for hypoglycemia; 14 neonates required treatment within the first 6 hours of life, 3 only had treatment for hypoglycemia after 6 hours of life, and 4 received both early and later treatment of hypoglycemia. Treatment of hypoglycemia included IV dextrose boluses or glucagon (IM or infusion). Of the 28 neonates
with hyperglycemia episodes since birth, 16 had episodes within the first 6 hours of life. Seven neonates received treatment for hypoglycemia prior to development of hyperglycemia. Four neonates required an insulin infusion for treatment of hyperglycemia and all insulin infusions were started after the first 6 hours of life.

3.2 Amplitude-integrated EEG

3.2.1 Study population

Of the 51 neonates recruited, 6 neonates were excluded from aEEG analysis (2 had no available interstitial glucose values, 3 had only single channel (C3-C4) recordings and 1 had no aEEG trace), leaving 45 eligible neonates. Demographic information is summarized in Table 3.1. Compared to neonates who maintained normoglycemia on CGM, neonates with hypoglycemia on CGM had higher mean gestational age and higher mean umbilical artery pH. One patient was not cooled (did not meet institutional criteria for therapeutic hypothermia) and 4 patients died during the neonatal period. Characteristics of excluded participants are found in Appendix Table 1.
**Table 3.1** Clinical and demographic data of the full study cohort, and subgroups of neonates with hypoglycemia and hyperglycemia, who were compared to those who maintained normoglycemia on continuous interstitial glucose monitoring

<table>
<thead>
<tr>
<th></th>
<th>FULL COHORT n=45</th>
<th>NORMOGLYCEMIA n=25</th>
<th>HYPOGLYCEMIA n=7</th>
<th>p value</th>
<th>HYPERGLYCEMIA n=11</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
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<tr>
<td>males</td>
<td>21</td>
<td>12</td>
<td>3</td>
<td>1.000</td>
<td>5</td>
<td>1.000</td>
</tr>
<tr>
<td>females</td>
<td>24</td>
<td>13</td>
<td>4</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Gestational age (weeks), mean (SD)</strong></td>
<td>39.54 (1.40)</td>
<td>39.19 (1.51)</td>
<td>40.53 (0.48)</td>
<td><strong>0.001</strong></td>
<td>39.35 (1.16)</td>
<td>0.746</td>
</tr>
<tr>
<td><strong>Birth weight (grams), mean (SD)</strong></td>
<td>3414.36</td>
<td>3468.80</td>
<td>3413.29</td>
<td>0.746</td>
<td>3373.09</td>
<td>0.470</td>
</tr>
<tr>
<td><strong>Birth length (cm), mean (SD)</strong></td>
<td>50.14 (3.15)</td>
<td>50.63 (3.16)</td>
<td>49.43 (3.77)</td>
<td>0.462</td>
<td>49.59 (3.14)</td>
<td>0.373</td>
</tr>
<tr>
<td><strong>Head circumference (cm), mean (SD)</strong></td>
<td>34.22 (1.31)</td>
<td>34.31 (1.47)</td>
<td>34.14 (0.85)</td>
<td>0.703</td>
<td>34.14 (1.33)</td>
<td>0.727</td>
</tr>
<tr>
<td><strong>Maternal diabetes, n (%)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Type II Diabetes Mellitus</td>
<td>5 (11)</td>
<td>4 (16)</td>
<td>0 (0)</td>
<td>0.552</td>
<td>1 (9)</td>
<td>1.000</td>
</tr>
<tr>
<td>Gestational Diabetes</td>
<td>4 (9)</td>
<td>3 (12)</td>
<td>0 (0)</td>
<td>1 (9)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Maternal hypertension, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maternal pre-eclampsia/eclampsia</td>
<td>2 (4.5)</td>
<td>2 (8)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
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<td><strong>Apgar score at 5 min, mean (SD)</strong></td>
<td>4.18 (2.39)</td>
<td>3.68 (1.82)</td>
<td>5.43 (3.10)</td>
<td>0.196</td>
<td>4.55 (2.51)</td>
<td>0.318</td>
</tr>
<tr>
<td><strong>Umbilical arterial cord pH, mean (SD)</strong></td>
<td>6.99 (0.18)</td>
<td>6.96 (0.16)</td>
<td>7.17 (0.17)</td>
<td><strong>0.026</strong></td>
<td>6.95 (0.20)</td>
<td>0.924</td>
</tr>
<tr>
<td><strong>Umbilical arterial cord BE, mean (SD)</strong></td>
<td>-15.13 (6.79)</td>
<td>-15.83 (6.32)</td>
<td>-9.00 (7.43)</td>
<td>0.077</td>
<td>-17.13 (6.83)</td>
<td>0.646</td>
</tr>
<tr>
<td><strong>Route of delivery, n (%)</strong></td>
<td></td>
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<tr>
<td>Vaginal delivery</td>
<td>20 (44.5)</td>
<td>13 (52)</td>
<td>2 (28.5)</td>
<td>0.402</td>
<td>5 (45.5)</td>
<td>1.000</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>25 (55.5)</td>
<td>12 (48)</td>
<td>5 (71.5)</td>
<td>6 (54.5)</td>
<td></td>
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<tr>
<td><strong>Sentinel event, n (%)</strong></td>
<td></td>
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<tr>
<td>Clinical seizures only</td>
<td>17 (38)</td>
<td>9 (36)</td>
<td>2 (28.5)</td>
<td>1.000</td>
<td>5 (45.5)</td>
<td>0.716</td>
</tr>
<tr>
<td>Electrographic and/or electroclinical seizures</td>
<td>28 (62)</td>
<td>14 (56)</td>
<td>5 (71)</td>
<td>0.671</td>
<td>7 (63.5)</td>
<td>0.729</td>
</tr>
<tr>
<td><strong>Seizures, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiepileptic medications, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lorazepam only</td>
<td>26 (58)</td>
<td>12 (48)</td>
<td>5 (71)</td>
<td>0.402</td>
<td>7 (63)</td>
<td>0.481</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>6 (13)</td>
<td>3 (12)</td>
<td>2 (28.5)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2AEDs</td>
<td>13 (29)</td>
<td>8 (32)</td>
<td>2 (28.5)</td>
<td>3 (27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Received opioids, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minutes of concomitant aEEG and continuous glucose monitoring, mean (SD)</td>
<td>3526.92</td>
<td>3135.60</td>
<td>3401.43</td>
<td>0.634</td>
<td>3563.18</td>
<td>0.345</td>
</tr>
<tr>
<td>(aEEG: 1280.02)</td>
<td>(1263.51)</td>
<td>(1264.65)</td>
<td>(1204.24)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2 subjects had both interstitial hypoglycemia and hyperglycemia events during concurrent aEEG monitoring
Continuous variables were analyzed with the student’s T-test, whereas categorical variables were analyzed with Fisher’s exact test
Significant p values are shown in bold
Hypoglycemia is defined as at least two interstitial glucose measurements less than or equal to 2.8 mmol/L and hyperglycemia is defined as at least two interstitial measurements greater than 8 mmol/L.
* Sentinel hypoxic event (e.g. cord prolapse, abruptio placenta) immediately before or during labor
** Electrographic and/or electroclinical seizures were identified by clinical team on aEEG or cEEG. Clinical seizures only had no seizures captured on aEEG or cEEG monitoring.
3.2.2 Prevalence of glucose derangements during aEEG monitoring

A mean of 55.6 hours (SD 20.4) of concurrent CGM and aEEG monitoring were available per neonate. Thirty-four episodes of glucose derangements were captured on CGM concurrent with aEEG monitoring; 16 episodes of hypoglycemia in 9 neonates (20%), and 18 episodes of hyperglycemia in 13 (29%). Hypoglycemia episodes had a median duration of 77.5 minutes (IQR 41.25 - 143.75) lasting up to 220 minutes. Hyperglycemia episodes had a median duration of 237.5 minutes (IQR 57.5 - 766.25) lasting up to 52.1 hours. During hypoglycemia episodes, the median interstitial glucose was 2.7 mmol/L (IQR 2.6 - 2.8, minimum 2.2 mmol/L), and during hyperglycemia episodes the median glucose was 14.4 mmol/L (IQR 10.2 - 19.3, maximum 22.8 mmol/L).

