Long-term neurodevelopmental and metabolic consequences of neonatal hyperglycaemia and early life protein intake

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Abstract

Neonatal hyperglycaemia is common in infants born very preterm, and is associated with adverse outcomes. However, it is not clear whether these associations are causal. The incidence of neonatal hyperglycaemia appears to decrease when early life protein intakes are increased, but associations between higher neonatal protein intakes and long-term outcomes are not well studied.

Our aim was to test the hypotheses that, in children born very preterm: i) tight glycaemic control for neonatal hyperglycaemia does not change metabolic outcomes compared with standard glycaemic control, but worsens neurodevelopment; ii) neonatal hyperglycaemia is associated with impaired metabolic, body composition and neurodevelopmental outcomes; and iii) increased neonatal protein intake is associated with improved metabolic outcomes, without changing neurodevelopment.

Eligible infants were born at <1,500 grams or <30 weeks' gestation, and admitted to the neonatal intensive care unit, National Women's Hospital, Auckland, NZ. Neonatal blood glucose concentrations and nutritional intakes were collected. At 7 years' corrected age children underwent standardised developmental assessments, intravenous glucose tolerance testing, and measurements of growth and body composition.

In the neonatal cohort (n=536), neonatal hyperglycaemia was associated with small, sick infants. A total of 129 children were assessed at 7 years' corrected age. In 57 with neonatal hyperglycaemia and randomised as neonates in a trial of tight vs. standard glycaemic control, tight glycaemic control did not change neurodevelopmental outcomes, but reduced fasting blood glucose concentrations, reduced height, and increased lean mass at 7 years. Children with neonatal hyperglycaemia (n=57) had worse neurodevelopmental outcomes than 54 non-hyperglycaemic matched preterm controls, but this difference did not persist after correction

for perinatal characteristics. Higher early protein intakes were associated with an increased risk of cerebral palsy and motor impairment at 7 years.

Neonatal hyperglycaemia, as currently managed, is not associated with altered neurodevelopmental outcomes in mid-childhood. A brief period of tight control of neonatal hyperglycaemia alters later growth and body composition, supporting the need for long-term follow up of neonatal interventions. The observed associations between increased early life protein intake and motor impairment in childhood require testing in a randomised control trial.

This work is dedicated in loving memory to my brother,

Christopher George Tottman

15th May 1989 – 16th February 2001

Acknowledgements

This thesis describes a cohort of children born very preterm and very low birth weight, and who received neonatal intensive care in Auckland, New Zealand. At the most difficult, frightening and uncertain time in their children's lives, these new parents consented to take part in research and, when contacted 7 years later, travelled from around the country to attend follow up assessments. Their desire to contribute to the advancement of neonatal care and to help other infants born preterm is humbling, and I am truly grateful to each and every family who gave up their time to be involved in this project.

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Table of Contents

List of Figures

List of Tables

Chapter 1. Introduction and literature review

1.1. Introduction

Infants born very preterm or at very low birth weight are at risk of developing high blood glucose concentrations in the neonatal period (Beardsall et al., 2010). Whether these high blood glucose concentrations contribute to neonatal mortality and morbidity, or to later adverse neurodevelopment and metabolic health, is not yet clear. Despite there being no agreed threshold at which high blood glucose concentrations may be described as pathological, or data with which to guide neonatal blood glucose management, neonatal hyperglycaemia is frequently diagnosed and treated with infusion of insulin or restriction of nutritional intakes (Alsweiler, Kuschel, & Bloomfield, 2007). Inadequate nutrient supply following preterm birth is likely to be one of the factors contributing to the high rates of faltering postnatal growth seen in very preterm infants (Grover, Khashu, Mukherjee, & Kairamkonda, 2008), and recent strategies to improve postnatal growth include provision of higher early protein intakes (Cormack & Bloomfield, 2013), with little data regarding the associated long-term outcomes.

This thesis explores whether tight glycaemic control for neonatal hyperglycaemia, neonatal hyperglycaemia itself, or changes to early neonatal macronutrient intakes, are associated with altered neurodevelopmental and metabolic outcomes in preterm infants at 7 years of age. It encompasses both neonatal observational studies and school-age follow up assessments undertaken as part of the Protein, Insulin and Neonatal Outcomes (PIANO) study.

This chapter provides an overview of the short- and long-term outcomes related to preterm birth, normal development of the endocrine pancreas, and the changes to glucose homeostasis that occur in the perinatal period. The factors relating to abnormal metabolic transition and the development of both hyperglycaemia and hypoglycaemia are reviewed, and the outcomes associated with abnormal neonatal glycaemia are explored. This chapter concludes by summarising fetal and neonatal protein metabolism, describes the associations between early

macronutrient intakes and later outcomes (including the potential for these relationships to be sex-specific), and briefly introduces the protein leverage hypothesis.

The aims and methods of the PIANO study are detailed in chapter 2. The subsequent chapters comprise published or submitted manuscripts detailing the results of the PIANO study. These results as a whole are discussed in chapter 10.

1.2. Preterm birth

The survival of infants born at the limits of viability or of extremely low birth weight (ELBW) is a modern phenomenon. Beginning with the simple act of placing an infant in a linen cupboard to keep warm (Christie & Tansey, 2001), the discipline of neonatology has grown to the point where even infants born at the end of the second trimester of pregnancy have a relatively good chance of survival into adulthood (Chow, 2013). However, increased preterm survival has not come without cost. Children born at extremely preterm gestations experience weeks or months of stressful intensive care, multiple medical procedures, and separation from their mothers during a period of what should be rapid fetal growth and development. The immediate complications of extremely preterm birth, such as intraventricular haemorrhage, periventricular leukomalacia, surfactant deficiency, sepsis, necrotising enterocolitis, and retinopathy of prematurity, have been the subject of a great deal of attention in a bid to improve neonatal health outcomes. Increasing survival rates of preterm neonates may be attributed to the research-driven improvements in neonatal care seen in the past 50 years.

In New Zealand, over 500 infants per year are admitted to a neonatal intensive care unit (NICU) having been born at < 32 weeks' gestation. Data collected from Australasian neonatal units show infants born at 24 weeks gestation have a 64% chance of survival to NICU discharge, with survival rates increasing to over 98% among infants born after 30 weeks of pregnancy (Chow, 2013). Infants born preterm are at increased risk of health problems

requiring hospital readmission after the neonatal period (Elder, Hagan, Evans, Benninger, & French, 1999). They are more likely to have cognitive, sensory or motor deficits diagnosed by 2 years of age compared to children born at term, with the frequency and severity of deficit increasing with decreasing gestational age (Moore et al., 2012). Although information is available on the progress of children born extremely preterm in the preschool years, the impact of extremely preterm birth on middle childhood, teenage and adult health outcomes is relatively less well studied.

1.3. Long-term consequences of preterm birth

Preterm birth is associated with increased mortality not only in infancy, but also in childhood and early adulthood (Crump, Sundquist, Sundquist, & Winkleby, 2011). Despite this, survival into adulthood of infants born prior to 30 completed weeks of pregnancy is now commonplace. This increasingly numerous group has allowed further research into the long-term consequences of extremely preterm birth, including its effects on growth, metabolism, cardiovascular risk factors, cognition, behaviour (including the prevalence of psychopathology) and sensory deficits.

Children born preterm at very or extremely low birth weights (< 1,500 grams and < 1000 grams) may be appropriately grown, large or small for gestational age. These fetal growth patterns are unlike those of low birth weight infants born at term gestations, the majority of whom are small for gestational age and have been subject to *in utero* metabolic deprivation; thus direct comparison of outcomes between term low birthweight and preterm infants is not advisable. Unlike term-born infants, preterm infants commonly experience inadequate postnatal nutrition and failure to match an optimal growth trajectory (Martin et al., 2009). Despite this, some preterm infants show periods of catch up growth in the neonatal period, such that by term corrected age they are an age appropriate weight and length. However, a more frequently described growth pattern is that of faltering neonatal growth, followed by a

significantly increased height velocity in the preschool years, reaching a plateau in middle childhood (Monset-Couchard & de Bethmann, 2000). A recent review has suggested that the timing of periods of growth failure and catch up growth may be related to both neurodevelopment and later metabolic health outcomes (Lapillonne & Griffin, 2013). Despite catch up growth, many ex-preterm children remain short for age throughout middle childhood (Knops et al., 2005). An additional period of catch up growth may occur during adolescence so that an appropriate adult stature is obtained, but on average, height *z*-scores at age 18 remain lower in those born extremely preterm compared to those born at term (Roberts et al., 2013). Increased adolescent height growth velocity could reflect early onset of the pubertal growth spurt in children born preterm, in whom a correlation between decreasing gestational age and advanced pubertal onset has been observed (Wehkalampi et al., 2011). Body composition may also be permanently altered by preterm birth, with adults born at preterm gestations and who experience postnatal catch up growth having a greater fat mass and higher BMI than their term compatriots (Euser et al., 2005), whilst slower accrual of bone mass in childhood and decreased peak bone mineral density in adulthood are seen in those born at less than 1,500 grams (Hovi et al., 2009).

Children born small for gestational age and who experience catch up growth have increased insulin resistance in childhood compared to those born at an appropriate weight (Veening, van Weissenbruch, Heine, & Delemarre-van de Waal, 2003). Insulin resistance is increased in childhood for infants born at low birth weight or preterm gestations (Hofman et al., 2004a). In a large follow up study of adults born at preterm gestations, increasing insulin resistance correlated with decreasing gestational age but was independent of birth weight *z*-score (Dalziel, Parag, Rodgers, & Harding, 2007). The same relationship was noted between decreasing gestational age and increasing systolic blood pressure; a relationship supported by findings in an older cohort of adults born at term and preterm gestations (Cooper, Atherton, & Power, 2009). These findings suggest that the risks of developing type-2 diabetes and

hypertension, and the subsequent increased morbidity and mortality accompanying these conditions, are likely to be higher in adults born preterm compared to those born at term. Pregnant, preterm-born adult women have higher rates of both hypertension and diabetes, and of overall pregnancy complications, than those born at term (Boivin et al., 2012).

Preterm birth, especially at very preterm gestations, is associated with disability and neurodevelopmental impairment in childhood. Rates of adverse neurodevelopmental outcome have been falling as neonatal care improves; however, only a minority of infants born at the limits of viability survive without impairment, the severity of which increases with decreasing gestational age (Moore et al., 2012). Neonatal follow-up programmes have traditionally assessed children at the ages of 2-4 years (Doyle et al., 2014), at which time diagnoses of cerebral palsy, sensory deficit, motor, language and cognitive impairment may be made with some certainty. However, cognitive development progresses during childhood, such that a low cognitive score on standardised testing in early childhood may not be predictive of cognitive impairment at the age of 8 years, in the absence of other sensory-motor deficit (Cartar et al., 2005).

Rates of global cognitive impairment in populations of ELBW or extremely preterm infants assessed at primary school age are high, with 24-56% of children reported as scoring below the average range on standardised testing (Hutchinson, De Luca, Doyle, Roberts, & Anderson, 2013; Saigal et al., 2003). More subtle neurodevelopmental impairments may become apparent later in childhood, when greater demands, such as the increasing academic and behavioural expectations that accompany progression through schooling, are placed on the child. School achievement scores are lower in preterm compared to term born children; in some cases over 50% of children born ELBW are identified as requiring special educational assistance (Saigal et al., 2003). Decreased educational achievement persists throughout the adolescent years (Buck, Msall, Schisterman, Lyon, & Rogers, 2000; Lagerstrom, Bremme,

Eneroth, & Janson, 1991) and adults born at even moderate preterm gestations are less likely than their term-born peers to complete secondary or tertiary education (Tanskanen et al., 2011).

One of the underlying causes of poor academic achievement in preterm populations may be a high incidence of behavioural problems, particularly attention difficulties (Jaekel, Wolke, & Bartmann, 2013). Children born preterm are more likely to show inattention than hyperactivity or combination disorders (Shum, Neulinger, O'Callaghan, & Mohay, 2008), although all types of attention difficulty are increased compared to those born at term (Lindstrom, Lindblad, & Hjern, 2011). Attention difficulties are associated with impaired measures of executive function (Mulder, Pitchford, Hagger, & Marlow, 2009), but may have variable impact upon global cognitive function scores depending on the assessment used. Significantly higher rates of anxiety and other emotional disorders, autistic spectrum disorders and other psychiatric diagnoses are also seen in the preterm compared to the term population in later childhood (Johnson et al., 2010). Increased psychiatric morbidity persists into adulthood, with those born small for gestational age at higher risk of both internalising and externalising disorders compared to those born at very low birth weight, where internalising disorders predominate (Lund et al., 2012).

Visual and auditory impairments are common in the extremely preterm population (Moore et al., 2012) and may result from prematurity itself or from unintended consequences of neonatal intensive care, such as oxygen or aminoglycoside toxicity. Retinopathy of prematurity (ROP) was historically associated with retinal detachment and high rates of substantial or complete visual loss in preterm survivors (Pollan, 2009). Attention to minimising oxygen toxicity, and rigorous neonatal screening and treatment programmes have reduced the number of catastrophic visual outcomes in preterm infants (Doyle & Anderson, 2005). Nevertheless, ROP remains a significant cause of visual impairment in children who were born preterm.

Myopia and strabismus, two of the most common visual anomalies seen in ex-preterm subjects (Leung, Thompson, Black, Dai, & Alsweiler, 2017), may both be associated with severe ROP. However, both are also seen in ex-preterm children who did not have a diagnosis of ROP in the neonatal period. In the case of myopia, absence of a history of ROP is associated with a later onset and milder severity of visual deficit (O'Connor & Fielder, 2008). Although the majority of significant visual deficits can be identified by the age of 2 years, visual problems may present or progress throughout the childhood years (Quinn et al., 2008) and adults born preterm may develop late onset visual loss (Fledelius & Jensen, 2011). Hearing loss is less commonly diagnosed than visual loss, but remains more frequent in infants born at preterm compared with term gestations (Martines, Martines, Mucia, Sciacca, & Salvago, 2013). Children born preterm may display delayed maturation of the auditory pathways such that some hearing is regained in early childhood (Hof et al., 2013). For those who do not recover sufficient hearing to promote speech development, treatment with traditional amplifying hearing aids or cochlear implants may be considered. Both hearing and visual loss are more common in ex-preterm children who have a diagnosis of cerebral palsy than those without neurological dysfunction. When cerebral palsy and severe visual impairment are present together, a negative relationship with long term survival is seen. This relationship is not evident in children with combined cerebral palsy and significant hearing loss (Hutton & Pharoah, 2002).

Despite the persistence of health, developmental, educational and psychological difficulties into adulthood following preterm birth, adults born preterm rate their own quality of life as similar that of adults born at term gestations, with no distinction between those born at extremely as compared to moderate preterm gestations despite an increased incidence of disability in the former group (Saigal).

1.4. Development of the endocrine pancreas

The human embryonic pancreas develops from the fusion of ventral and dorsal out-pouchings of the primitive gut during the seventh week of embryonic life (Villee, 1969). All pancreatic endocrine cell types (α (glucagon secreting), β (insulin secreting), δ (somatostatin secreting), ε (ghrelin secreting) and PP (pancreatic polypeptide secreting)) are present in the Islets of Langerhans at birth, alongside the cells of the exocrine pancreas (Andralojc et al., 2009). Expansion of islet β-cell mass during gestation occurs through the processes of neogenesis, replication and cell hypertrophy (Ackermann & Gannon, 2007). The perinatal period represents a critical window for the accumulation of β-cells, as the ability to create new β-cells through neogenesis appears to be limited beyond this point (Bouwens & Rooman, 2005). If sufficient β-cell mass is not accumulated during this critical period, or injury occurs to the mature pancreas, inadequate insulin secretion may result in disease, such as type-1 or type-2 diabetes mellitus (Ackermann & Gannon, 2007).

Knowledge regarding the process of endocrine cell development and hormone expression in the human fetus is limited by tissue supply; a study of 65 embryonic, fetal and neonatal human pancreatic specimens performed by Meier et al (2010) is the largest study to date. It revealed glucagon expression in the embryonic pancreas by the 8^{th} week of gestation. The expression of insulin was slightly delayed, appearing a week later and becoming abundant by the 12^{th} week. Both α and β-cells were present by the 9^{th} week of gestation, initially clustering along pancreatic ductal epithelium, then organising into early islet structures from the 12^{th} week. Islet cell organisation continued throughout fetal life, resulting in a mature central β-cell peripheral α-cell structure after the 26^{th} week of gestation. β-cell proliferation was present from the 9^{th} week onwards and remained consistent throughout the embryonic and fetal periods, with around 2.9% of the β-cell population expressing replication markers at any one time (Meier et al., 2010). Unlike the pattern seen in rodents (Scaglia, Cahill, Finegood, &

Bonner-Weir, 1997), human pancreatic endocrine cell structures do not appear to undergo a significant re-modelling period prior to birth, with expression of β-cell apoptotic markers stable throughout gestation (Meier et al., 2010). In neonatal rats, static β-cell mass between postnatal days 2-20 is followed by a rapid increase in mass from days 24-31, just before weaning (Scaglia et al., 1997). Findings in human pancreata suggest that β-cell proliferation increases after birth at term and continues until the second postnatal year, after which proliferation rates fall and are maintained throughout childhood (Gregg et al., 2012).

To overcome the difficulties of studying human pancreatic development, Blanco et al have performed a series of studies in baboons (Blanco et al., 2010; Quinn et al., 2012). In pancreas specimens taken from 12 prematurely delivered baboons, α, β and δ cell volumes remained constant as a percentage of the overall pancreatic volume at three preterm gestations, and islet cell structure became more organised throughout fetal life, as is also seen in the human (Quinn et al., 2012). In baboons killed immediately after birth at term, total pancreatic weights were similar to those of the preterm baboons, and pancreatic weight was consistent across gestation. However, in term baboons allowed to survive and feed freely for 5 days after birth, pancreatic weights were more than twice that of those killed at birth. This weight increase was not accompanied by an increased percentage of any one cell line, suggesting that both the endocrine and exocrine pancreas are involved in this expansion (Quinn et al., 2012). This rapid weight increase may reflect a period of vulnerability for the immature pancreas, and is a process also seen in the rat, albeit at a later postnatal age (Scaglia et al., 1997). Human studies suggest that increased proliferation of β-cells between birth and 2 years of age contributes to the establishment of the baseline pancreatic β-cell mass by 5 years of age (Gregg et al., 2012). It is not yet clear how preterm birth may affect this process in humans, but in sheep born preterm, the β-cell apoptosis to proliferation ratio is increased resulting in reduced relative and absolute β-cell mass in preterm lambs compared to term controls (Bansal et al., 2015).

Another period of pancreatic vulnerability may occur earlier in gestation, with baboon studies revealing pancreatic cells co-expressing insulin and trypsin, thus performing both endocrine and exocrine functions concurrently (Quinn et al., 2012). These cells were present at 65% of term gestation, but were not found in the term neonatal animal. In humans, cells co-expressing insulin/ glucagon/ somatostatin comprise up to 2% of the total cell volume at 12 weeks' gestation, with co-expressing cells becoming rare by full term gestation (Meier et al., 2010). These findings raise the possibility that a subpopulation of pancreatic cells may be bi- or pleuripotential beyond the embryonic period. If so, the potential exists for an early insult to the fetal pancreas to result in an abnormal distribution of mature endocrine cells due to alterations in the trajectory of pleuripotential cell maturation. This is an area of great scientific interest, as a population of stem-cells may allow β-cell regeneration in the mature pancreas and thus a potential cure for diabetes. Recent work (Banakh, Gonez, Sutherland, Naselli, & Harrison, 2012) has demonstrated the ability of mature mouse pancreatic cells, described as side population cells, to behave as endocrine cell progenitors, responding to β-cell injury by expansion, proliferation and differentiation into insulin secreting cells. Side population cells have been identified in both fetal and adult human pancreas (Zhang et al., 2005) and current research efforts are directed at investigating their potential use to restore insulin sufficiency in the diabetic pancreas.

The development of β-cell mass may be disrupted by an abnormal requirement for fetal insulin secretion, secondary to alterations in fetal nutritional supply (Henquin, 2000). In fetal sheep, chronic hypoglycaemia (a known complication of human intrauterine growth restriction) leads to reduced insulin secretion by β-cells in response to stimulation, but normal β-cell number, structure and insulin content (Rozance, Limesand, Zerbe, & Hay Jr, 2007). In contrast, fetal sheep exposed to hyperthermia-induced growth restriction show a markedly reduced β-cell mass secondary to decreased β-cell proliferation, accompanied by a disproportionate decrease in expression of insulin mRNA when compared to non-growth restricted controls. These

changes appeared to be specific to the β-cell line, as α-cell mass and glucagon expression were not reduced further than would be expected by the lower pancreatic weights seen in the growth restricted group (Limesand, Jensen, Hutton, & Hay, 2005). This finding suggests that β-cell development is plastic and responsive to the *in-utero* nutritional environment. Similar results have been reported in rat models, where maternal protein (Petrik et al., 1999) and calorie restriction (Dumortier et al., 2007) result in decreased β-cell mass in the offspring. The greater impact on β-cell mass by multi-nutrient as opposed to selective glucose restriction may be explained by the decreased sensitivity of β-cells to glucose at earlier gestations, when nutrients other than glucose play a larger role in stimulation of insulin secretion (Hellerström & Swenne, 1991). However, recent work has revealed that fetal pancreatic insulin secretion is increased by an infusion of amino acids to the fetal sheep, but this intervention does not impact the final β-cell mass (Gadhia et al., 2012). The effect of amino acid supplementation on insulin secretion in the growth restricted fetus is as yet unknown.

Maternal obesity also preferentially accelerates fetal β-cell development in the sheep (Ford et al., 2009), whilst maternal hyperglycaemia may increase or decrease fetal islet cell insulin content, dependent upon the severity of exposure (Green et al., 2012). β-cell proliferation may be directly promoted by increased circulating insulin concentration and indirectly promoted via its secondary effect of increasing production of insulin-like growth factors (IGF) 1 and 2 (Hill, Hogg, Petrik, Arany, & Han, 1999; Petrik et al., 1999). This has implications for insulin sufficiency in later life, as β-cells subject to excessive proliferation in early development may reach the limits of their capacity for renewal more rapidly, resulting in premature loss of insulin production (Gunasekaran, Hudgens, Wright, Maulis, & Gannon, 2012). In humans, children of mothers with gestational diabetes or obesity are more likely than the general population to display features of the metabolic syndrome (Chandler-Laney et al., 2012; Tam et al., 2010) and to develop type-2 diabetes mellitus in later life (Barker, 1999). Although insulin

resistance is responsible for some of this increased risk, the role of a prematurely exhausted β-cell mass cannot be discounted.

1.4.1 **Insulin**

Insulin, a polypeptide hormone comprising 51 amino acids arranged as A and B chains, is produced by β-cells from the 9th week of gestation onwards (Meier et al., 2010). The insulin precursor, preproinsulin, is constructed in the rough endoplasmic reticulum of the β-cell, cleaved to produce the three amino-acid chain proinsulin and transported to the cytoplasm where it is stored in secretory granules to await release. Insulin is formed from proinsulin by the dissolution of disulphide bonds, leaving the active insulin molecule (A and B chains) plus C-peptide within the β-cell granules (Belchez & Hammond, 2003; Uchizono, Alarcon, Wicksteed, Marsh, & Rhodes, 2007).

The mechanism for insulin release involves the transport of glucose across the β-cell membrane by the Glut-2 facilitative glucose transporter, followed by metabolism to produce adenosine triphosphate (ATP). ATP sensitive potassium channels on the β-cell membrane close, leading to the depolarisation-mediated opening of voltage-gated calcium channels on the cell membrane. The subsequent calcium influx promotes exocytosis of insulin storage granules (preferentially those most recently formed) (Uchizono et al., 2007) and insulin release (Henquin, 2000). Insulin release shows stimulus-secretion coupling via glucose stimulated insulin secretion (GSIS) (Henquin, 2000), and amino-acid stimulated secretion (AASIS) (Rozance, Limesand, & Hay, 2006). β-cell sensitivity to a glucose stimulus increases during gestation, and by term glucose is the dominant stimulus for insulin release (Aldoretta, Carver, & Hay Jr, 1998). Specific amino acids can also stimulate insulin release; leucine increases β-cell ATP production, and lysine and arginine directly depolarise the β-cell membrane (Green et al., 2012; Limesand, Rozance, Zerbe, Hutton, & Hay Jr, 2006). Insulin

release is also promoted by adrenergic stimulation of the β-cell mass via β2 receptors (Jackson, Piasecki, Cohn, & Cohen, 2000).

1.4.2 **Insulin-like growth factors**

The major determinant of fetal growth, the insulin–like growth factor axis, comprises the hormones insulin, IGF-1 and IGF-2, 4 receptor types and 6 main regulatory binding proteins (IGFBPs) (Rajaram, Baylink, & Mohan, 1997). IGFs -1 and -2 are structurally similar to the proinsulin molecule, each comprising an A and B amino acid chain connected by a C-peptide. Due to these similarities, there is cross-reactivity between insulin and the IGFs: insulin can activate its own receptor plus a hybrid IGF Type 1/ insulin receptor; IGF-2 activates both of these in addition to specific IGF Type 1 and Type 2 receptors; and IGF-1 acts on the insulin, Type 1 and Type 1/ insulin hybrid receptors (Randhawa & Cohen, 2005). IGF expression alters with development. IGF-2 is dominant in fetal life in experimental animals and humans. Its concentration decreases rapidly in the neonatal period and especially at the point of weaning, so that IGF-2 is nearly undetectable in the adult human. On the other hand, IGF-1 concentrations rise rapidly in the neonatal period, steadily in childhood, and peak during puberty (Fowden, 2003). Unlike insulin, both forms of IGF are expressed by a wide variety of fetal tissues, including the placenta (Li, Saunders, Gilmour, Silver, & Fowden, 1993), and have actions at both an endocrine and paracrine level.

IGF-1 is an important mediator of blood glucose regulation in the adult human, with effects upon glucogenesis and peripheral glucose uptake (Holt, Simpson, & Sonksen, 2003). In the fetus, IGF-1 promotes fetal protein accretion and modulates trans-placental amino acid and glucose supply, as demonstrated in the sheep (Bloomfield, van Zijl, Bauer, & Harding, 2002; Harding, Liu, Evans, & Gluckman, 1994). IGF-2 promotes cell division in fetal tissues; a capacity through which it influences the development of the pancreatic β-cell mass, inhibiting apoptosis and promoting cell survival (Petrik et al., 1999). It also has wider effects upon

placental nutrient transfer (Sferruzzi-Perri et al., 2011). Deficiency of either IGF-1 or IGF- 2 results in severe fetal growth restriction, as demonstrated in mice (DeChiara, Robertson, & Efstratiadis, 1991; Liu, Baker, Perkins, Robertson, & Efstratiadis, 1993) and reflected in humans with Laron syndrome, a form of growth deficiency (Laron, 2008).

1.4.3 **The role of insulin in the fetus**

Although primarily responsible for blood glucose regulation in the adult human, in the fetus insulin plays an important role in coordinating fetal growth with the available fetal nutrients (Hay Jr, 2006b), maintaining blood glucose homeostasis in the presence of a continuous but fluctuating maternal supply (Hay Jr, 2006a), and promoting protein (Shen et al., 2003) and glycogen accretion (Bourbon & Gilbert, 1981; Manns & Brockman, 1969).

The necessity of insulin for fetal growth may be appreciated in the congenital disorders of pancreatic agenesis (Baumeister, Engelsberger, & Schulze, 2005) and Donohue syndrome (de Bock, Hayes, & Semple, 2012), where insulin absence and critical insulin resistance respectively lead to severe intra-uterine growth restriction in the presence of an adequate fetal nutrient supply. Studies in experimental animals have confirmed the vital role insulin plays in regulating fetal growth (Fowden & Comline, 1984; Fowden, Hughes, & Comline, 1989). Following pancreatectomy in the final month of gestation, fetal sheep had an immediate plateau in growth (as determined through measurement of crown-rump length) and were born at a significantly lower birth weight, with shorter crown-rump length and shorter limb lengths than controls. This growth failure was not present in fetuses where an insulin infusion had been started immediately following pancreatectomy (Fowden et al., 1989). Movement of glucose from maternal to fetal circulations is determined by its trans-placental concentration gradient. This gradient is reduced in the hypoinsulinaemic fetus and fetal capacity to adjust its own glucose concentration to keep the gradient stable is limited. Thus the rate of glucose uptake from the maternal circulation is decreased (Fowden & Forhead, 2012) and the fetus is

dependent upon the maternal blood glucose concentration being maintained above its own. Glucose is the preferred fuel for fetal oxidative metabolism, the rate of which is maintained at the expense of fetal growth if substrate is insufficient (Hay Jr, 2006b). The fetus has some capacity for glucogenesis in many species (Fowden & Forhead, 2012), but diversion of nutrients from anabolic to oxidative pathways, followed by catabolism to maintain substrate supply will occur under hypoinsulinaemic conditions.

Administration of excess insulin does not increase growth in a linear fashion in either sheep (Fowden et al., 1989) or monkey (Susa et al., 1984) fetuses under normal nutritional conditions, but will increase glucose oxidation rates and growth in the presence of hyperglycaemia (Fowden & Comline, 1984). In a hypoglycaemic environment, both whole body and glucose specific oxidation rates are reduced despite excessive insulin concentrations (Brown & Hay, 2006). Human fetuses exposed to excess insulin concentrations in the presence of excess substrate display macrosomia, an overgrowth phenotype characteristic of infants of diabetic mothers. This growth may be disproportionate, with weight increasing at a greater rate than length (Persson, Pasupathy, Hanson, & Norman, 2012). Although sheep studies have indicated that insulin is necessary for normal skeletal growth, hyperinsulinaemia does not appear to promote increased skeletal growth in a consistent manner (Fowden et al., 1989). Detailed body composition analysis in monkey fetuses exposed to excess insulin show that the heart, liver and skeletal muscle are most susceptible to insulin-mediated growth (Susa et al., 1984), an effect that may be seen in humans where hypertrophic cardiomyopathy is a potentially fatal complication of fetal macrosomia (Mehta & Hussain, 2003; Sardesai, Gray, McGrath, & Ford, 2001). Increased neonatal fat deposition was clearly correlated with maternal fasting blood glucose concentration in an analysis of 479 non-diabetic pregnancies performed by Walsh et al (2011). Once again, the disproportionate response of different fetal tissue types to insulin was apparent, as neonatal length and head circumference measurements were unaffected.

The maintenance of fetal blood glucose concentrations by insulin is dependent upon the abundance and maturity of insulin-sensitive tissues, predominantly adipose tissue and skeletal muscle. Glucose uptake is mediated by the glucose transporter (Glut) proteins 1-5. Expression and distribution of Glut isoforms changes with increasing maturity. Glut-1, the most numerous glucose transporter in fetal tissue, decreases in prominence during the neonatal period as expression of Glut-4 increases (Devaskar & Mueckler, 1992). In the adult human, insulin acts to effect a translocation of the Glut-1(insulin independent) and Glut-4 (insulin sensitive) transporters from an intracellular to a trans-membrane position; this is also seen in the mature rodent. In the rat fetus, Glut-4 distribution is more highly weighted towards the trans-membrane position, but movement from the cell interior to this position in response to insulin stimulation is reduced compared to that in mature tissue (He, Thamotharan, & Devaskar, 2003). The translocation of Glut-4 appears to be a time-limited response, with studies in sheep revealing down-regulation of receptors after 24 hours despite an ongoing hyperinsulinaemic stimulus (Anderson et al., 2005). Both Glut-1 and Glut-4 transporter numbers were reduced in muscle but not liver in baboons delivered prematurely, with both transporter types increasing in number in line with increasing gestation (Blanco et al., 2010).

Although insulin promotes fetal protein accretion (Fowden, 1992; Shen et al., 2003), the mechanisms through which this occurs are less clear, and appear to be modulated by the fetal substrate environment and the actions of other hormones such as IGF-1 (Carver et al., 1997; Shen et al., 2003). Decreased protein synthesis (Milley, 1994) and inhibition of protein breakdown (Carver et al., 1997; Milley, 1994) have both been described as fetal responses to insulin. It is most likely that insulin exerts a greater effect upon protein breakdown than synthesis pathways, the net result being protein aggregation (Shen et al., 2003). Fetal amino acids also provide an important fuel source for oxidative metabolism (Carver et al., 1997; Hay Jr, 2006b). Manipulation of the substrate environment in fetal sheep, such that both glucose and amino acid concentrations remained stable during insulin infusion, led Milley (1994) to

conclude that exogenous insulin infusion results in lower plasma amino acid concentrations (using leucine as a marker) due to a decrease in protein breakdown, and net protein accretion. The utilisation of amino acids for oxidative metabolism was not altered when the fetal plasma amino acid concentration was maintained, but was reduced when amino acid concentrations were allowed to fall, leading to the conclusion that plasma amino acid concentration and not insulin concentration acts as the primary regulator of amino acid oxidative metabolism (Milley, 1994). However, this hypothesis was refuted by Thureen et al. (2000) and Brown and Hay (2006). Using broader panels of amino acids, they demonstrated that insulin promotes amino acid oxidative metabolism and protein aggregation independent of both plasma amino acid concentration and concurrent glucose oxidation rate in a physiological substrate environment.

Glycogen accretion occurs primarily within the last month of gestation in the human fetus, although small amounts of glycogen are formed from the first trimester onwards (Kalhan & Parimi). Glycogen synthesis is sensitive to a number of hormones, the most important being cortisol, glucagon and insulin. Insulin stimulates glycogen synthesis by pushing glucose into the direct synthesis pathway and increasing activity of the indirect pathway (Bismut & Plas, 1989). However, its most potent effect upon net glycogen accretion derives from its antagonism of glucagon, since the glucagon-insulin ratio is an important control mechanism to balance glycogenolysis with glycogen synthesis (Menuelle & Plas, 1991). A stable nutrient supply in the normal fetus promotes a stable glucagon-insulin ratio in favour of insulin and thus glycogen synthesis. It is the transition from fetal to neonatal life that pushes this balance toward glucagon and thus readies the infant for survival in an environment where the exogenous glucose supply is intermittent and potentially inadequate.

1.5. Fetal blood glucose homeostasis

In the absence of maternal glycaemic derangement, the human fetus receives a constant supply of glucose via insulin-independent facilitative diffusion across the placenta. This process utilises the glucose transporter proteins, especially Glut-1, which is expressed on the maternal placental surface of many mammals, including humans (Devaskar & Mueckler, 1992; Wooding, Dantzer, Klisch, Jones, & Forhead, 2007). In humans the poorly localised Glut-3 transporter, a protein with a high binding affinity for glucose which is important in early pregnancy but decreases in both number and influence by the mid-trimester, is likely to play a minor role in maternal-fetal glucose transfer (Larque, Ruiz-Palacios, & Koletzko, 2013). Maintenance of a trans-placental glucose concentration gradient in favour of the fetus is the result of ongoing placental and fetal glucose metabolism. The human placenta itself utilises up to 60% of the glucose taken up from the maternal circulation (Hauguel, Desmaizieres, & Challier, 1986). The remaining glucose is transferred to the fetus in order to maintain a blood glucose concentration significantly lower than the maternal concentration, but within a range approaching that of the adult human (Gennser & Nilsson, 1971). Placental glucose production, long thought to be impossible due to the supposed absence of active glucose-6-phosphate, may be a source of glucose to the fetus at term (Leonce et al., 2006). It is not yet known whether this is an important source of fetal glucose supplementation at earlier gestational ages in the human (Prendergast et al., 1999).

Multiple experiments using fetal sheep have demonstrated that under normal nutritional circumstances, fetal hepatic glucogenesis does not contribute to the fetal blood glucose concentration prior to term (Fowden, Mundy, & Silver, 1998). This finding may be explained by the immaturity of hepatic glycolytic and gluconeogenic enzyme activity. Glucose-6-phosphatase (G6Pase), the terminal enzyme in both hepatic glucogenic pathways, is present at low concentrations in both fetal sheep (Fowden et al., 1998) and fetal pig livers (Fowden,

Apatu, & Silver, 1995) during the majority of gestation. A rapid increase in G6Pase concentration occurs shortly prior to birth at term gestation, and is associated with both the peripartum cortisol surge and the commencement of significant endogenous fetal glucose production (Fowden, Mijovic, & Silver, 1993). An increase in G6Pase activity can be stimulated at earlier gestations by administration of corticosteroids (Fowden et al., 1993; Franko, Giussani, Forhead, & Fowden, 2007). Cortisol is a major determinant of hepatic enzyme maturity, and experiments with adrenalectomised sheep fetuses reveal a failure of endogenous glucose production in its absence (Fowden & Forhead, 2011). The activation of G6Pase appears to be dependent upon gestational age, with a more vigorous response to stimulation in late gestation reflecting the increasing maturity of hepatic enzyme synthesis. Significant hepatic glucogenesis can be seen at preterm gestations in sheep fetuses exposed to nutritional deprivation (DiGiacomo & Hay Jr, 1989). This is presumed to reflect the impact of prematurely elevated cortisol concentrations on hepatic enzyme maturation.

Term infants show a rapid increase in hepatic enzyme activity, with G6Pase concentrations reaching adult levels by the end of the first postpartum week (Burchell, Gibb, Waddell, Giles, & Hume, 1990). The persistent and non-suppressible hepatic glucose output seen in extremely preterm infants during periods of eu- or hyperglycaemia is in keeping with the immature human liver being capable of significant glucogenic activity (Chacko, Ordonez, Sauer, & Sunehag, 2011), despite evidence that the preterm infant (and indeed the preterm fetus) has low G6Pase concentrations similar to those seen in other immature mammals (Burchell et al., 1990). Despite low enzyme concentrations, the human fetal liver has the capacity for glucogenesis prior to the 20^{th} week of gestation (Adam, Schwartz, Rahiala, & Kekomäki, 1978; Gennser & Nilsson, 1971).

1.6. Metabolic adaptation

The defining feature of the metabolic transition from dependent fetus to independent neonate may be thought of as the moment of birth and the severance of the umbilical cord, resulting in an abrupt cessation of maternal glucose supply. However, the metabolic adaptation from fetus to neonate is in fact a process originating in the days to hours before birth and continuing until regular feeding is established in the neonatal period.