3.2.3 Amplitude-integrated EEG findings

3.2.3.1 aEEG measures during epochs of glucose derangements

Six-hour epochs of aEEG containing episodes of hypoglycemia did not differ from normoglycemic aEEG epochs for any of the measures assessed (background score, sleep cycling score or seizure score). Compared to normoglycemic epochs, aEEG epochs containing hyperglycemia displayed worse aEEG background scores, less sleep-wake cycling and more frequent seizures, which remained significant after adjusting for clinical markers of HIE (Table 3.2 and Figure 3.1). No seizures were recorded during epochs with hypoglycemia.
Table 3.2  Association of glucose derangements with aEEG scores during 6-hour epochs containing hypoglycemia or hyperglycemia

<table>
<thead>
<tr>
<th>Glucose derangements during epoch</th>
<th>Median (IQR)</th>
<th>Unadjusted Regression Slope (95% CI)</th>
<th>p value</th>
<th>Adjusted Regression Slope (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Worst background score during epoch</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>2 (1 – 3)</td>
<td>0.032 (-0.677 – 0.741)</td>
<td>0.929</td>
<td>0.211 (-0.435 – 0.857)</td>
<td>0.522</td>
</tr>
<tr>
<td>None</td>
<td>1 (1 – 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>3 (2 – 4)</td>
<td>0.971 (0.324 – 1.618)</td>
<td><strong>0.003</strong></td>
<td>1.120 (0.501 – 1.738)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Sleep cycling score during epoch</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>1 (1 – 1.75)</td>
<td>-0.024 (-0.252 – 0.204)</td>
<td>0.834</td>
<td>-0.020 (-0.226 – 0.186)</td>
<td>0.849</td>
</tr>
<tr>
<td>None</td>
<td>1 (1 – 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>2 (2 – 2)</td>
<td>0.586 (0.445 – 0.728)</td>
<td><strong>&lt;0.001</strong></td>
<td>0.587 (0.417 – 0.757)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Seizure score during epoch</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>0 (0 – 0)</td>
<td>-0.095 (-0.176 – -0.013)</td>
<td><strong>0.022</strong></td>
<td>-0.065 (-0.150 – 0.021)</td>
<td>0.139</td>
</tr>
<tr>
<td>None</td>
<td>0 (0 – 0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>0 (0 – 1)</td>
<td>0.394 (0.170 – 0.617)</td>
<td><strong>0.001</strong></td>
<td>0.433 (0.185 – 0.681)</td>
<td><strong>0.001</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for Apgar score, cord pH and cord base excess

<sup>b</sup>Reference category is normoglycemia

Significant p values are shown in bold

Hypoglycemia is defined as at least two interstitial glucose measurements less than or equal to 2.8 mmol/L and hyperglycemia is defined as at least two interstitial measurements greater than 8 mmol/L.
Figure 3.1 Bar graphs comparing aEEG scores with glucose derangement category during epochs of hypoglycemia, normoglycemia or hyperglycemia. a) worst background scores in epoch\(^a\); b) sleep-wake cycling scores in epoch\(^b\); c) seizure scores in epoch\(^c\)

\(^a\) Scoring system for background: Continuous normal voltage (CNV); Discontinuous normal voltage (DNV); Burst-suppression (BS); Continuous low voltage (CLV); Flat tracing (FT)

\(^b\) Scoring system for cycling: Developed sleep-wake cycling; Immature sleep-wake cycling; No sleep-wake cycling

\(^c\) Scoring system for seizures: No seizures; Single seizure; Repeated seizures; Status epilepticus
3.2.3.2 aEEG measures and severity of glucose derangements

Other glucose measures were analyzed to quantify the severity of glucose derangements during each 6-hour epoch (Table 3.3). Longer duration of hypoglycemia and greater area under the hypoglycemic curve were associated with worse aEEG background score, but no other measures were significant. Longer duration of hyperglycemia, higher maximum glucose values and higher mean glucose values were associated with worse aEEG background, less sleep-wake cycling and more frequent seizures. Greater area under the hyperglycemic curve was associated with worse aEEG background and less sleep-wake cycling.

Table 3.3 Association of severity of glucose derangements with aEEG scores

<table>
<thead>
<tr>
<th>Glucose measures during epoch</th>
<th>Worst BG score in epoch&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cycling score in epoch&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Seizure score in epoch&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted Regression Slope (95% CI)</td>
<td>Adjusted Regression Slope (95% CI)</td>
<td>Adjusted Regression Slope (95% CI)</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>p value</td>
<td>p value</td>
</tr>
<tr>
<td>Time hypoglycemic (hours)</td>
<td>0.299 (0.094 – 0.503)</td>
<td>0.065 (-0.054 – 0.184)</td>
<td>-0.023 (-0.059 – 0.013)</td>
</tr>
<tr>
<td>Minimum glucose (mmol/L)</td>
<td>0.101 (-0.131 – 0.333)</td>
<td>0.086 (-0.009 – 0.181)</td>
<td>0.001 (-0.034 – 0.035)</td>
</tr>
<tr>
<td>Mean glucose (mmol/L)</td>
<td>0.120 (-0.128 – 0.369)</td>
<td>0.098 (-0.006 – 0.201)</td>
<td>0.016 (-0.023 – 0.056)</td>
</tr>
<tr>
<td>Area under curve (hour*mmol/L)</td>
<td>1.235 (0.123 – 2.246)</td>
<td>0.165 (-0.367 – 0.700)</td>
<td>-0.147 (-0.351 – 0.056)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HYPOGLYCEMIA&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time hypoglycemic (hours)</td>
</tr>
<tr>
<td>Minimum glucose (mmol/L)</td>
</tr>
<tr>
<td>Mean glucose (mmol/L)</td>
</tr>
<tr>
<td>Area under curve (hour*mmol/L)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HYPERGLYCEMIA&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time hyperglycemic (hours)</td>
</tr>
<tr>
<td>Maximum glucose (mmol/L)</td>
</tr>
<tr>
<td>Mean glucose (mmol/L)</td>
</tr>
<tr>
<td>Area under curve (hour*mmol/L)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for Apgar score, cord pH and cord base excess
<sup>b</sup>Reference category is normoglycemia

Significant p values are shown in bold

Hypoglycemia is defined as at least two interstitial glucose measurements less than or equal to 2.8 mmol/L and hyperglycemia is defined as at least two interstitial measurements greater than 8 mmol/L.

BG: background
3.2.3.3 aEEG measures and glucose variability

The relationship of glucose variability with aEEG scores was also explored (Table 3.4). After adjusting for clinical markers of HIE, a greater standard deviation in interstitial glucose concentration was associated with worse sleep-wake cycling and more frequent seizures. Faster rates of increase in glucose concentration were associated with worse sleep-wake cycling, but faster rates of decrease were not.

**Table 3.4 Association of glucose variability with aEEG scores**

<table>
<thead>
<tr>
<th>Measure of glucose variability</th>
<th>Unadjusted Regression Slope (95% CI)</th>
<th>p value</th>
<th>Adjusted Regression Slope (95% CI)</th>
<th>p valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Worst background score in epoch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.470 (-0.054 – 0.995)</td>
<td>0.079</td>
<td>0.516 (-0.055 – 1.087)</td>
<td>0.077</td>
</tr>
<tr>
<td>Rate of glucose decrease (mmol/L/hour)</td>
<td>-0.407 (-1.411 – 0.596)</td>
<td>0.426</td>
<td>-0.309 (-1.296 – 0.679)</td>
<td>0.540</td>
</tr>
<tr>
<td>Rate of glucose increase (mmol/L/hour)</td>
<td>0.209 (-0.644 – 1.063)</td>
<td>0.631</td>
<td>0.375 (-0.613 – 1.364)</td>
<td>0.457</td>
</tr>
<tr>
<td><strong>Sleep cycling score in epoch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.348 (0.180 – 0.516)</td>
<td>&lt;0.001</td>
<td>0.370 (0.181 – 0.560)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rate of glucose decrease (mmol/L/hour)</td>
<td>-0.480 (-0.986 – 0.025)</td>
<td>0.063</td>
<td>-0.460 (-0.959 – 0.038)</td>
<td>0.070</td>
</tr>
<tr>
<td>Rate of glucose increase (mmol/L/hour)</td>
<td>0.379 (0.046 – 0.712)</td>
<td>0.026</td>
<td>0.359 (0.012 – 0.705)</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>Seizure score in epoch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.399 (0.204 – 0.594)</td>
<td>&lt;0.001</td>
<td>0.413 (0.188 – 0.639)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rate of glucose decrease (mmol/L/hour)</td>
<td>-0.165 (-0.444 – 0.114)</td>
<td>0.247</td>
<td>-0.165 (-0.445 – 0.115)</td>
<td>0.249</td>
</tr>
<tr>
<td>Rate of glucose increase (mmol/L/hour)</td>
<td>0.564 (-0.005 – 1.133)</td>
<td>0.052</td>
<td>0.465 (-0.126 – 1.057)</td>
<td>0.123</td>
</tr>
</tbody>
</table>

aAdjusted for Apgar score, cord pH and cord base excess

Note: Rates of glucose increase have positive slopes for rate of change and rates of glucose decrease have negative slopes for rates of change

Significant p values are shown in bold
3.3 Continuous EEG

3.3.1 Demographics and clinical variables

The first 30 neonates from the cohort of 51 neonates described above were included for cEEG analysis. There were 17 males and 13 females. Mean gestational age was 39.6 weeks (SD 1.25). The mean birth weight was 3351.1 grams (SD 623.63), birth length 50.14 (SD 3.22) and head circumference 34.0 cm (SD 1.40). Mean Apgar scores at 5 minutes was 4.3 (SD 2.3), arterial cord pH 6.97 (SD 0.18), and arterial cord base excess -15.46 (SD 7.53). The demographics of the subgroup are representative of the cohort of neonates included in the aEEG analysis described in detail above (p>0.05 for all demographic measures described).

All neonates received therapeutic hypothermia as per protocol and 2 patients died during the neonatal period. Twenty-one neonates had P3 and P4 electrodes added to the standard international 10–20 system of electrode placement modified for neonates. Clinical seizures only (not confirmed on aEEG or cEEG monitoring) or electrographic and/or electroclinical seizures identified by the clinical team on aEEG or cEEG were noted in 17 neonates. Three neonates received lorazepam only, 7 neonates received phenobarbital and 6 neonates received phenobarbital and further antiepileptic medications (levetiracetam, phenytoin or midazolam infusion).

3.3.2 Glucose derangements

There was a mean of 47.6 hours (SD 12.4) of concurrent CGM and cEEG monitoring available per neonate. Twenty-three episodes of glucose derangements were captured on CGM concurrent with cEEG monitoring; 9 episodes of hypoglycemia in 5 neonates (17%), and 14 episodes of hyperglycemia in 8 neonates (27%). Hypoglycemia episodes had a median duration of 45 minutes (IQR 20-120) lasting up to 220 minutes. Hyperglycemia episodes had a median duration of 212.5 minutes (IQR 57.5-540) lasting up to 20.9 hours. During hypoglycemia episodes, the median interstitial glucose was 2.7 mmol/L (IQR 2.6-2.8, minimum 2.2 mmol/L), and during hyperglycemia episodes the median glucose was 11.2 mmol/L (IQR 9-14.8, maximum 18.1 mmol/L).
3.3.3 Continuous EEG findings

Eight neonates had electrographic seizures recorded on continuous EEG monitoring. In these neonates, there was a median of 27.5 (IQR 6.0 -137.5) seizures per recording, ranging between 2 to 165 seizures. Seven neonates had only subclinical seizures on cEEG (87.5%) and one neonate had both clinical and subclinical seizures. Median seizure duration was 58 seconds (IQR 35-79.5), and maximum seizure duration recorded was 1043 seconds. Maximal extent of each seizures (all lobes involved) was recorded. Of the 469 electrographic seizures, 25% involved the frontal lobe, 53% involved the central and vertex regions, 54.6% involved the temporal lobes, and 16% the occipital lobes.

3.3.3.1 cEEG measures during epochs of glucose derangements

Five-minute epochs of cEEG containing episodes of hypoglycemia did not differ from normoglycemic epochs for background scores or seizures. No seizures were recorded during epochs with hypoglycemia. Compared to normoglycemia, cEEG epochs containing hyperglycemia displayed worse cEEG background scores, which remained significant after adjusting for clinical markers of HIE (Table 3.5 and Figure 3.2). No association was found between epochs containing hyperglycemia and seizures.
Table 3.5  Association of glucose derangements with cEEG scores during 5-minute epochs containing hypoglycemia or hyperglycemia

<table>
<thead>
<tr>
<th>Glucose derangement during epoch</th>
<th>Median (IQR)</th>
<th>Unadjusted Regression Slope (95% CI)</th>
<th>p value</th>
<th>Adjusted Regression Slope (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background score&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>0 (0 – 2)</td>
<td>-0.085 (-0.685 – 0.516)</td>
<td>0.782</td>
<td>0.504 (-0.335 – 1.342)</td>
<td>0.239</td>
</tr>
<tr>
<td>None</td>
<td>0 (0 – 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>3 (2 – 4)</td>
<td>1.842 (0.921 – 2.763)</td>
<td>&lt;0.001</td>
<td>1.621 (0.663 – 2.579)</td>
<td>0.001</td>
</tr>
<tr>
<td>Presence of seizures&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>0 (0 – 0)</td>
<td>-0.019 (-0.041 – 0.002)</td>
<td>0.077</td>
<td>0.026 (-0.018 – 0.070)</td>
<td>0.245</td>
</tr>
<tr>
<td>None</td>
<td>0 (0 – 0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>0 (0 – 0)</td>
<td>-0.002 (-0.037 – 0.033)</td>
<td>0.908</td>
<td>-0.020 (-0.082 – 0.042)</td>
<td>0.529</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for Apgar score, cord pH and cord base excess
<sup>b</sup>Reference category is normoglycemia
Significant p values are shown in bold

Hypoglycemia is defined as at least one interstitial glucose measurement less than or equal to 2.8 mmol/L and hyperglycemia is defined as at least one interstitial measurement greater than 8 mmol/L.

Figure 3.2  Bar graph comparing cEEG background scores with glucose derangement category during epochs of hypoglycemia, normoglycemia or hyperglycemia.
Examining reactivity, variability and state changes in 6-hour epochs, no association was found with the presence of hypoglycemia. However, hyperglycemia was associated with less cEEG variability and state changes (0.824; 95% CI 0.530-1.119; p<0.001), including after adjusting for clinical markers of hypoxia-ischemia severity (0.669; 95% CI 0.311-1.027; p<0.001).

3.3.3.2 cEEG measures and level of glucose

The association of the mean glucose level in each 5-minute epoch with background scores was also assessed (Table 3.6). Excluding epochs of hypoglycemia, higher glucose levels were also associated with worse cEEG background scores. Excluding epochs of hyperglycemia, lower glucose levels were surprisingly associated with better cEEG background scores.

<table>
<thead>
<tr>
<th>Glucose during epoch</th>
<th>BG score in epoch</th>
<th>Seizure score in epoch</th>
<th>BG score in epoch</th>
<th>Seizure score in epoch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Unadjusted</td>
<td>Adjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td></td>
<td>Regression</td>
<td>Regression</td>
<td>Regression</td>
<td>Regression</td>
</tr>
<tr>
<td></td>
<td>Slope (95% CI)</td>
<td>p value</td>
<td>Slope (95% CI)</td>
<td>p value</td>
</tr>
<tr>
<td>Mean glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HYPOGLYCEMIA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.263</td>
<td>0.017</td>
<td>0.012</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>(0.047 – 0.479)</td>
<td>(-0.003 – 0.026)</td>
<td>(0.042 – 0.421)</td>
<td>(-0.001 – 0.014)</td>
</tr>
<tr>
<td>HYPERGLYCEMIA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.260</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>(0.176 – 0.344)</td>
<td>(-0.001 – 0.008)</td>
<td>(0.169 – 0.307)</td>
<td>(-0.004 – 0.006)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for Apgar score, cord pH and cord base excess
<sup>b</sup>Reference category is normoglycemia
Significant p values are shown in bold
Hypoglycemia is defined as at least one interstitial glucose measurement less than or equal to 2.8 mmol/L and hyperglycemia is defined as at least one interstitial measurement greater than 8 mmol/L.
BG: background
3.3.3.3 cEEG measures in subgroup of patients with hyperglycemia episodes during continuous glucose monitoring

A subgroup analysis was performed examining the association of hyperglycemia with cEEG background scores including only the 8 patients that had episodes of hyperglycemia recorded on CGM monitoring. Epochs of hyperglycemia were still associated with worse background scores than epochs of normoglycemia in this subgroup. Higher mean glucose concentrations were also associated with worse cEEG background scores (Table 3.7, Figure 3.3).