Insulin and glucagon, the hormones responsible for fetal metabolic regulation, are integral to the process of metabolic adaptation. Stimulated by the catecholamine surge accompanying parturition (Sperling, Ganguli, Leslie, & Landt, 1984), the insulin-glucagon ratio alters in favour of catabolism and glucogenesis as glucagon concentrations increase (Girard et al., 1973). Glucagon secretion is further stimulated by the physiological drop in blood glucose concentration seen in the hours immediately following birth (Swenne, Ewald, Gustafsson, Sandberg, & Östenson, 1994). This drop is attenuated prior to the establishment of regular milk feeds by the onset of endogenous glucose production, stimulated by the increased glucagon concentration. Glucagon promotes both gluconeogenesis and glycogenolysis, but animal studies have shown its effects to be time-dependent, with a delay in the onset of glycogenolysis following the post-partum glucagon surge reflecting a period of maturation of the glucagon receptor in the first hours after birth (Kawai & Arinze, 1981; Snell & Walker, 1973). Insulin concentrations undergo a smaller but still significant change in the immediate postnatal period, with concentrations dropping rapidly after birth concomitant with falling blood glucose concentrations (Girard et al., 1973). Insulin acts upon hepatic glucose production by inhibiting the action of glucagon, so that there is not a direct correlation between plasma insulin and blood glucose concentrations.

Glycogenolysis is the major mechanism contributing to neonatal endogenous glucose supply in the human, with hepatic glycogen stores mobilised over the first day of life. However, even

under ideal circumstances (full term gestation, weight appropriate for gestational age, no hypoxic stress), neonatal glycogen reserves are depleted within 24 hours of birth, well before enteral feeding is fully established (Shelley & Neligan, 1966). Gluconeogenesis then becomes the dominant means of hepatic glucose output. Gluconeogenesis is present in healthy, term, human neonates as early as 3.5 hours of age (Sunehag, Gustafsson, & Ewald, 1996). Gluconeogenic activity in the neonatal period is limited by substrate availability rather than hepatic enzyme activity, unlike during the fetal period, where enzyme immaturity (especially that of PEPCK and G6Pase) is the limiting factor. The primary substrate for gluconeogenesis is glycerol (Sunehag et al., 1996); but alanine, glutamine, lactate and pyruvate may all be utilised (Kalhan & Parimi, 2000). Along with sufficient substrate, gluconeogenesis requires a direct hepatic supply of free fatty acids to act as co-factors for glucose production.

Given the requirement for free fatty acids, and the reliance on glycerol as a fuel, it is clear that the process of lipolysis is integral to the efficient production of glucose via gluconeogenesis. The rate of appearance of glycerol is a measure of lipolysis and shows that, like gluconeogenesis, lipolysis is active shortly after birth (Diderholm, Ewald, Ahlsson, & Gustafsson, 2007; Sunehag et al., 1996). The effectiveness of lipolysis is reliant upon the presence of adequate fat stores; lipolytic activity is reduced in the small-for-gestation infant (Diderholm et al., 2007). Lipolysis is also required for the formation of ketone bodies, which are important alternative cerebral fuels and glucose sparing agents in neonates where glucose supply is insecure. Catecholamines and thyroid stimulating hormone are potent stimulators of lipolysis, allowing for provision of cerebral metabolic substrates during periods of metabolic stress.

1.6.1 **Aberrant metabolic transition**

The mechanisms facilitating metabolic adaptation may fail in the infant in whom there is inadequate substrate deposition (as in intrauterine growth restriction) (Hawdon & Ward Platt,

1993), rapid substrate metabolism (anaerobic respiration due to perinatal hypoxia), or abnormal hormonal stimulus. This results in a failure to transition to a catabolic state and an increased risk of postnatal hypoglycaemia.

Aberrant metabolic transition may be demonstrated in the offspring of diabetic mothers, where prolonged fetal hyperglycaemic exposure leads to an increased fetal insulin concentration. At birth, insulin concentrations remain high, resulting in little change in the insulin-glucagon ratio despite a normal glucagon surge. Despite an excess of available substrate for glucogenesis (Baarsma et al., 1993), endogenous glucose production rates are decreased compared to those of infants born to non-diabetic mothers (Sunehag, Ewald, Larsson, & Gustafsson, 1997; Sunehag et al., 1996). Furthermore, there is poor correlation between endogenous glucose production rates and plasma glucose concentration in the infants of diabetic mothers, demonstrating an inability of hepatic glucose output to respond to metabolic demand (Sunehag et al., 1997). However, with the provision of appropriate supportive care, infants of diabetic mothers are able to respond to their new metabolic environment and by the end of the first post-partum week have blood glucose concentrations identical to those of infants born to non-diabetic mothers.

The preterm infant is at high risk of failed metabolic adaptation due to: a blunted catecholamine response to birth (Greenough, Lagercrantz, Pool, & Dahlin, 1987); suboptimal substrate provision secondary to low birth weight; immature hepatic enzyme expression; delayed establishment of enteral feeding (Bombell & McGuire, 2008); and increased metabolic stress secondary to exposure to the extra-uterine environment. In the sick preterm infant, these stressors may be compounded by respiratory distress or sepsis. Thus the preterm infant has multiple factors hindering a normal metabolic transition, and a prolonged period of metabolic vulnerability prior to the establishment of secure blood glucose homeostasis.

1.7. Neonatal blood glucose homeostasis

As in the adult, insulin is the major determinant of neonatal blood glucose concentration. However, the neonate is relatively insulin resistant compared to adults in both human and animal studies (Cowett & Tenenbaum, 1987; Farrag et al., 1997). The human neonate displays both unresponsiveness and insensitivity to insulin (Farrag et al., 1997). Insulin unresponsiveness, a decrement in the maximal response to a given insulin dose, suggests an aetiology distal to the insulin receptor, whilst insulin insensitivity, a rightwards shift in the dose-response curve, may be the result of pre-, intra- or post-receptor abnormalities (Ronald Kahn, 1978). Inability to suppress hepatic glucogenesis may occur, even if exogenous insulin is administered in supra-physiologic doses. However, insulin resistance is limited to the hepatic insulin response: in the peripheral tissues, insulin sensitivity is increased in the neonate compared to the adult (Farrag et al., 1997). This imbalance in insulin sensitivity may promote growth in the neonatal period provided there is adequate availability of glucogenic substrates. In the preterm infant, a paucity of insulin-sensitive peripheral tissue may exacerbate the effects of hepatic insulin resistance, leading to an increased propensity to inappropriately high blood glucose concentrations.

A period of low blood glucose concentration appears to be an integral part of the transition between fetal and neonatal life, acting as a stimulus to glucogenesis and an inhibitor to insulin production, promoting a catabolic state in the newborn. The point at which a low blood glucose concentration transitions from one that is physiological to one which may be described as pathological, or hypoglycaemic, is the subject of much debate (Cornblath et al., 2000; Thornton et al., 2015). Certainly, many studies have revealed otherwise healthy, term, appropriate birth weight infants have blood glucose concentrations with values less than 2.6mmol/L (a concentration widely taken as the threshold for hypoglycaemia) in the first hours after birth (Creery & Parkinson, 1953; Hawdon, Ward Platt, & Aynsley-Green, 1992;

Sunehag et al., 1996). However, once this initial low blood glucose concentration has been resolved by the mechanisms of metabolic adaptation, the neonate must maintain its blood glucose concentration whilst regular intermittent milk feeds are established, a process which may not be completed until the end of the first postpartum week (Neville et al., 1991). Neonatal glucose utilisation rates vary between 2.5- 6.0 mg/kg/min (Hay Jr & Sparks, 1985) and are driven largely by the approximate 3.7 mg/kg/min glucose requirement of the neonatal brain (Bougneres, 1987). Given that the glucose utilization rate is often in excess of a neonate's endogenous glucose production rate (Farrag et al., 1997), alternative metabolic fuels are required to bridge the gap between glucose supply and demand until enteral feeds are established.

1.7.1 **Alternative metabolic fuels**

The metabolically active ketone bodies β-hydroxybutyrate and acetoacetate are produced by the liver from free fatty acids derived from adipose tissue (Quant et al., 1998). Milk feeds also contribute free fatty acids in the form of triglycerides. However, this contribution is minimal in the first postnatal week during which ketone body concentrations are at their greatest, as colostrum volumes are small and fat content is relatively low. Ketone bodies influence glucose homeostasis via three mechanisms: direct replacement of glucose as a cerebral fuel; reduction in global glucose utilisation via down-regulation of peripheral enzyme activity; and reduction in substrate availability for gluconeogenesis (Robinson & Williamson, 1980). Their creation by the liver is the result of diversion of free fatty acids from an esterification pathway to one of oxidation, a process sensitive to low blood glucose concentration. Overall, the effect of increased ketone body concentrations is to modulate the glucose utilisation rate in states of glucose deficiency. Their ability to replace glucose as an oxidative fuel in the brain is thought to be important in the neonatal period, and they are frequently described as neuroprotective for the glucose deplete brain. However, knowledge regarding the cerebral metabolic rate for

ketone bodies has been extrapolated from animal experiments and those in fasted adult humans; its translation to the human neonate is uncertain.

Neonatal rats have greater cerebral uptake of ketone bodies than their adult counterparts (Dahlquist & Persson, 1976), secondary to a developmental window of increased blood brain barrier permeability to ketone bodies (Moore, Lione, Sugden, & Regen, 1976), but a similar process has not been demonstrated in the human. Preterm infants are able to produce ketone bodies at ~66% the rate of fed term infants (de Boissieu, Rocchiccioli, Kalach, & Bougneres, 1995), but are unable to mount a ketogenic response equivalent to that of the term infant when subject to acute hypoglycaemia (Hawdon et al., 1992). In the starved obese adult human, ketone bodies may account for up to 60% of the cerebral oxidative metabolic rate (Owen et al., 1967), and even in the non-fasted adult may account for 25% of the cerebral metabolic rate when exogenous supply increases the blood ketone body concentration. The use of ketone bodies appears to be slowly time-responsive, with greater utilisation after a 20 day fast compared to a 3 day fast in the adult. However, neonates fasted for 8 hours on the first day postpartum do not mount a significant ketogenic response in spite of falling glucose concentrations (Stanley, Anday, Baker, & Delivoria-Papadopolous, 1979), suggesting that hepatic ketogenesis undergoes a period of maturation and is less effective immediately after birth than in subsequent days. In addition, a modern study in term and near-term infants at risk of hypoglycaemia showed low plasma β-hydroxybutyrate concentrations within the first 48 hours following birth, suggesting that ketone bodies may not be neuroprotective during neonatal hypoglycaemia (Harris, Weston, & Harding, 2015)

Ketone bodies also have a non-oxidative role in the human brain, acting as precursors for neuronal myelin formation (Morris, 2005). This is an important activity in the preterm infant, where rapid brain development occurring *ex utero* includes myelination of white matter tracts.

It is not known if diversion of the limited pool of cerebral ketone bodies from an anabolic to an oxidative pathway affects brain growth and development in the preterm infant.

Lactate, alanine and glycerol are substrates for gluconeogenesis in the neonatal period. The most abundant of these immediately postpartum is lactate (Harris et al., 2015). Lactate is created by the anaerobic respiration of glycogen during birth and is present in the early neonatal period at concentrations greater than those of adults and older children (Hawdon et al., 1992). Animal studies show that, like ketone bodies, lactate can be used for gluconeogenesis, as an oxidative fuel by the neonatal brain and for cerebral lipogenesis (Vicario & Medina, 1992). Studies of neonatal rat brain have shown lactate to be oxidised at 10 times the rate of glucose and 3 times the rate of ketone bodies in the early neonatal period (Medina, 1985). Lactate is cleared rapidly from the circulation and its concentration in the blood declines with increasing postnatal age in neonatal rats (Girard et al., 1973), euglycaemic human infants (Hawdon et al., 1992) and infants at risk of hypoglycaemia (Harris et al., 2011). Lactate may account for up to 10% of the cerebral metabolic rate in adults with blood lactate concentrations within the physiological range, and up to 60% when lactate concentrations are increased to a supra-physiological level (Boumezber et al., 2010). Its contribution to the cerebral metabolic rate of the human neonate is not known. In rats, increasing ketone body concentrations result in a change from lactate to ketone body utilisation in brain tissue, increasing the availability of lactate as a gluconeogenic substrate (Vicario & Medina, 1992). However, suggestions that a similar process may limit the use of lactate as a direct oxidative fuel in humans are not supported by the recent finding that neonatal ketone body concentrations remain low after birth (Harris et al., 2015).

1.7.2 **The effect of enteral feeding**

Term infants have low mean blood glucose concentrations on the first day after birth, with lower blood glucose concentrations in breast fed compared with formula fed infants.

However, by the end of the first week, blood glucose concentrations are within the range seen in older children and do not differ between breast and formula fed infants (Hawdon et al., 1992). Unlike formula milk, the composition of breastmilk is dynamic, providing gluconeogenic substrates, fatty acids and glucose in quantities that display intra- and inter-daily variation (Neville et al., 1991). Term infants have an enhanced capacity for ketogenesis when compared to preterm infants or older children, and this capacity is further increased by breastmilk feeds (Hawdon et al., 1992). The mechanism for the promotion of ketogenesis is unknown; the provision of carnitine via the breastmilk has been suggested, but modern formula milks also contain carnitine (Rebouche & Paulson, 1986). It is possible that the relatively small volumes of breastmilk available compared to the neonatal metabolic substrate requirements lead to a state of semi-starvation, so that it is not breastmilk itself but lack of exogenous nutrition which promotes ketogenesis in these infants. This hypothesis is supported by studies in which adequate enteral nutrition is provided to the neonate from birth and blood ketone body concentrations are not increased (Anday, Stanley, Baker, & Delivoria-Papadopoulos, 1981; Harris et al., 2011).

In the preterm neonate, onset of enteral feeding is likely to be delayed, and many extremely preterm infants are dependent upon parenteral nutrition for long periods after birth (Bombell & McGuire, 2008). The absence of significant enteral milk feeds may play a role in the increased metabolic vulnerability of these infants, even in situations where adequate parenteral nutrition is being supplied. Enteral feeds stimulate the enteroinsular axis, a complex system of interacting gut hormones and peptides including the incretins Glucose-dependent Insulinotropic Peptide (GIP) and Glucagon-like Peptide type 1 (GLP-1). GIP, a hormone released by the jejunum in response to nutritional stimulus (Lucas, Bloom, & Aynsley Green, 1985), has been shown in both animal and adult human studies to promote insulin secretion, and dysfunctional GIP secretion (but not GLP-1 secretion) is associated with type-2 diabetes mellitus. GIP acts directly on the pancreatic β-cell via its receptor on the cell surface, leading

to closure of K_{ATP} channels and depolarisation of the β-cell membrane, initiating insulin secretion (Seino, Fukushima, & Yabe, 2010). GIP also has an effect upon β-cell survival, showing both anti-apoptotic and pro-proliferative actions such that its absence leads to a marked reduction in β-cell mass (Renner et al., 2010). In addition to the pancreas, GIP receptors are found on a variety of other tissues including fat, bone and brain, where it is particularly found in the hippocampal region.

In term newborn infants and enterally fed preterm infants, GIP concentrations increase after birth but in the early neonatal period do not show a response to bolus enteral feeding. By 24 days post-partum, however, GIP concentrations show a significant rise in response to a bolus feed (Lucas, Sarson, Bloom, & Aynsley-Green, 1980b). Feed-stimulated GIP increases are more pronounced in the formula fed than the breast fed infant (Lucas et al., 1980a) but this difference does not persist at 9 months of age (Salmenpera et al., 1988). In fasted preterm infants, GIP concentrations do not rise after birth despite the provision of intravenous glucose (Lucas et al., 1980b). The necessity of an enteral stimulus is supported by a study in full term neonates, where the administration of glucose-equivalent intravenous and enteral glucose loads has been compared. When given enteral glucose, infants showed increased GIP concentrations, increased peak plasma insulin concentrations but no significant differences in plasma glucose concentrations compared to those given an intravenous glucose infusion (King, Oliven, & Kalhan, 1989). The manner in which enteral feeds are delivered also appears to be important. A study of neonatal piglets (Stoll et al., 2012) fed with bolus enteral, continuous enteral or parenteral nutrition only, revealed impaired insulin sensitivity on intravenous glucose tolerance testing in continuously fed and parenterally fed piglets compared to piglets fed with bolus enteral nutrition, whether in elemental or non-elemental form. The reduction in insulin sensitivity became greater with increasing postnatal age. Piglets restricted to parenteral nutrition or continuous enteral feeds had lower intestinal weights and significantly lower plasma GIP and GLP-1 concentrations at 2 weeks post-partum

than bolus fed piglets (Stoll et al., 2012). Continuous enteral feeding also decreases peripheral protein synthesis compared to bolus enteral feeds (El-Kadi et al., 2012), potentially impacting muscle growth and the volume of insulin-sensitive tissue available to take up blood glucose.

1.8. Neonatal hyperglycaemia

In the normal, term infant, hyperglycaemia is an uncommon phenomenon; hypoglycaemia is the greatest risk during the postpartum metabolic transition. However, hyperglycaemia is common in sick, stressed and preterm infants (Lilien, Rosenfield, Baccaro, & Pildes, 1979), with the incidence of hyperglycaemia increasing with decreasing gestational age and birth weight (Iglesias Platas et al., 2009; Louik, Mitchell, Epstein, & Shapiro, 1985). Hyperglycaemia may affect 80% of very low birth weight infants (Beardsall et al., 2010) and the relationship between preterm birth and hyperglycaemia is also seen in animals such as the baboon (Blanco, McGill-Vargas, McCurnin, & Quinn, 2013).

Recent interest in the role of hyperglycaemia as a contributing factor to morbidity and mortality in the neonatal period has been driven by work performed with critically unwell adults. This revealed that high blood glucose concentrations were common even in adults with no history of diabetes, and that controlling blood glucose concentration reduced both morbidity and mortality (Van den Berghe et al., 2001). A number of studies subsequently have explored the relationship between mortality and blood glucose concentrations in adult intensive care patients and a variety of different treatment paradigms have been tested, most involving insulin to keep blood glucose concentrations within a predetermined range (Arabi et al., 2008; Zimmerman, Mlynarek, Jordan, Rajda, & Horst, 2004). However, there is ongoing controversy as to whether the risks of treating hyperglycaemia outweigh the benefits, given the high incidence of hypoglycaemia accompanying attempts to tightly control blood glucose concentrations (Gunst & Van den Berghe, 2010).

The concentration at which blood glucose may be described as hyperglycaemic is not clear and many different thresholds have been used both for its definition and for the initiation of treatment in the neonatal period (Alsweiler et al., 2007). Limits for neonatal blood glucose concentrations have been described based on statistical modelling (Alexandrou et al., 2010), observation of clinical importance (Binder, Raschko, Benda, & Reynolds, 1989), and from expert opinion (Sunehag & Haymond, 2002). Regardless of the variety of definitions used, increased neonatal blood glucose concentrations are associated with a number of neonatal morbidities, including retinopathy of prematurity (Chavez-Valdez, McGowan, Cannon, & Lehmann, 2011), intraventricular haemorrhage (Auerbach et al., 2013), sepsis (both fungal (Manzoni et al., 2006) and bacterial (Hirshberg, Larsen, & Van Duker, 2008)), and brain white matter injury (Alexandrou et al., 2010). Neonatal hyperglycaemia is also associated with increased neonatal mortality (Alexandrou et al., 2010; Hays, Smith, & Sunehag, 2006; Heimann et al., 2007). Whether these associations are a direct result of high blood glucose concentrations, or whether hyperglycaemia merely reflects a sick and vulnerable infant is not yet clear, but a recent study in preterm lambs randomised to hyperglycaemia (achieved using 50% dextrose infusion), treated hyperglycaemia (combined dextrose and insulin infusion) or control (saline infusion) found that lambs randomised to hyperglycaemia were more likely to have signs of infection (fever) and to die than lambs in the treated hyperglycaemia or control arms, suggesting that hyperglycaemia is a causative factor in these outcomes (Alsweiler, Harding, & Bloomfield, 2012a).

1.8.1 **Mechanisms promoting neonatal hyperglycaemia**

The phenomenon of increasing blood glucose concentrations with critical illness has long been considered an adaptive response, allowing uninterrupted glucose supply to organs at their most vulnerable (Goldstein & Elwyn, 1989). The physiological mechanisms underlying neonatal hyperglycaemia may be broadly divided into two categories: glucose over-supply and glucose

under-utilisation. Hyperglycaemia in an individual infant is likely to be multi-factorial, encompassing abnormalities in both supply and utilisation of glucose.

Increased glucose supply may be the result of exogenous infusions administered to the infant, inappropriate endogenous hepatic glucose production, or pathological hyper-catabolism, as seen in states of metabolic shock accompanying conditions such as sepsis or severe injury. Glucose, either as clear fluid or as part of a parenteral nutrition solution, is administered to all preterm infants prior to 30 weeks gestation due to the risk of failed metabolic transition and hypoglycaemia following birth (Cormack & Bloomfield, 2006). Increased glucose concentrations in intravenous fluids are linearly associated with increased blood glucose concentrations (Louik et al., 1985) and in older infants, restricting intravenous glucose administration is effective at preventing post-surgical hyperglycaemia (Verbruggen et al., 2011). However, the effect of exogenous glucose administration, if prescribed correctly, is likely to have less of an impact on blood glucose concentrations than endogenous glucose production in the preterm infant.

In the adult and older child, hepatic glucose production is sensitive to blood glucose concentration; glucose infusion at a rate just above the rate of endogenous glucose production rapidly suppresses up to 90% of hepatic glucose output (Saccà, Vitale, Cicala, Trimarco, & Ungaro, 1981). A reduction in hepatic glucose output of 50% also occurs when the insulin response to rising blood glucose concentrations is suppressed, showing that it is not just insulin, but glucose itself to which the liver responds (Saccà et al., 1981). However, in preterm infants, studies have repeatedly shown that endogenous glucose production is not fully suppressed in the presence of exogenous glucose infusions. (Chacko et al., 2011; Chacko & Sunehag, 2010). Even when supra-physiological glucose infusion rates are used, in lamb studies, hepatic gluconeogenesis is not fully suppressed (Cowett, Rapoza, & Gelardi, 1998). This reflects the physiology of the hyper-catabolic states seen in adults following sepsis or

severe trauma, where massive release of gluconeogenic substrates from global tissue breakdown drives glucose over-production (Frayn, 1985). In sick and preterm neonates, both stress and an inadequate nutritional intake after birth are common (Grover et al., 2008), resulting in a catabolic metabolism and poor growth, but an abundant supply of gluconeogenic substrates.

The ability to sense blood glucose concentrations is mediated by the Glut-2 transporter protein, both in liver and in pancreatic β-cells (Burcelin, Dolci, & Thorens, 2000). Hepatic Glut-2 expression is lower in preterm than in term infants. Animal studies reveal a rapid shift in the expression of hepatic glucose transporter protein isoforms shortly after birth, as the predominant fetal Glut-1 is replaced by the mature Glut-2 transporter (Lane, Crawford, Flozak, & Simmons, 1999). The hepatic glucose sensing system thus undergoes a developmental maturation after birth, although how this is affected by preterm birth is unclear. Portal vein carbohydrate delivery secondary to intestinal absorption may be an important maturational stimulus to hepatic glucose sensing (Burcelin et al., 2000; Zheng, Levitsky, Mink, & Rhoads, 1995). This stimulus is likely to be lacking in a preterm infant receiving only parenteral nutrition. Hyperglycaemia may itself induce changes in glucose transporter isoform expression, with animal studies revealing a decrease in expression of fetal hepatic Glut-1 after prolonged hyperglycaemic exposure (Das, Schroeder, Hay, & Devaskar, 1999).

In spite of an adequate or over supply of glucose, inefficient glucose utilisation is common and a significant contributor to hyperglycaemia in preterm and sick neonates. Preterm neonates have higher blood insulin concentrations during normoglycaemia than do term neonates (Mitanchez-Mokhtari et al., 2004). Although peripheral insulin sensitivity is normally increased in the neonate compared with the older child (Farrag et al., 1997), in the preterm neonate, a dearth of insulin sensitive tissues (Cooper et al., 2010) and immaturity of the insulin-sensitive glucose transporter Glut-4 contribute to the infant's inability to dispose of

high blood glucose concentrations. In addition, compared to those with normal blood glucose concentrations, hyperglycaemic preterm infants have an abnormal pancreatic secretory response to a glucose challenge, secreting large amounts of proinsulin, which is an ineffective glucose regulator (Mitanchez-Mokhtari et al., 2004). This insulin precursor should be converted into insulin within the β-cell granule prior to its release. Failure of this process in hyperglycaemic infants suggests the intra-β-cell enzymatic pathway is more immature than in non-hyperglycaemic preterm infants, where proinsulin concentrations correlate negatively with increasing gestational age (Hawdon, Hubbard, Hales, & Clark, 1995).

Sympathoadrenal stimulation may promote hyperglycaemia through its effects on insulin secretion, peripheral insulin resistance and hepatic glucose output. In the preterm infant, stressors such as birth asphyxia (Greenough et al., 1987), ventilation (Barker & Rutter, 1996), painful procedures (Anand et al., 1985) and environmental stimulation may all contribute to high circulating catecholamine concentrations. Noradrenaline, released by sympathetic nerve fibres, and adrenaline, released by the adrenal medulla, act upon adrenoreceptors. The α- and β- adrenoreceptor subtypes are distributed throughout the body. Liver, pancreatic, adipose and skeletal muscle adrenoreceptors are closely involved in the mediation of blood glucose homeostasis in the adult, with activation of the β_2 adrenoreceptor subtype being the most potent stimulus for hyperglycaemia. Hepatic β_2 adrenoreceptor stimulation induces hepatic glucose production through both glycogenolysis and gluconeogenesis, whilst skeletal muscle β_2 stimulation reduces insulin-dependent glucose uptake (although an increase in insulin-independent glucose uptake may occur concurrently and is a possible target in the treatment of type 2 diabetes mellitus). In adipose tissue, β-adrenoreceptor stimulation leads to lipolysis and the release of substrates for hepatic glucose production (Boyda, Procyshyn, Pang, & Barr, 2013). Although the contribution of specific adrenoreceptor activation in the preterm infant is not known, the effects of pharmacological blockade of β-adrenoreceptors on blood glucose concentrations suggests that their effects in the term infant are similar to those in the older

child (de Graaf et al., 2011). The use of either adrenaline or noradrenaline for inotropic support in the neonatal period may therefore increase the risk of hyperglycaemia.

Cortisol, produced by the adrenal cortex and a prominent counter-regulatory hormone, may be present at elevated concentrations in both the preterm and term neonate as part of the hormonal-metabolic stress response (Ward Platt, Anand, & Aynsley-Green, 1989). Infants displaying a stress response are at higher risk of developing hyperglycaemia, and have increased morbidity and mortality compared to non-stressed infants. Although cortisol concentrations are higher in stressed than non-stressed infants, hyperglycaemic stressed infants display a blunted cortisol response to a glucose infusion compared to stressed euglycaemic infants (Lilien et al., 1979). Unfortunately the appropriateness of weight for gestational age is not reported in Lilien's paper, as it is now known that small for gestational age infants display a blunted cortisol response to stress, likely due to developmental insults to the hypothalamus-pituitary-adrenal (HPA) axis (Schaffer et al., 2009). Cortisol acts via glucocorticoid receptors, the α- and β- subtypes of which are expressed in different ratios in the preterm and small-for-gestational-age infant compared to the term infant (Go et al., 2013). These receptors may additionally be stimulated by the administration of exogenous steroid such as occurs in the treatment of chronic lung disease in preterm infants (Doyle, Ehrenkranz, & Halliday, 2010). Glucocorticoid receptor stimulation has diverse effects as demonstrated in Cushing's syndrome, a diabetogenic, multi-system disorder, resulting from chronic cortisol over-exposure. With regard to glucose homeostasis: decreased pancreatic islet β-cell insulin secretion (Ling et al., 1998), increased hepatic gluconeogenesis, increased lipolysis, and decreased peripheral glucose uptake secondary to alterations in insulin-mediated Glut-4 translocation mobility (Dimitriadis et al., 1997) are all effects of glucocorticoid receptor activation resulting in high blood glucose concentrations.

Both glucagon and growth hormone are counter-regulatory hormones found at high concentrations during neonatal stress. Their production is stimulated via the HPA axis and both hormones act upon the liver to increase endogenous glucose production, and in peripheral muscle to antagonise the effect of insulin on glucose uptake. Growth hormone acutely activates lipolysis releasing free fatty acids for use as gluconeogenic substrates (Yuen, Chong, & Riddle, 2013). Growth hormone concentrations may be higher in the growth-restricted preterm infant due to relative growth hormone insensitivity secondary to low numbers of growth hormone receptors (Gluckman, Sizonenko, & Bassett, 1999).

Inflammatory cytokines, released as part of the systemic inflammatory response, are associated with hyperglycaemia in the critically ill adult patient (Nakamura et al., 2012). Neonates may display a fetal inflammatory response (often as the result of chorioamnionitis, a leading cause of preterm birth) or a systemic inflammatory response secondary to infectious or non-infectious stimuli; both are characterised by increased circulating concentrations of pro-inflammatory cytokines, including interleukin-6 (Romero, Gotsch, Erez, Vaisbuch, & Kusanovic, 2011). The mechanisms through which hypercytokinaemia may cause hyperglycaemia are not fully elucidated, but insulin resistance secondary to induction of counter-regulatory hormones appears to result from high circulating concentrations of TNF-α. Insect studies suggest that insulin resistance is an adaptive response to inflammation, forcing metabolic substrates away from growth pathways and into supporting the innate immune system during the initiation of lipid-dependent pro-inflammatory cascades (DiAngelo, Bland, Bambina, Cherry, & Birnbaum, 2009). Hyperglycaemia is strongly associated with sepsis in neonatal patients, with high blood glucose concentrations often preceding the onset of clinical signs of infection by several hours (Fanaroff et al., 1998).

1.8.2 **Effects of hyperglycaemia**

Human endothelial cells cultured in an environment where glucose concentrations are elevated to those seen in clinical states of hyperglycaemia demonstrate delayed cell replication, reduced proliferation, and increased cell death (Lorenzi, Cagliero, & Toledo, 1985; Lorenzi, Nordberg, & Toledo, 1987). Clinically, hyperglycaemia predominantly affects cells which do not have the ability to restrict trans-membrane glucose transport; these are the cell types most likely to be involved in the complications of long standing hyperglycaemia, such as vascular endothelium (Brownlee, 2005). There are a number of mechanisms by which raised intra-cellular glucose concentrations interfere with normal cell function: increased production of advanced glycation end products, which may deform normal protein structures rendering them functionally inept (Busch, Franke, Rüster, & Wolf, 2010); increased glucose movement through the polyol pathway, consuming co-factors and increasing cellular susceptibility to oxidative stress; altered gene expression through over activation of protein kinase C and *N*-acetyl glucosamine (Brownlee, 2005); and mitochondrial over-production of cell-damaging reactive oxygen species (Smart & Li, 2007).

Neonatal hyperglycaemia, especially when it occurs in a preterm infant, may disrupt the normal development of the endocrine pancreas. Animal experiments have shown that the developing pancreas is sensitive to increased blood glucose concentrations, such that prolonged hyperglycaemic exposure can lead to increased β-cell proliferation in the neonatal period (Ford et al., 2009). However, as previously described, the endocrine pancreas has a limited capacity for cell replication and renewal. Increased neonatal β-cell turn over may, in adult life, lead to reductions in β-cell mass and reduced insulin secretion. Furthermore, greater glucose exposure is correlated with the accumulation of reactive oxygen species in the β-cell, impairing function and increasing the likelihood of apoptosis (Green et al., 2012). The long term metabolic effects of neonatal hyperglycaemia in humans are not yet known, but in sheep

born preterm and made hyperglycaemic, β-cell mass and absolute β-cell numbers are reduced in infancy and in early adulthood, with hyperglycaemia promoting β-cell apoptosis and increasing the β-cell apoptosis to proliferation ratio above that seen in preterm controls (Bansal et al., 2015). Compared to those born at term, infants born preterm are, in later life, at increased risk of type-1 diabetes mellitus (Crump, Winkleby, Sundquist, & Sundquist, 2011), insulin resistance and type-2 diabetes mellitus (Hofman et al., 2004b), risks which are likely to reflect long term alterations to pancreatic function associated with preterm birth.

The effects of hyperglycaemia may not be limited to the period in which glucose concentrations are abnormally high. Hyperglycaemia can cause epigenetic changes, both *in vitro* and *in vivo* in mice (Brasacchio et al., 2009; El-Osta et al., 2008), resulting in persistent alterations in gene expression and perhaps explaining the "legacy effect" of reduced cardiovascular morbidity following a historic period of tight glycaemic control in type-2 diabetics (Chalmers & Cooper, 2008). Whether some specific genes are more susceptible to hyperglycaemia-mediated changes than others remains to be seen.

Neonatal hyperglycaemia may be clinically silent, requiring regular blood glucose monitoring to detect glucose concentrations above the normal range. However, once blood glucose concentrations breach the renal glucose threshold, tubular glucose reabsorption is impaired and glucose is lost into the urine. Glycosuria may result in excessive renal fluid and caloric loss, and potentially lead to increased plasma osmolarity and perturbations of blood sodium concentration (Hey, 2005).

In addition to the metabolic and electrolyte derangements caused by dehydration, the preterm infant is at increased risk of intracerebral vascular events secondary to perturbed intravascular osmolarity and altered blood flow to the structurally delicate vessels of the germinal matrix (Ghazi-Birry et al., 1997). An association has been demonstrated between increased amplitude and duration of exposure to high blood glucose concentrations and severe

intraventricular haemorrhage (IVH), (Auerbach et al., 2013) although no relationship was seen between blood osmolarity and IVH, throwing into doubt the possibility of this being a causal relationship. However, work with haemodynamically unstable infants post-cardiac surgery has demonstrated a correlation between high blood glucose concentrations and low cerebral oxygen saturation, a situation which may itself lead to alterations in cerebral blood flow (Zhang, Cai, & Li, 2012). However, this finding may not be directly translatable to the preterm population.

The association between hyperglycaemia and neonatal sepsis can be explained as a response to the inflammatory cascade, as detailed above. However, high blood glucose concentrations may themselves predispose an infant to infection, both through direct effects on the immune system (Turina, Fry, & Polk, 2005) and from increased opportunity for pathogens to survive in secretions contaminated with glucose, including those from the upper and lower respiratory tract (Baker et al., 2007; Wood, Brennan, Philips, & Baker, 2004). Clinical signs of sepsis may not always be a response to a foreign pathogen; neonates may mount a systemic inflammatory response to a number of inflammatory stimuli. *In vitro* studies show that, as in adults, preterm and term infants mount an increased pro-inflammatory response to a septic stimulus under hyperglycaemic conditions, with interleukin-8 production positively correlating with glucose concentrations (Temming et al., 2012). The association between hyperglycaemia and infection has also been demonstrated in preterm lambs made hyperglycaemic with dextrose infusions. Sepsis was a significant cause of mortality in hyperglycaemic animals and considered to be one of the driving factors behind the seemingly causal relationship between hyperglycaemia and increased lamb mortality in this study (Alsweiler et al., 2012a).

1.8.3 **Treating neonatal hyperglycaemia**

Although large trials of different glycaemic control strategies have taken place in critically unwell adult patients, there have been few trials of hyperglycaemia management in the

neonatal population. A number of retrospective, observational studies have been published (Binder et al., 1989; Heald, Abdel-Latif, & Kent, 2012; Verbruggen, Landzaat, Reiss, van Goudoever, & Joosten, 2012). As a group they are beset by the problems inherent in observational studies, such as lack of standardisation of patient selection, treatment thresholds, and treatment duration. Both restriction of glucose administration (and thus caloric intake) and insulin therapy have been attempted in the neonatal population, with initial observational reports suggesting that insulin administration appeared safe (Vaucher, Walson, & Morrow, 1982).

In 1991, a small randomised controlled trial was published comparing insulin infusion with caloric restriction in the treatment of hyperglycaemic ELBW infants. It was found that insulin infusion allowed more rapid advancement of parenteral nutrition than caloric restriction (perhaps unsurprisingly) and that insulin therapy was more efficacious in reducing blood glucose concentrations than caloric restriction. Infants treated with insulin showed greater weight gain, but no difference in linear growth or head circumference when compared to those who had been calorie restricted (Collins Jr et al., 1991). A further small randomised trial was reported in 1998, when once again insulin infusion was compared to restricted caloric intake in a total of 23 ELBW infants. Growth was not reported as an outcome, although the study found that infants treated with insulin had a higher caloric intake and more successful resolution of hyperglycaemia than those treated with caloric restriction (Meetze, Bowsher, Compton, & Moorehead, 1998). Hypoglycaemia was not reported as a complication of treatment in either trial.

Two larger randomised controlled trials have taken place in recent years. The Neonatal Replacement Insulin in Europe (NIRTURE) study, published in 2008, aimed to discover whether early insulin therapy to maintain blood glucose concentrations between 4-8 mM decreased mortality and morbidity in the VLBW population. Although not directly concerned

with the treatment of hyperglycaemia, this trial is important for its finding that despite 36% of the control group requiring insulin to treat hyperglycaemia, the treatment group had a significantly increased risk of hypoglycaemia. The trial was terminated early on the grounds of futility, but not before concerns were raised regarding an increase in parenchymal brain lesions and increased early mortality in the treatment group. Real-time ultrasound imaging did not support an increase in parenchymal lesions and mortality at term corrected age was found to be not different between the treatment and control groups despite improved glycaemic control in the treatment group (Beardsall & Dunger, 2008).

The only randomised controlled trial addressing tight glycaemic control in the VLBW population was published in 2012. The Hyperglycaemia in Neonates Trial (HINT) randomised 88 hyperglycaemic infants to tight (blood glucose maintained between 4-6 mM) or standard glycaemic control (blood glucose maintained between 8-10mM). The primary outcome was growth at 36 weeks' post menstrual age, with a number of secondary outcomes, including the incidence of hypoglycaemia and common neonatal morbidities. Insulin was used as the primary means to control blood glucose concentrations; infants randomised to tight glucose control were immediately started on an insulin infusion, whereas infants in the control group were only commenced on insulin if they met a number of conditions, including having a blood glucose concentration greater than 10mM. Once again, this trial found a significant increase in the incidence of hypoglycaemia in the treatment group, with 58% of infants randomised to tight glycaemic control having at least one blood glucose concentration less than 2.6mM. Tight glycaemic control improved both weight gain and head circumference at 36 weeks' post menstrual age, but had no effect on linear growth (Alsweiler, Harding, & Bloomfield, 2012b).