Table 3.7  Association of hyperglycemia and glucose levels with cEEG background scores in the subgroup of 8 neonates that had hyperglycemia recorded on CGM

<table>
<thead>
<tr>
<th>Glucose derangement during epoch</th>
<th>Median (IQR)</th>
<th>Unadjusted Regression Slope (95% CI)</th>
<th>p value</th>
<th>Adjusted Regression Slope (95% CI)sup</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background scoreb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2 (0 – 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>3 (2 – 4)</td>
<td>0.936 (0.005 – 1.866)</td>
<td>0.049</td>
<td>0.739 (0.038 – 1.439)</td>
<td>0.039</td>
</tr>
<tr>
<td>Mean glucose (mmol/L)</td>
<td>NA</td>
<td>0.141 (0.008 – 0.274)</td>
<td>0.038</td>
<td>0.117 (0.038 – 0.195)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

supAdjusted for Apgar score, cord pH and cord base excess
b Reference category is normoglycemia
Significant p values are shown in bold
Hyperglycemia is defined as at least one interstitial measurement greater than 8 mmol/L
NA; not applicable
Figure 3.3 Box plot comparing cEEG background scores with glucose level (mmol/L) in subgroup of 8 patients that had hyperglycemia recorded on CGM.

Ends of boxes represent IQR. Middle line represents median. Error bars represent 95% confidence intervals.
3.3.3.4 cEEG measures in subgroup of patients with hypoglycemia episodes during continuous glucose monitoring

Subgroup analysis was also performed examining the association of hypoglycemia with cEEG background scores including only the 5 patients that had episodes of hypoglycemia recorded during CGM monitoring (Table 3.8, Figure 3.4). Epochs of hypoglycemia were not associated with the background scores on univariate analysis. However, after adjusting for severity of HIE, epochs with hypoglycemia had better background scores. Lower mean glucose concentrations were also associated with better cEEG background scores.

**Table 3.8:** Association of hypoglycemia and glucose levels with cEEG background scores in subgroup of the 5 neonates that had hypoglycemia recorded on CGM

<table>
<thead>
<tr>
<th>Glucose derangement during epoch</th>
<th>Median (IQR)</th>
<th>Unadjusted Regression Slope (95% CI)</th>
<th>p value</th>
<th>Adjusted Regression Slope (95% CI)(^a)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0 (0 – 2)</td>
<td>-0.272 (-1.236 – 0.692)</td>
<td>0.581</td>
<td>-0.602 (-0.814 – -0.391)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>0 (0 – 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Mean glucose (mmol/L)            | NA           | 0.454 (0.015 – 0.892)               | 0.043   | 0.159 (0.063 – 0.255)                   | 0.001   |

\(^a\)Adjusted for Apgar score, cord pH and cord base excess  
\(^b\)Reference category is normoglycemia  
Significant p values are shown in bold  
Hyperglycemia is defined as at least one interstitial measurement greater than 8 mmol/L  
NA: not applicable
Figure 3.4 Box plot comparing cEEG background scores with glucose level (mmol/L) in subgroup of 5 patients that had hypoglycemia recorded on CGM.

Ends of boxes represent IQR. Middle line represents median. Error bars represent 95% confidence intervals.
Chapter 4

4 General discussion

4.1 Discussion

Among neonates with encephalopathy, epochs of hyperglycemia (>8 mmol/L), but not hypoglycemia (2.2 to 2.8 mmol/L), were temporally associated with worse global brain function and greater seizure frequency on aEEG monitoring, even after adjusting for clinical markers of HI severity. Greater variability in glucose concentration was also temporally correlated with worse sleep-wake cycling and more frequent seizures. Epochs of hyperglycemia were also temporally associated with worse global brain function on cEEG, even after adjusting for clinical markers of HI severity. Hyperglycemia was also associated with less variability and state changes on cEEG as well. These observations provide new insights into the dynamics of glucose homeostasis and brain function, and highlight the potential importance of hyperglycemia on impaired brain function.

In this cohort of neonates with encephalopathy, epochs with hyperglycemia were significantly associated with worse EEG background scores. These findings are consistent with previous studies in other age cohorts. In extremely preterm neonates, moderate hyperglycemia was significantly associated with greater discontinuity on aEEG (Wikstrom et al., 2011). Another prospective study in premature neonates found high blood glucose was associated with depressed cerebral activity on aEEG (Granot et al., 2012). Schumacher et al. with continuous EEG monitoring showed increased blood glucose was associated with decreased total absolute band power during the first 3 days of life in premature infants (Schumacher et al., 2014). A study using CGM and simultaneous cEEG monitoring in children with type 1 diabetes demonstrated that asymptomatic hyperglycemia was associated with changes in the power spectra of various EEG frequency bands (Rachmiel et al., 2016).

EEG background abnormalities and their recovery are predictive of long-term neurodevelopmental outcome in neonates with HIE (Azzopardi & Toby study group, 2014; Chandrasekaran et al., 2017; Dunne et al., 2017; Skranes et al., 2017; Spitzmiller et al., 2007;
Thoresen et al., 2010; van Laerhoven et al., 2013). A meta-analysis of 8 studies before the advent of therapeutic hypothermia showed a pooled sensitivity of 91% and specificity of 88% of early severe aEEG tracings to predict poor neurodevelopmental outcome (Azzopardi & Toby study group, 2014; Spitzmiller et al., 2007). With therapeutic hypothermia, a persistently abnormal aEEG background at 48 hours remains an important prognostic indicator (Chandrasekaran et al., 2017; Thoresen et al., 2010). And, although therapeutic hypothermia has changed the time-frame, aEEG and EEG are still the best objective predictive tools to identify the highest-risk patients for poor outcome (Bonifacio et al., 2015). Several other EEG features have been associated with unfavorable outcome including, background amplitude of less than 30 mcV, IBI of greater than 60 seconds, and electrographic seizures (Kontio et al., 2013; Menache et al., 2002; Murray et al., 2009; Obeid et al., 2017; Pressler et al., 2001; Shah et al., 2014b). Discontinuous patterns with IBI lasting 10-60 seconds may lead to either favorable or unfavourable outcomes after HIE (Murray et al., 2009; Nevalainen et al., 2017). Quantitative automated analysis of EEG can improve the objectivity of EEG assessment in newborns and may improve prognostic capabilities. Quantitative EEG measures that have been associated with outcomes included EEG discontinuity (Dunne et al., 2017), total suppression time (Flisberg et al., 2011), and novel features of bursts such as measures of burst area and duration and their interrelationships (Iyer et al., 2014).

In our cohort, hyperglycemia and greater glucose variability (as measured by SD and rate of glucose increase), were also associated with worse sleep-wake cycling scores. Neonatal sleep-wake cycling is a marker of brain function and enhances prediction of long-term neurodevelopmental outcomes in neonates with HIE (Osredkar et al., 2005; Thoresen et al., 2010). A recent multimodality neuromonitoring study in neonates at risk for cerebral dysfunction showed that inefficient neonatal sleep patterns were independent predictors of 18-month neurodevelopmental outcome (Shellhaas et al., 2017).

We demonstrate that hyperglycemia and greater glucose dispersion (as measured by SD) are associated with seizure burden on aEEG, although not necessarily time-locked to the 5-minute epochs with hyperglycemia on cEEG. There is growing evidence that higher seizure burden is associated with worse short and long-term outcomes, adjusting for confounders including seizure etiology and illness severity (Glass et al., 2016; Kharoshankaya et al., 2016; McBride et al., 2000; Pisani et al., 2007; Pisani et al., 2008; Srinivasakumar et al., 2015). Studies
in neonates undergoing therapeutic hypothermia for HIE show a relationship between higher seizure burden and worse MRI injury scores in univariate (Glass et al., 2011; Nash et al., 2011; Srinivasakumar et al., 2015; van Rooij, Toet, et al., 2010) and multivariable analyses (Dunne et al., 2017; Shah et al., 2014b).

Electrographic seizures have been observed in 30-65% of neonates with HIE (Bashir et al., 2016; Glass et al., 2011; Glass et al., 2014; Kharoshankaya et al., 2016; Nash et al., 2011; Srinivasakumar et al., 2015; Wusthoff et al., 2011) and were identified in 27% of our cohort on cEEG. Our cohort includes neonates with all severities of encephalopathy (mild to severe) including neonates with early improvement of aEEG background and no clinical seizures, who may not be monitored with cEEG during routine clinical practice. While twelve patients had cEEG requested by the clinical neurology consultation service, 18 cEEGs were research studies (one of which became a clinical study after electrographic seizures were recorded). In neonates with seizures, a median of 27.5 (IQR 6.0-137.5) seizures were identified per recording with a median seizure duration of 58 seconds (IQR 35-79.5). The seizure number and duration in our cohort is similar to a previously described cohort of 23 neonates with HIE and seizures that received therapeutic hypothermia. They recorded a median seizure duration of 125 seconds (IQR 82-238) and total median seizure number of 26 (IQR 13-86) per neonate (Lynch et al., 2015).

There is increasing evidence of an association between hyperglycemia and neurodevelopmental outcomes in term and preterm neonates. Hyperglycemia is common in preterm neonates and has been demonstrated to be associated with increased mortality, short-term morbidities (Alexandrou et al., 2010; Bermick et al., 2016; Hays et al., 2006; Kao et al., 2006) and with long-term outcomes, including white matter reduction on term equivalent MRI scan (Alexandrou et al., 2010) and neurological and behavior problems at age 2 years (van der Lugt et al., 2010). In a cohort of well babies at risk for hypoglycemia, higher glucose levels were associated with neurosensory impairment and cognitive delay at 2 years of age (McKinlay et al., 2015). And in neonates with HIE, hyperglycemia has also been shown to adversely affect long-term outcome in two small retrospective studies (Chouthai et al., 2015; Spies et al., 2014), as well as a post-hoc analysis of the CoolCap therapeutic hypothermia trial in which both hypo- and hyperglycemia were common and associated with unfavorable neurodevelopmental outcome by age 18 months (Basu et al., 2016). Longer duration of hyperglycemia and higher peak glucose levels have also been associated with mortality in the PICU (Srinivasan et al., 2004).
The relationship between glucose levels and brain injury is likely complex. A study in well babies had demonstrated that less glucose stability and steeper increase in glucose concentrations following hypoglycemia in the first 12 hours after birth were associated with neurosensory impairments at age 2 years (McKinlay et al., 2015), and development of neurosensory impairment between ages 2 and 4.5 years (McKinlay, Alsweiler, et al., 2017). Another retrospective cohort of term HIE found that glucose variability was associated with severe neurodevelopmental disability (Al Shafouri et al., 2015). An association between glucose variability and mortality was likewise shown in very low birth weight preterm neonates (Fendler et al., 2012), term neonates and children admitted to the pediatric ICU (Hirshberg et al., 2008; Rake et al., 2010; Wintergerst et al., 2006) and adult ICU (Ali et al., 2008; Egi et al., 2006; Hermanides et al., 2010; Pidcoke et al., 2009). Long-term outcomes are being assessed in our cohort to identify whether the acute changes seen on aEEG and cEEG associated with hyperglycemia and glucose variability are predictive of long-term neurodevelopmental outcomes.

Perhaps surprisingly, in our primary analysis we did not observe any associations between hypoglycemia (2.2-2.8 mmol/L) and brain function. This may be because episodes of hypoglycemia were generally shorter in duration (median 77.5 minutes) than hyperglycemic episodes (median 237.5 minutes). Still, the majority of hypoglycemic episodes on CGM were not clinically identified and treated. In our secondary analysis, a longer duration of hypoglycemia and greater area under the hypoglycemic curve were associated with worse background scores, suggesting that the duration and severity of hypoglycemia may indeed be important.

Neonatal hypoglycemia has been associated with more severe neonatal encephalopathy (Basu et al., 2009) and studies looking at long-term outcomes have shown hypoglycemia in neonates with HIE to be associated with worse neurodevelopmental outcomes (Basu et al., 2016; Salhab et al., 2004; Tam et al., 2012). However, those studies in which aEEG has been used in newborns to examine acute neurophysiological changes have been unable to detect changes during hypoglycemia (Harris et al., 2011; Stenninger et al., 2001) except for a single case report of one critically ill neonate (Hellstrom-Westas et al., 1989). In terms of conventional EEG, there are reports of children with recurrent hypoglycemia, who showed increased slow activity on EEG with glucose concentrations ≤2.3 mmol/L which improved with normalization of glucose concentrations (Haworth et al., 1960; Pavone et al., 1980). In diabetic children, an increase in
low-frequency EEG activity was observed when glucose concentrations decreased ≤4.0 mmol/L (Bjorgaas et al., 1998). Canine models of insulin-induced newborn hypoglycemia demonstrated progressive EEG slowing with declining glucose concentration (Vannucci et al., 1981) and suggest that the EEG response to hypoglycemia may depend not only on the concentration of glucose in the brain but also on the presence of associated metabolic derangements (Vannucci et al., 1980). In HIE, a neonate’s ability to produce and use alternative fuels may not be sufficient to compensate for low glucose levels, making them particularly vulnerable to hypoglycemia induced brain injury (Harris et al., 2015; Harris et al., 2011; Stanley et al., 2015). Nonetheless, looking in our cohort, we still did not identify EEG changes with hypoglycemia. Our data in this regard may suggest that either our current management protocols for hypoglycemia are adequate to maintain brain function, or the impact of hypoglycemia on brain function is not immediately apparent by visual EEG analysis. Furthermore, it was noted in the subgroup of five neonates with hypoglycemia captured on CGM, that lower mean glucose concentrations were associated with better cEEG background scores. Potential explanations for this finding include the possibility that qualitative EEG grading may not be an adequate method to capture the changes seen on EEG with hypoglycemia. Previous studies suggest progressive EEG slowing occurs as glucose levels decrease, with progression to a burst suppression pattern only after a severe decrease in glucose levels, for example to ≤0.3 mmol/L in newborn dogs (Bjorgaas et al., 1998; Vannucci et al., 1981). In our cohort, interstitial glucose concentrations during hypoglycemia episodes recorded on CGM were between 2.2 to 2.8 mmol/L, although several early severe hypoglycemia episodes (<2.2 mmol/L) were recorded by standard intermittent glucose testing in the first 6 hours of life prior to commencement of CGM, aEEG and cEEG monitoring. Accordingly, the potential neurophysiological changes that occur at glucose concentrations below 2.2 mmol/L could not be assessed in this study. Additionally, quantitative frequency and spectral power analysis may be a more sensitive method to detect changes during hypoglycemia. Furthermore, differences in distribution of glucose disturbances over time may also contribute. Whereas hyperglycemia events were more widely distributed over the course of CGM monitoring, hypoglycemia events occurred later during CGM monitoring. Although several neonates had early hypoglycemia events prior to CGM insertion, the hypoglycemia events recorded on CGM started at a mean of 48.2 hours of life (SD 13.8, ranging from 32.5 to 76 hours of life) and occurred later than hyperglycemia events which started at a mean of 29.5 hours of life (SD 19.4, ranging from 7.7 to 74 hours, p=0.016). EEG background can improve over the course of
therapeutic hypothermia although it also may remain persistently severe or worsen over the course of monitoring (Nash et al., 2011; Pressler et al., 2001; Sewell et al., 2018). Future analyses will account for evolution over time.

Our findings are consistent with a growing body of evidence that it is important to consider the potential harm of allowing permissive hyperglycemia when trying to prevent hypoglycemia (Lear et al., 2017; McKinlay et al., 2015; McKinlay, Alsweiler, et al., 2017). Several mechanisms have been proposed to explain the association of hyperglycemia with poor outcomes during critical illness, including: dyslipidemia, inflammatory cytokine production, endothelial dysfunction, hypercoagulation (Van den Berghe, 2004), and accelerated glucose toxicity leading to metabolic disturbances and increased cellular apoptosis (Quagliaro et al., 2003; Van den Berghe, 2004). The overproduction of superoxide by the mitochondrial electron-transport chain may underlie the mechanisms implicated in glucose-mediated vascular damage (Brownlee, 2001). It has also been postulated that increased glucose variability would generate more reactive oxygen species due to hyperglycemia-induced oxidative stress (Hirsch et al., 2005). Greater glucose variability has been associated with elevated markers of oxidative stress in adolescents (Dasari et al., 2016). Preclinical studies using cell culture and rodent models have shown that neuronal death was triggered by glucose reperfusion rather than hypoglycemia itself, and that hyperglycemia following hypoglycemia can worsen neuronal injury (Ennis et al., 2015; Suh et al., 2007). These findings suggest that the rate of correction of hypoglycemia as well as prevention of rebound hyperglycemia may be important.