Thus, the only large randomised controlled trials of insulin use in preterm infants have both raised concerns regarding the incidence of hypoglycaemia secondary to tight glycaemic

control, and neither showed an improvement in morbidity or mortality related to improved glycaemic control (although the HINT trial was not powered to detect significant differences in mortality). The impact of treating hyperglycaemia on long term metabolic health is unknown. In lamb studies, treating neonatal hyperglycaemia with insulin may reduce β-cell apoptosis, but does not appear to preserve overall pancreatic β-cell mass in adulthood (Bansal et al., 2015). If a similar reduction in β-cell apoptosis were to occur in humans, insulin treatment of neonatal hyperglycaemia may decrease the risk of later insulin insufficiency. However, the finding of increased weight gain without concomitant linear growth in the HINT trial raises the possibility of insulin-mediated alteration in body composition, increased abdominal adiposity and a potential increase in the risk of insulin resistance and metabolic syndrome in later life (a risk already increased in the preterm compared to the term population). Only limited data are yet available on the long term neurodevelopmental outcomes of treating hyperglycaemia in preterm infants (van der Lugt, Smits-Wintjens, van Zwieten, & Walther, 2010). It is possible that any neuroprotective effect of tight glycaemic control, as suggested by improved head growth (Cheong et al., 2008), is outweighed by the potential for neural damage secondary to more frequent episodes of hypoglycaemia (Burns, Rutherford, Boardman, & Cowan, 2008; Lucas, Morley, & Cole, 1988) .

1.9. Neonatal hypoglycaemia

Low blood glucose concentrations are most common in neonates during the period of metabolic transition immediately following birth. Over half of at risk infants (inappropriate growth for gestational age, preterm or infant of a diabetic mother) suffer at least one episode of neonatal hypoglycaemia, defined as a blood glucose concentration of < 2.6 mM (Harris, Weston, & Harding, 2012). Historically, newborn infants were thought to tolerate hypoglycaemia well, and treatment for hypoglycaemia was instigated at lower blood glucose concentrations in the neonatal population than in older children or adults (Shelley & Neligan,

1966). However, work in infants and children by Koh in the 1980's revealed that, in some subjects, sensory evoked potentials altered below a blood glucose threshold of 2.6 mM (Koh, Aynsley-Green, Tarbit, & Eyre, 1988). Recognition that clinically asymptomatic hypoglycaemia might be detrimental to brain development and long term neurodevelopmental outcomes followed shortly after (Lucas et al., 1988). The screening of at risk infants for hypoglycaemia in the hours after birth is now standard practice in most neonatal units, and hypoglycaemia is recognised as being both a cause (Tam et al., 2008) and a promoter (Martinez-Biarge et al., 2012) of neonatal brain injury.

In the early neonatal period, improved glucose delivery via increased cerebral blood flow (Pryds, Christensen, & Friis-Hansen, 1990) and cerebral usage of alternative fuels such as ketone bodies (Hawdon et al., 1992), may preserve brain energy usage and thus neuronal function during hypoglycaemia. This is demonstrated in healthy adults, where infusion with either lactate or β-hydroxybutyrate lowers the blood glucose concentration at which neuroglycopoenic symptoms and cognitive dysfunction occur (Cryer, Gerich, Mitrakou, Mokan, & Veneman, 1994). However, the availability of alternative cerebral fuels is not a universal finding, particularly in the first 24-48 h after birth (Harris et al., 2011) and it is possible that their protective role has been overstated.

Insulin inhibits lipolysis (Kamel, Norgren, Persson, & Marcus, 1999), preventing release of fatty acids and thus inhibiting both hepatic gluconeogenesis and ketone body formation during hypoglycaemia. In neonatal sheep, concentrations of alternative cerebral fuels such as lactate and β-hydroxybutyrate are suppressed during insulin-induced hypoglycaemia (Harris, Battin, Williams, Weston, & Harding, 2009). In infants being treated with insulin for hyperglycaemia, hypoglycaemia is likely to occur in the setting of an inappropriately high blood insulin concentration, resulting in suppression of the normal counter-regulatory responses to a low blood glucose concentration. Thus hypoglycaemia occurring during insulin

therapy may be more dangerous to the neonatal brain than hypoglycaemia occurring during the metabolic transition.

The preterm infant at term corrected age normally responds to a non-insulin-induced hypoglycaemic episode by increasing plasma growth hormone and cortisol concentrations; if the episode is prolonged or severe, adrenaline and lactate concentrations also increase (Jackson, Williams, Burchell, Coughtrie, & Hume, 2004). Unlike in the adult, where glucagon release is an integral part of the counter-regulatory response to hypoglycaemia, preterm neonates at term do not appear to increase their plasma glucagon concentrations even when hypoglycaemia is prolonged or severe (Jackson et al., 2004). Blunting of the glucagon response to hypoglycaemia is a feature which differentiates older children with congenital hyperinsulinaemia from those with ketotic hypoglycaemic disorders (Hussain, Bryan, Christesen, Brusgaard, & Aguilar-Bryan, 2005), and it is possible that in the preterm infant, impaired glucagon release is a feature of all hypoglycaemic episodes, not just those precipitated by excess insulin. Term and late-preterm human infants with congenital hyperinsulinaemia demonstrate an impaired cortisol response to hypoglycaemia (Hussain, Hindmarsh, & Aynsley-Green, 2003), suggesting that this may also be a feature of insulin-induced hypoglycaemia in preterm infants.

Hypoglycaemia may itself modify counter-regulatory responses to hypoglycaemia, such that in diabetic patients, recurrent hypoglycaemia is a recognised risk factor for autonomic nervous system failure, adreno-medullary impairment and hypoglycaemic unawareness (Cryer, Davis, & Shamoon, 2003). Recurrent hypoglycaemia is reported as a complication of neonatal insulin treatment, affecting 16% of infants treated with tight glycaemic control in the largest randomised controlled study (Alsweiler et al., 2012b). This subset of infants may therefore be at even greater risk of hypoglycaemic injury.

Thus, in neonates treated with insulin for hyperglycaemia, hypoglycaemic episodes may be accompanied by a failure to mount an adequate counter-regulatory response, resulting in failure of endogenous glucose production, absence of alternative cerebral fuels and increased vulnerability of the neonatal brain to injury secondary to energy depletion.

1.9.1 **Hypoglycaemic brain injury**

Failure to meet cerebral energy requirement results in primary neuronal injury through apoptosis of cells unable to maintain ATP dependent trans-membrane transport systems and thus cell membrane integrity (Rodríguez De Lores Arnaiz, 2007). Additional injury occurs due to the release of excitotoxic amino acids from disrupted cells and increased susceptibility to glutamate induced excitotoxicity due to persistent stimulation of neuronal glutamate receptors (Suh, Hamby, & Swanson, 2007). In neonatal piglets, mitochondrial production of reactive oxygen species occurs during insulin-induced hypoglycaemia (McGowan, Chen, Gao, Trush, & Wei, 2006), whilst *in vitro* mature human neurones generate reactive oxygen species at the time of glucose reperfusion, resulting in high levels of oxidative stress and neuronal death (Suh, Gum, Hamby, Chan, & Swanson, 2007). Unlike in the adult, where coma and loss of cerebral electrical discharges are thought to be a necessary precursor to permanent neuronal loss (Auer, 2004), neonates presenting with brain injury secondary to hypoglycaemia may display a variety of signs. Whilst neuroglycopoenic coma has been reported, seizures are a much more common accompaniment to damaging neonatal hypoglycaemia (Montassir et al., 2009), and apnoea, vomiting and abnormal movement patterns have also been reported. However, many of these signs are also seen in normal infants and are not specific for hypoglycaemia (Lucas et al., 1988). It is hypothesised that the most metabolically active brain areas are those most susceptible to hypoglycaemic injury, and thus the areas of the brain involved in an insult may also be determined by gestational age.

In the term infant, a specific distribution of parieto-occipital brain injury is described as a consequence of symptomatic hypoglycaemia (Tam et al., 2008). Encompassing the metabolically active visual cortex, this distribution of injury appears to be age dependent, with children older than 6 months preferentially displaying basal ganglia injury and children older than 22 months displaying temporal-parietal cortical injury in response to a significant hypoglycaemic event (Gataullina et al., 2013). However, the anatomical distribution of hypoglycaemic brain injury in the neonate is not restricted to the parietal and occipital regions. Magnetic Resonance Imaging (MRI) has revealed damage to a variety of other areas including the basal ganglia, thalamus, posterior limb of the internal capsule and cortex; in one study, less than a third of hypoglycaemic injury was associated with the classic parieto-occipital distribution (Burns et al., 2008). Despite the differences in injury locality, a very high incidence of brain white matter involvement is consistently described in relation to neonatal hypoglycaemic injury (Burns et al., 2008; Filan, Inder, Cameron, Kean, & Hunt, 2006; Montassir et al., 2009; Tam et al., 2008).

Preterm infants are at much higher risk of brain injury and subsequent neurodevelopmental impairment than are their term counterparts, and the incidence of brain injury increases with decreasing gestational age (Volpe, 2009). The aetiology of brain injury in the preterm infant is likely to be multi-factorial and there have been no studies of the specific contribution of hypoglycaemia to preterm brain injury. However, it is known that in the term infant, hypoglycaemia in combination with a hypoxic-ischaemic insult results in injury to the corticospinal tracts, with the severity of injury greater than that expected from the degree of asphyxia alone (Tam et al., 2012). In this report, the parieto-occipital injury distribution was not seen, resulting in the suggestion that this may be a feature of isolated hypoglycaemia only. Preterm infants are also at significant risk of hypoxic-ischaemic brain injury, although the distribution of injury differs from that seen in the term infant. Classically, the periventricular white matter is involved with cystic degradation leading to a loss of white matter volume and

decreased neuronal connectivity. This may be accompanied by ventricular dilatation or porencephalic cyst formation secondary to intraventricular haemorrhage precipitated by the same hypoxic-ischaemic insult (Back et al., 2001). Preterm infants affected by periventricular leukomalacia are at high risk of impaired motor function in later life (Harvey et al., 2013). If hypoglycaemia worsens periventricular white matter injury, preterm infants so affected may be at even higher risk of impaired neurological outcome.

The preterm brain displays a more limited capacity for blood-to-brain glucose transport than the term infant, secondary to a reduced number of GLUT-1 transporters. Glucose transport across the blood-brain-barrier is correlated with blood glucose concentration and is greater than the cerebral metabolic rate for glucose when blood glucose concentrations remain within the euglycaemic range. In a small study of preterm infants, cerebral glucose transport fell to match the cerebral metabolic rate for glucose at a blood glucose concentration of 3 mM (Powers, Rosenbaum, Dence, Markham, & Videen, 1998). Cerebral glucose metabolism is globally decreased in the preterm compared to the term infant, with the thalamus displaying the greatest glucose use in both cases. However, in comparison with the early preterm infant, in the term infant the occipital region has a disproportionately larger increase in glucose metabolic rate compared to other brain areas (Shi et al., 2012). Maturational differences in regional brain glucose metabolism might account for the differing injury patterns seen secondary to hypoglycaemic insult at different developmental stages.

Neonatal hypoglycaemia may not only cause specific brain injury, but also impact global neuronal development. Oligodendrocyte precursor cells undergo a number of morphological changes before reaching the mature, myelin producing state. Present at varying localities and at differing maturation states within the preterm brain, oligodendrocyte precursors display decreased resistance to metabolic and oxidative stress, with alterations in specific vulnerabilities as the oligodendrocyte passes through its 4 maturational stages (Back, Riddle,

& McClure, 2007). White matter myelination increases rapidly from 32 weeks of gestation onward, with progression from central to peripheral areas and in an anterior to posterior direction. Rapid myelination continues for the first 3 months after birth. A slower increase in cerebral white matter myelination is displayed until 2 years of age, and of cerebellar myelination until 3 years (Yap et al., 2013). It is possible that in the preterm brain, both hyper and hypoglycaemia particularly impact the oligodendrocyte population of a maturational stage most vulnerable to metabolic insult. Thus, the distribution and severity of glycaemic injury will vary from infant to infant depending on gestational age and brain maturation state. Immature oligodendrocytes subjected to hypoxic-ischaemic injury display decreased process formation, poor cell differentiation and impaired myelin producing capacity, resulting in hypomyelination of the white matter (Volpe, Kinney, Jensen, & Rosenberg, 2011). It is likely that a similar sequence of events would result from a purely metabolic insult. White matter abnormalities are predictive of later motor impairment, with severity of impairment related to both the degree of injury (Spittle et al., 2011) and the gestational age at birth (Harvey et al., 2013).

1.9.2 **Long term consequences of neonatal hypoglycaemia**

Neonates who suffer brain injury as a result of hypoglycaemia may have an increased incidence of seizure disorders, motor and cognitive impairment, and visual impairment (Burns et al., 2008; Filan et al., 2006; Montassir, Maegaki, Ohno, & Ogura, 2010). Less clear is the effect of hypoglycaemia on the long term outcome of otherwise well neonates who do not have symptoms associated with their hypoglycaemic episode.

The most widely cited study on the neurodevelopmental impact of neonatal hypoglycaemia was published almost 30 years ago (Lucas et al., 1988). This observational study of 661 infants <1850g birth weight revealed that repeated blood glucose concentrations less than 2.6 mM in the first 72 hours of life were correlated with decreased scores on standardised

developmental testing at 18 months of age. Additionally, a linear relationship was seen between increasing frequency of hypoglycaemia and decreased developmental scores. Similar results were seen in a retrospective study of 107 hypoglycaemic infants, where lower blood glucose concentration and longer duration of hypoglycaemia were linearly correlated with both presence of hypoglycaemic symptoms and decreased mental and psychomotor indices at 12 months of age. However, the presence of hypoglycaemic symptoms appeared to be the most important predictor of impaired long term outcome; infants with asymptomatic hypoglycaemia did not show any differences in developmental indices compared to age-matched euglycaemic controls (Singh et al., 1991).

The conclusion that moderate neonatal hypoglycaemia is detrimental to long term outcome has not been universally supported. An observational study of 15-year old adolescents who were born at <32 weeks gestation compared 45 infants who had prolonged hypoglycaemia <2.6 mM to matched normoglycaemic controls. It revealed no differences in motor or cognitive outcome between groups (Tin, Brunskill, Kelly, & Fritz, 2012). A further study following neonates and older children who were managed with tight glycaemic control during critical illness also showed that, despite an increased incidence of hypoglycaemia, there were no differences in standardised neurodevelopmental tests from those managed using a standard protocol. In fact, tight glycaemic control was associated with better motor co-ordination and cognitive flexibility scores (Mesotten et al., 2012). However, it should be noted that no subgroup analysis was performed for neonatal participants, who are the population most at risk of hypoglycaemia in other studies of tight glycaemic control (Verbruggen et al., 2012).

Subtle neurodevelopmental impairment has been linked to asymptomatic hypoglycaemia in infants of diabetic mothers. Compared to healthy controls, infants of diabetic mothers had similar motor and psychological function at follow up, but had increased scores on ratings of minimal brain dysfunction; scores were significantly higher in those who had experienced

hypoglycaemia (Stenninger, Flink, Eriksson, & Sahlèn, 1998). Given that the infants involved in this study had *in utero* exposure to a diabetic environment, these results may not be translatable to other groups of infants with hypoglycaemia.

The Children with Hypoglycaemia and their Later Development (CHYLD) study is a prospective cohort study of over 500 New Zealand neonates born at late preterm and term gestations, and who were at risk of hypoglycaemia in the neonatal period. Participating infants had their blood glucose concentrations measured from birth to postnatal day 7 using intermittent or continuous blood glucose monitoring. Hypoglycaemia was defined as a blood glucose concentration < 2.6 mM and infants were managed to keep blood glucose concentrations above this threshold. Two followed-up assessments have subsequently been performed in this cohort. At 2 years of age, infants who had hypoglycaemia in the neonatal period had similar rates of neurosensory impairment to infants who did not become hypoglycaemic, and there was no relationship between minimum blood glucose concentration, duration of hypoglycaemia or recurrence of hypoglycaemia and either neurosensory impairment or processing difficulty (McKinlay et al., 2015). On repeat assessment at 4.5 years of age, hypoglycaemia continued to show no association with neurosensory impairment, but was associated in a dose-dependent manner with an increased risk of impaired executive function and worsened visual-motor performance (McKinlay et al., 2017), suggesting that routine neurodevelopmental follow-up performed at 2 years may be too early to detect differences in development mediated by neonatal hypoglycaemia.

1.10. Glucose variability

Sustained abnormal blood glucose concentrations are known to be deleterious to health, and persistent hyperglycaemia is linked to the accelerated development of macro- and microvascular complications in patients with diabetes mellitus (Hanssen, 1997). The potential contribution of glucose variability to the development of such complications was highlighted

by the Diabetes Control and Complications Trial (Diabetes Control and Complications Trial Research Group, 1993). Published in 1993, this trial randomised 1441 patients with known type-1 diabetes to intensive or standard insulin treatment in order to assess the effect of tight glycaemic control on the development of diabetic retinopathy; the results were strongly in favour of an intensive insulin regimen. Some patients randomised to the standard insulin regimen had a greater risk of progression of diabetic retinopathy than those in the intensively treated group despite comparable glycaemic control measures. Percentage glycosylated haemoglobin (HbA1c), the validated measure of glycaemic control in diabetes, did not appear to be fully predictive of this risk. It was hypothesised that the increased post-prandial blood glucose fluctuation seen in the standard treatment group was contributing to this increased risk, but was too subtle to be affecting HbA1c levels (Brownlee & Hirsch, 2006).

The mechanisms by which high glucose variability results in micro- and macro-vascular damage are thought to be similar to those seen in sustained hyperglycaemia: namely increased protein glycosylation, induction of oxidative stress and increased programmed cell death (Lorenzi et al., 1985). Sun (Sun, Xu, Sun, Sun, & Wang, 2010b) discovered that human retinal endothelial cells (HRECs) displayed increased expression of vascular endothelial growth factor (VEGF- a peptide strongly implicated in the development of diabetic retinopathy and known to be up-regulated under conditions of oxidative stress (Aiello & Wong, 2000)) when cultured in high glucose concentrations compared to those cultured under normoglycaemic conditions. HRECs exposed to alternating high and normal glucose concentrations showed a further significant increase in VEGF expression and other markers of cell oxidative stress over and above that of the high glucose culture group. Another study (Risso, Mercuri, Quagliaro, Damante, & Ceriello, 2001) exposing human umbilical vein endothelial cells (HUVECs) to similar culture media conditions (normal, high, or fluctuating glucose concentrations) found decreased expression of the anti-apoptotic Bcl-2 protein, increased expression of the pro-apoptotic Bax protein and increased HUVEC apoptosis at 14

days in the fluctuating glucose group compared to the high or normal groups. Further studies using human renal cells (Jones, Saunders, Qi, & Pollock, 1999) and adipocytes (Sun et al., 2010a) have also found fluctuating glucose concentrations to be more damaging than sustained high concentrations.

Fluctuations in blood glucose concentration are used in human subjects to provide a measure of glucose variability. These fluctuations are most easily measured with a continuous glucose monitor (CGM) (Whitelaw, Choudhary, & Hopkins, 2011). However, there is as yet no universally accepted measure of glucose variability, and a variety of different definitions have been proposed (Rodbard, 2012). At its simplest, glucose variability has been described as the standard deviation around a subject's mean blood glucose value. This measure has the advantage of being simple both to compute and interpret, but does not take into account the timing of blood glucose measurements (if continuous monitoring is not used), nor the skewed distribution of blood glucose values (McCall & Kovatchev, 2009). In the absence of a transformation to a Gaussian distribution, glycaemic variability may also be described using non-parametric measures such as median and inter-quartile range (Hirshberg et al., 2008). Transformation of blood glucose measurements to a Gaussian distribution allows the application of parametric statistical analysis, and may be performed using a logarithmic transformation (Kovatchev, Cox, Gonder-Frederick, & Clarke, 1997), with or without correction to predetermined euglycaemic limits. Standard deviation or coefficient of variance (correcting for mean blood glucose concentration) may be more appropriately derived from symmetrically distributed data (Siegelaar, Holleman, Hoekstra, & DeVries, 2010). Clinically useful risk scores for the prediction of hyper and hypoglycaemia in diabetic patients have been developed using symmetrical blood glucose concentration distributions. The Low Blood Glucose Index (LBGI) and High Blood Glucose Index (HBGI) are measures of the frequency and magnitude of glycaemic excursions beyond predetermined blood glucose limits. Although predictive of future episodes, these are measures of glycaemic control rather than glycaemic

variability (Kovatchev et al., 1998; Rodbard, 2009). A similar method has been used to calculate the average daily risk range (ADRR), a predictor of both hyper and hypoglycaemia. In diabetic patients, ADRR correlates with the future risk of both hyper and hypoglycaemia, unlike standard deviation of glycaemia, which is weakly predictive of future hyperglycaemia but does not correlate with future hypoglycaemia (Kovatchev, Otto, Cox, Gonder-Frederick, & Clarke, 2006). The limiting factor in calculating ADRR is the need for at least 3 blood glucose values within a 24 hour period; continuously sampled blood glucose concentrations are preferable (Farhy et al., 2011). Although ADRR has been used as a measure of glycaemic variability in children with type-1 diabetes, ADRR scores were found to be more accurate when calculated using continuous rather than intermittent glucose monitoring techniques (Patton, Midyett, Dolan, & Powers, 2012). There is currently no evidence to support the use of ADRR as a measure of glycaemic variability among critically ill non-diabetic children.

Specific measures of blood glucose variability have been developed for use with continuous blood glucose monitoring systems. Continuous overlapping net glycaemic action (CONGA) takes the standard deviation of the difference between blood glucose concentrations separated by a known time period *n* (McDonnell, Donath, Vidmar, Werther, & Cameron, 2005). Intra-day glycaemic variation can thus be calculated, with the value of *n* determined by the operator. Other calculations using the rate of change of blood glucose concentration, such as the standard deviation rate of change (SDRC) and absolute average rate of change (AARC) take advantage the ability of CGMs to aggregate blood glucose concentrations over a pre-determined time period before making comparisons, limiting the effect of small glycaemic variations and improving correlation with clinical indices of blood glucose lability (Whitelaw et al., 2011). Once again, these methods have been developed entirely for use in diabetic patients, with no literature to support their use in critically ill, non-diabetic children.

Despite the plausibility of glucose variability as a contributor to macro- and microvascular complications in diabetic subjects through the activation of mitochondrial reactive oxygen pathways (Brownlee, 2005), translation of laboratory findings to the clinical setting has not been straightforward. Monnier (Monnier et al., 2006) showed a significant correlation between increased glucose variability measured using CGM, and oxidative stress measured using urinary excretion of free 8-iso prostane, in type-2 diabetic subjects. This relationship persisted after adjustment for other measures of glycaemic control. However, these results were not replicated in studies of type-1 diabetic subjects (Monnier et al., 2010; Wentholt, Kulik, Michels, Hoekstra, & DeVries, 2008), nor of type-2 diabetics with good overall glycaemic control (Siegelaar, Barwari, Kulik, Hoekstra, & DeVries, 2011). A further detriment to the status of glucose variability as an important factor in the causation of diabetic complications occurred in 2008, when statisticians revisited the results of the Diabetes Control and Complications Trial (Diabetes Control and Complications Trial Research Group, 1993). The application of a flawed statistical model was found to have underemphasised the contribution of HbA1c to the development of diabetic retinopathy, and with new data modelling, reduction in HbA1c levels were found to be responsible for 96% of the differences between the intensive and standard treatment groups (Lachin et al., 2008), suggesting any contribution from glucose variability was likely to be minimal.

Glucose variability has found credibility as an important factor in the contribution of glycaemic control to mortality and morbidity in critically ill non-diabetic patients (Krinsley, 2010). Glucose variability measures correlate with adverse outcome among adults admitted to intensive care units with severe burns (Farhy et al., 2011), sepsis (Ali et al., 2008), trauma (Dossett et al., 2008) and brain injury (Matsushima et al., 2012). Similar results are seen in the paediatric population (Rake, Srinivasan, Nadkarni, Kaptan, & Newth, 2010). A retrospective analysis in 1094 patients admitted to a single Paediatric Intensive Care Unit (PICU), using a glucose variability index calculated from the absolute difference in time

separated blood glucose concentration samples, stratified by quintile, found that children in the highest quintile of glucose variability accounted for 15.1% of total deaths, whereas children in the lowest quintile accounted for only 1.3% of the total deaths. In addition, of children who died, 67% had both maximum blood glucose concentrations in the highest quintile and minimum blood glucose concentrations in the lowest quintile, suggesting marked fluctuations of blood glucose concentrations in this group. (Wintergerst et al., 2006) Another retrospective study of PICU admissions also reported that length of PICU stay, number of nosocomial infections and mortality were all increased in children classified as having glucose variability, where glucose variability was defined as having at least one hyperglycaemic and one hypoglycaemic blood glucose concentration (Hirshberg et al., 2008).

There is little research evaluating the effects of glucose variability among the preterm and low birth weight neonatal populations. A recent study of 95 infants weighing less than 1,500 grams at birth used log-transformed data to calculate mean and standard deviation of blood glucose concentrations (Fendler, Walenciak, Mlynarski, & Piotrowski, 2012), as well as dividing subjects into quintiles based on raw blood glucose concentrations as has been previously described (Hirshberg et al., 2008; Wintergerst et al., 2006). Both mean and standard deviation of blood glucose concentrations were associated with increased mortality, with standard deviation alone remaining significant after multivariate modelling.

1.11. Nutrition in the preterm infant

Growth of a preterm infant is dependent on the provision of adequate nutrition. The traditional goal of neonatologists has been for preterm infant growth trajectories to match those of fetuses at comparable gestations, resulting in a normally grown infant at term equivalent age. It remains unclear whether this represents the optimum growth trajectory, as the long term consequences of attempting to manipulate the growth of preterm infants are poorly studied. As previously described, abnormal growth patterns (either faltering or

excessive) during critical periods of development are not only associated with an increased incidence of neonatal morbidity and mortality, but may result in a greater likelihood of adverse health outcomes in later life (Lapillonne & Griffin, 2013; Toschke, Grote, Koletzko, & von Kries, 2004).

Preterm infants frequently exhibit faltering growth during a prolonged postnatal period of suboptimal nutrient delivery and high metabolic risk (Cormack & Bloomfield, 2006). Concerns regarding fluid overload, ammonia toxicity and difficulties with venous access may all inhibit provision of adequate nutritional support, with some neonatal units reporting delays of up to seven days in achieving full delivery of prescribed parenteral nutrients (Grover et al., 2008). Immaturity of the gastrointestinal tract necessitates the use of parenteral nutrition in all extremely preterm and many very preterm infants, but parenteral delivery of amino acids is less efficient than via the enteral route, due to high urinary nitrogen losses (Kashyap & Heird, 2011; Zlotkin, Bryan, & Anderson, 1981). In addition, parenteral nutrition does not appear as effective in reducing proteolysis in comparison to enteral nutrition delivered to preterm infants (Denne, 2007). Enteral feeds, ideally comprising maternal breastmilk, may not be fully established for days or weeks after birth. Even breastmilk may not provide adequate nutrition to the youngest, sickest infants; breastmilk fortification to increase protein, carbohydrate and calorie content is therefore common practice (Harding et al., 2013). The ideal macronutrient compositions of the intravenous nutritional solutions administered to preterm infants also are as yet unknown. Attempts to replicate an *in utero* trans-placental nutrient profile are hampered by an incomplete understanding of human fetal metabolism, and do not account for the need to support an acute phase protein response to the unique environmental and physiological stressors impacting the preterm infant (Kashyap & Heird, 2011). Nevertheless, it is clear that inadequate protein intakes are associated with poor neonatal growth (Cormack & Bloomfield, 2013).

1.11.1 **Perinatal protein metabolism**

Amino acids are actively transported across the human placenta by shared specific transporters and exchangers (Cleal et al., 2007), and are utilised by the fetus for protein synthesis, gluconeogenesis, and as substrates for oxidation. As in the older human, amino acids may be essential, non-essential, or conditionally essential to the fetus. Significant variations are seen in the rates at which amino acids are transported to the fetus, with leucine (a promotor of protein synthesis and gluconeogenic substrate) and phenylalanine (an aromatic amino acid) the most rapidly taken up by human fetuses in stable isotope studies (Galan, Marconi, Paolini, Cheung, & Battaglia, 2009). The contribution of individual amino acids to fetal growth is not well understood, as uptake rates may not necessarily reflect usage. Estimating amino acid utilisation generally requires the modelling of a mixed free amino acid "pool" and does not take into account the rapidity of turn-over of different protein types (Matthews & van Goudoever, 2011), nor is there an ability to discriminate between dispersal to oxidation or protein synthesis pathways (Van Den Akker et al., 2011). However, it is known that hypotyrosinaemia secondary to immaturity of the phenylalanine hydroxylase pathway, exogenous insulin usage or inadequate exogenous phenylalanine or tyrosine supply (Mayes, Tan, & Morgan, 2014; Roberts, Ball, Filler, Moore, & Pencharz, 1998), are associated with poor growth even when total protein delivery is adequate (Kashyap & Heird, 2011).

In the preterm infant, a total protein intake of at least 3.5g/kg/day is thought to be required to replicate fetal protein accretion rates of 2g/kg/day (Embleton, Morgan, & King, 2015). This does not take into account *ex utero* stress, during which production of acute phase proteins may increase amino acid requirements (particularly of aromatic amino acids) and promote hypercatabolism (Kashyap & Heird, 2011). Clinical guideline groups have revised their recommended protein intakes upwards as concerns regarding the safety of early amino acid administration have been resolved (Ibrahim, Jeroudi, Baier, Dhanireddy, & Krouskop, 2004;

Vlaardingerbroek et al., 2013). Current guidelines recommend an early, aggressive approach to parenteral nutrient delivery with a protein intake target of 4 - 4.5g/kg/day (Agostoni et al., 2010; Koletzko et al., 2005b). Even when delivered, this nitrogen load cannot be maximally utilised without an adequate concomitant energy intake. However, the optimum calorie to protein intake ratio is not yet known (Kashyap & Heird, 2011). Protein catabolism appears to be prevented when the ratio of total energy to protein delivery is at least 34 kcal per gram (Lucas, Baker, & Morley, 1993). Delivery of sufficient protein, lipid and energy remains difficult to achieve in clinical practice, especially in the smallest, sickest infants (Cormack & Bloomfield, 2006; Grover et al., 2008). Inadequate early nutritional intake may rapidly result in catabolism, leading to weight loss, decreased or absent linear growth and slowing of head growth. Unlike term infants, preterm infants appear unable to suppress proteolysis in response to exogenous amino acids (Poindexter, Karn, Leitch, Liechty, & Denne, 2001), contributing to an imbalance between proteolysis and protein accretion, and promoting catabolism. Once established, nutritional deficits may persist for weeks or months and significantly impair postnatal growth (Embleton, Pang, & Cooke, 2001).

1.11.2 **Associations between neonatal macronutrient intakes and outcomes**

Early initiation of parenteral nutrition has been associated with decreased postnatal weight loss, reduced time to regain birth weight, and improvement in length and head circumference measures at 40 weeks postmenstrual age (Dinerstein et al., 2006). Higher protein intakes have been specifically linked to improvement in measures of length (Olsen, Harris, Lawson, & Berseth, 2014) and head circumference (Morgan, McGowan, Herwitker, Hart, & Turner, 2014), which may be used as a proxy for brain volume in the absence of imaging data and is known to relate to improved neurodevelopmental outcomes (Cheong et al., 2008; Franz et al., 2009). However, improvements in growth have not been consistently reported in association with increased protein intake strategies (Moyses, Johnson, Leaf, & Cornelius, 2013). One

randomised trial reported that preterm infants randomised to a higher early protein intake had worse neonatal growth outcomes (Balasubramanian, Nanavati, & Kabra, 2013). The reported reduction in neonatal morbidities associated with increased neonatal protein intakes may be an indirect cause of improvement in neurodevelopmental outcomes (Ehrenkranz et al., 2011; Mahaveer, Grime, & Morgan, 2012).

Long-term metabolic health may be programmed by early life protein intakes, in keeping with the theory of developmental origins of health and disease. In a study of 37 children born very preterm, insulin sensitivity was decreased compared to term born controls but did not correlate with early life protein intake. However it was noted that neonatal protein intakes were universally inadequate in this preterm cohort (Regan, Cutfield, Jefferies, Robinson, & Hofman, 2006). In a study of intrahepatocellular lipid (IHCL) accumulation (a marker for metabolic syndrome in the adult population) preterm infants were found to have increased IHCL at term equivalent age compared to term-born controls. Once again, neonatal protein intakes were inadequate in this study, with actual intakes below those recommended. Inadequate protein intake was accompanied by an excessive lipid intake, and lipid intakes but not protein intakes were weakly correlated with IHCL concentrations (Vasu et al., 2013). It is likely that macronutrient imbalance, with excessive carbohydrate and lipid-based caloric provision associated with inadequate protein, may be important in determining the long term effects of neonatal nutrition.

Whilst under-nutrition and inadequate protein intakes have complicated studies in preterm infants, healthy children born at term and fed high protein diets in infancy and early childhood demonstrate accelerated weight gain, increased adiposity, and are at increased risk of becoming overweight or obese by school age (Koletzko et al., 2005a; Parizkova & Rolland-Cachera, 1997; Toschke et al., 2004). A large randomised control trial of high and low protein infant formula milks fed to well term infants in the first year of life revealed that low protein

formula was associated with decreased weight gain without a concomitant decrease in linear growth at 1 year of age, and lower body mass index at 2 years of age (Koletzko et al., 2009) and at 6 years of age (Weber et al., 2014) suggesting that lower (but still adequate) protein intakes in early childhood may be protective against the later development of overweight or obesity.

1.11.3 **Sex-specific responses to neonatal nutritional intakes**

Preterm infants randomised to receive enteral feeds with higher protein and lipid content had improved neonatal growth measures and improved cognitive outcomes at both 18 months and 8 years of age, with male children selectively demonstrating a marked improvement in verbal intelligence quotient (VIQ) scores (Lucas, Morley, & Cole, 1998; Lucas et al., 1990). The increase in VIQ scores persisted into adolescence and was found to correlate with larger brain caudate nucleus volumes in boys who had received higher postnatal protein, energy and lipid intakes (Isaacs et al., 2008), suggesting that nutritional intakes may affect structural brain development in preterm male infants. Intriguingly, sex-specific changes to outcomes associated with early life nutrition have also been reported in animal (Berry, Jaquiery, Oliver, Harding, & Bloomfield, 2016) and human studies (Lauritzen et al., 2016; Ou et al., 2014), including different patterns of association between early nutrition and later neurodevelopment in boys and girls born preterm (Christmann et al., 2017). The underlying reasons for these observed differences are unknown, and few studies of neonatal nutrition analyse outcomes separately by sex.

1.11.4 **The protein leverage hypothesis**

In 2005, Simpson and Raubenheimer hypothesised that protein was the key dietary macronutrient responsible for regulation of human appetite and overall energy intake. A physiologically predetermined protein intake (protein appetite) and not calorie intake

determined satiety in individuals, and excessive caloric consumption occurred when food protein content was diluted. This protein leverage hypothesis has been suggested as one of the driving factors behind the increasing incidence of overweight and obesity in adults (Simpson & Raubenheimer, 2005). As BMI increases, so does the amount of dietary protein required to satisfy protein appetite and reach satiety, and this positive feedback loop may thus enhance the development of the metabolic syndrome (Gosby, Conigrave, Raubenheimer, & Simpson, 2014).

Interestingly, in children, protein and energy intakes at 10 months of age correlated with intakes of the same nutrients at 8 years of age, whilst no such correlation was seen with either fat or carbohydrate intakes, suggesting that the protein may also be an important mediator of food choice and satiety in childhood (Deheeger, Akrout, Bellisle, Rossignol, & Rolland-Cachera, 1996). It is unknown whether protein intakes during critical periods of metabolic development may impact lifelong protein appetite and thus, according to the protein leverage hypothesis, alter metabolic risk.

Chapter 2. Protein, Insulin and Neonatal Outcomes: The PIANO study

2.1. Introduction to the PIANO study

There are currently few reliable data regarding the effects of hyperglycaemia, its treatment with insulin, or early life protein intakes on long term metabolic, growth and developmental outcomes in infants born very preterm or at very low birth weights. We therefore undertook the Protein, Insulin and Neonatal Outcomes (PIANO) study at the Liggins Institute, University of Auckland between December 2012 and March 2016, with the aim of testing the following hypotheses:

1. In preterm infants with neonatal hyperglycaemia, tight glycaemic control will not change metabolic outcomes compared with standard glycaemic control, but will worsen neurodevelopmental outcomes;
2. Neonatal hyperglycaemia is associated with impaired metabolic function, body composition and neurodevelopmental outcome in childhood; and
3. Increased neonatal protein intake is associated with improved metabolic outcomes without adverse effects on neurodevelopmental outcomes.

The PIANO study aimed to test these hypotheses by evaluating the neurodevelopmental, growth, metabolic and body composition outcomes at 7 years' corrected age in infants with birth weight <1,500 grams or < 30 weeks' gestational age, and admitted to the neonatal intensive care unit (NICU), National Women's Hospital, Auckland, New Zealand, between July 2005 and October 2008. A total of 536 infants were eligible for the PIANO study, from whom we recruited three overlapping follow-up cohorts, with a small number of children belonging to more than one group:

2.1.1 **Cohort 1: Hyperglycaemia in neonates trial (HINT) participants**

Between July 2005 and October 2008, a randomised controlled trial of tight glycaemic control to manage neonatal hyperglycaemia (Alsweiler et al., 2012b) recruited 88 infants with neonatal hyperglycaemia (defined as 2 consecutive blood glucose concentrations >8.5 mM no less than 4 hours apart). The 43 infants randomised to tight glycaemic control were managed to achieve target blood glucose concentrations of 4–6 mM, whereas the 45 infants randomised to receive standard treatment were managed to achieve target blood glucose concentrations of 8–10 mM. The primary outcome of the trial was linear growth at 36 weeks' postmenstrual age, which was found to be reduced in the tight glycaemic control group compared to those receiving standard treatment.

2.1.2 **Cohort 2: Matched non-hyperglycaemic preterm controls**

During the period of recruitment to the HINT trial (Alsweiler et al., 2012b), 448 infants < 1,500 grams or < 30 weeks' gestation were admitted to the NICU and did not develop neonatal hyperglycaemia as defined by the trial entry criteria. A hierarchical matching procedure was undertaken to select children from this cohort as non-hyperglycaemic preterm controls, in whom outcomes could be compared with hyperglycaemic infants in a matched-cohort study.