4.2 Limitations

Our study has several limitations. Because our study is observational, it does not establish a causal relationship between episodes of hyperglycemia and abnormal brain function. Hyperglycemia may be considered as part of the physiological response to stress (Davidson et al., 2008; Dungan et al., 2009) and whether hyperglycemia is associated with worse brain function or rather with more severe HIE and therefore worse EEG scores cannot be concluded. A subgroup analysis demonstrated that epochs with hyperglycemia were still associated with worse
cEEG background scores in the subgroup of neonates that had all had episodes of hyperglycemia on CGM, strengthening our findings.

Further, accounting for clinical severity of HIE is challenging in this cohort because the clinical signs of hypoglycemia and HIE are overlapping. Signs of encephalopathy may be due to either hypoxia-ischemia or hypoglycemia. Even attempting to separate these factors on MRI can be complex, as hypoglycemia has been shown to be associated with worsened HIE-related brain injury in the corticospinal tracts (Tam et al., 2012). Also, initial clinical assessment of mild neonatal encephalopathy can still frequently be associated with MRI abnormalities (Walsh et al., 2017). We thus chose to adjust for Apgar scores, umbilical artery pH and base deficit.

In addition, other potential unmeasured confounders may exist such as carbon dioxide and pH levels which have been associated with aEEG changes in premature infants (Granot et al., 2012; Lingappan et al., 2016; Wikstrom et al., 2011). Also, the aEEG tracings were graded in 6-hour epochs due to low temporal resolution of aEEG because of time compression of the EEG signal. cEEG analysis allows better temporal resolution although there may still be EEG features altered during glucose derangements that cannot be captured by a visual scoring system.

Finally, although the continuous interstitial glucose monitors have been found to show good agreement between interstitial and blood glucose measurements (Bailey et al., 2015; Harris et al., 2010) and allow us to determine the exact duration of glucose derangements, there are still limitations of the sensors outside of 2.2 to 22.2 mmol/L. Moreover, studies have reported that interstitial glucose concentrations can have a variable “lag” behind the blood glucose concentrations by as long as 20 minutes due to delays in diffusion of glucose from the vascular into the interstitial space, as well as patient-specific and instrumental factors. There is variability in this time lag documented between studies. Variability in the lag may also be noted depending on the phase of change in the glucose, in that there may be increasing positive error as the blood glucose concentrations decrease and increasing negative error when the glucose concentrations are rising. Although, studies have also documented good agreement between the intermittent and continuous glucose measurements during the baseline, decline and hypoglycemic periods yet in the recovery phase the blood glucose levels can be significantly higher than the interstitial glucose measurements (Boyne et al., 2003; Caplin et al., 2003; D. L. Harris et al., 2009; Kovatchev et al., 2009; Rebrin et al., 1999; Schmelzeisen-Redeker et al., 2015; Shah et al.,
Consequently, an unknown and variable delay may exist between the intermittent and continuous glucose measurements and shifting the CGM data could be further explored.

Please see future directions section to see how some of the limitations discussed will be overcome in future analyses.

4.3 Conclusions

Our data give credence to our hypotheses that glucose derangements in neonates with encephalopathy are associated with abnormal brain background activity and increased electrographic seizures on aEEG and cEEG. More specifically, \textit{hyperglycemia (>8 mmol/L), but not hypoglycemia (2.2-2.8 mmol/L),} was temporally associated with worse global brain function and seizures on aEEG as well as worse brain function on cEEG. Glucose variability was associated with seizures and impaired sleep-wake cycling. However, it remains to be determined whether these associations represent a causal link between hyperglycemia, glucose variability and brain function and whether these immediate perturbations in brain function are associated with worse long-term outcomes following HIE in and of themselves. Our results are in keeping with growing evidence in the literature that hyperglycemia and glucose variability are associated with worse long-term neurodevelopmental outcomes (Al Shafouri et al., 2015; Basu et al., 2016; Chouthai et al., 2015; McKinlay et al., 2015; McKinlay, Alsweiler, et al., 2017; Salhab et al., 2004; Spies et al., 2014; Tam et al., 2012). Further analyses with longitudinal modeling to account for time series data and explore temporal correlations of glucose derangements with EEG changes are ongoing. Future studies are needed to clarify optimal treatment approaches for hypo- and hyperglycemia, in particular the optimal rate of glucose correction while maintaining brain function, in order to prevent subsequent brain injury in this high-risk population.
Chapter 5

5 Future directions

5.1 Continuous EEG monitoring - Qualitative analysis

The Neurological Outcome of Glucose in Neonatal encephalopathy (NOGIN) study is an ongoing prospective cohort study using continuous glucose monitoring, early MRI scans and long-term follow-up to resolve major knowledge gaps in our understanding of the neurological consequences of neonatal glucose derangements. Continuous glucose monitors provide average glucose values every 5 minutes and EEG monitoring provides a method that can detect acute and ongoing changes that may occur with either hypo- or hyperglycemia or glucose instability. EEG provides an approach to monitoring the developing brain with excellent temporal resolution, in the millisecond range, through direct access to brain electrical activity. It can assess dynamic oscillatory activities and brief transient cortical events. Furthermore, the scalp and skull impedance in neonates is relatively low, thus decreasing smearing of EEG signals from volume conduction and enhancing the spatial accuracy of brain activity recorded from scalp electrodes (Dan et al., 2015). Analysis of 30 cEEGs in our cohort demonstrated the feasibility of detecting acute changes using this method. Over fifty more neonates have been recruited and recruitment is ongoing. Further analysis of these cEEGs using the qualitative measures described in this study for scoring background, reactivity and state changes, and seizures will be performed, as well as the addition of quantitative EEG analysis as discussed below.
5.2 Continuous EEG monitoring - Quantitative EEG analyses

5.2.1 Previous studies validating use of quantitative EEG analyses

Quantitative automated analysis of EEG features may improve the objectivity of EEG assessment in newborns. Several studies have compared quantitative EEG features with neuroimaging findings and neurodevelopmental outcomes in neonates with HIE.

Several features of burst suppression have been assessed. A study calculated EEG discontinuity with a novel algorithm and showed that higher mean discontinuity at 24h and 48 h were associated with severe cerebral tissue injury on MRI and unfavorable neurodevelopmental outcome at 24 months old (Dunne et al., 2017). Quantitative analysis of the total suppression length has also been associated with death or neurodevelopmental disability. The authors argue that short artefacts can be mistakenly classified as bursts with an automated burst suppression classifier and can significantly influence the mean IBI but would only slightly reduce the total suppression length (Flisberg et al., 2011). Iyer et al. examined the distributions of burst area and duration and their interrelationships, demonstrating their ability to predict neuroimaging and neurodevelopmental outcomes in a cohort of 20 neonates with HIE. They had extracted 3 novel burst metrics for each EEG: mean burst duration and its coefficient of variation, scaling exponent of the cumulative distribution function of the burst areas in each recording, and slope value based on the relationship between burst duration and burst area (Iyer et al., 2014).

Other quantitative measures have also been assessed in cohorts of neonates with HIE. The spectral frequency content of bursts occurring during burst suppression can be differentiated from those during a normal tracé alternant pattern. There is a lower amount of low-frequency activity in periods of burst suppression, with the relative amount of low- and high-frequency activity being the parameter that best discriminated between the groups (Thordstein et al., 2010). A retrospective study showed that total EEG power calculated at a mean age of 8.9 h, was significantly higher in neonates with no or mild injury on MRI compared to those with moderate/severe MRI injury (Jain et al., 2017). In a preterm fetal sheep model of HIE, early hypothermia started within 30 minutes of asphyxia was associated with faster recovery of spectral edge frequency, reduced seizure burden, and less suppression of EEG power and amplitude and corresponded with reduced neuronal loss and microglial induction in the striatum on pathological examination (Wassink et al., 2015). Temko et al. developed a support vector
machine classifier for prediction of outcome in neonates with HIE using a multimodal combination of routine clinical markers, EEG features and heart rate parameters. They evaluated for neurodevelopmental outcome prediction at 24 months in newborn infants with HIE and identified 12 multimodal features that provided promising prediction results, 9 of which were EEG features and 3 were clinical features. EEG achieved the highest performance as a predictor of clinical outcome (Temko et al., 2015).

Quantitative EEG measures have also been used to assess whether EEG changes occur with changes in core body temperature during mild therapeutic hypothermia for HIE. A study using both visual and quantitative EEG measures during rewarming in 15 neonates with HIE showed that rewarming was associated with a worsening of visual EEG scores which corresponded to an increase in EEG discontinuity, and was more pronounced in newborns with severe than moderate HIE. They also examined absolute magnitude and relative spectral magnitudes and noted an increase in the relative magnitude of slower delta and a decrease in higher frequency theta and alpha waves with rewarming (Birca et al., 2016). However, another study that assessed several quantitative features of the aEEG in 10 neonates with HIE found that severity of HIE but not core body temperature affected the quantitative EEG features (Burnsed et al., 2011).

As with visual analysis, maturational changes are seen in several quantitative features with increasing gestational age in preterm neonates with normal neurological follow-up, including maturational changes in spectral measures with a shift from lower to higher frequencies, decrease IBI length and length of discontinuous activity (periods with high interburst-burst ratio) and increase in continuous activity (Niemarkt et al., 2010; Niemarkt et al., 2011) as well maturational changes in spectral power in EEG bursts which are believed to correspond to ongoing gyration and postnatal white matter maturation (Jennekens et al., 2012). Minimal changes in the power spectrum in the first 3 days of life are also noted in healthy term neonates with normal neurocognitive function at 4 years of age. When comparing the first 6 hours of life to 48 to 72 hours of life, there is overall higher relative power spectrum in the delta frequency band and lower alpha/delta ratio at 6 hours of life (Castro Conde et al., 2017). Thus, gestational age and temporal evolution since birth should be considered in analysis of quantitative measures of discontinuity and spectral measures.
Of note, quantitative EEG measures such as amplitude, continuity (skewness, kurtosis and discontinuity) and frequency content are associated with visual interpretation of the EEG background. A study had 2 expert electroencephalographers visually grade EEGs of 54 neonates into 4 categories and showed that a combination of quantitative EEG measures could accurately predict EEG/HIE grade (Korotchikova et al., 2011). Another study developed a cerebral health index score using multiple components of the EEG (power spectrum and spectral edge frequency, entropy, volatility and sub-band spectral entropy) and compared it to clinical measures of severity of HIE (based on Sarnat scores and visually interpreted EEG). The cerebral health index score was able to distinguish between normal controls and moderate and severe encephalopathy and normal versus severe EEG groups (Hathi et al., 2010). Other groups have also used quantitative EEG features to develop automatic grading scales of the degree of abnormality of the neonatal EEG (Lofhede et al., 2010; Stevenson et al., 2013) as well as automated classification of sleep cycling (or referred to with the more general term, brain activity cycling) (Stevenson et al., 2014).

Furthermore, detrended fluctuation analysis (DFA) has been discussed for CGM as a method for analyzing scaling behavior in time series and can also be used to investigate the scale-free amplitude modulation of ongoing neuronal oscillations (Hardstone et al., 2012). In a complex system, scale-free dynamics can give rise to long-range temporal correlations, which can be assessed from the EEG using DFA. Multifractal DFA (MF-DFA) can characterize time series with multiple co-existent dynamic processes that may give rise to the temporal local fluctuations, including of both small and large magnitudes (Matic et al., 2015). In a cohort of term neonates with HIE the feasibility of DFA and MF-DFA and its correlation to EEG background grades was assessed. They demonstrated that DFA could distinguish different grades of abnormality in the background EEG activity in neonates with HIE, and MF-DFA was superior to conventional DFA in distinguishing between EEG grades. MF-DFA parameters were also significantly correlated to IBI intervals. They had visually inspected a large number of DFA plots and noted that the trends were often multiphasic, with very clear “crossover points” (where there was a change of slope in the fluctuation function), which are suggestive that the EEG signal might exhibit multifractal behavior. They thus determined that MF-DFA better represents neonatal EEG dynamics (Matic et al., 2015).
Considering glucose derangements, few previous studies have examined the acute EEG changes associated with hypo- or hyperglycemia, as previously discussed, some of which used quantitative EEG measures. A study in young children that was able to detect changes during hypoglycemia performed quantitative spectral analysis of EEG during a gradual decline in glucose concentrations induced using insulin. At a plasma glucose of 4 mmol/L, an increase in delta and theta amplitudes were seen in both groups. At 3 mmol/L, there was a further and more widespread increase in low-frequency EEG activity, with greater slowing and epileptiform discharges seen in the diabetic than nondiabetic children (Bjorgaas et al., 1998). Several studies detected changes with hyperglycemia using quantitative EEG measures. In extremely preterm neonates, moderate hyperglycemia was significantly associated with greater discontinuity on aEEG, measured using an automated algorithm to measure average IBI from 10-minute artifact-free epochs of EEG recordings before each blood sample (Wikstrom et al., 2011). Schumacher et al. measured on continuous EEG recordings the total absolute band power using spectral EEG analysis and showed increased blood glucose concentrations were associated with decreased total absolute band power during the first 3 days of life in premature infants (Schumacher et al., 2014). A study using CGM and simultaneous cEEG monitoring in children with type 1 diabetes demonstrated that asymptomatic hyperglycemia was associated with changes in the power spectra of the different frequency bands during wakefulness and sleep (Rachmiel et al., 2016).

Therefore, several quantitative EEG features have been validated in term neonates with HIE, are predictive of neuroimaging findings and neurodevelopmental outcomes, and correlate with visual EEG grading scales. Quantitative EEG analysis may provide a more objective measure than visual analysis, as well as additional detail and novel measures of EEG complexity. For example, our visual EEG classification would not capture an increase in slow frequencies or increase in IBI duration which have been previously reported with hypo- and hyperglycemia. Thus, the addition of quantitative EEG analysis in our future analyses will provide the opportunity to assess acute changes in brain activity associated with glucose derangements in neonates with HIE beyond the features captured in our visual background grading.
5.2.2 Application of quantitative EEG analyses in the NOGIN study

Several quantitative EEG features will be assessed in our cohort through a collaborative project with Dr. Sampsa Vanhatalo, a pediatric neurophysiologist at the University of Helsinki. The signal will be segmented into 5-minute non-overlapping epochs corresponding to the 5-minute epochs of mean glucose data available from the continuous interstitial glucose monitor. The quantitative features are calculated on each epoch for each channel and then averaged across channels. Quantitative EEG features being assessed will include mean amplitude of the epoch (mcV), mean frequency of the epoch (Hz) (Stevenson et al., 2013), measures of spectral power from different frequency ranges (delta 1 [0.5-2Hz], delta 2 [2-4 Hz], theta [4-7 Hz], alpha [7-12 Hz], and beta [12-30 Hz]) (O'Toole et al., 2016), activation synchrony index (measure of interhemispheric synchrony) (Rasanen et al., 2013), IBI (seconds), duration of bursts (sec), DFA (Kantelhardt et al., 2001) and MF-DFA (Ihlen, 2012).

The relationship of hypo- or hyperglycemia with the qualitative and quantitative EEG findings will be analyzed, adjusting for clinical markers of HIE severity (Apgar scores, umbilical artery pH and base deficit) as well as gestational age and antiseizure medications. Longitudinal modeling will be used to account for time series data and explore temporal correlations of glucose levels with EEG changes. The visual and multiple quantitative EEG features will be assessed to investigate which variables fit better with the changes in glucose over time. We will incorporate cubic splines in the GEE models to characterize the possibly non-linear relationship between cEEG features and glucose levels, in particular to investigate the possibility of threshold effects. Phase shifting of the CGM data will be used to explore the delay that may exist between the intermittent and continuous glucose measurements.
5.3 Short and long-term outcomes

Both short and long-term outcomes will be assessed in our cohort (Figure 5.1) to identify if the neurophysiological changes associated with glucose derangements are associated with later outcomes. Previous studies have shown a relationship between EEG background abnormalities with brain injury on MRI in neonatal encephalopathy (Biagioni et al., 2001; Briatore et al., 2013; Dunne et al., 2017; Shah et al., 2006), and MRI patterns of injury are predictive of long term outcomes (Biagioni et al., 2001; Dalmazzo et al., 2011). However, EEG findings have not yet been linked to outcome in our cohort. Short-term outcomes will include a standardized neurological examination and MRI at 3-5 days (including 3-dimensional T1 and T2-weighted sequences, diffusion tensor imaging and MR spectroscopy).

EEG background abnormalities and recovery have also been shown to be predictive of long-term neurodevelopmental outcome in neonates with HIE (Azzopardi & Toby study group, 2014; Chandrasekaran et al., 2017; Dunne et al., 2017; Skranes et al., 2017; Spitzmiller et al., 2007; Thoresen et al., 2010; van Laerhoven et al., 2013). Long-term outcomes are being assessed in our cohort to identify if the acute changes seen on aEEG and cEEG associated with hyperglycemia and glucose variability are predictive of long-term neurodevelopmental outcomes. At 18 and 36 months, children undergo full neurological examination and standardized tests of visual function by a pediatric neurologist, and cognitive and neurodevelopmental outcomes will be assessed by a pediatric neuropsychologist using several measures to assess for language, cognitive, behavioral, visual and motor function.