2.1.3 **Cohort 3: Audit of protein intakes**

In January 2007, in response to international guidelines recommending higher protein intakes for very low birth weight infants (Koletzko et al., 2005b), a new nutritional protocol was introduced to the NICU. The aims of the new protocol were to standardise prescribing of intravenous nutrition by use of an online calculator, limit total fluid intakes in the first postnatal week, and increase daily protein intakes to meet international recommendations. To

monitor the effect of this change in practice on neonatal fluid and macronutrient intakes, an audit of 80 preterm infants with birth weight <1,500 grams and with full nutritional intake data from postnatal days 1-30 was undertaken, including 40 infants treated under each of the old and new nutrition protocols (Cormack, Bloomfield, Dezoete, & Kuschel, 2011). Infants' neonatal growth, neonatal morbidity and neurodevelopmental outcome (measured using Bayley III assessment at 18 months' corrected age) were assessed. This audit revealed that infants treated under the new protocol received less fluid and more protein in the first postnatal week, but these changes were not associated with any differences in neonatal growth, morbidity or 18 month neurodevelopmental outcomes.

The change in nutrition protocol was observed to coincide with a decrease in the incidence of hyperglycaemia in the NICU. However, this was not an outcome included in the original audit. As all infants admitted to the NICU were exposed to the change in nutrition protocol, the analysis of outcomes at 7 years corrected age was expanded to not only include the original audit participants, but also those other PIANO participants who had full nutrition data available for the first postnatal week.

2.2. Ethics and consent

Ethics approval for the PIANO study was obtained from the NZ Health and Disability Ethics Committee (NTY/12/05/035) and the Auckland District Health Board Research Review Committee (ADHB 5486). Informed, written consent was obtained from the child's parent or legal guardian prior to commencing the assessment, and verbal assent was sought from the child.

2.3. Tracing

Children were traced through hospital records, general practitioners, family contacts and social media. Once contact details had been found, a letter was sent to the child's caregivers providing them with an information sheet and inviting them to take part in the study. If no response occurred within 2 weeks, a follow up phone-call was made, followed by a home visit if these measures did not result in contact.

All children were invited to the Liggins Institute at the University of Auckland for assessment at 7 years corrected age ± 6 months. A few families were unable to attend the Institute; these children were seen at home or school, and underwent a partial assessment including growth, physical examination, developmental assessment and visual acuity.

2.4. Assessment format

Participants attended the Liggins Institute fasted and with local anaesthetic cream applied to the antecubital fossae. Assessors were blind to which neonatal cohort the child belonged to. Growth measurements were taken, then an intravenous cannula was placed and a frequently sampled intravenous glucose tolerance test (IVGTT) performed. Following the IVGTT, the child underwent protein satiety testing, after which breakfast was served. The child next took part in developmental testing, blood pressure monitoring and dual x-ray absorptiometry (DXA) scanning, followed by a break for lunch. Magnetic Resonance Imaging (MRI) of the brain was performed in the early afternoon, then visual and physical examinations, including assessments of pubertal status and dermal scarring. Eye-drops to dilate the pupils were inserted, and the assessment concluded with visual assessment and retinal photography. During the assessment, the child's parent/caregiver was asked to complete a number of questionnaires and if English was their first language, an objective literacy test (Peabody picture vocabulary test fourth edition, Pearson, USA).

2.4.1 **Physical examination**

Cardiovascular, respiratory, neurological and musculoskeletal systems were examined by a paediatrician using standard techniques. Size and location of scarring was assessed visually, with any scar > 2 centimetres in length classed as large. Pubertal development was assessed as outlined by Marshall and Tanner (1969; 1970), with breast or genital development of stage 2 or above being considered puberty.

2.4.2 **Blood pressure**

Blood pressure was measured using an oscillometric system and suitably sized cuff (Dinamap ProCare 100, GE). The child was asked to recline quietly for 5 minutes prior to measurement. Systolic, diastolic and mean blood pressures were recorded 3 times, averaged, and converted to a *z*-score (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

2.4.3 **Anthropometry**

Children were weighed without shoes and wearing light clothing only, using a calibrated digital scale (Tamita baby+mommy 1582, Wedderburn, NZ) to the nearest 100 grams.

Height was measured three times with shoes, hats and hair decorations removed, using a calibrated stadiometer (Holtain Ltd, Dyfed, UK). Measurements were recorded to the last completed millimetre. The three recorded measurements were compared and any measure varying by more than 5 mm from the other two was excluded. Measures were then averaged to give a final height.

Sitting height was measured three times to the nearest completed millimetre with the child sitting on a stool of known height. The three recorded measurements were compared and any

measure varying by more than 5 mm from the other two was excluded. Measures were averaged to give a final sitting height.

Head circumference was measured three times to the nearest completed millimetre using a non-stretch measuring tape passed around the widest occipito-frontal circumference, and averaged to give a final head circumference.

Abdominal circumference was measured three times to the nearest millimetre using a non-stretch measuring tape passed around the circumference of the abdomen halfway between the lower edge of the ribcage and the anterior superior iliac crest. Measurements were averaged to give a final abdominal circumference.

2.4.4 **Intravenous glucose tolerance test**

Children were asked to attend the Liggins Institute fasted for a period of at least 9 hours. Topical anaesthetic cream (Emla 5% cream, AstraZeneca Ltd, NZ) was sent to the child's caregivers with instructions to apply the cream to both antecubital fossae one hour prior to their appointment time. A 22-gauge cannula (BD Insyte, Becton Dickinson Infusion Therapy Systems, USA) was inserted within the anaesthetised field, secured and attached to a three-way tap and giving set flushed with heparinised saline (1 unit heparin sodium per millilitre sodium chloride 0.9%).

At time 0 minutes, a 0.3 g/ kg dose of glucose was given intravenously over 60 seconds. At 20 minutes, 0.015 IU/ kg Insulin (Actrapid, Novo Nordisk A/S, Denmark) was given intravenously over 30 seconds. Blood was taken for analysis at -20, -10, -1, 2, 4, 5, 6, 8, 10, 12, 14, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90 and 100 minutes. Blood samples were collected in sterile containers primed with 68 IU lithium heparin (BD Vacutainer, BD,

UK) and immediately placed onto ice. At the conclusion of the test, samples were spun at 2540 rpm for 10 minutes at 4 C and plasma frozen at -18 C until analysis.

2.4.5 **Protein satiety testing**

Children were randomised, using computer generated random numbers, to receive one of two chocolate milkshakes with "high" or "low" protein content, but similar fat and energy contents (Table 2-1). Each 100ml of milkshake contained: 86ml Anchor UHT trim milk (Fonterra Brands, NZ), 10ml chocolate flavouring (Supreme Milkshake Syrup, Finest foods, NZ) and either 5g whey protein isolate (Beneprotein, Nestlé HealthCare Nutrition Inc., USA) or 4.7g maltodextrin (Polycal, Nutricia Ltd., UK). Assessors, parents and children remained blind to the content of the milkshake.

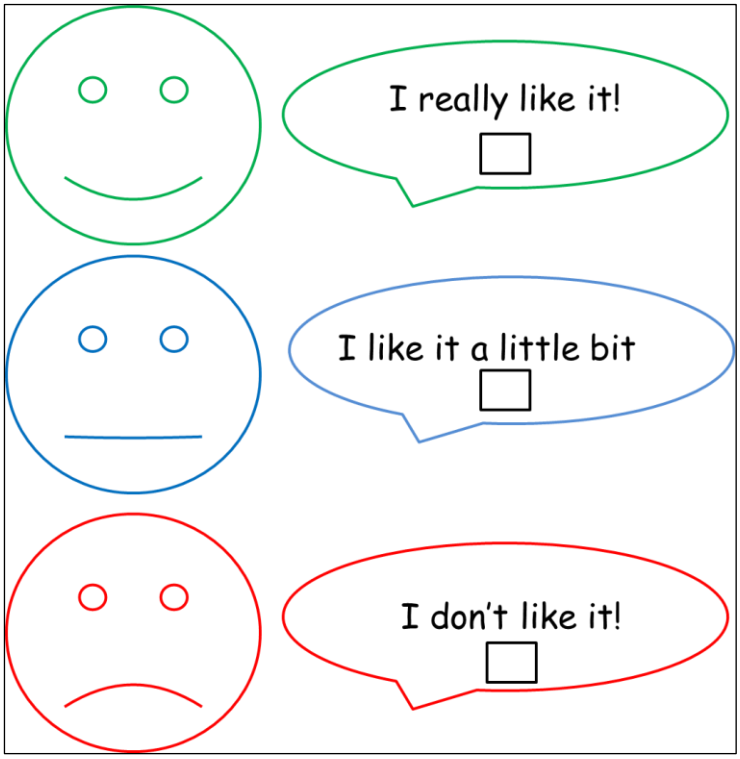
Table 2-1: Milkshake composition

	Protein (g)	Carbohydrate (g)	Fat (g)	Energy (kcal)	Energy from protein* (%)
High protein shake (100ml)	7.7	8.6	0.1	66	47
Low protein shake (100ml)	3.2	13.1	0.1	66	19

* assuming 4 kcal/gram protein

Milkshakes (total 700ml) were presented to children in brightly-coloured opaque cups with integral straws of known weight. Using a standard script, children were told to drink as much

Figure 2-1: Milkshake palatability



or as little as they wanted, and reminded that a full breakfast would be provided in 5 minutes. Parental involvement with milkshake drinking was discouraged. Milkshake palatability was assessed using a 3-point visual Likert scale (Figure 2-1).

The aim of this protocol was to test the protein leverage hypothesis, by determining whether fasted ex-preterm children limit milkshake intake by protein or energy intake, and if the amount of protein imbibed differed in children with different protein intakes in early life. The results of protein satiety testing are not reported in this thesis.

2.4.6 **Developmental testing**

Children were assessed by a certified developmental assessor trained in the use of standardised tests and overseen by a Clinical Psychologist. Cognitive and motor development were assessed using: Wechsler Intelligence Scale for Children Fourth edition Australian (WISC-IV Australian, Pearson, USA); Movement Assessment Battery for Children second edition (MABC-2, Pearson, USA); Beery-Buktenica Developmental Test of Visual-Motor Integration (Pearson, USA); and four subtests of the Test of Everyday Attention for Children (Sky Search, Score!, Creature Counting and Sky Search DT; TEA-Ch, Pearson, USA). Assessments were performed in a standard sequence and scored by the assessor. With parental consent, developmental assessments were video-recorded and reviewed by a Clinical Psychologist for ongoing training purposes.

2.4.7 **Parental questionnaires**

Parents or caregivers were asked to complete the Behavior Rating Inventory of Executive Function (BRIEF), Achenbach System of Empirically Based Assessment (ASEBA) child behavior checklist, Child Health Questionnaire, Modified Health Utilities Index 2 scale, and a demographic questionnaire.

Table 2-2: Developmental assessments

Test	Subtest	Outcome measures	Global outcome measure
Wechsler Intelligence Scale for Children 4th edition Australian	Block design	Perceptual reasoning	Full scale intelligence quotient
	Picture concepts		
	Matrix reasoning		
	Similarities	Verbal comprehension	
	Vocabulary		
	Comprehension		
	Digit span	Working memory	
	Letter-Number sequencing		
	Coding	Processing speed	
	Symbol search		
Movement Assessment Battery for Children 2nd edition	Placing pegs (both hands)	Manual dexterity	Total motor score
	Threading lace		
	Drawing trail		
	2-handed catch	Aiming and catching	
	Beanbag throw		
	One-board balance (both legs)	Balance	
	Forward heel-toe walking		
	Hopping on mats (both legs)		
Beery-Buktenica Developmental Test of Visual-Motor Integration	Beery Visual Motor Integration		
	Visual perception		
	Motor coordination		
Test of Everyday Attention for Children	Sky Search	Selective attention	
	Score!	Sustained attention	
	Creature Counting	Shifting attention	
	Sky Search DT	Dual attention	

2.4.8 **Body composition**

Bone mineral density, lean and fat tissue-mass distribution and body fat percentage were measured using dual-energy X-ray absorptiometry (Lunar Prodigy, utilising enCORE software, both GE, USA). The system was calibrated using equipment supplied by the manufacturer, children were positioned appropriately and a total body sequence performed.

2.4.9 **Visual assessment**

Vision testing was performed by an optometrist. Visual acuity, stereopsis and ocular motility were measured using standard techniques. Visual motion coherence was assessed using a bespoke computer program. Following instillation of cyclopentolate (Cyclopentolate hydrochloride 1%) to achieve cycloplegia, optical coherence tomography, retinal photography and auto refraction were performed.

Only visual acuity is reported in this thesis.

2.4.10 **Magnetic resonance imaging**

Structural and functional magnetic resonance imaging (MRI) of the brain was performed using a 3-T MAGNETOM Skyra scanner (Siemens AG, Erlangan, Germany) located at the Centre for Advanced MRI at the University of Auckland. Children were habituated to the scanner before being appropriately positioned with a 32-channel head coil (Siemens) and field mapping performed. The following structural imaging sequences were run: high resolution T1 (with fat suppression), T2 (susceptibility weighted and fluid inversion images), T2*-weighted and diffusion tensor imaging (DTI) sequences. Functional imaging was performed using blood oxygen level dependent (BOLD) T2*-weighted sequences, performed during resting state and during a global motion visual stimulus. Anatomical scans were read

by a paediatric radiologist blinded to the participants' details and clinical course. Scores were given for grey and white matter appearance, evidence of cystic change and volume loss.

The results of MRI scanning are not reported in this thesis.

2.4.11 **Academic progress**

Participants' teachers were contacted and asked to complete the BRIEF and ASEBA teacher forms. National Standards data were requested for each participant. School socioeconomic decile ranking was obtained from the Ministry of Education.

2.5. Historical data collection

2.5.1 **Neonatal nutrition and health outcomes**

Electronic medical records were accessed and the following data extracted: estimated date of delivery, daily fluid and nutrition intakes for the first 28 days after birth, and length, weight and head circumference measures during the first 28 days and at 36 weeks' post-menstrual age. Energy and macronutrient intakes were calculated using the reference data given in appendix A. All measured blood glucose concentrations were obtained from birth to 36 weeks' postmenstrual age. Neonatal morbidity and mortality outcomes were taken from the Australia New Zealand Neonatal Network (ANZNN) codes or directly from the medical records.

2.5.2 **Growth and development in early childhood**

Records from the routine developmental surveillance offered to all very preterm and VLBW infants were obtained from the child's local District Health Board. In addition, growth, audiology and developmental data recorded at the time of a nationally provided pre-school

health and developmental assessment (the B4 School Check- offered free of charge to all 4 year old children in New Zealand) were requested from the Ministry of Health.

2.6. Laboratory methods

Plasma frozen following completion of the frequently sampled IVGTT was thawed, agitated and re-spun. Insulin and glucose concentrations were measured in all samples except those taken at 100 minutes. Cortisol concentrations were measured in samples taken at 19, 30, 40, 50, 60, 70, 80, 90 and 100 minutes. Plasma glucose concentrations were measured on a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) by enzymatic colorimetric assay (Roche Diagnostics, Mannheim, Germany). Plasma insulin and cortisol concentrations were measured using electrochemiluminescence immunoassays (ECLIA) on an Eclysys 2010 immunology analyser (Hitachi High Technologies Corporation, Tokyo, Japan) utilising insulin and cortisol assay kits (Roche Diagnostics, Mannheim, Germany). Glucose and insulin concentrations were entered into the MinMod Millennium software package (MinMod Inc., California, USA) for analysis.

2.7. Statistical methods

All study data were entered into a secure database and imported to SAS version 9.4 for final data merging and coding. Statistical analyses were performed using JMP vs 10.0 or 11.2.0, or SAS v 9.4 (SAS Institute Inc., Cary, NC, USA). Statistical analysis plans were prespecified for each sub-cohort nested within the PIANO study, and are described individually in the following chapters.

2.8. PIANO study recruitment

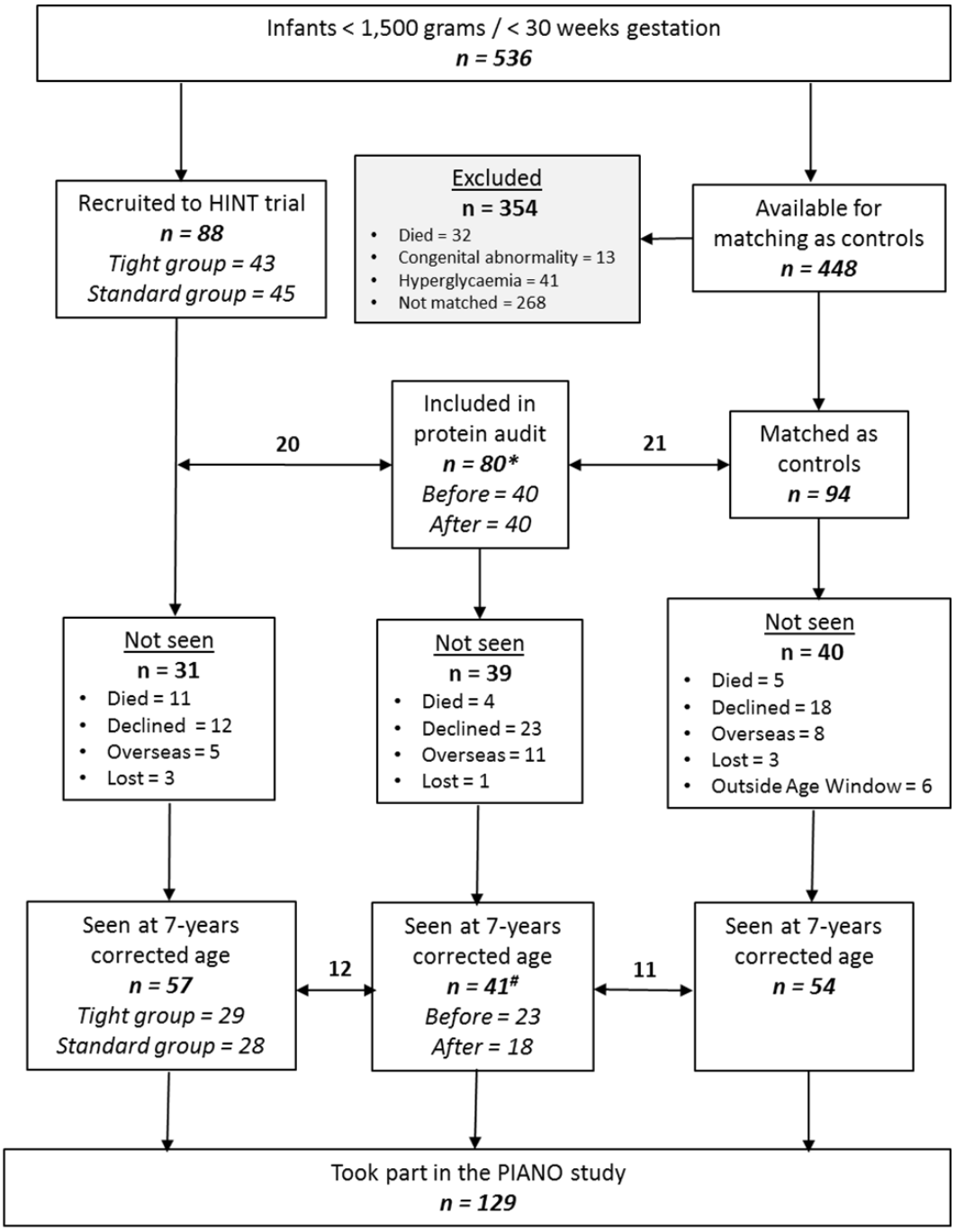
A total of 536 infants born at <30 weeks' gestation or <1,500 g birth weight were admitted during the period July 2005 – October 2008, and were considered for inclusion in the neonatal observational studies and the active PIANO follow up cohorts (Figure 2-2). The baseline neonatal cohort had a median birth weight of 1130g and a median gestational age of 29 completed weeks (Table 2-3). Eighty-eight eligible infants were randomised to the HINT trial, and 94 matched controls were recruited from the 448 infants available for matching. Eighty infants were included in the protein audit, of whom 20 were also HINT participants and 21 were also matched controls. This gave a total of 221 infants available for follow up, including 88 HINT participants, 94 matched controls and 39 additional infants included in the protein audit. Ninety-two children were not seen at 7 years due to having died, declined, moved overseas, lost (no contact made) or outside our prespecified age window of 7 years' corrected age ± 6 months. Thus, a total of 129 children were assessed at 7 years' corrected age (Figure 2-2).

Table 2-3: Perinatal characteristics of the PIANO study neonatal baseline cohort

	PIANO neonatal cohort n = 536
Birth weight (g)	1130 (860 – 1340)
Birth weight *z*-score	-0.29 (-0.99 – 0.39)
Gestational age (completed weeks)	29 (26 – 30)
Male sex	295 (55)
Any antenatal steroids	467 (87)
Maternal ethnicity	
Māori	121 (23)
Pacific Island	57 (11)
Asian	106 (20)
NZ European / Other	252 (46)
Congenital anomaly	22 (4)

Data are median (IQR) or n (%)

Figure 2-2: STROBE diagram of PIANO study recruitment and participation



* Includes 20 infants from the HINT trial, 21 matched control infants and 39 additional infants only in the audit group.
# Includes 12 infants from the HINT trial, 11 matched control infants and 18 additional infants only in the audit group.

2.9. Introduction to the results of the PIANO study

This thesis comprises manuscripts, either published or in various stages of the submission process, which detail the results of 3 neonatal and 4 school-age follow-up studies undertaken within the PIANO study. Taken together, these studies aim to further our understanding of the contribution of neonatal glycaemia and nutrition to the long-term neurodevelopment and health of children born very preterm; a group at high risk of adverse outcomes (Hutchinson et al., 2013).

Although high and low blood glucose concentrations frequently occur in very preterm, very low birth weight infants (Beardsall et al., 2010), it is not clear why some infants tightly regulate their blood glucose concentrations in the neonatal period, whilst others have a wide range of blood glucose concentrations. Furthermore, it is not clear whether the pattern of blood glucose concentrations has any independent associations with later outcomes, or whether an infant's blood glucose concentrations are merely markers of the degree of illness and prematurity, and thus their ability to maintain homeostasis. We undertook the study described in chapter 3 to further explore the characteristics of infants with different patterns of blood glucose concentrations and to explore whether these different patterns were independently associated with different outcomes. In this manuscript we reported the underlying perinatal characteristics of infants with high and low blood glucose excursions, and explored associations between measures of glycaemia such as mean blood glucose and glucose variability, mortality and morbidity in the neonatal period, and survival without neurodevelopmental impairment at 2 years' corrected age.

As the incidence of hyperglycaemia in the National Women's NICU was observed to decrease after the introduction of a new nutrition protocol, we performed a retrospective, observational study, reported in chapter 4, to test whether this observation was correct. We also aimed to

explore any interactions between different neonatal macronutrient intakes and blood glucose concentrations, to determine which component of the changed nutrition protocol might be associated with changes in the incidence of abnormal blood glucose concentration. This information may be useful in contributing to evidence to guide the formulation of nutritional guidelines for infants at risk of hyperglycaemia.

Despite advances in neonatal intensive care, girls born preterm have consistently better short and long term outcomes than boys born at the same gestation (Hintz et al., 2006; Stevenson et al., 2000). There is some evidence that girls and boys respond differently to neonatal nutritional intakes (Isaacs et al., 2008; Lucas et al., 1990). We therefore explored the presence of interactions between neonatal nutrition, infant sex, and neonatal and 2-year outcomes in our relatively large neonatal cohort, as described in chapter 5.

The subsequent results chapters describe the findings from follow-up assessments of the PIANO cohort performed at 7 years' corrected age. The study reported in chapter 6 tests the hypothesis that tight glycaemic control of neonatal hyperglycaemia improves metabolic and body composition outcomes, but worsens neurodevelopment at school age. It reports long term outcomes of hyperglycaemic infants randomised to tight or standard glycaemic control in the HINT Trial (Alsweiler et al., 2012b). The study reported in chapter 7 tests the hypothesis that hyperglycaemia itself contributes to adverse long-term outcomes using a matched cohort study of children who did and did not develop neonatal hyperglycaemia.

The study described in chapter 8 tests the hypothesis that increased early protein intakes are associated with improved metabolic outcomes, without changing neurodevelopment at 7 years' corrected age. We report the outcomes of children assessed at 7 years' corrected age who were exposed to two different nutritional protocols in the neonatal period, and explore associations between actual early macronutrient intakes and later neurodevelopmental outcomes.

We originally intended to undertake a comparison of the incidence of scarring between children randomised to tight or standard glycaemic control who are reported in chapter 6. However, it became clear during the follow-up period that the burden of scarring in very preterm children at school age was high, and that there were no contemporary data regarding the incidence or severity of scarring in children born very preterm, and certainly none from a population including a significant number of non-caucasians. Thus, the study described in chapter 9 became a descriptive analysis of scarring in the very preterm population at school age.

I was involved in the conceptualisation of the PIANO study, and sat on the study Steering Group from its initiation. I wrote the study protocol, applied for and obtained ethics approval, created the record forms used during the assessments and planned the assessment format. I performed contact tracing and organised assessment bookings, performed frequently sampled glucose tolerance tests, growth measures, physical and neurological examinations, and protein satiety testing with assistance from a research nurse. I assisted with the development of the visual assessment and MRI protocols, and with the performance of these tests in individual participants, although these assessments were primarily undertaken by others. I administered and scored the MABC-2, Beery-Buktenica Developmental Test of Visual Motor Integration, Peabody Picture Vocabulary Test, and assisted with the Wechsler Intelligence Scale for Children 4th edition (Australian) and the Test of Everyday Attention for Children administered by a developmental assessor. I performed 70% of laboratory analyses, entered all IVGTT results to MinMod software, and performed all analyses of glucose-insulin metabolism. I assisted with neonatal data collection and performed all calculations of macronutrient intakes. I assisted with data cleaning and data entry following the 7-year assessments, drafted the statistical analysis plans for each component of the PIANO study with assistance from the study statisticians, and attended weekly data meetings to plan and perform statistical analyses. I undertook the majority of the statistical analyses for chapters 3-5 and chapter 9, and assisted

with the statistical analyses for chapters 6-8, which were largely undertaken by the study statisticians.

Chapter 3. Relationship between measures of neonatal glycemia, neonatal illness, and 2-year outcomes in very preterm infants

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Tottman AC, Alsweiler JM, Bloomfield FH, Pan M & Harding JE.

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This manuscript is presented as published in the Journal of Pediatrics, and uses American English and metric mass units. To convert mg/dL to mM, divide by 18.

3.1. Introduction

Infants born very preterm and at very low birth weights are at risk of adverse neurodevelopmental and health outcomes (Hutchinson et al., 2013; Wood et al., 2005). Some of these outcomes have been linked to the metabolic vulnerability that is associated with preterm birth. Specifically, preterm infants are at risk of both hypoglycemia and hyperglycemia (Beardsall et al., 2010; Fendler et al., 2012; Mitanchez, 2007). Neonatal hypoglycemia has been associated with brain injury (Burns et al., 2008), visual impairment (Tam et al., 2008), and impaired cognitive performance in childhood (Lucas et al., 1988). In very preterm infants, hyperglycemia has been associated with increased rates of sepsis (Kao et al., 2006), retinopathy of prematurity (Blanco, Baillargeon, Morrison, & Gong, 2006), intraventricular hemorrhage (Hays et al., 2006) and abnormal neurologic examination at 2 years of age (van der Lugt et al., 2010). Neonates with hyperglycemia commonly are treated with insulin (Alsweiler et al., 2007), which substantially increases their risk of hypoglycemia (Alsweiler et al., 2012a; Beardsall et al., 2008) and is likely to increase glucose variability (van der Lugt et al., 2010).

In critically ill adults and children, high glucose variability is itself associated with increased morbidity and mortality (Dossett et al., 2008; Krinsley & Preiser, 2015; Pisarchik, Pochepen, & Pisarchyk, 2012; Rake et al., 2010). However, it is not clear if this relationship is causal or if increasing glucose variability merely signals deteriorating homeostatic function secondary to illness. Relationships between glucose variability and mortality have been observed in very low birth weight infants (Fendler et al., 2012), term neonates, and children undergoing intensive care (Hirshberg et al., 2008; Wintergerst et al., 2006). It is not known if these relationships can be explained by the associated degree of absolute hypoglycemia or hyperglycemia, or whether glucose variability alone relates to outcomes, even in normoglycemic neonates.

The purpose of this study was to characterize very preterm infants with different glucose profiles in the first week after birth, and to investigate the relationships between neonatal glucose profiles (absolute glycemia, mean blood glucose concentration, and glucose variability), neonatal illness, and developmental outcomes at 2 years of age.

3.2. Methods

Eligible infants were born weighing <1500 g or at <30 weeks of gestation and admitted to the National Women's Health, Auckland City Hospital neonatal intensive care unit (NICU) from July 2005 to October 2008. Infants were excluded if they were admitted to the NICU after 24 hours of age, died or were discharged before day 7, or had significant congenital abnormality.

The usual clinical practice during the study period was to start intravenous 10% dextrose at 60–90 mL/kg/day as soon as possible after birth. Standardized amino acid solutions were introduced once central venous access was obtained and increased gradually over the first week. Maternal expressed breastmilk was the preferred enteral feeding and was introduced as soon as available. Enteral feeds were increased at up to 30 mL/kg/day. Hyperglycemia was managed by reducing the glucose infusion rate or initiation of an insulin infusion to maintain blood glucose concentration of 72-180 mg/dL (4-10 mmol/L).

Intrauterine growth was determined using birth weight *z*-scores (Fenton & Kim, 2013). Maternal ethnicity was prioritized (Ministry of Health, 2004) and socioeconomic status determined from maternal address (quintile 5 is most deprived) (Salmond, Crampton, & Atkinson, 2007).

All blood glucose concentrations were measured on a blood gas analyzer (ABL 700, Radiometer Ltd, Copenhagen, Denmark), and recorded from birth until the end of postnatal day 7. For 8 participants, blood glucose concentrations measured before NICU admission were also collected (<0.01% of all blood glucose concentrations). Infants with <3 recorded

blood glucose concentrations were excluded. All available blood glucose concentrations for each infant were log-transformed to approximate a normal distribution, the mean and standard deviation of the mean calculated, and back-transformed to give a mean blood glucose concentration and a measure of glucose variability.

To determine the effect of absolute glycemia on outcomes, infants were categorized by the occurrence of absolute glycemic excursions during the first week after birth as follows: normoglycemic (a single blood glucose concentration of 38-45 mg/dL [2.1-2.5 mmol/L], or a single blood glucose concentration of 155-180 mg/dL [8.6-10.0 mmol/L], with all other measures 47–153 mg/dL [2.6-8.5 mmol/L]); hypoglycemic (blood glucose concentration of ≤45 mg/dL [2.5 mmol/L] on ≥2 measures >1 hour apart, or any blood glucose concentration ≤36 mg/dL [2.0 mmol/L]); hyperglycemic (blood glucose concentration ≥155 mg/dL [8.6 mmol/L] on ≥2 measures >1 hour apart, or any blood glucose concentration ≥182 mg/dL [10.1 mmol/L]); unstable (≥1 blood glucose concentration ≤45 mg/dL [2.5 mmol/L] and ≥1 blood glucose concentration ≥155 mg/dL [8.6 mmol/L]).

During the study period, written guidelines to guide practice used these blood glucose concentration thresholds, based on the limited data available (Alsweiler et al., 2007; Lucas et al., 1988). Glycemic categories were thus defined to reflect clinical practice, aiming to identify infants in whom treatment of glucose excursions was most likely to have taken place. Infants who experienced a single blood glucose concentration slightly outside the normal range were unlikely to have been treated and were therefore included in the normoglycemic group.

Neonatal morbidity and mortality up to 2 years of age were obtained from the clinical records. Survival without neonatal morbidity was defined as the absence of any of death, chronic lung disease (Chow, 2013), retinopathy of prematurity (grade 3 or 4) ("An international classification of retinopathy of prematurity. Prepared by an international committee.," 1984),

intraventricular hemorrhage (grade III or IV) (Papile, Burstein, Burstein, & Koffler, 1978), necrotizing enterocolitis (Bell stage ≥2)(Chow, 2013), or periventricular leukomalacia (Chow, 2013).

Findings were obtained from routine developmental surveillance at 2 years, including Bayley II or Bayley III assessment. Where standardized assessment was not performed, information was obtained from the child's usual doctor. Development was categorized as normal, mild impairment (motor score <-1 SD), moderate impairment (cognitive score -1 to -2 SD or mild-moderate cerebral palsy without cognitive impairment or impaired vision requiring spectacles or conductive hearing loss requiring aids), or severe impairment (cognitive score <-2 SD or severe cerebral palsy or bilateral blindness or sensorineural hearing loss requiring hearing aids)(National Women's Health, 2015).

3.3. Statistical analyses

Data were analyzed using JMP V10 (SAS Institute, Cary, North Carolina). Glycemic groups were compared used ANOVA with the Tukey *post hoc* correction for multiple comparisons, or Wilcoxon rank-test with Dunn's *post hoc* test. Categorical data were analyzed using the X^2 test. Logistic or linear regression were used to investigate relationships between glycemia category, mean blood glucose concentration, glucose variability, and neonatal and 2-year outcomes. Variables where $P < .1$ in bivariate analyses (gestational age, birth weight, birth weight *z*-score, ethnicity, socioeconomic quintile, and Clinical Risk Index in Babies 2 [CRIB II] score (Parry, Tucker, & Tarnow-Mordi, 2003)) were considered for inclusion in a multivariable model. After assessment for collinearity, variables included in the final multivariable model were gestational age, birth weight *z*-score, and socioeconomic quintile. Relationships between measures of neonatal glycemia and outcome at 2 years of age were additionally adjusted for the type of assessment performed at 2 years of age (Bayley II, Bayley III, other). Data are presented as n (%), median (range) or OR and 95% CI.

Ethical approval was obtained from the Northern B ethics committee (NTY/12/05/035) and institutional approval from the Auckland District Health Board (ADHB 5486).

3.4. Results

During the study period, 536 eligible infants were admitted to the NICU, of whom 443 (83%) had glucose profiles available for analysis and 346 (65%) had a 2-year developmental assessment available (Figure 3-1).

Of participants whose glucose profiles were available for analysis, 287 of the443 (65%) were categorized as normoglycemic (Table3-1). Infants in the hypoglycemic category (42 /443, 9%) were of similar gestational age and birth weight to those in the normoglycemic category. Infants in the hyperglycemic category (73/443, 16%) were of lower gestational age, lower birth weight, and had higher CRIB II scores than those in both the normoglycemic and hypoglycemic categories. Infants in the unstable category (41/443, 9%) were similar to hyperglycemic infants in gestational age and CRIB II score, but had lower birth weights than infants in all other glycemia categories. Infants in the unstable and hypoglycemic categories had lower birth weight *z*-scores than those in the normoglycemic and hyperglycemic categories (Table 3-1).

Infants in the hyperglycemic category were more likely to be Māori and less likely to be New Zealand European, whereas infants in the hypoglycemic category were less likely to be Māori and more likely to be of other ethnicities. There were no differences in socioeconomic quintiles between glycemia categories (Table3-1).

Blood glucose measurements were performed most frequently in infants in the unstable category and least frequently in those in the normoglycemic category (Table3-1). Infants in the hyperglycemic category had the highest mean blood glucose concentration and those in the

hypoglycemic category had the lowest mean blood glucose concentration. Glucose variability was greatest in infants in the unstable and hypoglycemic categories (Table 3-1).

Infants in the normoglycemic and hypoglycemic categories had shorter neonatal stays and were less likely to die or to have severe retinopathy of prematurity, necrotizing enterocolitis, late-onset infection, or chronic lung disease, or to go home on oxygen than infants in the hyperglycemic and unstable categories. However, the incidence of intraventricular hemorrhage and periventricular leukomalacia was similar in all glycemia categories (Table 3-2). Eighty-five percent of infants (245/287) in the normoglycemic category achieved the composite outcome of survival without neonatal morbidity. The odds of this outcome was similar for infants in the hypoglycemic category (35/42 [84%]; OR, 0.86; 95% CI, 0.38–2.22; P = .73), but much less likely for infants in the hyperglycemic category (44/73 [60%]; OR, 0.26; 95% CI,0.15–0.46; P < .0001) and the unstable category (21/41 [51%]; OR, 0.18; 95% CI, 0.09–0.36; P < .0001).

Of the 346 infants for whom 2-year developmental assessment data were available, 56 (16%) had neurodevelopmental impairment. Severe neurodevelopmental impairment was uncommon, but moderate neurodevelopmental impairment was more frequent than mild (Table 3-2). Impairment was uncommon in infants in the normoglycemic and hypoglycemic categories, but more common in those in the hyperglycemic and unstable categories. The composite outcome of survival without neurodevelopmental impairment was achieved by 290 of 365 of the whole cohort (79%), and 195 of 233 infants (84%) in the normoglycemic category. The odds of survival without impairment was similar for infants in the hypoglycemic category (30/33 [91%]; OR ,1.95; 95% CI, 0.65–8.41; P = .29), but was much less likely for infants in the hyperglycemic category (42/62 [68%]; OR, 0.41; 95% CI, 0.21–0.78; P = .006) and the unstable category (23/37 [62%]; OR, 0.32; 95% CI, 0.15–0.69; P = .003) (Table 3-3).

In univariate analyses, both glycemia category and mean blood glucose concentration were associated with survival without neonatal morbidity, and survival without neurodevelopmental impairment at 2 years of age (Table 3-3). Glucose variability was associated with survival without neonatal morbidity, but not with survival without neurodevelopmental impairment at 2 years (Table 3-3). After adjustment for gestational age, birth weight *z*-score, socioeconomic quintile, and type of assessment at 2 years, there were no significant associations between glycemia category, mean blood glucose concentration, or glucose variability and either survival without neonatal morbidity or survival without neurodevelopmental impairment at 2 years of age (Table 3-3).

3.5. Discussion

In this cohort of very preterm and very low birth weight infants, absolute glycemic excursions were strongly associated with birth weight, gestational age, intrauterine growth, and illness severity as indicated by CRIB II scores. In general, infants with normal growth were more likely to have normoglycemic glucose profiles, whereas hypoglycemia was seen in more growth-restricted infants, and hyperglycemia was more likely in smaller and sicker infants. Unstable glucose profiles were more likely in small and sick infants who also had lower birth weight *z*-scores.