The optimal timing for assessing children to accurately predict development in childhood and beyond remains debated. Environmental and educational experiences and supports may alter developmental trajectories but deficits may also emerge that may not have been evident at
younger ages. The greater academic and psychosocial demands placed on children as they grow older can reveal neurodevelopment abnormalities potentially associated with glucose derangements that occurred in the neonatal period. The CHYLD study performed initial follow-up at 2 years and hypoglycemia was not associated with neurodevelopmental impairments (McKinlay et al., 2015). However, at 4.5 years old, the investigators found that neonatal hypoglycemia was associated with an increased risk of low executive function and low visual motor function, with the highest risk in those children that had severe, recurrent, or clinically undetected hypoglycemia (McKinlay, Alsweiler, et al., 2017). Similarly, in children with congenital heart disease the investigators showed that postoperative electrographic seizures were not associated with worse outcomes on the BSID at 1 year of age, except in the subset of children with frontal-onset seizures (Gaynor et al., 2006). Whereas at 4 years, postoperative electrographic seizures were associated with worse executive function and impaired social interactions or restricted behaviours (Gaynor et al., 2013). Furthermore, although the BSID is a reliable instrument and in particular the subdomain scores can predict later performance (Soysal et al., 2014), in both term and premature populations, measures of cognitive function during infancy have been shown to have poor correlation with later childhood IQ and may overestimate neurocognitive impairments (Aylward, 2004; Hack et al., 2005; Illingworth et al., 1959). Thus, follow-up at 3 years and beyond will be important in determining whether delays persist into early childhood.

5.4 Future directions - Summary

This study has demonstrated that acute neurophysiological changes can be detected with glucose derangements in neonates with encephalopathy. Findings in this study support growing evidence that it is important to consider the potential harm in allowing permissive hyperglycemia while trying to prevent hypoglycemia. In several of the previous trials with intensive insulin therapy for hyperglycemia management there has been an increased incidence of hypoglycemia seen (Agus et al., 2017; Alsweiler et al., 2012; Macrae et al., 2014; Vlasselaers et al., 2009) and inconsistent effects on glucose variability (Siegelaar et al., 2010). CGM provide an advantage by providing continuous glucose trending information that can be acted on rapidly and may protect
against sudden unexpected decreases in blood glucose. However, there is currently limited evidence for treating newborns based on this monitoring. Knowledge about the degree and duration of hypo- and hyperglycemia that correlate with changes in global brain function will aid in determining clinically significant target glucose ranges in neonates, leading to the development of evidence-based algorithms for glucose management and effective neuroprotection. How management algorithms effect the rate of glucose correction and glycemic variability and subsequent long-term outcomes will be important to consider. The ultimate goal is to decrease brain injury and improve long-term outcomes of neonatal encephalopathy.
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# Appendix 1 - Appendix Table 1

Demographic data of the study cohort; for participants included in aEEG analysis and for participants excluded for incomplete data

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<td>3 ♀: 3 ♂</td>
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<td><strong>Gestational age (wks), mean (SD)</strong></td>
<td>39.54 (1.40)</td>
<td>39.86 (1.52)</td>
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<td><strong>Birth weight (grams), mean (SD)</strong></td>
<td>3414.36 (548.24)</td>
<td>2745 (487.38)</td>
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<tr>
<td><strong>Birth length (cm), mean (SD)</strong></td>
<td>50.14 (3.15)</td>
<td>47.92 (2.11)</td>
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<td><strong>Head circumference (cm), mean (SD)</strong></td>
<td>34.22 (1.31)</td>
<td>33.17 (1.37)</td>
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<td><strong>Maternal diabetes, n (%)</strong></td>
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<tr>
<td>Type II Diabetes Mellitus</td>
<td>5 (11)</td>
<td>1 (17)</td>
<td>0.548</td>
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<tr>
<td>Gestational Diabetes</td>
<td>1 (2)</td>
<td>0 (0)</td>
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<tr>
<td><strong>Maternal hypertension, n (%)</strong></td>
<td>4 (9)</td>
<td>0 (0)</td>
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<td>Maternal pre-eclampsia/ eclampsia</td>
<td>2 (4.5)</td>
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<td><strong>Apgar score at 5 min, mean (SD)</strong></td>
<td>4.18 (2.39)</td>
<td>4.50 (1.08)</td>
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<td><strong>Umbilical arterial cord pH, mean (SD)</strong></td>
<td>6.99 (0.18)</td>
<td>6.99 (0.23)</td>
<td>0.987</td>
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<tr>
<td><strong>Umbilical arterial cord BE, mean (SD)</strong></td>
<td>-15.13 (6.79)</td>
<td>-13.33 (11.37)</td>
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<td><strong>Route of delivery, n (%)</strong></td>
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<td>Vaginal delivery</td>
<td>20 (44.5)</td>
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<td>Cesarean section</td>
<td>25 (55.5)</td>
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<td><strong>Sentinel event, n (%)</strong></td>
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<td></td>
<td>17 (38)</td>
<td>4 (67)</td>
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<td><strong>Seizures, n (%)</strong></td>
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<td>Clinical seizures only</td>
<td>28 (62)</td>
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<td>Electrographic and/or electoclinical seizures</td>
<td>12 (26.5)</td>
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<td><strong>Antiepileptic medications, n (%)</strong></td>
<td>26 (58)</td>
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<td>Lorazepam only</td>
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<td>Phenobarbital</td>
<td>13 (29)</td>
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<td>≥2AEDs</td>
<td>7 (15.5)</td>
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Continuous variables were analyzed with the student’s T-test, whereas categorical variables were analyzed with Fisher’s exact test. Significant p values are shown in bold.

*Electrographic and/or electoclinical seizures were identified by clinical team on aEEG or cEEG. Clinical seizures only had no seizures captured on aEEG or cEEG monitoring.

**The birth weight was significantly lower in excluded neonates and may be related to greater difficulty with CGM insertion in smaller neonates.
Appendix 2 - Contributions

The author was responsible for the writing and preparation of this original thesis. All of the work presented, including the planning, data analysis, interpretation of results and writing of the original research, was performed by the author, with the guidance and expertise of the individuals listed below. The following contributions to the work in this thesis are formally and inclusively acknowledged:

**Dr. Emily Tam** conceived and developed study design, data collection, provided guidance with analytic approach and interpretation of results, and provided important feedback and detailed revisions of the thesis.

**Dr. Cecil Hahn** provided guidance with study design, analytic approach, interpretation of results, provided expertise for development of EEG scoring and review, supervision for clinical review and reporting of cEEG studies, and provided important feedback and detailed revisions of the thesis.

**Dr. Rollin Brant** provided guidance for statistical analyses.

**Dr. Steven Miller** and **Dr. Aideen Moore** provided guidance with study design and analytic approach. **Dr. Vann Chau** provided guidance with study design, analytic approach and contributed help with clinical review and reporting of cEEG studies.

**Daphne Kamino** was responsible for overall study set-up and management, including managing REB submissions, protocol development, subject recruitment, REDCap database design & data management of all study data and helped with cEEG coordination. **Ashley LeBlanc**, **Angela Thompson**, and **Giselle Da Rocha** contributed help with clinical data collection.

**Drs. Ayako Ochi, Tina Go, Dragos Nita and Ala Birca** contributed help with clinical review and reporting of cEEG studies. The **Neurophysiology technologists including Roy Sharma** applied electrodes and started EEG recordings for all study subjects.

**The Acute Care Transport Services team including Marc LePine and Michelle Manning** recruited all study subjects and **Matthew Keyzers** for study recruitment and storage of aEEG files.

This work was supported by a CIHR grant awarded to **Dr. Emily Tam**, as well as the Savoy Epilepsy Foundation Studentship and Research Training Competition (RESTRACOMP) Graduate Scholarships and Research Fellowship awarded to Dr. Elana Pinchefsky.
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Title: Outcomes following electrographic seizures and electrographic status epilepticus in the pediatric and neonatal ICUs

Author: Eliana Pinchefsky and Cecil Hahn

Publication: Current Opinion in Neurology

Publisher: Wolters Kluwer Health, Inc.

Date: Apr 1, 2017

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