We found that the absolute glycemia category, mean blood glucose concentration, and glucose variability were all associated with survival without neonatal morbidity. Glycemia category and mean blood glucose concentration, but not glucose variability, also were associated with survival without neurodevelopmental impairment at 2 years. However, after correction, none of these indicators of the glucose profile remained associated with either neonatal or 2-year outcomes. These data suggest that early life glucose profiles and the measured outcomes are both related to the underlying characteristics of very preterm infants, and that the neonatal glucose profile may merely reflect metabolic stability as a marker for an infants' ability to

cope with allostatic load. This finding raises the possibility that attempts to improve glycemic stability, for example by insulin treatment of hyperglycemia, may have limited potential to improve either short- or long-term outcomes in these smallest infants. However, because our cohort were all undergoing intensive neonatal care, including management of abnormal glycemic excursions, it is also possible that the lack of association between glycemic indicators and outcomes in this study may reflect the effectiveness of this management in minimizing the effect of any potential glycemia-related mortality and morbidity.

We found that infants in the normoglycemic category had the least glucose variability, and unexpectedly, glucose variability was greatest in infants in the hypoglycemic and unstable categories rather than the hyperglycemic category. This finding may be due to our use of a log transformation to correct for the non-normal distribution of blood glucose concentrations before calculating glucose variability. This reduces the impact of hyperglycemic blood glucose excursions (which are not numerically limited) on glucose variability (Kovatchev et al., 1997).

Infants in the hypoglycemic category had the lowest mean blood glucose concentration, as well as high glucose variability. They were more likely to survive without neonatal morbidity and to survive without neurodevelopmental impairment at 2 years of age than infants in the unstable category, who also suffered absolute hypoglycemia and had similar glucose variability. Thus, the major difference in glycemia indices between infants in the hypoglycemic and unstable categories was the higher mean blood glucose concentration in the unstable group. This finding suggests that higher mean blood glucose concentration may be a better early marker of neonatal and 2-year outcomes among very preterm infants with fluctuating blood glucose concentrations, which include hypoglycemia.

Our findings differ from those in a previous study using a similar method to calculate glucose variability(Fendler et al., 2012), in which glucose variability but not mean blood glucose

concentration was associated with early neonatal mortality. This difference may in part be due to our exclusion of infants who died during the study period, thus eliminating the increasing instability of blood glucose concentrations associated acutely with the dying process (Pisarchik et al., 2012) and giving an improved estimate of inherent glucose variability in this cohort of very low birth weight infants. Another reason for the differences between our study and previous studies may be that a greater number of blood glucose concentration measurements are performed in infants with abnormal glycemic excursions. If study entry requires a minimum number of daily blood glucose concentrations, there is a potential selection bias towards infants with more unstable glucose homeostasis. Selection bias may also explain the very high neonatal mortality rate reported in a previous study (23%) (Fendler et al., 2012), compared with 16 neonatal deaths of 443 infants (4%) enrolled in our study and 34 neonatal deaths of 536 infants (6%) in the total cohort of very low birth weight infants admitted to our nursery during the study period.

The higher rate of late-onset infection in infants in the hyperglycemic and unstable glycemia categories, but not the hypoglycemic category, is consistent with reports that high blood glucose concentrations are associated with sepsis in both animal (Alsweiler et al., 2012a) and human (van der Lugt et al., 2010) studies. However, because infants in the hyperglycemic and unstable glycemia categories were also the most preterm and smallest in the cohort, these factors may have contributed to the higher incidence of late-onset infection in these infants.

We did not have access to continuous glucose monitors in this study. Intermittent blood glucose sampling is usual neonatal practice, but may miss up to 25% of hypoglycemic episodes in at-risk infants (McKinlay et al., 2015). The less frequently the blood glucose concentration is measured, the less likely it is that an abnormal result will be detected; thus, there may be infants in the normoglycemic category (in whom the fewest blood glucose concentration measures were performed) who would be reclassified if more blood glucose

concentration measurements had been made. However, this is unlikely to change our conclusions given that glycemia category was not associated with outcome after adjustment for gestational age, birth weight *z*-score, and socioeconomic status at birth.

It is possible that assessment at 2 years of age is too early to predict relationships between early life glycemia and long-term neurodevelopmental outcome. There is increasing evidence that even brief episodes of hypoglycemia in the neonatal period may impact later learning ability (Kaiser et al., 2015; Kerstjens, Bocca-Tjeertes, de Winter, Reijneveld, & Bos, 2012), and motor disorders other than severe cerebral palsy may also be missed at 2 years of age, especially if assessment is performed using the Bayley III (Burakevych et al., 2016). Further assessment at school age of children born very preterm may help elucidate the associations between early glycemia and neurodevelopmental outcome. However, randomized trials of glycemic management strategies will be needed to determine causality.

3.6. Conclusion

Early life glucose profiles are associated strongly with neonatal characteristics of very preterm infants. Measures of neonatal glycemia are markers for neonatal vulnerability and, after correction for gestational age, birth weight *z*-score and socioeconomic status at birth, do not predict neonatal illness or outcomes at 2 years of age.

3.7. Figure and tables

Figure 3-1: Flow diagram of participants in the neonatal period and at 2 years of age

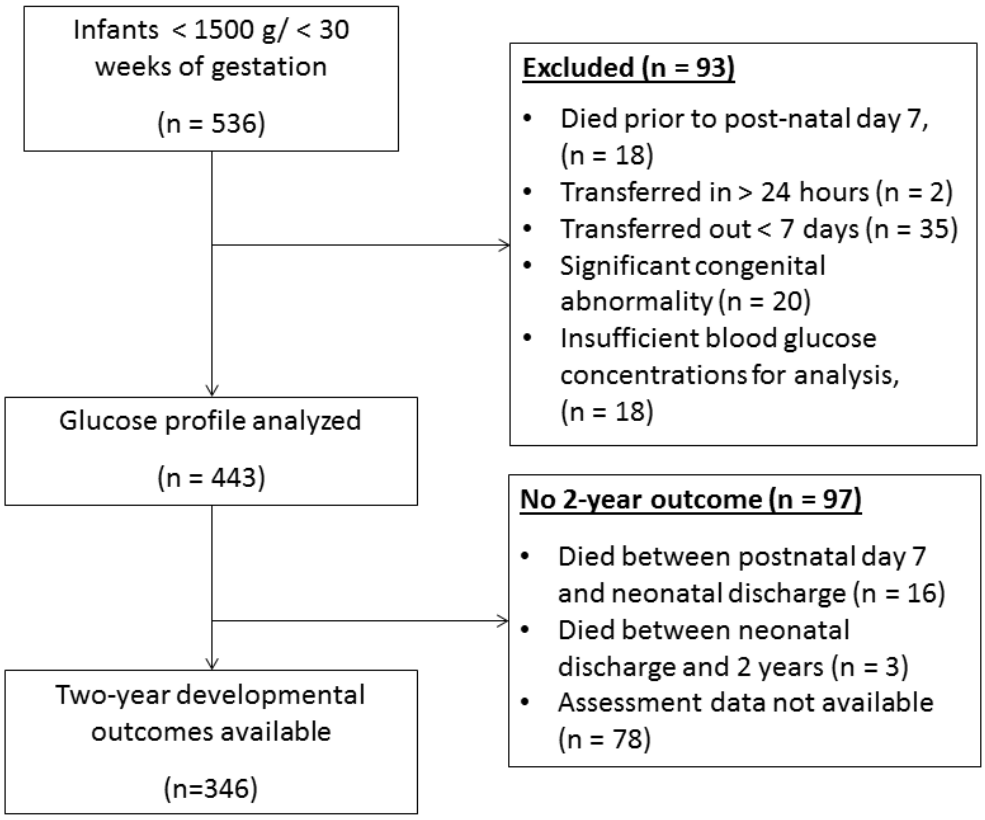

Table 3-1: Demographic and blood glucose characteristics of infants in different neonatal glycaemia categories

	Glycemia Category				Total
	Normoglycemic	**Hypoglycemic**	**Hyperglycemic**	**Unstable**	
Number of infants	287 (65)	42 (9)	73 (16)	41 (9)	443
No antenatal steroids	16 (6)	1 (2)	10 (14)	2 (5)	29 (7)
Maternal diabetes	21 (7)	2 (5)	3 (4)	3 (7)	29 (7)
Multiple birth	76 (26)	10 (24)	21 (29)	8 (20)	115 (26)
Outborn	17 (6)	1 (2)	8 (11)	2 (5)	28 (6)
Gestational age *** (completed weeks)	29 (23 – 35)	29 (25 – 34)	26‡¶ (23 – 32)	26‡¶ (24 - 31)	28 (23 – 35)
Birth weight (g) ***	1170 (500 – 1730)	1140 (450 – 1645)	890 (600 – 1580) ‡	740 (540 – 1380)‡¶$	1100 (450 – 1730)
Birth weight *z*-score ***	0.0 (-2.8 – 2.2)	-0.9 (-3.3 – 1.8) ‡	0.1 (-1.7 – 2.2) ¶	-0.6 (-2.0 – 1.1) †$	-0.1 (-3.3 – 2.2)
Male	159 (55)	22 (51)	45 (62)	18 (44)	244 (55)
CRIB II score ***	7 (2 – 16)	6 (2 – 13)	11 (3 – 16) ‡¶	11 (3 – 17) ‡¶	8 (2 – 17)
5-minute Apgar score	9 (2 – 10)	9 (1 – 10)	9 (3 – 10)	8 (3 – 10) ‡‖	9 (1 – 10)

Table 3-1 continued…	**Normoglycemic**	**Hypoglycemic**	**Hyperglycemic**	**Unstable**	**Total**
Ethnicity *					
Māori	56 (20)	4 (10)	26 (36)	9 (22)	95 (21)
Pacific Island	41 (14)	4 (10)	10 (14)	4 (10)	59 (14)
NZ European	132 (46)	20 (48)	17 (23)	23 (56)	192 (43)
Other	58 (20)	14 (33)	20 (27)	5 (12)	97 (22)
Socioeconomic status					
Least deprived quintile	33 (12)	5 (13)	7 (10)	3 (8)	48 (11)
Most deprived quintile	80 (29)	9 (23)	23 (32)	15 (38)	127 (30)
No. of blood glucose measures ***	9 (3 – 45)	10 (3 – 44)	24 (3 – 42) ‡¶	31 (9 – 65) ‡¶$	11 (3 – 65)
Mean blood glucose *** (mg/dL)	86 (54 – 128)	70 (41 – 103) ‡	126 (77 – 200) ‡¶	103 (70 – 162) ‡¶$	90 (41 – 200)
Glucose variability *** (mg/dL)	22 (19 – 29)	27 (23 – 46) ‡	24 (21 – 40) ‡¶	28 (24 – 39) ‡$	23 (19 – 46)

Data are median (range) or number (%). * P <.05, ** P <.01, *** P <.0001 for the overall comparisons across glycemic categories.

† P <.05 and ‡ P < .01 for comparison with the normoglycemic category.

‖ P < .05 and ¶ P < .01 for comparison with the hypoglycemic category.

§ P < .05 and $ P < .01 for comparison with the hyperglycemic category.

Table 3-2: Neonatal clinical outcomes and neurodevelopmental outcomes at 2 years for children in different neonatal glycemia categories

	Glycemia Category				Total
	Normoglycemic n = 287	**Hypoglycemic n = 42**	**Hyperglycemic n = 73**	**Unstable n = 41**	**n = 443**
Died prior to neonatal discharge **	6 (2)	0	6 (8)	4 (10)	16 (4)
Ever received insulin ***	17 (6)	3 (7)	46 (63)	20 (49)	86 (20)
Intraventricular hemorrhage (grades III-IV)	19 (7)	5 (12)	7 (10)	2 (5)	33 (8)
Late onset infection ***	21 (7)	3 (7)	18 (25)	11 (28)	53 (12)
Necrotizing enterocolitis *	7 (2)	2 (5)	6 (8)	4 (10)	19 (4)
Retinopathy of prematurity **	7 (2)	1 (2)	8 (11)	7 (17)	23 (5)
Chronic lung disease ***	34 (12)	6 (14)	24 (33)	16 (39)	80 (18)
Home oxygen ***	28 (10)	4 (10)	17 (24)	12 (29)	61 (14)
Periventricular leukomalacia	3 (1)	0	2 (3)	3 (8)	8 (2)
Survival without neonatal morbidity ***	245 (85)	35 (84)	44 (60)	21 (51)	345 (78)
Duration of stay (d) ***	61 (20 – 158)	52 (14 – 121)	87 (34 – 158) ‡¶	99 (48 – 189) ‡¶	65 (14 – 189)
Children assessed at 2 years of age [a]	225 (81)	33 (79)	55 (83)	33 (89)	346 (82)

Table 3-2 continued…	**Normoglycemic n = 287**	**Hypoglycemic n = 42**	**Hyperglycemic n = 73**	**Unstable n = 41**	**Total n = 443**
Assessment type at 2 years of age [b]					
Bayley II	31 (14)	5 (15)	7 (13)	9 (27)	52 (15)
Bayley III	182 (81)	26 (79)	40 (73)	20 (61)	268 (77)
Other	12 (5)	2 (6)	8 (15)	4 (12)	26 (8)
Any neuro-developmental impairment at 2 years of age* [b]	30 (13)	3 (9)	13 (24)	10 (30)	56 (16)
Severity of impairment at 2 years of age [b]					
Mild	2 (1)	1 (3)	2 (4)	2 (6)	7 (2)
Moderate	23 (10)	2 (6)	9 (16)	7 (21)	41(12)
Severe	5 (2)	0	2 (4)	1 (3)	8 (2)

Data are median (range) or number (%). * $P < .05$, ** $P < .01$, *** $P < .0001$ for the overall comparisons across glycemic categories

‡ $P < .01$ for the comparison with the normoglycemic group

¶ $P < .01$ for the comparison with the hypoglycemic group

a: Data are n (%) of children potentially available for assessment at 2 years of age

b: Data are n (%) of children assessed at 2 years of age

Table 3-3: Relationships between neonatal glycemia characteristics, survival without neonatal morbidity and survival without neurodevelopmental impairment at 2 years of age, before and after adjustment

Variable	Survival without neonatal morbidity				Survival without neurodevelopmental impairment at 2 years of age			
	Parameter estimate	**SE**	**OR (95% CI)**	***P* value**	**Parameter estimate**	**SE**	**OR (95% CI)**	***P* value**
Glucose variability (mg/dL)	-0.06	0.03	0.94 (0.88 – 1.00)	.05	-0.03	0.04	0.97 (0.91 – 1.05)	.45
	0.02	*0.05*	*1.02 (0.93 – 1.13)*	*.68*	*0.01*	*0.05*	*1.01 (0.92 – 1.11)*	*.91*
Mean blood glucose concentration (mg/dL)	-0.02	0.00	0.98 (0.97 – 0.99)	<.0001	-0.02	0.00	0.98 (0.97 – 0.99)	.003
	-0.00	*0.00*	*1.00 (0.98 – 1.01)*	*.78*	*0.00*	*0.00*	*1.00 (0.98 – 1.02)*	*.99*
Glycemia category [a]								
Hypoglycemia	-0.08	0.22	0.86 (0.38 – 2.22)	.73	0.33	0.32	1.95 (0.65 – 8.41)	.29
	0.04	*0.31*	*1.08 (0.34 – 3.99)*	*.89*	*0.33*	*0.34*	*1.94 (0.57 – 9.09)*	*.31*
Hyperglycemia	-0.67	0.15	0.26 (0.15 – 0.46)	<.0001	-0.45	0.16	0.41 (0.21 – 0.78)	.006
	-0.03	*0.19*	*0.93 (0.45 – 1.99)*	*.86*	*-0.12*	*0.21*	*0.79 (0.35 – 1.84)*	*.57*
Unstable	-0.86	0.18	0.18 (0.09 – 0.36)	< .0001	-0.57	0.19	0.32 (0.15 – 0.69)	.003
	0.13	*0.24*	*1.28 (0.50 – 3.37)*	*.61*	*0.02*	*0.28*	*1.04 (0.36 – 3.18)*	*.95*

Italic script shows values adjusted for gestational age, birth weight *z*-score and socioeconomic quintile at birth. For survival without neurodevelopmental impairment at 2 years of age, values are additionally adjusted for assessment type. a: Normoglycemia is the reference value

Chapter 4. Relationships between early nutrition and blood glucose concentrations in very preterm infants

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4.1. Introduction

Infants born very preterm or very low birth weight are at high risk of neonatal morbidity and mortality (Costeloe et al., 2012; Saigal & Doyle, 2008). The sudden cessation of placental nutrient supply, difficulties in providing adequate parenteral nutrition and stress-induced hyper-catabolism may all contribute to a prolonged period of postnatal under-nutrition and subsequent growth faltering (Embleton et al., 2001; Grover et al., 2008). In addition, the smallest infants are at risk of hyperglycaemia, which is associated with a number of morbidities including intraventricular haemorrhage (Auerbach et al., 2013), sepsis (Alsweiler et al., 2012a; Manzoni et al., 2006) and reduced white matter volumes (Alexandrou et al., 2010). Hyperglycaemia is often treated with an infusion of insulin. However, this practice significantly increases the risk of hypoglycaemia (Alsweiler et al., 2012b; Beardsall & Dunger, 2008), which can itself damage the developing brain (Burns et al., 2008).

In January 2007, a new nutritional protocol was introduced to the neonatal intensive care unit (NICU), Auckland City Hospital, New Zealand. The aim was to restrict early fluid intake while increasing early lipid, protein and energy intakes to meet nutritional recommendations for very and extremely low birth weight infants (Cormack et al., 2011). Following introduction of the new protocol, the incidence of insulin-treated neonatal hyperglycaemia appeared to decrease (Alsweiler et al., 2012b). We therefore sought to determine whether changes in early macronutrient intakes were associated with changes in neonatal glycaemia and, if so, to determine which specific macronutrients contributed to this.

4.2. Methods

Infants born < 30 weeks' gestation or < 1,500 grams birth weight and admitted to the NICU within 24 hours of birth were eligible for inclusion in this retrospective observational study. Infants transferred out within the first 7 postnatal days were excluded from the study, as were

infants with significant congenital anomaly, infants who changed from the old to the new nutritional protocol during the first 7 days, and those who had fewer than 3 blood glucose concentrations measured within the first week. Birth weight, length, gestation, sex, antenatal steroid exposure and maternal diabetes status were obtained from the medical record. Growth velocity from birth to day 7, and from birth to day 28, was calculated using the exponential method (Patel, Engstrom, Meier, Jegier, & Kimura, 2009). Weight, length and head circumference *z*-scores were calculated using reference data (Fenton & Kim, 2013). Deaths occurring prior to discharge from a neonatal service were recorded. Sepsis and chronic lung disease (CLD) were defined as per the Australia and New Zealand Neonatal Network (Chow, 2013).

4.2.1 **Nutrition protocol changes**

The changes to the nutrition protocol have been published elsewhere (Cormack et al., 2011). Briefly, under the old protocol, all infants were started on an intravenous infusion of 10% dextrose or a standardised solution of amino acids 20 $g.L^{-1}$ in 10% dextrose at 60-90 $ml.kg^{-1}.d^{-1}$. The total fluid volume increased by 30 $ml.kg^{-1}.d^{-1}$ to a target of 180 $ml.kg^{-1}.d^{-1}$. Under the new protocol, infants <1,000 g birth weight with central venous access received a standardised solution of amino acids 67.9 $g.L^{-1}$ in 15% dextrose at 30 $ml.kg^{-1}.d^{-1}$ plus additional 10% dextrose for the first 2 days; all other infants commenced on a standardised solution of amino acids 38 $g.L^{-1}$ in 10% dextrose. Fluid volumes started at 60 $ml.kg^{-1}.d^{-1}$ and increased by 15 $ml.kg^{-1}.d^{-1}$ for the first four days and 30 $ml.kg^{-1}.d^{-1}$ thereafter, to a target of 180 $ml.kg^{-1}.d^{-1}$. In both cohorts, parenteral lipid was introduced at 1 $g.kg^{-1}.d^{-1}$ when central access was gained, and increased by 1 $g.kg^{-1}.d^{-1}$ daily to a target 3 $g.kg^{-1}.d^{-1}$. Maternal expressed breastmilk (EBM) was the preferred enteral feed, started as soon as available and increased by 20 - 30 $ml.kg^{-1}.d^{-1}$. Standard term formula was the preferred initial enteral feed if maternal EBM was

not available. Once full enteral volumes were reached, human milk fortifier (Nutriprem, Nutricia, NZ) was added or preterm formula introduced.

4.2.2 **Nutritional intakes and glucose profiles**

All recorded enteral and parenteral intake (excluding blood products) for the first 28 days were obtained from the medical record, excluding the calendar day of birth which was of variable length. Actual daily macronutrient intakes were calculated using relevant reference values (chapter 11, appendix A) and the highest, most recent infant weight. Macronutrient intakes were then averaged for days 1-7 (week 1) and days 1-28 (month 1). 10% enteral feeds were defined as achieved on the day when ≥ 10% of the total fluid intake was via the enteral route, 50% enteral feeds when ≥ 50% of the total fluid intake was via the enteral route, and 100% enteral feeds when enteral feeds were ≥ 150 $ml.kg^{-1}.d^{-1}$ or no further IVN was given (Cormack, Embleton, van Goudoever, Hay Jr, & Bloomfield, 2016). All blood glucose concentrations (BGC) were measured using the glucose oxidase method on a blood gas analyser (ABL 700, Radiometer, Copenhagen). These were collected from the electronic medical record, divided into time periods (week 1: birth to the end of day 7, and month 1: birth to the end of day 28), and used to calculate the proportion of measurements in different ranges. Hypoglycaemia was defined as any BGC < 2.6 mM and hyperglycaemia as any BGC > 8.5 mM. All BGC values for each baby were log transformed to calculate mean and standard deviation (defined as glucose variability) and back-transformed for reporting. The highest value of glucose lost in the urine was recorded for each of days 1 - 7 where urine had been tested for glycosuria using urine test strips (Multistix reagent strips, Bayer Health care, Bayer, Auckland, New Zealand). Results were categorised as no glycosuria (negative for glucose), mild (trace or 1+ glucose) or significant glycosuria (≥ 2+ glucose).

4.2.3 **Statistical methods**

The baseline characteristics and macronutrient intakes of infants exposed to the old and new nutritional protocols were compared using Student's t-test for continuous variables, Wilcoxon's test for non-normally distributed continuous variables, or chi-squared for categorical variables. Relationships between hypo- and hyperglycaemia, and macronutrient intakes, energy intakes and time to establishment of enteral feed volumes were investigated using logistic regression, with and without adjustment for gestational age and birth weight *z*-score. The level of significance was 0.05, and Bonferroni correction was made for multiple comparisons. Data are presented as number (%), mean (SD), median (IQR), or odds ratio (95% Confidence Intervals (CI)). Ethical (NTY/09/98/EXP/AM02) and institutional (A+4984) approvals were gained for this study.

4.3. Results

From July 2005 – October 2008, 536 infants < 30 weeks' gestation or < 1,500 grams birth weight were admitted to the NICU. Of these, 79 were excluded, leaving 457 infants, of whom 190 (42%) were exposed to the old nutritional protocol (Old Protocol) and 267 (58%) to the new protocol (New Protocol) (Figure 4-1). Baseline and clinical characteristics were similar in Old Protocol and New Protocol infants, although those in the New Protocol group tended to have more chronic lung disease (P=0.05) (Table 4-1).

Complete records of nutrition intakes were available for 443 / 457 (97%) infants in week 1 and 419 / 457 (92%) infants in month 1. Compared to Old Protocol, New Protocol infants received less fluid, fat, carbohydrate and energy, but more protein and a higher protein-to-energy ratio in both week 1 and month 1 (Table 4-2). The days on which 10% and 50% enteral feeds were achieved were similar, but New Protocol infants took longer to achieve 100% enteral feeds (Table 4-2).

BGCs were available for all infants, and the number of BGCs measured in week 1 and month 1 were similar for Old and New Protocol infants (Table 4-2). Eighty-five percent of hypoglycaemia and 75% of hyperglycaemia occurred in week 1. Compared to Old Protocol infants, New Protocol infants had similar rates of hypoglycaemia, but were less likely to experience hyperglycaemia both in week 1 and month 1. However, the proportion of infants requiring insulin treatment was similar in both groups (Table 4-2). Mean BGCs were lower in New Protocol infants in both week 1 and month 1, but there was no difference in glucose variability. A urine test for glycosuria was recorded on half the days eligible for testing; this proportion was not different between Old Protocol and New Protocols. New Protocol infants were more likely to have a negative test for glycosuria, and less likely to have a mild glycosuria, but the rate of significant glycosuria was similar in both groups (Table 4-2).

Analysis of the distribution of week 1 BGCs showed that New Protocol infants had a larger proportion of BGCs in the range 2.6 – 3.9 mM, and a smaller proportion in the ranges 6.1 – 8.5 mM and >8.5 mM. The proportions of BGCs <2.6 mM and 4 – 6 mM were similar in Old and New Protocol infants (Table 4-2).

In the whole cohort, weight growth velocity was negative from birth to day 7 and positive to day 28, and was similar in Old and New Protocol infants over both time periods. Changes in *z*-scores for weight, length and head circumference from birth to 36 weeks' post-menstrual age were also similar for the two groups (Table 4-2).

In week 1, hypoglycaemia was recorded in a total of 129/457 (28%) infants, and hyperglycaemia in 151/457 (33%) infants. Macronutrient intake, energy intake, and protein to energy ratio were not associated with hypoglycaemia (Table 4-3). However, each $g.kg^{-1}.d^{-1}$ increase in protein and fat approximately halved the odds of hyperglycaemia. Carbohydrate intake and protein to energy ratio were not independently associated with hyperglycaemia, but after correction for gestational age and birth weight *z*-score, the odds of hyperglycaemia

increased with each $g.kg^{-1}.d^{-1}$ carbohydrate intake, and decreased with increasing $g.100kcal^{-1}$ protein to energy ratio. Increased energy intake was associated with a 5% decrease in the odds of hyperglycaemia, but this relationship did not persist after adjustment for gestational age and birth weight *z*-score (Table 4-3). In month 1, all macronutrients and energy intakes were associated with reductions in hyperglycaemia, although the association with carbohydrate intake did not persist after adjustment for gestational age and birthweight *z*-score. Protein and fat intakes were associated with reductions in hypoglycaemia in unadjusted but not adjusted analyses (Table 4- 3).

Increased time to achieve 10% enteral feeds was associated with increased odds of both hypoglycaemia and hyperglycaemia in week 1 (Table 4-3). Each extra day taken to achieve 100% enteral feeds also was associated with an approximate 10% increase in the odds of hypoglycaemia, and a 20% increase in the odds of hyperglycaemia in month 1 (Table 4-3).

On multivariate analysis adjusting for birthweight *z*-score, gestational age and intake of protein, fat and carbohydrate within the first week, all macronutrients were significantly associated with the odds of week 1 hyperglycaemia (protein 0.37 (0.21 – 0.64), P = 0.0004, fat 0.54 (0.39 – 0.73), P < 0.0001, carbohydrate 1.36 (1.18 – 1.59), P < 0.0001, all $g.kg^{-1}.d^{-1}$). Odds ratios were essentially unchanged for the relationship between month 1 macronutrient intakes and month 1 hyperglycaemia, although the P-values increased as confidence intervals widened (protein 0.26 (0.06 – 1.04), P = 0.06, fat 0.58 (0.31 – 1.04), P = 0.07, carbohydrate 1.34 (1.07 – 1.70), P = 0.01).

4.4. Discussion

Extremes of blood glucose concentrations are common in very preterm infants, and both hypo and hyperglycaemia have been linked to adverse outcomes in both the short and long term (Alexandrou et al., 2010; Auerbach et al., 2013; Kaiser et al., 2015; Lucas et al., 1988). The

introduction of a new nutrition policy to our unit was associated with a reduction in mean blood glucose concentration and a reduced incidence of hyperglycaemia and glycosuria. There were no changes in the incidence of hypoglycaemia, and measures of early growth were also unchanged. Unexpectedly, the New Protocol group took one day longer to achieve 100% enteral feeds. The change in nutrition protocol did not include changes to the enteral feeding policy, and there was no difference in the usage of formula milk between Old and New Protocol infants, thus the reason for the difference in time to achieve 100% enteral feeds remains unclear.

New Protocol infants received less carbohydrate and energy than Old Protocol infants and experienced less hyperglycaemia. Extremely low birth weight infants have been reported to be at particular risk of developing hyperglycaemia during intravenous glucose infusion (Louik et al., 1985), and early clinical and animal work suggested that persistent hepatic gluconeogenesis, abnormal pancreatic function and impaired peripheral insulin response may underlie this association (Chacko et al., 2011; Cooper et al., 2010; Cowett et al., 1998; Mitanchez-Mokhtari et al., 2004). This has led to the common practice of reducing carbohydrate load in response to neonatal hyperglycaemia (Alsweiler et al., 2007). However, the evidence that neonatal hyperglycaemia is influenced by intravenous glucose infusion rate is mixed, with no association found between intravenous glucose infusion rates and hyperglycaemia in 188 very low birth weight infants recruited to the Neonatal Insulin Therapy in Europe (NIRTURE) trial (Beardsall et al., 2008). There may be multiple risk factors for carbohydrate intolerance in these infants, confounding observable relationships between carbohydrate intake and hyperglycaemia.

New Protocol infants also received more protein than Old Protocol infants, particularly in the first week after birth, and experienced less hyperglycaemia. Indeed, when protein, fat and carbohydrate intakes were considered in combination, increased protein intake had the

strongest association with reduced odds of hyperglycaemia. This is consistent with the findings of a previous study (Mahaveer et al., 2012) where an increase in early life parenteral protein intake alone was associated with a significant reduction in the occurrence of insulin-treated neonatal hyperglycaemia. These associations may be partially explained by potentiation of insulin secretion by individual amino-acids such as arginine (Burgess, Morgan, Mayes, & Tan, 2014).

New Protocol infants received less fat than Old Protocol infants and experienced less hyperglycaemia, despite the finding that, on multivariate analysis, each additional $g.kg^{-1}.d^{-1}$ of fat intake was associated with approximately halving the odds of hyperglycaemia. Intravenous lipid infusion enhances hepatic gluconeogenesis in isotope studies (van Kempen et al., 2006), but lipid is also the most energy-dense of the macronutrients, providing energy which may prevent proteolysis, lipolysis, and the proliferation of gluconeogenic substrates thought to occur under conditions of metabolic stress (Frayn, 1985; Holm, Hörbrand, Mayr, Henckel von Donnersmarck, & Mühlbauer, 2004). Thus, the effect of lipid on blood glucose concentrations might depend on an infant's overall nutritional status. In our study, the increase in protein intake of New Protocol infants compared with Old Protocol infants was proportionately much greater than the decrease in fat intake (protein +16% vs fat -3% in week 1), again suggesting that increased protein intake was likely to have made a greater contribution to the reduction in hyperglycaemia than the decrease in fat intake.

In the NIRTURE trial higher intravenous lipid infusion rates were an independent risk factor for the development of moderate hyperglycaemia, although the average lipid intake in NIRTURE was approximately 2 $g.kg^{-1}.d^{-1}$ lower than that received by infants in our study (Beardsall et al., 2008). A retrospective study of an enhanced parenteral nutrition protocol in 343 ELBW infants also reported an association between neonatal hyperglycaemia and higher combined lipid, protein and carbohydrate intakes (Stensvold, Strommen, Lang, & et al., 2015).

However, infants in the enhanced parenteral nutrition group received an average of 0.5 $g.kg^{-1}.d^{-1}$ less protein, 0.9 $g.kg^{-1}.d^{-1}$ less lipid, and 0.9 $g.kg^{-1}.d^{-1}$ less carbohydrate than our New Protocol group, making it difficult to draw comparisons between the studies.

We did not find any associations between hypoglycaemia and macronutrient or energy intake in our cohort. As any hypoglycaemia is likely to be treated clinically with intravenous dextrose, this may confound analysis of any relationship between glucose intakes and hypoglycaemia. Nevertheless, although the new nutrition protocol was not associated with an increase in the incidence of hypoglycaemia, it did increase the proportion of blood glucose concentrations within the range 2.6 – 3.9 mM. The threshold of blood glucose at which hypoglycaemia should be diagnosed is the subject of ongoing debate (Adamkin & Committee on Fetus and Newborn, 2011; Thornton et al., 2015), with recent evidence suggesting even a single blood glucose concentration <2.5 mM in the neonatal period is associated with worse performance in literacy testing at school age (Kaiser et al., 2015)(17). Maintenance of blood glucose concentrations in the 4 – 6 mM range in preterm infants is associated with improved long-term neurodevelopmental outcomes (Tottman et al. Submitted for publication), and the proportion of blood glucose concentrations in this range was not altered by the change in nutrition protocols. Thus, it is unclear if the shift towards lower BGCs associated with the New Protocol could impact long term neurodevelopmental outcomes.

Despite the observed reduction in hyperglycaemia in the New Protocol group, the number of infants treated with insulin was not different. Significant glycosuria is one of the criteria for initiation of insulin treatment in our unit, and the incidence of significant glycosuria was not altered by the New Protocol. Conditions causing blood glucose concentrations high enough to result in significant glycosuria, or the need for insulin, may reflect external stressors to the infant, such as sepsis or surgery, and be less susceptible to nutritional modification.

Our finding that a longer time to achieve 10% enteral intake was associated with an increase in both hyper- and hypoglycaemia may suggest that enteral feeding may help stabilise blood glucose concentrations in very preterm infants. There are a number of possible mechanisms for this. Enteral feeding stimulates the entero-insular axis and, in the term infant, may be necessary for the production of Glucose-dependent Insulinotropic Peptide (GIP) (King et al., 1989). GIP is an incretin which acts to depolarise the beta-cell membrane and stimulate insulin secretion (Seino et al., 2010), potentially improving an infant's responsiveness to increasing blood glucose concentrations. Small, early enteral feed volumes may also have a maturational effect on hepatic Glut-2 receptors via the direct delivery of carbohydrate to the liver via the portal venous system (Burcelin et al., 2000; Zheng et al., 1995), thus allowing for more accurate hepatic sensing of blood glucose concentrations, and potentially dampening hepatic gluconeogenesis which contributes to the risk of hyperglycaemia in very low birth weight infants (Chacko & Sunehag, 2010). Enteral feeds may also improve arginine synthesis by small-intestine enterocytes, thus promoting insulin secretion (Wu, Jaeger, Bazer, & Rhoads, 2004).

Alternatively, the association between 10% enteral feeds and more stable blood glucose concentrations may reflect enteral feeding being withheld or delayed in very small or very sick infants (Bombell & McGuire, 2008), so that infants who are more clinically stable and who are less likely to have abnormal glycaemia are quicker to feed. Nevertheless, the relationships between hyper- and hypoglycaemia and achievement of 10% enteral feeds remained after adjustment for gestational age and birth weight *z*-score, suggesting that the effect is independent of these factors.

Because this study is retrospective and observational, it has a number of limitations. Differences in the incidence of hyperglycaemia between the two cohorts might be explained by other changes in intensive care management during the study time period, although we are

not aware of any concurrent changes in unit policy that would impact upon neonatal glycaemia. In addition, we have assessed combined macronutrient intakes via the parenteral and enteral routes, and it is possible that route of intake is an important mediator of the effects of individual macronutrients. However, as all of our cohort received parenteral nutrition, and achieved 50% enteral feeds only at the end of the first week, it is unlikely that this has had a major effect our analysis.

Strengths of this study are the relatively large number of infants included, collection of all recorded enteral and parenteral intakes, and recalculation of macronutrient and energy intakes per kilogram every time the infant was weighed, allowing for accurate calculation of all nutrition received.

In conclusion, a change in nutrition protocol resulting in reduced fat, carbohydrate and energy intakes, and higher protein intake, was associated with lower mean blood glucose concentrations, less hyperglycaemia and less glycosuria, without changing the incidence of hypoglycaemia or growth in the neonatal period. Protein was the macronutrient most strongly associated with the reduction in hyperglycaemia. More rapid attainment of 10% enteral feeds was also associated with more stable blood glucose concentrations and may assist with the management of neonatal glycaemia in very preterm infants

4.5. Figure and tables

Figure 4-1: Flow diagram of participants in the nutrition study

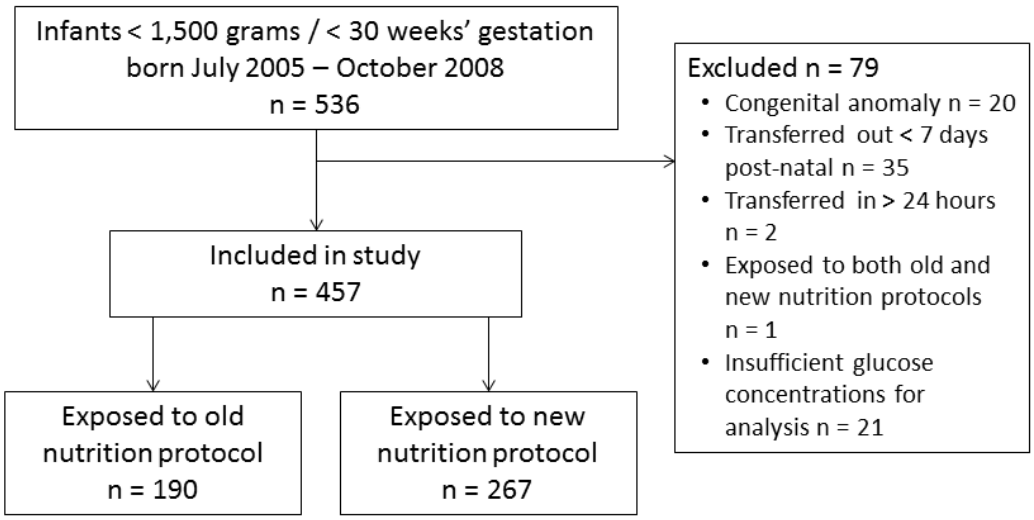

Table 4-1: Baseline characteristics of Old Protocol and New Protocol infants

	Old Protocol n = 190	**New Protocol n = 267**	***P*-value**
Gestational age (weeks)	28 (26 – 30)	28 (26 – 29)	0.6
No antenatal steroids	14 (6)	22 (8)	0.9
Maternal diabetes	12 (6)	17 (6)	1.0
Multiple birth	51 (27)	66 (25)	0.7
Birth weight (g)	1118 (833 – 1346)	1080 (860 – 1304)	0.6
Birth weight *z*-score	-0.18 ± 0.07	-0.16 ± 0.06	0.7
CRIB II score	7 (5 – 11)	8 (5 – 10)	0.7
Male sex	108 (57)	144 (54)	0.6
Ethnicity			0.8
Māori	39 (21)	62 (23)	-
Pacific Island	25 (13)	35 (13)	-
Asian	36 (19)	59 (22)	-
European / Other	90 (47)	111 (41)	-
Grade III/ IV intraventricular haemorrhage	6 (4)	13 (5)	0.4
Sepsis	25 (13)	40 (15)	0.6
Chronic lung disease	25 (13)	54 (20)	0.05
Died prior to discharge	12 (6)	17 (6)	0.9

Data are number of infants (%), median (IQR) or mean ± standard deviation.

Table 4-2: Macronutrient intakes, enteral feeds, blood glucose, glycosuria and growth in Old Protocol and New Protocol infants

	Old protocol n = 190	**New protocol n = 267**
Week 1 nutrition [a]		
Fluid ($ml.kg^{-1}.d^{-1}$)	147 ± 14	123 ± 13****
Protein ($g.kg^{-1}.d^{-1}$)	2.5 ± 0.4	2.9 ± 0.4****
Fat ($g.kg^{-1}.d^{-1}$)	3.8 ± 1.0	3.7 ± 0.7*
Carbohydrate ($g.kg^{-1}.d^{-1}$)	12.1 ± 1.4	10.1 ± 1.3****
Energy ($Kcal.kg^{-1}.d^{-1}$)	89 ± 13	82 ± 9****
Protein : Energy ratio ($g.100kcal^{-1}$)	2.9 ± 0.3	3.7 ± 0.5****
Month 1 nutrition [b]		
Fluid ($ml.kg^{-1}.d^{-1}$)	167 ± 9	159 ± 10****
Protein ($g.kg^{-1}.d^{-1}$)	3.37 ± 0.34	3.44 ± 0.27*
Fat ($g.kg^{-1}.d^{-1}$)	6.2 ± 0.8	6.0 ± 0.8
Carbohydrate ($g.kg^{-1}.d^{-1}$)	15.3 ± 1.4	14.4 ± 1.4****
Energy ($Kcal.kg^{-1}.d^{-1}$)	129 ± 14	124 ± 13***
Protein : Energy ratio ($g.100kcal^{-1}$)	2.6 ± 0.1	2.8 ± 0.2****
Week 1 glucose profiles		
BGCs per infant (n)	9 (6 – 22)	12 (7 – 21)
Mean BGC (mM)	5.6 ± 1.4	4.9 ± 1.2****
Glucose variability (mM)	1.3 ± 0.2	1.3 ± 0.2
[§] Proportion of BGCs within range		
< 2.6 mM	0.03 ± 0.05	0.04 ± 0.10
2.6 – 3.9 mM	0.13 ± 0.15	0.22 ± 0.21****
4.0 – 6.0 mM	0.46 ± 0.25	50 ± 0.22
6.1 – 8.5 mM	0.28 ± 0.22	0.18 ± 0.18****
> 8.5 mM	0.11 ± 0.16	0.06 ± 0.12***
Glycosuria tested (% of days)	51 ± 34	49 ± 32
[†] No glycosuria (% of tests)	75 ± 32	86 ± 23***
[†] Mild glycosuria (% of tests)	18 ± 24	10 ± 16***
[†] Significant glycosuria (% of tests)	7 ± 15	4 ± 13
Hyperglycaemia in week 1	80 (42)	71 (27)***
Hypoglycaemia in week 1	51 (27)	78 (29)

Table 4-2 continued…		
Month 1 glucose profiles		
BGCs per infant	16 (9 – 39)	19 (10 – 37)
Mean BGC (mM)	5.5 ± 1.1	5.0 ± 1.1****
Glucose variability (mM)	1.3 ± 0.1	1.3 ± 0.2
Hyperglycaemia in month 1	96 (51)	105 (39)*
Hypoglycaemia in month 1	60 (32)	92 (34)
Ever received insulin	36 (19)	53 (20)
Day of achievement of % enteral feeds		
10%	3 (2 – 4)	3 (2 – 5)
50%	6 (5 – 8)	7 (5 – 9)
100%	8 (6 – 11)	9 (8 – 11)*
Received any formula milk	69 (36)	89 (33)
Growth		
Week 1 growth velocity ($g.kg^{-1}.d^{-1}$)	-4.4 ± 8.4	-4.1 ± 9.6
Month 1 growth velocity ($g.kg^{-1}.d^{-1}$)	12.0 ± 3.4	11.7 ± 3.6
Change in *z*-score birth to 36 weeks'		
Weight [c]	-1.06 ± 1.14	-1.07 ± 1.15
Length [d]	-1.13 ± 1.27	-1.16 ± 1.32
Head circumference [e]	-0.97 ± 1.36	-0.85 ± 1.22

Data are number of infants (%), mean ± standard deviation or median (IQR). BGC = blood glucose concentration.

* P< 0.05, ** P< 0.01, *** P< 0.001, ****P< 0.0001 for the comparison with Old Protocol.

§ P < 0.01 and † P < 0.02 significant due to Bonferroni correction for multiple comparisons.

Due to missing data a: Old Protocol n = 184, New Protocol n = 259. b: Old Protocol n=175, New Protocol n = 244. c: Old Protocol n = 153, New Protocol n = 208 d: Old Protocol n = 128 New Protocol n = 174 e: Old Protocol n = 138, New Protocol n = 182.

Table 4-3: Unadjusted and adjusted odds ratios for hypoglycaemia and hyperglycaemia in week 1 and month 1, per unit increase in macronutrient, energy and enteral intakes in week 1 and month 1

	Hypoglycaemia in week 1 n = 129		**Hyperglycaemia in week 1 n = 151**	
	Unadjusted	**† Adjusted**	**Unadjusted**	**† Adjusted**
Protein ($g.kg^{-1}.d^{-1}$)	1.28 (0.80 – 2.08)	1.29 (0.80 – 2.11)	0.46 (0.28 – 0.73)**	0.47 (0.23 – 0.79)**
Fat ($g.kg^{-1}.d^{-1}$)	0.81 (0.64 – 1.03)	1.00 (0.77 – 1.30)	0.40 (0.30 – 0.53) ****	0.54 (0.40 - 0.74)****
Carbohydrate ($g.kg^{-1}.d^{-1}$)	1.04 (0.93 – 1.17)	1.09 (0.96 – 1.23)	1.08 (0.96 – 1.21)	1.25 (1.09 – 1.44)**
Energy ($kcal.kg^{-1}.d^{-1}$)	1.00 (0.99 – 1.02)	1.01 (0.99 – 1.03)	0.95 (0.94 – 0.97)****	0.98 (0.96 – 1.00)
Protein to energy ratio ($g.100kcal^{-1}$)	1.15 (0.81 – 1.63)	1.07 (0.74 – 1.55)	0.90 (0.64 – 1.26)	0.61 (0.41 – 0.89)*
Achieved 10% enteral feeds (d)	1.10 (1.02 – 1.19) *	1.09 (1.00 – 1.18) *	1.23 (1.13 – 1.35)****	1.16 (1.06 – 1.28)**
Achieved 50% enteral feeds (d)	1.05 (0.99 – 1.11)	1.05 (0.98 – 1.12)	1.23 (1.15 – 1.32)****	1.14 (1.06 – 1.22)***
	Hypoglycaemia in month 1 n = 152		**Hyperglycaemia in month 1 n = 201**	
	Unadjusted	**† Adjusted**	**Unadjusted**	**† Adjusted**
Protein ($g.kg^{-1}.d^{-1}$)	0.47 (0.24 – 0.90)*	0.61 (0.30 – 1.24)	0.13 (0.06 – 0.26)****	0.21 (0.09 – 0.49)***
Fat ($g.kg^{-1}.d^{-1}$)	0.66 (0.51 – 0.84)***	0.77 (0.59 – 1.01)	0.38 (0.27 – 0.51)****	0.56 (0.39 – 0.78)***
Carbohydrate ($g.kg^{-1}.d^{-1}$)	0.95 (0.83 – 1.09)	1.02 (0.88 – 1.18)	0.81 (0.71 – 0.93)**	0.99 (0.84 – 1.17)
Energy ($kcal.kg^{-1}.d^{-1}$)	0.98 (0.97 – 1.00)*	0.99 (0.97 – 1.01)	0.95 (0.94 – 0.97)****	0.98 (0.96 – 1.00)*
Achieved 100% enteral feeds (d)	1.09 (1.04 – 1.16)***	1.08 (1.02 – 1.15)**	1.23 (1.16 – 1.31)****	1.07 (1.01 – 1.16)*

Data are odds ratios (95% confidence intervals) † Adjusted for gestational age and birth weight *z*-score. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

Chapter 5. Relationships between nutrition, growth and neurodevelopment in preterm infants are sex-specific

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5.1. Background

Infants born very preterm or of very low birth weight are at high risk of faltering growth in the neonatal period (Martin et al., 2009). Supplying adequate nutrition to allow approximation of *in utero* growth trajectories is a recommended objective for neonatal practitioners (Koletzko et al., 2005b), and undernutrition in the neonatal period is associated with adverse outcomes (Ehrenkranz et al., 2006; Embleton et al., 2001). However, the optimal amount, composition and method of delivery of nutrition for preterm babies is not yet clear. Boys born preterm are consistently reported to have worse outcomes than girls, including neonatal morbidity (Cuestas, Bas, & Pautasso, 2009; Kent, Wright, Abdel-Latif, & New South Wales Australian Capital Territory Neonatal Intensive Care Units Audit Group, 2012), mortality (Stevenson et al., 2000) and long-term neurodevelopment (Hintz et al., 2006; Kent et al., 2012). Nutrition provided to the fetus may impact upon the risk of adverse long-term metabolic outcomes (Barker, 1999), and a number of studies, both human (Lucas et al., 1998) and animal (Berry et al., 2016), have shown intriguing differences in female and male responses to altered neonatal nutrition. It is therefore possible that sex-specific responses to early neonatal nutrition may explain some of the differences in outcome between girls and boys born preterm.

5.2. Aim

This purpose of this retrospective, observational cohort study is to determine whether relationships between neonatal nutritional intakes, neonatal growth and survival without neurodevelopmental impairment at 2 years of age are different in girls and boys born very preterm.

5.3. Methods

In 2007, the Newborn Intensive Care Unit (NICU) at National Women's Health, Auckland, NZ introduced a new nutritional protocol. The aim of this protocol was to reduce early fluid

intake, increase protein intakes and standardise parenteral nutrition prescribing practices for very preterm infants. A previously published audit of 80 surviving infants suggested that the new protocol was successful in achieving these aims, but was not associated with any differences in postnatal growth or 18 month neurodevelopmental outcomes (Cormack et al., 2011). The change in nutritional protocol also affords the opportunity to examine the relationships between early nutrition and outcomes in a larger cohort of infants with wide variation in their nutritional intakes.

5.3.1 **Participants**

Infants born from 2005 – 2008 at <30 weeks' gestation or <1,500 grams birthweight, and admitted to the NICU were identified from unit records. This included the 80 infants previously audited (Cormack et al., 2011). Infants with significant congenital anomalies, those transferred into the unit later than 24 hours after birth, and those transferred on or before the 7th postnatal day were excluded.

5.3.2 **Neonatal data**

All recorded enteral and parenteral fluid intakes (excluding blood products) from birth until the end of postnatal day 28 were collected from the medical records. Intakes from the calendar day of birth were excluded due to its variable duration. Parenteral, enteral and total macronutrient and energy intakes were calculated using reference data (see chapter 11, appendix A), and the current, highest recorded weight was used to calculate intakes per kilogram, which were then averaged over days 1-7 (week 1) and days 1-28 (month 1). The days on which 10% and 50% of total fluid intakes were via the enteral route were identified, as was the day 100% (full) enteral feeds were achieved (Cormack et al., 2016). If full enteral feeds were not established by day 28, or the infant died before full feeds were reached, enteral feeding was reported as not established.

Antenatal steroid exposure, birth plurality, sex, maternal ethnicity (Ministry of Health, 2004), place of birth and the presence of prolonged rupture of the fetal membranes (>24 hours) were collected from the neonatal record and CRIB II score was calculated (Parry et al., 2003). Weight, head circumference and length measures at birth and at 36 weeks' postmenstrual age were converted to *z*-scores (Fenton & Kim, 2013). Growth velocity was calculated using the exponential method (Patel et al., 2009).

5.3.3 **Neonatal outcomes**

Neonatal morbidities included length of stay (from birth to discharge from a neonatal facility), intraventricular hemorrhage grade III or grade IV (Papile et al., 1978), retinopathy of prematurity stage 3 or 4 ("An international classification of retinopathy of prematurity. Prepared by an international committee.," 1984), chronic lung disease (oxygen or mechanical ventilation at 36 weeks' postmenstrual age), sepsis (a positive blood, urine or cerebrospinal fluid culture necessitating antibiotic treatment)(Chow, 2013), and necrotizing enterocolitis Bell Stage ≥ 2 (Bell et al., 1978). Deaths between birth and 2 years were recorded.

5.3.4 **Neurodevelopmental outcomes at 2 years**

Children underwent routine neurodevelopmental assessment at 2 years' corrected age (± 6 months) using the Bayley II, Bayley III or non-standardized assessment. The primary outcome, survival without neurodevelopmental impairment, includes children who were alive and had no neurodevelopmental impairment on assessment at 2 years. Children with neurodevelopmental impairment were further categorised as having mild (motor score <-1 SD), moderate (cognitive score -1 to -2 SD, or mild-moderate cerebral palsy without cognitive impairment, or impaired vision requiring correction, or conductive hearing loss requiring aids) or severe impairment (cognitive score <-2 SD or severe cerebral palsy or bilateral blindness or sensorineural hearing loss requiring aids) (National Women's Health, 2015).

5.3.5 **Statistical analysis**

Analysis was performed in JMP v11.2.0 (SAS Institute Inc., USA). Descriptive data are presented as number (%), mean ± SD or median (IQR) as appropriate. Between girls and boys, continuous data were compared using ANOVA, or Wilcoxon's test for non-normal distributions. Categorical data were compared using Chi-Squared or Fisher's Exact tests. The level of significance was taken as 0.05 throughout, with no correction for multiple comparisons. The associations between nutrition parameters, sex and the selected outcomes were explored using multiple logistic regression analysis. Gestational age and birth weight *z*-score were included in all models *a priori* as confounders that may influence neonatal nutrition, growth, and outcome at 2 years. Analyses were additionally adjusted for neonatal morbidities found to be different between girls and boys ($P<0.05$), and outcomes at 2 years were also adjusted for the type of assessment (Bayley II, Bayley III, other). Nutritional components found to have significant associations with both outcome and sex were further explored by estimating the odds ratios for the outcome for quantiles of nutritional intake separately in girls and boys.

5.4. Results

A total of 536 infants were admitted to the NICU July 2005 – October 2008, of whom 478 (89%) were included in this study and 263 (55%) were boys (Figure 1).

Girls and boys were similar in gestational age at birth, birth weight, birth weight *z*-score, CRIB II score, ethnicity, antenatal steroid exposure and birth plurality (Table 5-1). Girls were less likely than boys to have necrotizing enterocolitis (3 (1%) vs. 16 (6%), $P = 0.009$) and sepsis (22 (10%) vs. 46 (17%), $P = 0.02$), but not other neonatal illnesses. Average protein, fat, carbohydrate and energy intakes were lower during week 1 than during month 1, and were

similar in girls and boys (Table 5-1). Girls and boys achieved enteral feeds at similar ages, with breastmilk comprising the majority of enteral feedings for all infants (Table 5-1).

All infants lost weight in the first week after birth, reflected in an average growth velocity of -4.0 (-9.0 – 0.9) $g.kg^{-1}.d^{-1}$ from birth to day 7 (Table5-2). Growth velocity was similar in girls and boys from birth to day 7 and to day 28. *Z*-score change from birth to 36 weeks' postmenstrual age could be calculated for weight in 374 / 478 (78%), head circumference in 332 / 478 (69%), and length in 312 / 478 (65%). The cohort as a whole did not achieve intrauterine-equivalent growth rates, with negative changes in *z*-scores for all growth parameters from birth to 36 weeks' postmenstrual age (Table 5-2). In adjusted models, no nutritional component was associated with change in weight, head circumference or length *z*-scores from birth to 36 weeks' postmenstrual age.

By 2 years of age, 38 (8%) of the cohort had died, and 81 (17%) did not have a neurodevelopmental assessment, so the composite outcome of survival without neurodevelopmental impairment could be determined in 180 / 215 (84%) girls and 217 / 263 (83%) boys (Table 5-2). Neurodevelopmental impairment occurred in 56 / 359 (16%) children assessed at 2 years, was similar in girls and boys, and was moderate in the majority of cases. Girls were more likely than boys to achieve the composite outcome of survival without neurodevelopmental impairment (147 (82%) vs. 156 (72%), $P = 0.02$) (Table 2).

In unadjusted, multivariate logistic regression analysis week 1 fat, but not protein or carbohydrate intake, was positively associated with survival without neurodevelopmental impairment (OR (95%CI) 1.56 (1.14 – 2.15), $P = 0.005$). A similar relationship was seen in girls where fat (5.36 (2.59 – 12.60), $P < .001$) but not protein or carbohydrate was again associated with survival without neurodevelopmental impairment. In contrast, there were no significant relationships between week 1 intake of any of the 3 macronutrients and survival without neurodevelopmental impairment in boys (Data not shown).

In adjusted models, energy, fat, and enteral feed volumes in week 1, and energy and enteral feed volumes in month 1, were each associated with survival without neurodevelopmental impairment at 2 years and had significant sex interactions (Table 5-3). These relationships were similar when the outcome of neurodevelopmental impairment only was examined (Table 5-3). In girls, week 1 fat, energy and enteral fluid intakes were all positively associated with survival without neurodevelopmental impairment at 2 years, but there were no associations between these nutritional intakes and outcome in boys (Figure 5-2). Similarly, month 1 enteral feed volumes were positively associated with survival without neurodevelopmental in girls but not boys (Figure5-2). Although energy intake in month 1 was associated with survival without neurodevelopmental impairment in both girls and boys, significantly increased odds of this outcome were seen for the highest quartile of energy intake in girls but only for the second quartile of energy intake in boys (Figure 5-2).

5.5. Discussion

Neonatal nutrition is an important modifier of short and long-term outcomes of infants born preterm, but unlike for all other age groups (Capra & and members of the working party, 2006), current recommendations for neonatal nutritional intakes do not differ by sex (Agostoni et al., 2010). In our cohort of infants born very preterm or at very low birth weight, we observed that associations between neonatal nutrition, growth and neurodevelopmental outcomes are different in girls and boys. These data support the hypothesis that preterm infants may have sex-specific nutritional requirements in the neonatal period.

Such an hypothesis is supported by evidence from animal (Hinde, 2007) and human (Powe, Knott, & Conklin-Brittain, 2010; Thakkar et al., 2013) studies that maternal breastmilk composition differs by offspring sex, providing higher concentrations of lipid and energy to boys. There is also evidence that supplementation of enteral feeds after preterm birth leads to sex-specific differences in growth and neurodevelopmental outcomes, with preterm boys who

receive supplemented feeds showing bigger changes in short and long-term growth (Berry et al., 2016; Fewtrell et al., 2004), and improvements in verbal and overall IQ scores (Lucas et al., 1998), compared to girls.

In our study, fat intake in the first postnatal week was strongly associated with survival without neurodevelopmental impairment in girls but not boys. The reason for the association between fat intake and outcome in girls, and the absence of this association in boys, is not clear, but could be due to differences in the requirements for, and metabolism of, fatty acids in early life. At one month of age, term-born girls have a higher percentage body fat and less fat-free mass than boys (Fields, Krishnan, & Wisniewski, 2009). Healthy infant girls have higher serum concentrations of triglycerides, cholesterol and low density lipoproteins than boys (Dathan-Stumpf et al., 2016) and also different lipid compositions of the vernix (which is dissolved into the amniotic fluid by surfactant proteins then swallowed by the fetus) (Narendran, Wickett, Pickens, & Hoath, 2000; Nishijima et al., 2012), with girls tending towards longer-chain hydrocarbon components (Mikova et al., 2014). Further, a long term study of docosahexaenoic acid supplementation to preterm neonates showed improved executive function only in girls (Collins et al., 2015), whereas fish oil supplementation to lactating mothers of term infants was associated with increased diastolic blood pressure and delayed puberty at 13 years only in boys (Lauritzen et al., 2016). Together, these findings suggest sexual dimorphism in lipid requirement and metabolism, and hence potentially nutritional fat requirements in early life.

We observed an association between week 1 energy intake and survival without neurodevelopmental impairment in girls but not boys; findings similar to those recently reported in a cohort of 112 two-year olds born very preterm and exposed to high or low protein and energy intakes in the first 2 postnatal weeks (Christmann et al., 2017). As this association mirrors the association between week 1 fat intake and outcome, it is difficult to

distinguish whether energy or fat (which is the most energy-dense macronutrient) has the more important effect. We did not find any associations between week 1 or month 1 protein intakes and survival without neurodevelopmental impairment; an unexpected finding as increased early amino acid provision has previously been reported to improve survival without major disability in boys, but reduce cognitive scores in surviving girls (van den Akker, te Braake, Weisglas-Kuperus, & van Goudoever, 2014).

Enteral feed volumes in week 1 and month 1 were strongly associated with survival without neurodevelopmental impairment in girls but not boys. Achievement of enteral feeds may be delayed in infants who are small and sick (Bombell & McGuire, 2008), but given the similarities in gestational age, birth weight, and CRIB II score between the sexes, delays in enteral feeding would be expected to affect girls and boys equally, and our data show that there were no differences between girls and boys for time to achieve 10%, 50% and full enteral feeds. Girls have been shown to have a larger increase in insulin-like growth factor-1 (IGF-1) concentrations than boys in response to higher enteral protein intakes (Closa-Monasterolo et al., 2011). As IGF-1 is a vital regulator of brain and central nervous system development (Hellström et al., 2016), increased sensitivity of girls' IGF-1 axis to enteral feeding is another possible mechanism underlying the associations between enteral feeding and neurodevelopmental outcome in our cohort. Most of the enteral feed provided to infants in our cohort was maternal expressed breastmilk, and it is possible that sex-related differences in maternal breastmilk composition resulted in differential outcomes between preterm girls and boys.

Weight is the variable most commonly used to estimate adequacy of neonatal nutrition, yet the change in weight *z*-score between birth and 36 weeks was not associated with any component of nutritional intake in either sex. In term infants, weight is sensitive to relative lean mass, and weight may also change in response to hydration status. The use of weight as a marker for

nutritional status also may be confounded through the presence of hypoalbuminemia, resulting in edema in undernourished infants. Crown-heel length is difficult to measure accurately, especially in infants who are unable to tolerate significant handling. Thus, although linear growth is attenuated by undernutrition (Gat-Yablonski & De Luca, 2017), length measures in preterm infants may not accurately reflect body composition (Kiger, Taylor, Wagner, Finch, & Katikaneni, 2016).

It is possible that the interactions we have described between sex, fat and energy are secondary to our use of standard values to estimate enteral macronutrient intakes, and if actual, sex-specific breastmilk composition was known, these associations may no longer be observed. The volumes of enteral feed received by the cohort in the first week represent less than 50% of infants' nutritional intakes, but enteral feeds provide the majority of nutrition in the first month, thus any disparities between estimated and actual enteral macronutrient intakes are likely to be most important during this time period. Our findings in this retrospective study may reflect reverse causation, and the associations described may merely reflect that infants with good outcomes grow better and feed better in the neonatal period, but this seems an unlikely explanation for our findings of sex-specific relationships with outcome. Further, although this is a relatively large cohort, the number of infants with poor outcome at 2 years is small. However, we prespecified an analysis plan specifically searching for sex-specific interactions and our findings are statistically robust.

5.6. Conclusions

Infants born very preterm have sex-specific associations between nutrition and outcomes. We found that higher early fat and enteral feed intakes were associated with improved outcome to 2 years in girls, but not boys. Prospective trials of neonatal nutrition should consider that outcomes may diverge by infant sex, and should therefore be adequately powered to report

outcomes separately in girls and boys and examine sex interactions. Future recommendations for nutrition of preterm infants may need to be sex-specific

5.7. Figures and tables

Figure 5-1: Flow diagram of participants in the neonatal period and at 2 years

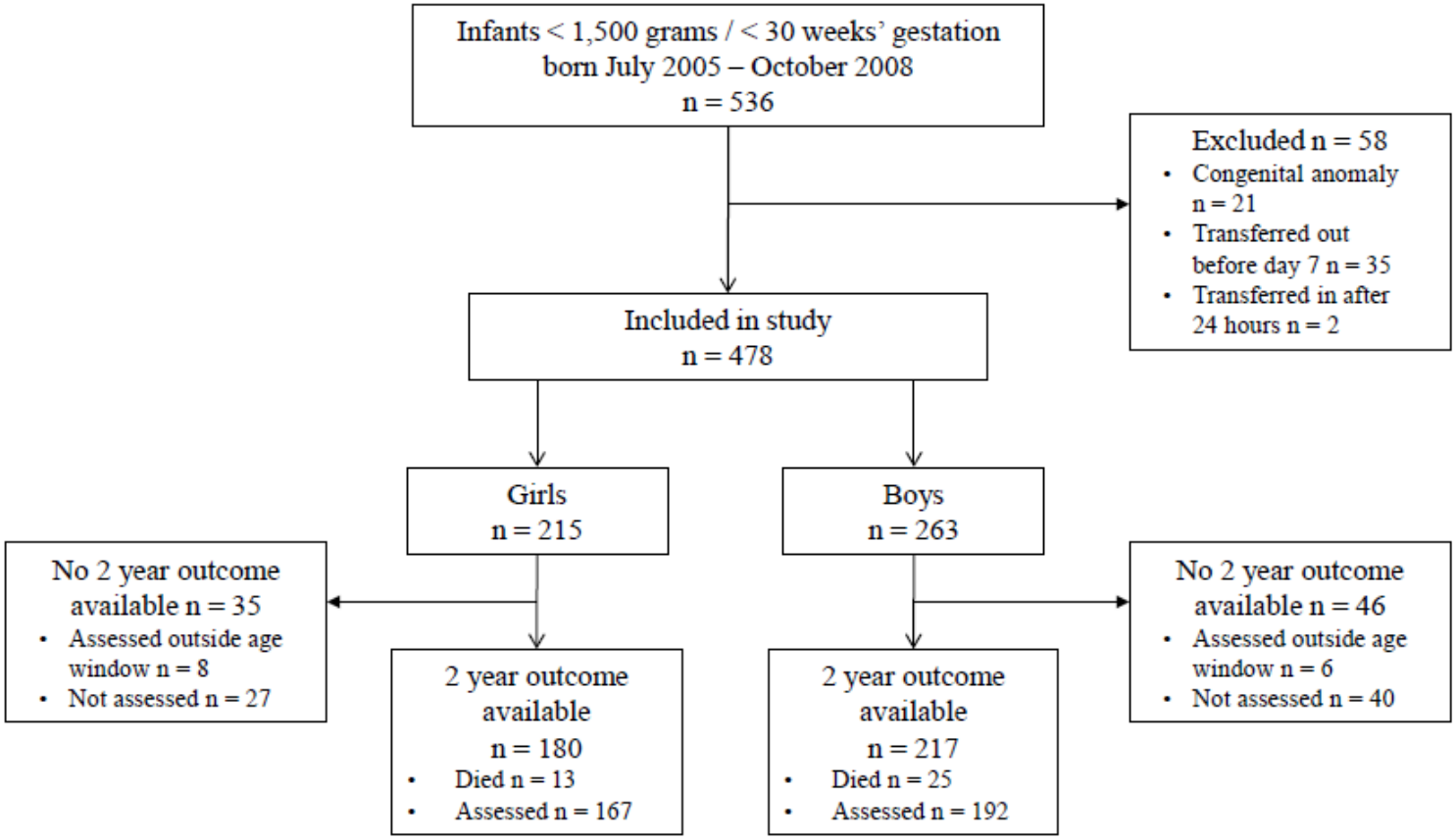
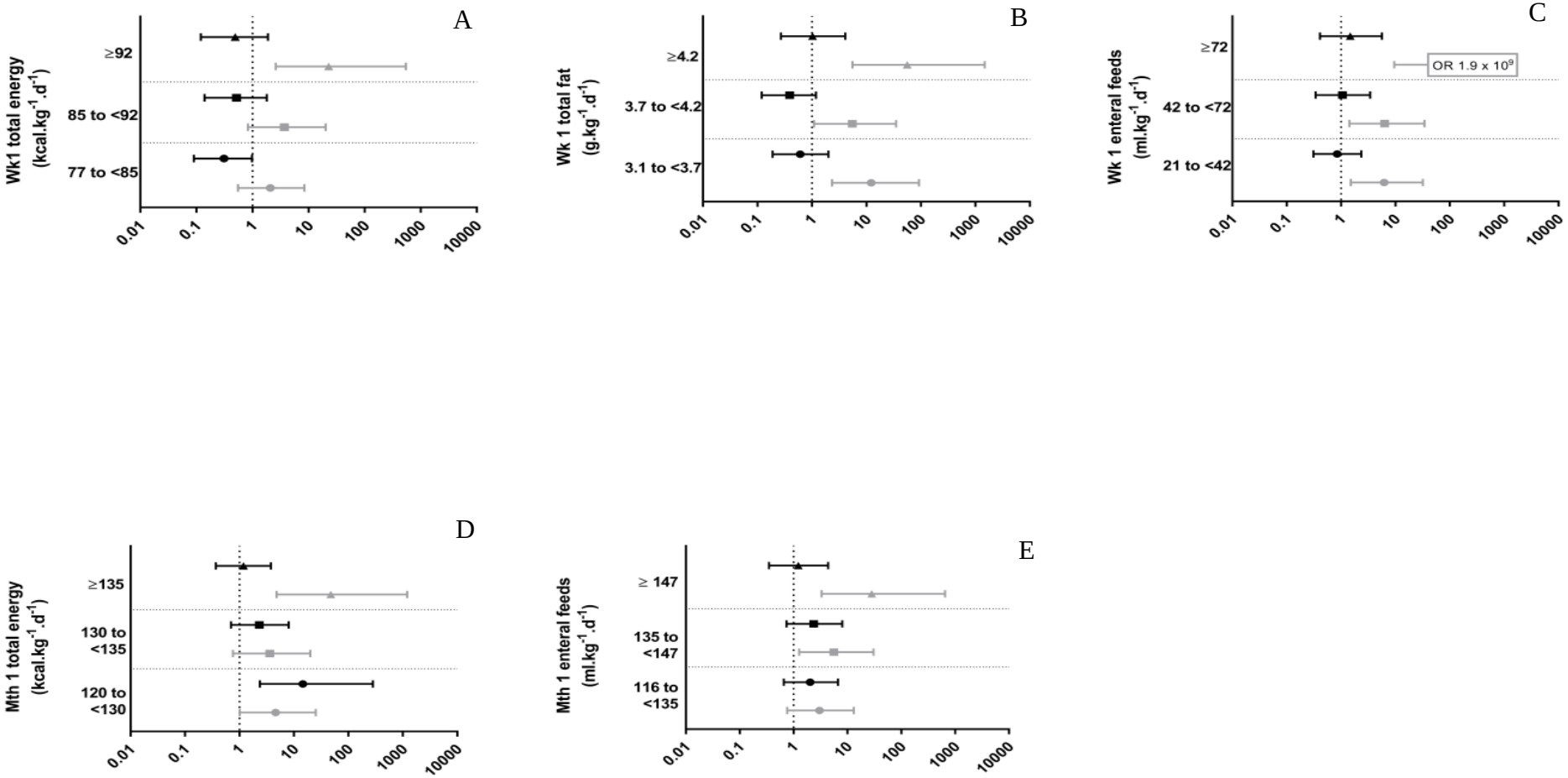


Figure 5-2: Odds of survival without neurodevelopmental impairment at 2 years in girls and boys for quartiles of nutrient intakes in week 1 and month 1



Adjusted odds of survival without neurodevelopmental impairment at 2 years in girls (grey) and boys (black) in association with quartiles of nutrient intakes, where the reference values is the first quartile. Circles denote quartile 2, squares quartile 3 and triangles quartile 4.

A: Week 1 energy intake (reference value is energy intake < 77 kcal.kg^{-1}.d^{-1}) Girls X^2 = 8.84, P = 0.03. Boys X^2 = 4.00, P = 0.27.

B: Week 1 fat intake (ref. is fat intake < 3.1 g.kg^{-1}.d^{-1}). Girls X^2 = 18.91, P <.001. Boys X^2 = 4.20, P = 0.24.

C: Week 1 enteral feed volume (ref. is enteral feed volume < 21 ml.kg^{-1}.d^{-1}). Girls X^2 = 15.96, P = 0.001. Boys X^2 = 0.70, P = 0.87.

D: Month 1 energy intake (ref. is energy intake < 120 kcal.kg^{-1}.d^{-1}). Girls X^2 = 12.82, P = 0.005. Boys X^2 = 12.14, P = 0.007.

E: Month 1 enteral feed volumes (ref. is enteral feed volume < 116 ml.kg^{-1}.d^{-1}). Girls X^2 = 10.99, P = 0.01. Boys X^2 = 3.02, P = 0.39.

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Table 5-1: Perinatal characteristics and nutrition received by girls and boys

	Girls n = 215	**Boys n = 263**	***P* value**
Gestational age (weeks)	28 (26 – 30)	28 (26 – 29)	0.76
Any antenatal steroids	196 (91)	245 (94)	0.26
Outborn	15 (7)	18 (7)	0.96
Prolonged rupture of membranes	61 (28)	67 (25)	0.53
Multiple birth	51 (24)	69 (26)	0.51
Māori/ Pacific Islander	73 (34)	89 (34)	0.98
CRIB II score	7 (5 – 11)	8 (5 – 10)	0.29
Birth weight (g)	1059 ± 278	1107 ± 269	0.06
Birth weight *z*-score	-0.19 ± 0.94	-0.20 ± 1.00	0.94
Birth length (cm)	36.5 ± 3.5	36.6 ± 3.2	0.72
Birth length *z*-score	-0.07 ± 1.15	-0.19 ± 1.06	0.27
Birth head circumference (cm)	25.9 ± 2.3	26.2 ± 2.3	0.19
Birth head circumference *z*-score	0.16 ± 1.02	0.10 ± 1.07	0.52
Length of neonatal stay (days)	69 ± 28	70 ± 30	0.78
Hyperglycemia treated with insulin	36 (17)	55 (21)	0.29
Stage 3/4 retinopathy of prematurity	7 (5)	16 (9)	0.28
Grade III/IV intraventricular hemorrhage	12 (6)	9 (3)	0.25
Chronic lung disease	32 (15)	48 (18)	0.34
Home oxygen	26 (12)	35 (13)	0.51
Sepsis	22 (10)	46 (17)	0.02
Necrotizing enterocolitis	3 (1)	16 (6)	0.009
Day enteral feeds achieved			
10%	3 (2 – 4)	3 (2 - 4)	0.97
50%	6 (5 – 8)	7 (5 – 9)	0.71
100%	9 (7 – 11)	9 (7 – 11)	0.59
Enteral feeds not established at d28	13 (6)	22 (8)	0.33
Total protein intake ($g.kg^{-1}.d^{-1}$)			
Week 1[a]	2.7 ± 0.4	2.7 ± 0.4	0.71
Month 1[b]	3.4 ± 0.3	3.4 ± 0.3	0.08

Table 5-1 continued…	**Girls n = 215**	**Boys n = 263**	***P* value**
Total fat intake ($g.kg^{-1}.d^{-1}$)			
Week 1[a]	3.8 ± 0.8	3.7 ± 0.9	0.40
Month 1[b]	6.2 ± 0.8	6.0 ± 0.9	0.07
Total carbohydrate intake ($g.kg^{-1}.d^{-1}$)			
Week 1[a]	10.9 ± 1.7	11.0 ± 1.7	0.57
Month 1[b]	14.7 ± 1.8	14.7 ± 1.4	0.94
Total energy intake ($kcal.kg^{-1}.d^{-1}$)			
Week 1 [a]	85 ± 11	85 ± 11	0.82
Month 1 [b]	127 ± 14	125 ± 14	0.07
Week 1 enteral intakes [a]			
Total enteral feeds ($ml.kg^{-1}.d^{-1}$)	48 ± 31	46 ± 33	0.57
Breastmilk ($ml.kg^{-1}.d^{-1}$)	44 ± 30	43 ± 30	0.87
Protein ($g.kg^{-1}.d^{-1}$)	0.8 ± 0.6	0.8 ± 0.6	0.73
Fat ($g.kg^{-1}.d^{-1}$)	1.8 ± 1.2	1.7 ± 1.3	0.59
Carbohydrate ($g.kg^{-1}.d^{-1}$)	3.4 ± 2.3	3.3 ± 2.6	0.66
Month 1 enteral feeds ($ml.kg^{-1}.d^{-1}$) [b]	128 ± 32	125 ± 35	0.25

Data are number (%), median (IQR) or mean ± SD.

Due to missing data a: girls n = 206 (96%), boys n = 256 (97%). b: girls n = 194 (90%) boys n = 241 (92%).

Table 5-2: Growth and 2-year outcomes in girls and boys

	Girls n = 215	**Boys n = 263**	***P* value**
Growth velocity			
Birth to day 7 ($g.kg^{-1}.d^{-1}$) [a]	-4.2 ± 9.4	-4.2 ± 8.7	0.94
Birth to day 28 ($g.kg^{-1}.d^{-1}$) [b]	12.0 ± 3.9	11.7 ± 3.3	0.47
Z-score change birth to 36/40			
Weight [c]	-1.04 ± 0.69	-1.07 ± 1.43	0.84
Length [d]	-1.05 ± 0.99	-1.19 ± 1.53	0.37
Head circumference [e]	-0.90 ± 1.03	-0.89 ± 1.47	0.95
Assessed at 2 years of age	**n = 167**	**n = 192**	
Assessment type			
Bayley II	26 (16)	28 (15)	0.58
Bayley III	126 (75)	152 (79)	
Other	15 (9)	12 (6)	
Neurodevelopmental impairment	20 (12)	36 (19)	
Mild	5 (3)	3 (2)	0.08
Moderate	13 (8)	28 (15)	
Severe	2 (1)	5 (3)	
Died before 2 years of age	**13 (6)**	**25 (10)**	**0.16**
Survived without neurodevelopmental impairment	**147 (82)**	**156 (72)**	**0.02**

Data are number (%), median (IQR) or mean ± SD. Due to missing data a: girls n=173, boys n=212. b: girls n=133, boys n=161. c: girls n=175, boys n=199. d: girls n= 142 boys n=170. e: girls n=154, boys n=178.

Table 5-3: Associations between early nutrient intakes, sex and nutrient-sex interaction terms with early growth and 2-year outcomes

	Change in weight z score birth to 36 weeks[$] n = 374			Change in length z score birth to 36 weeks[$] n = 312			Change in head circumference z score birth to 36 weeks[$] n = 332			Neurodevelopmental impairment at 2 years† n = 359			Survival without neurodevelopmental impairment at 2 years† n = 397		
	Nutrient	**Sex**	**Inter action**	**Nutrient**	**Sex**	**Inter action**	**Nutrient**	**Sex**	**Inter action**	**Nutrient**	**Sex**	**Inter action**	**Nutrient**	**Sex**	**Inter action**
Week 1															
Total energy ($kcal.kg^{-1}.d^{-1}$)	0.00 (0.01) 0.38	0.02 (0.05) 0.62	0.00 (0.00) 0.52	-0.00 (0.01) 0.44	0.08 (0.06) 0.20	0.00 (0.01) 0.67	0.00 (0.01) 0.38	0.01 (0.06) 0.83	0.01 (0.01) 0.05	-0.04 (0.02) 0.03	-0.38 (0.18) 0.03	-0.03 (0.01) 0.02	0.04 (0.02) 0.03	0.38 (0.18) 0.03	0.03 (0.01) 0.02
Total protein ($g.kg^{-1}.d^{-1}$)	0.12 (0.15) 0.41	0.02 (0.05) 0.63	-0.10 (0.11) 0.36	-0.04 (0.15) 0.81	0.08 (0.06) 0.21	-0.18 (0.15) 0.23	0.12 (0.15) 0.41	0.02 (0.06) 0.78	-0.07 (0.15) 0.64	-0.12 (0.40) 0.77	-0.31 (0.17) 0.06	-0.50 (0.39) 0.21	0.12 (0.40) 0.77	0.31 (0.17) 0.06	0.50 (0.39) 0.21
Total fat ($g.kg^{-1}.d^{-1}$)	0.07 (0.08) 0.35	0.02 (0.05) 0.65	0.12 (0.06) 0.03	-0.01 (0.08) 0.91	0.08 (0.06) 0.21	0.13 (0.07) 0.07	0.07 (0.08) 0.35	0.01 (0.06) 0.88	0.23 (0.07) 0.002	-0.71 (0.26) 0.005	-0.47 (0.20) 0.02	-0.71 (0.24) 0.003	0.72 (0.26) 0.005	0.47 (0.20) 0.02	0.71 (0.24) 0.003
Total carbohydrate ($g.kg^{-1}.d^{-1}$)	0.02 (0.04) 0.65	0.02 (0.05) 0.61	-0.04 (0.03) 0.16	-0.03 (0.04) 0.37	0.08 (0.06) 0.20	-0.05 (0.04) 0.14	0.02 (0.04) 0.65	0.01 (0.06) 0.81	-0.02 (0.04) 0.62	-0.04 (0.10) 0.70	-0.29 (0.16) 0.07	-0.02 (0.09) 0.82	0.04 (0.10) 0.70	0.29 (0.16) 0.07	0.02 (0.09) 0.82
Enteral feeds ($ml.kg^{-1}.d^{-1}$)	0.00 (0.00) 0.13	0.02 (0.05) 0.63	0.00 (0.00) 0.25	-0.00 (0.00) 0.95	0.08 (0.06) 0.21	0.00 (0.00) 0.10	0.00 (0.00) 0.13	0.01 (0.06) 0.85	0.01 (0.00) 0.001	-0.02 (0.01) 0.003	-0.54 (0.22) 0.01	-0.02 (0.01) 0.008	0.02 (0.01) 0.003	0.55 (0.22) 0.01	0.02 (0.01) 0.008

Table 5-3 continued...	**Change in weight z score birth to 36 weeks[$] n = 374**			**Change in length z score birth to 36 weeks[$] n = 312**			**Change in head circumference z score birth to 36 weeks[$] n = 332**			**Neurodevelopmental impairment at 2 years† n = 359**			**Survival without neurodevelopmental impairment at 2 years† n = 397**		
	Nutrient	**Sex**	**Inter action**	**Nutrient**	**Sex**	**Inter action**	**Nutrient**	**Sex**	**Inter action**	**Nutrient**	**Sex**	**Inter action**	**Nutrient**	**Sex**	**Inter action**
Month 1															
Total energy ($kcal.kg^{-1}.d^{-1}$)	0.00 (0.01) 0.66	0.03 (0.05) 0.49	0.01 (0.00) 0.6	-0.00 (0.01) 0.56	0.10 (0.06) 0.12	0.00 (0.01) 0.49	0.00 (0.01) 0.66	0.01 (0.06) 0.89	0.02 (0.00) 0.001	-0.03 (0.01) 0.02	-0.37 (0.18) 0.04	-0.03 (0.01) 0.02	0.03 (0.01) 0.02	0.37 (0.18) 0.04	0.03 (0.01) 0.02
Total protein ($g.kg^{-1}.d^{-1}$)	0.41 (0.22) 0.06	0.04 (0.05) 0.48	0.21 (0.17) 0.22	0.11 (0.23) 0.64	0.10 (0.06) 0.12	0.01 (0.22) 0.97	0.41 (0.22) 0.06	0.01 (0.06) 0.86	0.51 (0.22) 0.02	-0.12 (0.40) 0.77	-0.31 (0.17) 0.06	-0.50 (0.39) 0.21	0.12 (0.40) 0.77	0.32 (0.17) 0.06	0.50 (0.39) 0.2
Total fat ($g.kg^{-1}.d^{-1}$)	0.09 (0.09) 0.31	0.03 (0.05) 0.57	0.17 (0.07) 0.01	-0.00 (0.10) >.99	0.10 (0.06) 0.13	0.08 (0.09) 0.42	0.09 (0.09) 0.31	0.00 (0.06) 0.95	0.32 (0.09) <.001	-0.45 (0.20) 0.03	-0.34 (0.17) 0.05	-0.32 (0.18) 0.08	0.45 (0.20) 0.03	0.34 (0.10) 0.05	0.33 (0.18) 0.08
Total carbohydrate ($g.kg^{-1}.d^{-1}$)	-0.06 (0.04) 0.12	0.03 (0.05) 0.48	0.01 (0.03) 0.74	-0.06 (0.04) 0.19	0.10 (0.06) 0.13	0.02 (0.04) 0.57	-0.06 (0.04) 0.12	0.02 (0.06) 0.71	0.05 (0.04) 0.17	-0.10 (0.11) 0.39	-0.34 (0.17) 0.05	-0.15 (0.10) 0.13	0.10 (0.11) 0.39	0.34 (0.17) 0.05	0.15 (0.10) 0.13
Enteral feeds ($ml.kg^{-1}.d^{-1}$)	0.00 (0.00) 0.49	0.02 (0.05) 0.70	0.01 (0.00) 0.004	0.00 (0.00) 0.58	0.07 (0.06) 0.24	0.00 (0.00) 0.04	0.00 (0.00) 0.49	0.00 (0.00) >.99	0.01 (0.00) <.001	-0.01 (0.01) 0.03	-0.34 (0.18) 0.05	-0.01 (0.00) 0.02	0.01 (0.01) 0.03	0.34 (0.17) 0.05	0.01 (0.00) 0.02

Data are parameter estimate (SE), *P* value. $ *P* adjusted for gestational age, birth weight *z*-score, necrotizing enterocolitis and sepsis, and † *P* additionally adjusted for type of assessment at 2 years.

Chapter 6. Long term outcomes of hyperglycemic preterm infants randomized to tight glycemic control

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6.1. Introduction

Hyperglycemia is common in infants born very preterm or at very low birth weight (Beardsall et al., 2010), and is associated with an increased risk of mortality and neonatal morbidities (Alexandrou et al., 2010; Auerbach et al., 2013; Chavez-Valdez et al., 2011; Hirshberg et al., 2008). In critically unwell adults, hyperglycemia is commonly controlled by restricting glucose intake or administration of insulin (Van Hooijdonk, Mesotten, Krinsley, & Schultz, 2016). However, in preterm infants, restriction of caloric intake may result in faltering growth, and insulin infusion increases the risk of hypoglycemia (Beardsall et al., 2008), both of which are associated with poor neurodevelopmental outcomes (Burns et al., 2008; Ehrenkranz et al., 2006). Exposure to neonatal hyperglycemia or exogenous insulin during critical developmental periods may also affect pancreatic development (Bansal et al., 2015; Green et al., 2012) thereby increasing the risk of glucose intolerance in later life; a risk already increased in children (Hofman et al., 2004b) and adults (Kajantie, Osmond, Barker, & Eriksson, 2010) born preterm, potentially due to loss of β-cell mass (Gunasekaran et al., 2012).

In a previous randomized trial (Alsweiler et al., 2012b) we found that infants randomized to tight glycemic control for neonatal hyperglycemia were more likely to be treated with insulin at a higher dose and for longer durations compared with those randomized to standard treatment. However, the 2 groups had similar carbohydrate intakes, and there were no between-group differences in the rates of common neonatal morbidities or mortality. Tight glycemic control resulted in improved weight gain and head circumference growth, but not linear growth, by 36 weeks' postmenstrual age (PMA). Greater head circumference growth is associated with increased brain volume and may be associated with improved neurodevelopmental outcome (Cheong et al., 2008). However, infants randomized to tight glycemic control also had a 3-fold higher incidence of hypoglycemia (Alsweiler et al., 2012b). Thus, with tight glycemic control in preterm infants, there may be a trade-off between reduced

morbidity from reduced hyperglycemia and brain injury caused by hypoglycemia. The finding of increased weight gain without associated linear growth suggested that tight glycemic control may also alter neonatal body composition, potentially increasing the long-term risks of glucose intolerance and metabolic syndrome in these children.

6.2. Methods

In the Hyperglycemia in Neonates Trial (HINT) (anzctr.org.au: ACTRN12614000492651), conducted in 2005-2008, a total of 88 hyperglycemic preterm infants (<1,500 g or <30 weeks' gestation) were randomized to standard glycemic control (blood glucose concentrations maintained <180 $mg.dL^{-1}$ [<10.0 mM] or tight glycemic control (blood glucose concentrations maintained < 155 $mg.dL^{-1}$ [<8.6 mM] or 72 – 108 $mg.dL^{-1}$ [4–6 mM] if on insulin) (Alsweiler et al., 2012b). The primary outcome was linear growth rate to 36 weeks' PMA. All surviving children randomized in the HINT trial were eligible to participate in this follow up study. Families were traced and invited to attend an assessment at 7 years ± 6 months corrected age.

Data on birth weight, sex, birth plurality, gestational age, survival, blood glucose concentrations, insulin dosing, and fluid and nutritional intakes were taken from the electronic neonatal medical record. Maternal ethnicity was prioritized (Ministry of Health, 2004), and *z*-scores for measurements at birth, 28 days postnatal age and 36 weeks' PMA were calculated (Fenton & Kim, 2013). Socioeconomic deprivation (NZ Deprivation Index) at birth was derived from the maternal pregnancy booking address (Salmond et al., 2007). Macronutrient intakes for the first 28 postnatal days were calculated. The Clinical Risk Index in Babies, Version 2 score was used as a measure of neonatal illness severity (Parry et al., 2003). The neonatal morbidities of intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, retinopathy of prematurity, early and late onset sepsis, major neonatal surgery, chronic lung disease and discharge with home oxygen were defined in accordance with Australia and New Zealand Neonatal Network criteria (Chow, 2013).

Assessments were conducted at the Liggins Institute research clinic, University of Auckland, or at a location convenient for the participant. Caregivers gave written consent and children gave verbal assent to assessment. All assessors were blind to the neonatal randomization status of participants. Ethical approval was obtained from the Northern B ethics committee (NTY/12/05/035).

Weight, height, sitting height, head circumference and abdominal circumference were measured and used to generate *z*-scores (De Onis et al., 2007). Leg length was calculated from standing and sitting heights.

Body composition was assessed using dual energy x-ray absorptiometry (Lunar prodigy utilising enCORE software, GE Healthcare, Chicago, Illinois). Bone mineral density, fat mass and lean mass were adjusted for height.

A modified frequently sampled glucose tolerance test was performed (Cutfield, Bergman, Menon, & Sperling, 1990). Glucose concentrations were measured using enzymatic colorimetric assay (Hitachi 902 autoanalyzer, Hitachi, Tokyo, Japan), and insulin concentrations using electrochemiluminescence immunoassay (Eclysis 2010 immunology analyser; Hitachi). Fasting insulin and glucose concentrations were taken as the average of 3 baseline samples. Bergman's minimal model (MinMod, Millennium Software, Los Angeles, California) was used to calculate insulin sensitivity, acute insulin response to glucose, glucose effectiveness and disposition index. The glucose disappearance constant was calculated as well (Bergman, 1989).

Trained assessors administered following standardized developmental tests: Wechsler Intelligence Scale for Children, Fourth Edition, Australian; the Beery-Buktenica Developmental Test of Visual Motor Outcomes; the Movement Assessment Battery for Children, 2nd Edition (MABC-2), and the Test of Everyday Attention in Children using the

Sky search, Score!, Creature counting and Sky search DT subtests only. Raw scores were transformed to age-scaled or standard scores as appropriate.

Caregivers were asked to complete the Behavior Rating Inventory of Executive Function, Child Health Questionnaire, Modified Health Utilities Index 2 scale, Achenbach System of Empirically Based Assessment child behaviour checklist, and demographic questionnaire. The child's teacher was asked to complete the BRIEF and Achenbach System of Empirically Based Assessment teacher forms. Global executive composite T-scores ≥60 on the parent and teacher completed BRIEF were described as impaired home function and impaired classroom function respectively.

Children were examined by a pediatrician. Cerebral palsy was categorised using the Gross Motor Function Classification Scale (GMFCS) (Palisano et al., 2000). Presenting visual acuity was assessed by an optometrist using a crowded logMAR chart and scored by letter (McGraw & Winn, 1993). Blood pressure was measured using oscillometric methods with the child semi-reclined. The average of three measures was taken and converted to *z*-scores (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Average systolic or diastolic blood pressures ≥95th percentile for sex, age and height were defined as hypertension, and ≥90th percentile as prehypertension (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

6.2.1 **Statistical analysis**

Statistical analyses were performed using SAS v.9.4 and JMP v.11.2.0 (SAS Institute Inc., Cary, North Carolina). The primary outcome was survival without neurodevelopmental impairment, defined *a priori* as any of: full scale IQ standard score more than 1 SD below the mean, or MABC-2 total score ≤5th centile, or cerebral palsy, or visual acuity of 6/60 (1.0

logMAR) or worse in the best eye, or deaf requiring hearing aids. Secondary outcomes included individual components of the primary outcome, executive function, growth, glucose metabolism, blood pressure, body composition, health, and quality of life outcomes.

Descriptive data are presented as number (%), mean (standard deviation) or median (IQR). Continuous variables were compared between groups using a 2-sample *t* test or Wilcoxon test if not normally distributed. Categorical data were compared using exact methods. Primary and secondary outcomes were compared between treatment groups using unadjusted and adjusted linear regression models, and presented as odds ratios or mean differences between the groups, with 95% confidence intervals and *P* value. The presence of twins was considered a cluster effect in the primary analysis.

Because randomization in the HINT trial was stratified by sex and weight for gestational age (small for gestational age or not) all analyses were adjusted for these variables. In addition, gestational age, NZ Deprivation Index at birth, birth plurality, and protein intake in the first 14 postnatal days were pre-specified potential confounders and assessed for balance between the groups in the follow-up cohort. Only birth plurality was found to differ by more than 10% between groups and was included as a covariate in the final analyses.

6.3. Results

Of the 88 infants randomized, 11 (13%) had died, leaving 77 (88%) eligible for assessment at 7 years. Of those 77 children, 57 (65% of those randomized and 74% of those potentially eligible) were assessed at 7 years. The primary outcome was thus available for 68 children (77% of those randomized), including 33 of the 45 (73%) randomized to standard glycemic control (standard group) and 35 of the 43 (81%) randomized to tight glycemic control (tight group) (Figure 6-1).

Children assessed at 7 years were similar to those who were eligible but not assessed except for lower *z*-scores for weight, length and head circumference at birth (Table 6-1). Only weight *z*-score remained significantly different at 36 weeks PMA.

There was no difference in the primary outcome of survival without neurodevelopmental impairment between the tight and standard groups (Table 6-2). Neurodevelopmental impairment was common, but there were no differences between randomization groups (Table 6-3).

Overall, the cohort achieved significant linear catch-up growth between 36 weeks PMA and 7 years' corrected age, with a mean (SD) increase in length *z*-score of 1.71 (1.07). However, the tight group were on average 4 cm (or 0.9 SD) shorter than the standard group (Table 6-3) and had less linear catch-up growth between 36 weeks PMA and 7 years' corrected age [post hoc analysis mean length *z*-score change of 1.43 (1.04) vs. 2.06 (1.03) SD; P =.03]. Post hoc analysis revealed that although both sitting height and leg length were reduced in the tight group, only the average 2.3 cm (or 0.7 SD) difference in leg length was statistically significant (P = .02).

Abnormal blood pressures were seen in 7 of the 57 children (12%) assessed at 7 years, including 5 (9%) with hypertension and 2 (4%) with prehypertension, with no difference between randomization groups (Table 6- 3).

The mean fasting glucose concentration was lower in the tight group [84.6 (6.30) $mg.dL^{-1}$ vs. 90.0 (5.58) $mg.dL^{-1}$, P = .02], although all fasting measures were within the normal range (Table 6-3). These findings were unchanged after adjustment for body weight and lean mass at age7 years (data not shown).

Fat mass, lean mass and bone mineral density were not different between randomization groups. After correction for height, lean mass was greater in the tight group [mean, 18.7 (1.1)

kg vs. 17.6 (0.9) kg, *P* <.01), but there remained no differences between groups in fat mass or bone mineral density (Table 6-3).

6.3.1 **Exploratory analyses**

The mean proportion of blood glucose concentrations in the target 72 – 108 $mg.dL^{-1}$ range between birth and 36 weeks' PMA was significantly higher in the tight group [42.5% (10.0%) vs 35.8% (12.3%), *P* = .03). An increasing proportion of neonatal blood glucose concentrations in this range was associated with a greater chance of survival without neurodevelopmental impairment at age 7 years (aOR, 1.07; 95% CI, 1.01–1.13; *P* = .01). An increasing proportion of neonatal blood glucose concentrations in this range was also associated with reduced risks of death at 7 years (aOR, 0.93; 95% CI, 0.87 – 1.00; *P* = .04); neurodevelopmental impairment (aOR, 0.94; 95% CI, 0.88 – 1.00; *P* = .04); full scale IQ more than 1SD below the mean (aOR, 0.93; 95% CI, 0.88–0.99; *P* = .02); but not of MABC-2 score $\leq 5^{th}$ percentile. (aOR, 0.96; 95% CI, 0.91–1.01; *P* = .10).

There were no associations between height or height *z*-score at 7 years and total neonatal insulin dose, change in insulin-like growth factor 1 concentration between trial entry and 36 weeks' gestation or insulin-like growth factor 1 concentration at 36 weeks' gestation (data not shown).

6.4. Discussion

Tight glycemic control of hyperglycemic preterm infants did not alter the primary outcome of survival without neurodevelopmental impairment at 7 years corrected age, despite increasing the incidence of neonatal hypoglycemia. However, children in the tight glucose control group were shorter and had more height-adjusted lean mass compared with those in the standard glucose control group. The tight group also had lower fasting blood glucose concentrations,

but did not differ from the standard group in other measures of glucose tolerance or insulin metabolism.

In exploratory analyses, increased proportions of blood glucose concentrations within the 72 – 108 mg.dL^{-1} range (the target range for infants in the tight group while treated with insulin) was associated with improved survival without neurodevelopmental impairment in the whole cohort. Nonetheless, the tight group, which had a greater proportion of blood glucose concentrations within this range, did not exhibit improved outcomes. This finding may reflect the practical difficulties of achieving tight control of neonatal blood glucose concentrations using bedside titration of insulin infusions. Even in the tight group, only 43% of blood glucose concentrations fell within the 72 – 108 mg.dL^{-1} range, which was only a slightly greater proportion than seen in the standard group (36%). It is possible that strategies to improve the implementation of tight glycemic control, such as computer-assisted insulin dosing or continuous glucose monitoring, might increase the proportion of blood glucose concentrations within the 72 – 108 mg.dL^{-1} range, potentially improving neurodevelopmental outcomes. However, less-unwell infants, who are likely to have better developmental outcomes, exhibit more stable glucose homeostasis in the neonatal period (Tottman, Alsweiler, Bloomfield, Pan, & Harding, 2017). Thus, an increased proportion of blood glucose concentrations within the 72 – 108 mg.dL^{-1} range may be simply a marker for more stable infants with greater likelihood of a good outcome, in whom increasing the proportion of time in this range is unlikely to change long-term neurodevelopment.

In the neonatal period, infants randomized to tight glycemic control had reduced linear growth to the end of the trial at 36 weeks' PMA, determined by knemometry, although absolute lengths were not different between the 2 groups (Alsweiler et al., 2012b). Our current findings suggest that this difference in growth trajectory persisted after the end of the trial, with shorter

stature at age 7 years, owing primarily to shorter leg length, and on average 0.6 SD less linear catch-up growth between 36 weeks' PMA and 7 years in the tight group.

Several possible mechanisms may contribute to this persistent difference in linear growth. Leg growth, via the epiphyseal growth plate, is the primary means of height accrual in the pre-pubertal child, and leg length is reduced in conditions of undernutrition, metabolic stress or severe illness (Bogin, Silva, & Rios, 2007; Gunnell, Smith, Frankel, Kemp, & Peters, 1998). Thus leg length may be reduced in the tight group secondary to the increased rate of hypoglycemia seen in this group, although this effect would not be expected to persist beyond the period of insulin treatment. Bone is an insulin sensitive tissue and osteoblast differentiation (which increases bone formation) might be promoted by exogenous insulin dosing (Oldknow, MacRae, & Farquharson, 2015). However, we did not find any associations between neonatal insulin dose and height at 7 years.

Our finding that infants randomized to tight glycemic control had increased weight but not length in the neonatal period led to concern that tight glycemic control may change body composition, potentially through increased fat mass (Alsweiler et al., 2012b). However at 7 years, children in the tight group unexpectedly had greater height-adjusted lean mass without a difference in fat mass. These long-term alterations in body composition are in keeping with findings in burned children randomized to receive insulin or no insulin post-burn injury, where insulin-randomized patients had more lean mass at 12 months post-treatment (Finnerty et al., 2014).

Given the changes in body composition, the implications of altered linear growth in our cohort are not clear. Because height *z*-scores in the tight group lie closer to the population mean compared with those in the standard group, it is possible that the standard group is showing relatively accelerated linear growth, a prepubertal growth pattern associated with increased accrual of adipose tissue and markers of cardio-metabolic risk (Haugaard et al., 2016). If so,

pubertal peak growth velocity may occur earlier (Luo, Cheung, He, Albertsson-Wikland, & Karlberg, 2003), and result in a relative reduction in adult height in the standard group, which may be associated with impaired long-term metabolic health (Mueller & Pereira, 2015).

Despite concerns that increased rates of neonatal hypoglycemia in the tight control group might result in visual cortex injury (Filan et al., 2006), no child was blind. As all infants were receiving intensive care, with close management of blood glucose concentration, it may be that no infant remained hypoglycemic long enough for permanent visual cortex damage to become evident. Posterior brain magnetic resonance imaging abnormalities associated with neonatal hypoglycaemia are not consistently accompanied by evidence of vision loss (Paudel et al., 2017), and could explain why we have not captured any functional effects in our assessments.

The lower fasting blood glucose concentrations in the children randomized to tight glycemic control may be related to the greater height-adjusted lean mass found in this group, possibly reflecting increased availability of skeletal muscle tissue for glucose disposal (Abdulla et al., 2014). However, if this were the case, greater glucose effectiveness, a measure of insulin-independent glucose uptake, would also be expected. Because neither glucose effectiveness nor any other measure of glucose tolerance or insulin metabolism differed between the 2 groups, the finding of decreased fasting blood glucose concentrations in the tight control group could be a type II error owing to multiple comparisons. Because all fasting blood glucose measures were within the normal range, further assessment of insulin-glucose metabolism after puberty is needed to determine the clinical significance, if any, of this finding.

We achieved a follow up rate of 77% for the primary outcome. The children assessed at 7 years corrected age were similar to the children not assessed in all perinatal characteristics except for weight, length and head circumference *z*-scores. Raw weight, length and head circumference measures were not different between children assessed and not assessed at 7 years, and it is unlikely that the differences in *z*-scores alone had a meaningful influence on

our results. Thus the cohort of children assessed is likely to be representative of those originally randomized.

The sample size for this follow-up study was predetermined by the size of the original inception cohort, and had limited power to detect differences between groups in the primary outcome of survival without neurodevelopmental impairment, the outcome we considered likely the most important to both parents and health professionals caring for infants with hyperglycemia. A retrospective power calculation found that this study had 80% power to detect a 36% difference in the primary outcome of survival without neurodevelopmental impairment, a substantially larger difference than the observed 7% difference between the 2 groups. Nevertheless, our findings report a unique cohort of children assessed in mid-childhood after a randomized trial, and no other similar data are available on the effects of management of neonatal hyperglycaemia on long-term outcomes.

Only 37% of hyperglycemic preterm infants randomized to a trial of neonatal glycemic control survived without neurodevelopmental impairment to 7 years corrected age, and randomization to tight glycemic control did not change this outcome. However, tight glycemic control for a relatively brief period up to 36 weeks' PMA caused decreased linear growth and altered body composition 7 years later.

6.5. Figure and tables

Figure 6-1: Recruitment of participants to the follow up study

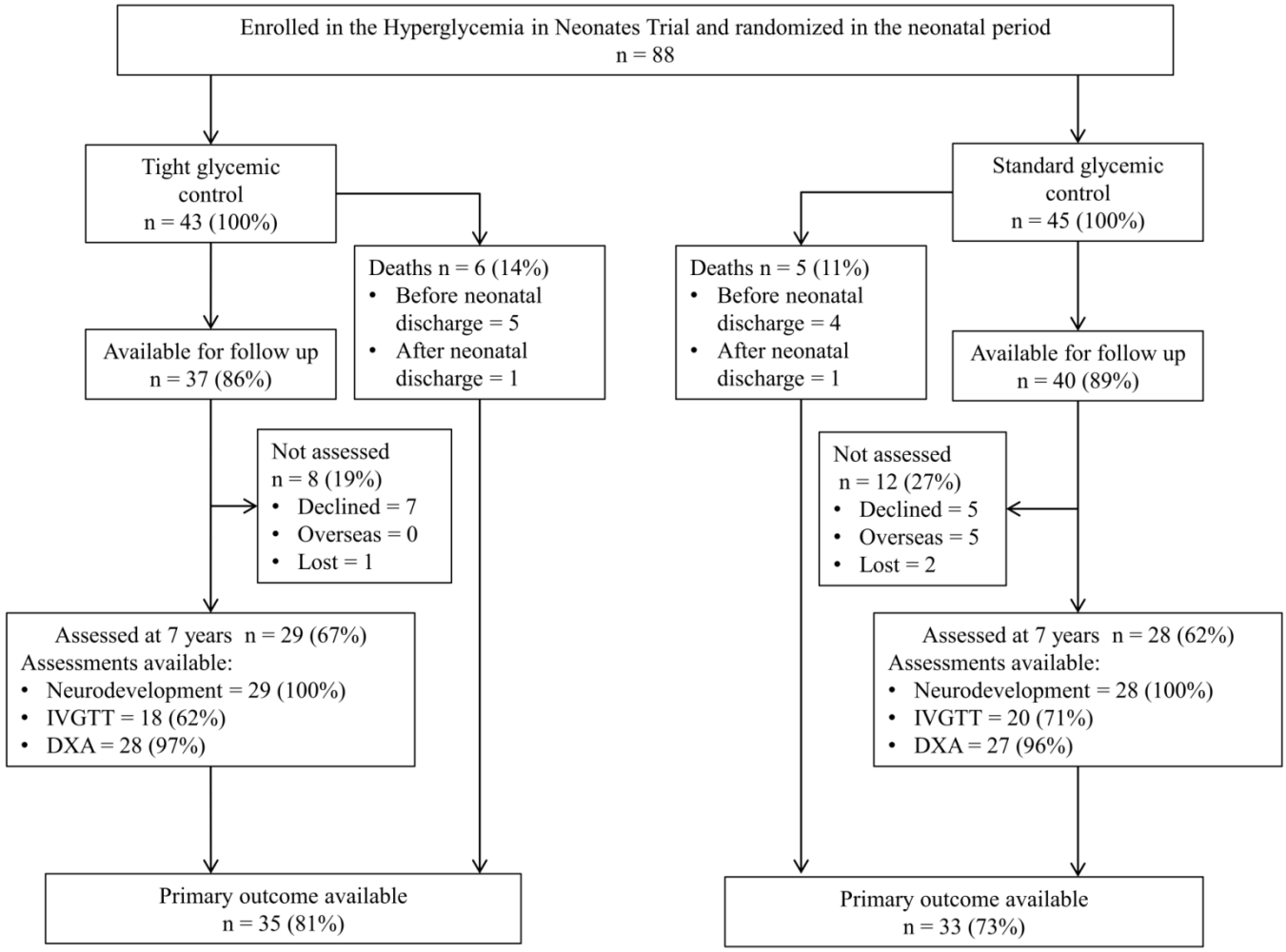

Table 6-1: Perinatal characteristics of children eligible for follow up at 7 years who were and were not assessed and who were randomized to tight or standard glycemic control

	Total randomized n = 88	**Eligible, not assessed n = 20**	**Assessed at 7 years n = 57**	
			Tight n = 29	**Standard n = 28**
Maternal diabetes	4 (5%)	1 (5%)	2 (7%)	0
Multiple pregnancy	32 (36%)	8 (40%)	12 (41%)	8 (29%)
Prioritised ethnicity				
Māori	32 (36%)	7 (35%)	10 (34%)	10 (36%)
Pacific Island	14 (16%)	7 (35%)	4 (14%)	3 (11%)
Asian	17 (19%)	3 (15%)	8 (28%)	3 (11%)
NZ European / Other	25 (29%)	3 (15%)	7 (24%)	12 (43%)
Deprivation index				
Most deprived decile	18 (20%)	5 (25%)	8 (28%)	3 (11%)
Least deprived decile	6 (7%)	0	4 (14%)	2 (7%)
Received any antenatal steroids	77 (88%)	19 (95%)	24 (83%)	25 (89%)
Inborn	80 (91%)	16 (80%)	28 (97%)	26 (93%)
Male	42 (48%)	9 (45%)	11 (38%)	14 (50%)
CRIB II score	12 (4)	13 (4.5)	11 (3)	12 (3)
Gestational age (weeks)	25 (24 - 27)	25 (24 – 26)	25 (25 – 27)	25 (25 – 27)

Table 6 – 1 continued…	**Total randomized n = 88**	**Eligible, not assessed n = 20**	**Assessed at 7 years n = 57**	
			Tight n = 29	**Standard n = 28**
Birth measurements				
Weight (g)	793 (693 – 905)	851 (700 – 993)	725 (670 – 840)	825 (723 – 920)
Weight *z*-score	0.07 (-0.59 – 0.54)	0.45 (0.14 – 0.77) †	-0.10 (-0.53 – 0.49)	0.07 (-0.87 – 0.62)
Crown-heel length (cm)	33.0 (31.5 – 34.5)	34.0 (31.0 – 37.0)	32.5 (31.0 – 34.0)	34.0 (32.0 – 34.5)
Length *z*-score	-0.03 (-0.91 – 0.66)	0.45 (-0.06 – 1.31) †	-0.15 (-0.97 – 0.58)	-0.26 (-0.97 – 0.66)
Head circumference (cm)	23.5 (22.5 – 25.0)	24.0 (22.6 – 26.0)	23.3 (22.1 – 24.0)	24.0 (23.0 – 25.0) *
Head circumference *z*-score	0.09 (-0.57 – 0.88)	0.82 (0.07 – 1.20) †	-0.38 (-1.03 – 0.62)	0.58 (-0.21 – 0.88)
Small for gestational age	10 (11%)	1 (5%)	4 (14%)	4 (14%)
Intraventricular hemorrhage (grade III /IV)	8 (9%)	2 (10%)	3 (10%)	1 (4%)
Periventricular leukomalacia	2 (2%)	0	0	0
Necrotizing enterocolitis	3 (3%)	0	1 (4%)	0
Retinopathy of prematurity (grade III/IV)	13 (15%)	3 (15%)	6 (21%)	3 (11%)
Early onset sepsis	1 (1%)	1 (5%)	0	0
Late onset sepsis	24 (27%)	7 (35%)	7 (24%)	5 (18%)
Major neonatal surgery	12 (14%)	2 (10%)	5 (17%)	2 (7%)
Chronic lung disease	35 (40%)	9 (45%)	13 (45%)	11 (39%)

Table 6 – 1 continued…	**Total randomized n = 88**	**Eligible, not assessed n = 20**	**Assessed at 7 years n = 57**	
			Tight n = 29	**Standard n = 28**
Postnatal corticosteroid exposure	29 (33%)	7 (35%)	8 (28%)	11 (39%)
Month 1 Protein intake ($g.kg^{-1}.day^{-1}$)[a]	3.3 (0.3)	3.2 (0.3)	3.3 (0.3)	3.3 (0.3)
Month 1 Energy intake ($Kcal.kg^{-1}.day^{-1}$)[a]	120 (14)	120 (11)	118 (16)	124 (11)
36 week measurements				
Weight *z*-score	-0.95 (-1.7 – -0.51)	-0.62 (-1.17 – -0.38) †	-1.09 (-1.80 – -0.60)	-1.22 (-1.98 – -0.54)
Length *z*-score	-1.56 (-2.14 – -0.94)	-1.28 (-1.87 – -0.74)	-1.62 (-2.26 – -0.81)	-1.61 (-2.11 – -1.03)
Head circumference *z*-score	-1.13 (-1.65 – -0.44)	-1.25 (-1.52 – -0.32)	-1.11 (-1.79 – -0.51)	-1.29 (-1.60 – -0.48)
Duration of neonatal stay (days)	97 (81 – 115)	106 (88 – 125)	99 (85 – 116)	93 (79 – 109)
Discharged with home oxygen	24 (27%)	5 (25%)	12 (41%)	7 (25%)
Died				
Prior to neonatal discharge	9 (10%)	0	0	0
Post neonatal discharge	2 (2%)	0	0	0

Data are n (%) or mean (SD) or median (IQR). † $P < .05$ for the comparison with children assessed at 7 years. * $P < .05$ for the comparison with the tight glycemic control group.

a: For the number with full nutrition data: Total randomized n = 79; Eligible not assessed n = 17; Assessed, randomized to tight glycemic control n = 28; Assessed, randomized to standard glycemic control n = 28.

Table 6-2: Primary outcome and components of the primary outcome for children assessed at 7 years who were randomized to tight or standard glycemic control

	Tight n = 35	**Standard n = 33**	**OR (95% CI)**	**aOR [§] (95% CI)**
Survived without neurodevelopmental impairment	14 (40%)	11 (33%)	1.33 (0.46 – 3.66)	1.14 (0.40 – 3.20)
Died	6 (17%)	5 (15%)	1.16 (0.29 – 4.57)	1.38 (0.35 – 5.42)
Assessed at 7 years	**n = 29**	**n = 28**		
Neurodevelopmental Impairment	15 (52%)	17 (61%)	0.69 (0.24 – 2.01)	0.75 (0.25 – 2.21)
WISC full scale IQ < 85	12 (41%)	14 (50%)	0.71 (0.24 – 2.04)	0.74 (0.25 – 2.20)
Movement ABC-2 total score ≤ 5th centile	9 (31%)	9 (32%)	0.95 (0.31 – 2.88)	0.99 (0.29 – 3.43)
Cerebral palsy	3 (10%)	3 (11%)	0.96 (0.17 – 5.38)	1.02 (0.18 – 5.74)
Blind	0	0	-	-
Deaf	0	1 (4%)	-	-

Data are n (%).

[§] Adjusted for sex, small for gestational age and multiple birth.

Table 6-3: Neurodevelopmental, growth, blood pressure, glucose tolerance, body composition and quality of life outcomes for children assessed at 7 years who were randomized to tight or standard glycemic control

	Tight n = 29	Standard n = 28	OR or mean difference (95% CI)	aOR or mean difference [§] (95% CI)
Cognitive outcomes				
Cognitive impairment	12 (41%)	14 (50%)	0.71 (0.24 – 2.04)	0.74 (0.25 – 2.20)
Mild	9 (31%)	11 (39%)	-	-
Moderate	1 (3%)	3 (11%)	-	-
Severe	2 (7%)	0	-	-
Full scale IQ score	84 (15)	87 (15)	-3.25 (-10.77 – 4.29)	-3.50 (-11.09 – 4.08)
Motor outcomes				
Motor impairment	11 (37%)	16 (57%)	0.46 (0.16 – 1.32)	0.45 (0.15 – 1.36)
At risk	2 (7%)	7 (25%)	-	-
Severe	9 (31%)	9 (32%)	-	-
Motor ABC-2 total score	7 (4)	7 (4)	0.20 (-1.50 – 1.91)	0.11 (-1.58 – 1.79)
Impaired visual motor integration	6 (21%)	6 (21%)	1.00 (0.27 – 3.77)	0.84 (0.20 – 3.63)

Table 6-3 continued…	**Tight n = 29**	**Standard n = 28**	**OR or mean difference (95% CI)**	**aOR or mean difference [§] (95% CI)**
Executive function				
Impaired attention	24 (83%)	23 (82%)	1.39 (0.36 – 5.34)	2.54 (0.52 – 12.55)
Impaired classroom function [a]	7 (25%)	2 (8%)	4.00 (0.74 – 21.4)	4.19 (0.74 – 23.9)
Impaired home function	5 (17%)	10 (36%)	0.38 (0.10 – 1.35)	0.38 (0.10 – 1.42)
Growth				
Weight (kg)	24.6 (8.5)	26.1 (7.4)	-1.53 (-4.93 – 1.88)	-1.10 (-4.52 – 2.32)
Weight *z*-score	-0.12 (1.60)	0.35 (1.54)	-0.46 (-1.17 – 0.25)	-0.36 (-1.06 – 0.34)
Height (cm)	121.3 (6.3)	125.1 (5.4)	-3.8 (-6.5 – -1.1)**	-3.4 (-6.0 – -0.73)*
Height *z*-score	-0.22 (1.17)	0.47 (1.01)	-0.70 (-1.19 – -0.20)**	-0.63 (-1.12 – -0.14)*
Sitting height (cm)	66.6 (3.8)	68.1 (2.6)	-1.56 (-3.25 – 0.13)	-1.45 (-3.00 – 0.11)
Sitting height *z*-score	0.23 (1.25)	0.70 (0.80)	-0.48 (-1.02 – 0.07)	-0.48 (-0.99 – 0.04)
Leg length (cm)	54.8 (3.2)	57.0 (3.6)	-2.25 (-3.99 – -0.51)*	-1.99 (-3.54 – -0.43)*
Leg length *z*-score	-0.58 (3.18)	0.13 (1.11)	-0.71 (-1.24 – -0.16)*	-0.65 (-1.14 – -0.16)**
Head circumference (cm)	51.4 (1.9)	51.7 (1.9)	-0.36 (-1.2 – 0.5)	-0.26 (-1.14 – 0.61)
Head circumference *z*-score	-1.28 (1.34)	-1.07 (1.26)	-0.20 (-0.82 – 0.42)	-0.19 (-0.82 – 0.46)

Table 6-3 continued…	**Tight n = 29**	**Standard n = 28**	**OR or mean difference (95% CI)**	**aOR or mean difference [§] (95% CI)**
BMI	16.4 (3.9)	16.5 (3.5)	-0.05 (-1.72 – 1.61)	0.10 (-1.57 – 1.77)
BMI *z*-score	0.03 (1.58)	0.09 (1.56)	-0.06 (-0.79 – 0.67)	0.04 (-0.66 – 0.74)
Abnormal BMI	8 (28%)	7 (25%)	1.14 (0.37 – 3.51)	1.36 (0.42 – 4.40)
Abdominal circumference (cm)	58.3 (10.9)	58.5 (9.0)	-0.20 (-5.35 – 4.9)	0.20 (-4.06 – 4.45)
Z-score change birth to 7 years				
Weight	0.05 (1.34)	0.44 (1.46)	-0.39 (-1.04 – 0.26)	-0.30 (-0.99 – 0.40)
Length/ height	0.09 (1.21)	0.68 (1.15)	-0.59 (-1.20 – 0.02)	-0.56 (-1.14 – 0.02)
Head circumference	-1.13 (1.58)	-1.39 (1.41)	0.25 (-0.56 – 1.07)	0.30 (-0.53 – 1.12)
Z-score change 36 weeks' PMA to 7 years.				
Weight	1.08 (1.66)	1.57 (1.54)	-0.49 (-1.25 – 0.26)	-0.39 (-1.18 – 0.41)
Length/ height	1.43 (1.04)	2.06 (1.03)	-0.63 (-1.16 – -0.12)*	-0.52 (-1.00 – -0.04)*
Head circumference	-0.06 (1.27)	-0.03 (1.33)	-0.02 (-0.73 – 0.68)	0.06 (-0.60 – 0.71)
Blood pressure				
Systolic blood pressure (mmHg)	98 (10)	97 (10)	1.49 (-3.63 – 6.61)	1.77 (-3.23 – 6.78)
Systolic blood pressure *z*-score	0.19 (0.86)	-0.13 (0.92)	0.31 (-0.15 – 0.77)	0.31 (-0.14 – 0.76)

Table 6-3 continued…	**Tight n = 29**	**Standard n = 28**	**OR or mean difference (95% CI)**	**aOR or mean difference [§] (95% CI)**
Diastolic blood pressure (mmHg)	56 (6)	56 (6)	-0.07 (-2.93 – 2.80)	-0.29 (-3.26 – 2.66)
Diastolic blood pressure *z*-score	-0.14 (0.54)	-0.21 (0.55)	0.08 (-0.19 – 0.35)	0.05 (-0.23 – 0.33)
Mean blood pressure (mmHg)	71 (8)	70 (6)	0.51 (-3.37 – 4.39)	0.11 (-3.76 – 3.99)
Mean blood pressure *z*-score	0.05 (1.12)	-0.02 (0.91)	0.07 (-0.50 – 0.65)	0.01 (-0.56 – 0.59)
Abnormal blood pressure	4 (13%)	3 (10%)	1.28 (0.26 – 6.30)	1.30 (0.24 – 6.89)
Hypertension	3 (10%)	2 (7%)	-	-
Prehypertension	1 (3%)	1 (3%)	-	-
Glucose metabolism	**n = 18**	**n = 20**		
Fasting blood glucose concentration ($mg.dL^{-1}$)	84.6 (6.30)	90.0 (5.58)	-4.5 (-8.10 – -0.72)*	-3.6 (-6.84 – -0.36)*
Fasting insulin concentration ($mIU.L^{-1}$)	6.15 (6.1)	7.35 (6.7)	-1.20 (-5.15 – 2.75)	-0.87 (-4.89 – 3.14)
Glucose effectiveness ($x10^{-2}.min^{-1}$)	3 (1)	3 (1)	0.34 (-0.34 – 1.00)	0.17 (-0.50 0- 0.83)
Glucose disappearance constant	3.06 (1.20)	2.91 (1.23)	0.15 (-0.64 – 0.94)	-0.10 (-0.79 – 5.9)
Insulin sensitivity $((mU/L)^{-1}.min^{-1})$	7.25 (5.38)	8.53 (3.84)	-1.29 (-4.23 – 1.66)	-1.68 (-4.04 – 0.68)

Table 6-3 continued…	**TIGHT n = 29**	**STANDARD n = 28**	**OR or mean difference (95% CI)**	**Adjusted OR or mean difference § (95% CI)**
Acute insulin response to glucose ($mU.L^{-1}.min$)	443 (344 – 602)	381 (195 – 840)	334 (-383 – 1051)	283 (-339 – 906)
Disposition index	2867 (2257 – 4501)	2476 (1964 – 6781)	-298 (-1806 – 1209)	-331 (-1596 – 934)
Body composition	**n = 28**	**n = 27**		
Fat mass (kg)	5.24 (5.50)	5.99 (5.09)	-0.75 (-3.00 – 1.51)	-0.80 (-3.08 – 1.47)
Height adjusted fat mass (kg)	-	-	0.49 (-1.47 – 2.44)	-0.15 (-2.06 – 1.77)
Android/gynoid fat ratio	0.33 (0.11)	0.33 (0.13)	0.006 (-0.06 – 0.07)	-0.002 (-0.06 – 0.06)
Lean mass (kg)	18.09 (3.54)	18.82 (2.76)	-0.72 (-2.24 – 0.80)	-0.40 (-1.79 – 1.00)
Height adjusted lean mass (kg)	-	-	0.97 (0.12 – 1.82) *	1.06 (0.25 – 1.87) **
Bone mineral density ($g.cm^{-2}$)	0.67 (0.07)	0.69 (0.06)	-0.02 (-0.05 – 0.01)	-0.02 (-0.05 – 0.01)
Height adjusted bone mineral density ($g.cm^{-2}$)	-	-	0.004 (-0.02 – 0.03)	0.0006 (-0.03 – 0.03)
Caregiver reported quality of life	**n = 28**	**n = 28**		
Family cohesiveness score	85 (85 – 100)	85 (60 – 93)	7.86 (-2.24 – 17.96)	8.41 (-1.23 – 18.04)
Global health score	79.8 (21.8)	80.5 (20.4)	-0.71 (-11.95 – 10.52)	1.71 (-9.39 – 12.82)
HUI2 dead-healthy scale	0.9 (0.1)	0.9 (0.1)	0.008 (-0.04 – 0.05)	0.007 (-0.04 – 0.05)

Data are n (%) or mean (SD) or median (IQR). § Adjusted for sex, small for gestational age and multiple birth.

* $P < .05$, ** $P < .01$ for the comparison with the tight glycemic control group. a: n = 28 for children randomized to tight glycemic control, n = 26 for children randomized to standard glycemic control.

Chapter 7. Neonatal hyperglycemia is not associated with outcome at 7 years in children born very preterm: A matched cohort study

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7.1. Introduction

Neonatal hyperglycemia is a common complication of prematurity (Beardsall et al., 2010) and is associated with a number of adverse neonatal outcomes (Alexandrou et al., 2010; Auerbach et al., 2013; Kao et al., 2006). It is not yet established whether these associations are due to hyperglycemia itself, or whether hyperglycemia is acting as a marker for an unwell, metabolically vulnerable and thus high-risk infant. In preterm lambs, experimental hyperglycemia causes sepsis and increased mortality (Alsweiler et al., 2012a), as well as exacerbating the changes in adult pancreatic β-cell mass caused by preterm delivery (Bansal et al., 2015), suggesting that hyperglycemia is causally related to later metabolic impairment. Infants born preterm are at high risk of adverse neurodevelopmental (Hutchinson et al., 2013) and metabolic outcomes (Dalziel et al., 2007; Hofman et al., 2004b), but any additional contribution of neonatal hyperglycemia to these outcomes has been difficult to establish.

7.2. Aim

To determine the effect of neonatal hyperglycemia on neurodevelopment, growth, body composition and glucose metabolism in school age children born very preterm.

7.3. Methods

From 2005 – 2008, a randomized controlled trial (RCT) of tight glycemic control for neonatal hyperglycemia was carried out at the Neonatal Intensive Care Unit (NICU), National Women's Health, Auckland, New Zealand (Alsweiler et al., 2012b). Infants were eligible to participate in the RCT if they were born < 30 weeks' gestation or < 1,500 grams birth weight, had hyperglycemia (defined as consecutive blood glucose concentrations > 8.5 mM (153 $mg.dL^{-1}$) at least 4 hours apart), were not at imminent risk of death and did not have significant

congenital abnormality likely to affect growth and development. A total of 88 infants with neonatal hyperglycemia were randomised, 43 to tight glycemic control (target blood glucose concentration 4 – 6 mM [72 – 108 mg.dL^{-1}]) and 45 to standard glycemic control (target blood glucose concentration 8 – 10 mM [144 – 180 mg.dL^{-1}]). For this matched cohort study, each infant who participated in the RCT and was known to have survived to 7 years' corrected age was matched to an infant who did not meet the criteria for entry to the RCT, was admitted to the NICU during the trial recruitment period and survived to hospital discharge. Matching criteria were decided *a priori* and were applied on a hierarchical basis as follows: sex, gestational age (± 1 week), birth weight *z*-score (± 0.5 SD) (Fenton & Kim, 2013), date of birth (± 3 months), deprivation index at birth (low, medium, high) (Salmond et al., 2007), birth plurality (single/multiple). We had intended to also match on clinical risk index in babies (CRIB II) score (Parry et al., 2003) . However, there was almost no overlap in the range of CRIB II scores between cases and potential controls, so this criterion could not be applied for matching.

As families with children who had taken part in the neonatal RCT were considered more likely to take part in this follow-up study than those who had not participated in a neonatal trial, where possible, more than one control was matched per case to allow for a predicted imbalance in participation rates between groups.

Ethical approval for this study was obtained from the Northern B ethics committee (NTY/12/05/035). Caregivers gave written informed consent and children gave written or verbal assent to participation.

7.3.1 **Assessment**

Children were assessed at 7 years' corrected age, as previously reported (Tottman et al. Submitted for publication). Assessments included: Wechsler Intelligence Scale for Children

4th Edition (Australian) (WISC-IV), Movement Assessment Battery for Children 2nd Edition (MABC-2), Beery-Buktenica test of visual motor integration, Test of Everyday Attention in Children (TEA-Ch)- Sky Search (selective attention), Score! (sustained attention), Creature counting (shifting attention) and Sky Search DT (dual task attention) subtests only (All Pearson, Texas, USA); neurological examination and functional motor assessment (Palisano et al., 2000) by a pediatrician; visual acuity assessment (McGraw & Winn, 1993) by an optometrist; anthropometric measures using standard techniques with results averaged and converted to *z*-scores (De Onis et al., 2007); body composition using dual x-ray absorptiometry (Lunar prodigy utilising enCORE software, both GE Healthcare, USA); blood pressure using an oscillometric method and converted to *z*-scores (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004); and a modified frequently sampled glucose tolerance test (Cutfield et al., 1990), with fasting glucose and insulin concentrations taken as the average of three baseline samples, and Bergman's minimal model (Bergman, 1989) utilizing MinMod Millennium software (v. 6.02, Millennium, USA) to calculate measures of glucose-insulin metabolism. Caregivers and teachers were asked to complete the following standardised questionnaires: Behavior rating inventory of executive function (BRIEF: PAR Inc., Florida, USA); ASEBA child behaviour checklist (ASEBA CBCL: ASEBA, Vermont, USA), and child health questionnaire (CHQ: Healthactchq Inc, Massachusetts, USA- completed by caregivers only). Caregivers were also asked to complete a demographic questionnaire. All assessment was performed blind to participants' neonatal glycemia status.

7.3.2 **Outcomes**

The primary outcome was neurodevelopmental impairment at 7 years' corrected age, defined as any of: WISC-IV full scale IQ <85 (<-1SD); MABC-2 total score ≤5th centile; cerebral palsy; blind (presenting visual acuity 6/60 or worse in the best eye), or deaf requiring aids.

Secondary outcomes include individual components of the primary outcome, impaired attention (age-scaled score <-1SD on any TEA-Ch subtest), impaired executive function (global executive composite T-score ≥65 on the caregiver (home) or teacher (classroom) BRIEF), global behaviour *z*-score (CHQ) and total problem score (CBCL), growth, abnormal blood pressure (prehypertension defined as an averaged systolic or diastolic blood pressure ≥ 90th percentile, and hypertension ≥95th percentile for sex and height (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004)), glucose-insulin metabolism and measures of body composition.

7.3.3 **Statistical methods**

Analyses were performed using SAS v.9.4 and JMP v.11.2.0. Significance level was set at 5% with no adjustment for multiple comparisons. Continuous variables are summarised using mean (standard deviation) or median (inter-quartile range), and compared between groups using two-sample t-test or Wilcoxon test for non-normal data. Categorical variables are summarised as frequencies and percentages, and compared between groups using Chi-square test or Fishers' exact test with small counts <5.

Outcomes assessed at 7 years' corrected age were compared between groups using generalised linear regression models with an appropriate link function, both unadjusted and adjusted for all matching criteria (considered *a priori* as potential confounders). Results are presented as mean differences for continuous variables, or odds ratios for categorical variables with 95% confidence intervals. In the primary analysis, the presence of twins in the cohort was considered as a cluster effect using generalised estimating equations.

7.4. Results

Of 88 infants randomised in the RCT, 77 (88%) survived to 7 years' corrected age and 57/77 were assessed (74% of survivors) (HYPER group). Their outcomes at 7 years have been

reported previously (Tottman et al, submitted for publication). A total of 94 infants were identified as matched controls, of whom 5 were discovered to have died and 54/89 (61% of survivors) were assessed at 7 years (CONTROL group) (Figure 7-1).

HYPER children assessed at 7 years were more likely to be inborn and to have lesser weight, length and head circumference *z*-scores at birth than those not assessed (Table 7-1). Only the difference in weight *z*-score persisted at 36 weeks' post menstrual age. There were no differences in perinatal characteristics between CONTROL children assessed at 7 and those not assessed. However, despite matching, there were many differences in perinatal characteristics between the HYPER and CONTROL groups. At birth, HYPER children were less likely to be boys, had lower gestational ages, lower Apgar scores and higher CRIB-II scores than CONTROL children. HYPER children also weighed less, were shorter and had smaller head circumferences at birth, 28 days and 36 weeks' postmenstrual age. HYPER children had more chronic lung disease and a longer duration of neonatal stay than CONTROL children, but other neonatal morbidities were similar between groups (Table 7-1). As expected, HYPER children had higher mean blood glucose concentrations prior to 36 weeks' postmenstrual age than CONTROL children, and no CONTROL child had hyperglycemia or received insulin (Table7-1).

HYPER children were almost twice as likely as CONTROL children to have neurodevelopmental impairment at 7 years, and to have an IQ score <85 (Table 7-2). However, these differences were no longer significant after adjustment for matching variables. Children in both groups had similar rates of motor scores $\leq 5^{th}$ centile, cerebral palsy (which was mild in 8/9 (89%) cases), blindness and deafness (Table 7-2).

Full scale IQ scores were low in the cohort as a whole (mean ± SD, 89 ± 16). HYPER children had full scale IQ scores an average of 7 points lower than CONTROL, and also had lower processing speed and perceptual reasoning scores (Table 7-3). Only the differences in

perceptual reasoning scores persisted after adjustment. Motor ABC-2 total scores were lower in the HYPER group compared to CONTROL, but again this difference did not persist after adjustment. Minimum threshold performance to allow administration of any of the 4 TEA-Ch sub-tests was not achieved by 3/57 (5%) HYPER and 3/54 (6%) CONTROL children. The incidence of impaired attention was 95/105 (90%) of those who could be tested, and was similar in both groups. Sky Search DT scores were better in the HYPER than the CONTROL group (6±5 vs. 4±4, P=0.02), but there were no differences in dual task attentional impairment scores. Teachers and caregivers perceived HYPER and CONTROL children to have similar rates of impaired executive function in the classroom and home environments. After adjustment for confounders, caregivers rated HYPER children as having better global behaviour *z*-scores and lower total behavioural problem scores than CONTROL children (Table 7-3).

HYPER and CONTROL groups were similar in size at 7 years, although the cohort as a whole had small head circumferences, with mean head circumference *z*-score -1.09 ± 1.43 (Table 4). Blood pressure, fasting blood glucose concentrations, insulin sensitivity, glucose effectiveness and fasting insulin concentrations were also similar between groups. HYPER children had more height-adjusted fat mass than CONTROL, but this difference was no longer significant after adjustment for confounders. Lean mass and bone mineral density were similar between groups (Table7-4).

7.5. Discussion

In this matched cohort study, children who experienced neonatal hyperglycemia had a higher rate of neurodevelopmental impairment at school age than those who had not experienced hyperglycemia, with lower full scale IQ, processing speed and perceptual reasoning scores on standardised testing, and lower motor performance scores. However, there were also important differences between hyperglycemic and control children in their underlying

perinatal characteristics, with infants who developed hyperglycemia being of earlier gestation, lower birthweight and more unwell at birth than those who did not develop hyperglycemia. Once these differences were taken into account, only the decrement in perceptual reasoning scores remained significant.

There are few reports regarding the impact of neonatal hyperglycemia on neurodevelopmental outcomes. A retrospective study showed reduction in normal neurological and behavioral outcomes at 2 years in insulin-treated hyperglycemic preterm infants compared to matched non-hyperglycemic controls (van der Lugt et al., 2010). However, two additional retrospective reviews revealed no adverse effect of neonatal hyperglycemia on standardised developmental testing at 1 year (Heald et al., 2012) and 2 years of age (Ramel et al., 2013). We also did not find any associations between neonatal hyperglycemia and cognitive outcome, with the exception of the subdomain of perceptual reasoning. The perceptual reasoning domain in the WISC-IV comprises measures of visual processing and fluid reasoning (Keith, Fine, Taub, Reynolds, & Kranzler, 2006), and in teenagers, higher perceptual reasoning scores are positively associated with frontal lobe volumes (Schilling et al., 2013). There is some evidence from children with Type 1 diabetes mellitus that hyperglycemic exposure is associated with changes to the brain in the regions of the cuneus / precuneus and prefrontal cortex (Arbelaez, Semenkovich, & Hershey, 2013). However, children with type 1 diabetes are older at hyperglycemia onset, have a high incidence of recurrent hypoglycemia and may suffer diabetic ketoacidosis, making interpretation of comparisons with children affected by neonatal hyperglycemia difficult. High neonatal blood glucose concentrations in the context of respiratory acidosis are associated with abnormal amplitude integrated electronic encephalograph recordings during the first 72 hours after birth in infants born <28 weeks' gestation, suggesting that neonatal hyperglycemia may have immediate deleterious effect on cerebral function (Granot, Meledin, Richardson, Friger, & Shany, 2012). However, it is not clear that this has any prognostic significance for later cognitive ability.

Children born preterm are known to be at risk of attention problems, with a behavioral phenotype featuring inattention more prominently than hyperactivity (Samuelsson et al., 2017), and specific deficiencies in dual task attention (Delane et al., 2016). The cohort as a whole had a very high incidence of attentional impairment but, although the hyperglycemic group had lower scores in the Sky Search DT domain than controls, there was no overall difference in impaired attention between the groups.

Nevertheless, children exposed to neonatal hyperglycemia had better global behavioral *z*-scores and reduced total problem scores on caregiver questionnaires compared to controls. Adolescents and young adults born extremely preterm are reported to have a characteristic internalising, compliant behavioral phenotype(Hille et al., 2008; Samuelsson et al., 2017). Since the children exposed to neonatal hyperglycemia were born earlier and of lower birthweight than controls, their caregivers' reports of reduced behavioral problems may reflect a greater degree of this compliant phenotype in mid-childhood.

We did not find any differences in growth measures between children exposed to hyperglycemia and controls. In a previous retrospective study of 80 preterm infants (Ramel et al., 2013), those exposed to neonatal hyperglycemia were heavier, longer and had bigger heads at term corrected age than those who did not become hyperglycemic. However, by 15 months' corrected age, those who did not become hyperglycemic were bigger in all growth measures, and this difference persisted at 2 years. Without interim growth measures, we cannot determine whether neonatal hyperglycemia altered the infantile growth trajectories of our cohort, but it is reassuring that neonatal hyperglycemia does not appear to be associated with different growth or body composition by 7 years' corrected age.

It is also reassuring that insulin secretion, sensitivity and glucose tolerance did not appear to be altered in children who experienced neonatal hyperglycemia. Human pancreatic beta-cell mass increases rapidly after the 20^{th} week of gestation and continues to expand, albeit more

slowly, in the immediate postnatal period (Bouwens & Rooman, 2005). Beta-cells are responsive to the fetal glycemic environment, with chronic maternal hyperglycemia shown to decrease, and pulsatile maternal hyperglycemia to increase, pancreatic beta-cell mass in fetal sheep (Frost, Zehri, Limesand, Hay Jr, & Rozance, 2012) . Human fetuses exposed to hyperglycemia *in utero* also demonstrate altered beta-cell function in childhood (Bush et al., 2011), and preterm birth is known to decrease insulin sensitivity in adulthood (Dalziel et al., 2007) (Mathai et al., 2012). Our cohort is at risk of insulin resistance in adulthood, and thus would be expected to have reduced insulin sensitivity in mid-childhood compared to infants born at term (Hofman et al., 2004b). However, the finding that insulin sensitivity was unchanged in the hyperglycemic group suggests that neonatal hyperglycemic exposure does not significantly increase the risk of later insulin resistance above the risk caused by preterm birth itself.

This is the largest and oldest cohort of children with neonatal hyperglycemia to be reported and thus provides unique information regarding the long-term outcomes of neonatal hyperglycemia. As we have previously shown (Tottman et al., 2017) infants with hyperglycemia are the smallest, sickest infants in the NICU and despite our efforts to match hyperglycemic with control infants, there were persistent differences in baseline characteristics between the two groups. After adjustment for these characteristics the odds ratio for the primary outcome decreased and was no longer statistically significant, but the 95% confidence intervals were wide (2.36(1.08-5.14) vs 1.91(0.71-5.08)). This may reflect the size of the cohort, which was determined by that of the original RCT and, whilst sufficient to detect large effects, may have limited power to detect small but clinically important differences.

We did not have access to continuous glucose monitoring in the neonatal period, and although not clinically identified or managed as having neonatal hyperglycemia, children matched as non-hyperglycemic controls may have had large excursions of neonatal blood glucose

concentrations, including occasional hyperglycemia, without meeting the entry criteria for the RCT (Alsweiler et al., 2012b).

Hyperglycemic infants were randomised to treatment with tight or standard glycemic control, and this treatment may have confounded our results. However, all infants in the hyperglycemic group were managed to keep neonatal blood glucose concentrations <10 mM (180 mg.dL^{-1}), and it is possible the lack of an association between neonatal hyperglycemia and outcomes in our study reflects the success of this management strategy. Further, we have previously reported that the only differences between randomisation groups were shorter standing height, lower fasting glucose and increased lean mass in the tight control group (Tottman et al. Submitted for publication).

7.6. Conclusion

At 7 years corrected age, children born very preterm who became hyperglycemic in the neonatal period had a higher incidence of neurodevelopmental impairment than children who did not become hyperglycemic. However, neonatal hyperglycemia occurs in children who are smaller and sicker at birth than those who do not become hyperglycemic. After correction for these factors, neonatal hyperglycemia is no longer independently associated with neurodevelopmental, growth or metabolic outcomes in childhood.

7.7. Figure and tables

Figure 7-1: Flow diagram of participants in the matched control study

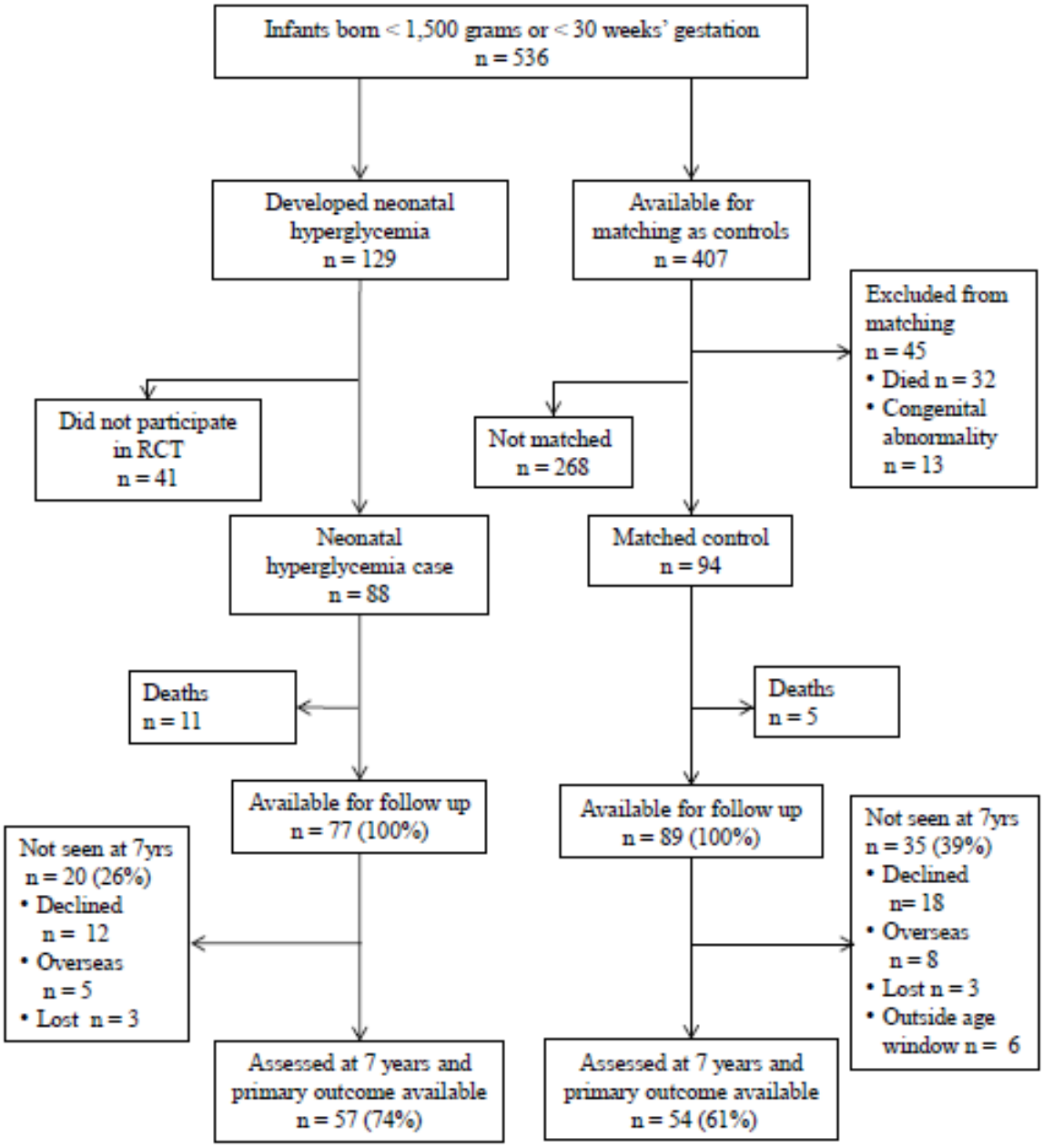

Table 7-1: Perinatal characteristics of children eligible for assessment who were and were not assessed at 7 years corrected age

	Neonatal hyperglycemia n = 77		**Controls n = 89**		**HYPER vs. CONTROL *P* value**
	HYPER n = 57	**Not assessed n = 20**	**CONTROL n = 54**	**Not assessed n = 35**	
Inborn	54 (95%)	16 (80%)	48 (89%)	34 (97%)	0.31
Male sex	25 (44%)	9 (45%)	34 (63%)	21 (60%)	0.04
Multiple birth	20 (35%)	8 (40%)	13 (24%)	5 (14%)	0.20
Received antenatal steroids	49 (86%)	19 (95%)	51 (94%)	34 (97%)	0.80
CRIB-II score	12 (10 to 13)	13 (10 to 15)	9 (8 to 10)	9 (8 to 10)	<0.001
Apgar score					
1 minute	5 (3 to 7)	6 (4 to 7)	6 (4 to 8)	6 (4 to 8)	0.03
5 minute	8 (7 to 9)	8 (7 to 9)	8 (7 to 9)	8 (8 to 9)	0.09
Gestational age (weeks)	25 (25 to 27)	25 (24 to 26)	26 (26 to 28)	27 (26 to 29)	0.007
Birth measurements					
Weight (g)	804 ± 151	884 ± 233	1010 ± 222	991 ± 233	<0.001
Weight *z*-score	-0.13 ± 0.91*	0.47 ± 0.90	0.32 ± 0.85	0.03 ± 0.97	0.002
Crown-heel length (cm)	33.1 ± 2.3	34.3 ± 3.3	35.7 ± 2.5	35.6 ± 2.5	< 0.001

Table 7-1 continued…	**Neonatal hyperglycemia n = 77**		**Controls n = 89**		**HYPER vs. CONTROL *P* value**
	HYPER n = 57	**Not assessed n = 20**	**CONTROL n = 54**	**Not assessed n = 35**	
Length *z*-score	-0.29 ± 1.15*	0.36 ± 1.08	0.26 ± 0.92	0.06 ± 1.06	0.009
Head circumference (cm)	23.7 ± 1.6	24.4 ± 2.1	25.1 ± 1.8	25.1 ± 1.5	< 0.001
Head circumference *z*-score	-0.02 ± 1.14*	0.67 ± 1.21	0.42 ± 0.95	0.12 ± 1.11	0.03
Birth weight *z*-score <-2SD	8 (14%)	1 (5%)	3 (6%)	4 (11%)	0.14
Intraventricular hemorrhage (grade III/ IV) [35]	4 (7%)	2 (10%)	2 (4%)	0 (0%)	0.44
Periventricular leukomalacia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
Necrotizing enterocolitis (≥ Bell stage 2) [36]	1 (2%)	0 (0%)	4 (7%)	1 (3%)	0.20
Retinopathy of prematurity (grade III/ IV) [37]	9 (16%)	3 (15%)	4 (7%)	1 (3%)	0.24
Early onset sepsis [38]	0 (0%)	1 (5%)	1 (2%)	2 (6%)	0.49
Late onset sepsis [38]	12 (21%)	7 (35%)	7 (13%)	4 (11%)	0.32
Major neonatal surgery [38]	7 (12%)	2 (10%)	3 (6%)	1 (3%)	1.0
Chronic lung disease [38]	24 (42%)	9 (45%)	12 (22%)	12 (34%)	0.03
Length of neonatal stay (days)	95 (81 to 115)	106 (88 to 125)	85 (67 to 99)	78 (59 to 95)	0.01
Discharged with home oxygen	19 (33%)	5 (25%)	11 (20%)	7 (20%)	0.14

Table 7-1 continued…	**Neonatal hyperglycemia n = 77**		**Controls n = 89**		**HYPER vs. CONTROL *P* value**
	HYPER n = 57	**Not assessed n = 20**	**CONTROL n = 54**	**Not assessed n = 35**	
36 week measurements					
Weight (g)	2193 ± 408	2354 ± 366	2408 ± 323	2454 ± 380	0.006
Weight *z*-score	-1.21 ± 0.96*	-0.78 ± 0.85	-0.67 ± 0.8	-0.58 ± 0.85	0.003
Length (cm)	42.9 ± 2.3	43.2 ± 2.7	45.0 ± 1.9	44.4 ± 1.8	< 0.001
Length *z*-score	-1.6 ± 0.91	-1.41 ± 0.99	-0.83 ± 0.76	-1.03 ± 0.77	< 0.001
Head circumference (cm)	30.8 ± 1.4	30.9 ± 1.7	31.4 ± 1.3	32.0 ± 1.3	0.03
Head circumference *z*-score	-1.19 ± 0.90	-1.06 ± 1.08	-0.79 ± 0.92	-0.40 ± 0.92	0.04
Glycemia (birth to 36 weeks')					
Hyperglycemia	57 (100%)	20 (100%)	0 (0%)	0 (0%)	-
Mean blood glucose concentration (mM)	6.6 ± 0.9	6.6 ± 0.9	5.2 ± 0.6	5.3 ± 0.7	<0.001
Received insulin	44 (77%)	15 (75%)	0 (0%)	0 (0%)	<0.001

Data are n (%), median (IQR) or mean ± SD.

* $P < 0.05$ for the comparison with neonatal hyperglycemia cases not assessed

Table 7-2: Primary outcome and its components in HYPER and CONTROL children at 7 years

	HYPER (n = 57)	**CONTROL (n = 54)**	**Odds Ratio (95% CI)**	***P* value**	**†Adjusted OR (95% CI)**	**†Adjusted *P* value**
Neurodevelopmental Impairment	32 (56%)	19 (35%)	2.36 (1.08 to 5.14)	0.03	1.91 (0.71 to 5.08)	0.20
Full scale IQ <85	26 (46%)	14 (26%)	2.40 (1.07 to 5.36)	0.03	2.01 (0.69 to 5.83)	0.20
Movement ABC-2 score $\leq 5^{th}$ centile	18 (32%)	14 (26%)	1.32 (0.59 to 2.93)	0.50	0.86 (0.33 to 2.21)	0.75
Cerebral palsy	6 (11%)	3 (6%)	2.00 (0.48 to 8.38)	0.34	2.42 (0.48 to 12.29)	0.29
Blind	0 (0%)	0 (0%)	-	-	-	-
Deaf requiring aids	1 (2%)	1 (2%)	-	-	-	-

Data are n (%).

†Odds ratios adjusted for sex, gestational age, birthweight *z*-score, deprivation index, multiple birth and CRIB II score.

Table 7-3: Cognitive, motor, executive function and behavioral outcomes in HYPER and CONTROL children at 7 years

	HYPER n = 57	**CONTROL n = 54**	**OR or mean difference (95% CI)**	***P* value**	**†Adjusted OR or mean difference (95% CI)**	**†Adjusted *P* value**
Cognitive outcomes						
Full scale IQ score	85 ± 15	92 ± 15	-6.6 (-12.4 to -0.8)	0.03	-2.3 (-7.7 to 3.0)	0.39
Verbal comprehension score	93 ± 14	96 ± 13	-3.1 (-8.4 to 2.2)	0.26	1.3 (-4.5 to 7.1)	0.66
Perceptual reasoning score	84 ± 13	93 ± 14	-9.3 (-14.4 to -4.2)	<0.001	-6.9 (-11.6 to -2.1)	0.005
Working memory score	88 ± 14	91 ± 15	-2.6 (-7.8 to 2.6)	0.33	0.4 (-5.6 to 6.5)	0.89
Processing speed score	89 ± 14	96 ± 14	-7.0 (-12.4 to -1.6)	0.01	-4.8 (-10.7 to 1.0)	0.11
Motor Outcomes						
Motor ABC-2 total score	7 ± 3	9 ± 4	-1.4 (-2.7 to -0.1)	0.04	-0.7 (-1.9 to 0.6)	0.29
Balance and co-ordination score	8 ± 3	9 ± 3	-0.6 (-1.8 to 0.6)	0.32	0.1 (-1.0 to 1.1)	0.92
Motor dexterity score	7 ± 3	8 ± 4	-1.2 (-2.7 to 0.2)	0.09	-1.2 (-2.7 to 0.2)	0.10
Aiming and catching score	10 ± 3	10 ± 3	-0.8 (-1.8 to 0.2)	0.11	-0.1 (-1.4 to 1.2)	0.86
Impaired visual motor integration	12 (21%)	10 (19%)	1.2 (0.5 to 3.0)	0.74	0.5 (0.2 to 1.5)	0.22
Visual motor integration score	91 ± 8	92 ± 8	-0.8 (-3.8 to 2.2)	0.61	-0.1 (-3.4 to 3.2)	0.96
Executive Function Outcomes						
"Sky search" score [a]	6 ± 3	7 ± 3	-1.2 (-2.5 to 0.1)	0.06	-0.8 (-2.2 to 0.7)	0.30
"Score!" score [b]	6 ± 4	7 ± 3	-0.6 (-1.8 to 0.6)	0.34	-0.1 (-1.7 to 1.6)	0.96
"Creature counting" score [c]	5 ± 3	7 ± 3	-1.1 (-2.3 to 0.1)	0.06	0.2 (-1.2 to 1.5)	0.81

Table 7-3 continued…	**HYPER n = 57**	**CONTROL n = 54**	**OR or mean difference (95% CI)**	***P* value**	**†Adjusted OR or mean difference (95% CI)**	**†Adjusted *P* value**
"Sky search DT" score [d]	6 ± 5	4 ± 4	1.8 (-0.1 to 3.7)	0.06	2.5 (0.4 to 4.7)	0.02
Impaired attention [e]	47 (87%)	48 (89%)	0.4 (0.1 to 1.8)	0.24	0.1 (0.0 to 1.1)	0.07
Selective	25 (44%)	18 (33%)	-	-	-	-
Sustained	31 (54%)	21 (39%)	-	-	-	-
Shifting	29 (51%)	26 (48%)	-	-	-	-
Dual task	23 (40%)	36 (67%)	-	-	-	-
Behavioral outcomes						
Impaired executive function						
Classroom	9 (16%)	6 (11%)	1.5 (0.5 to 4.4)	0.43	0.8 (0.2 to 3.4)	0.77
Home	15 (26%)	8 (15%)	2.0 (0.8 to 5.2)	0.15	1.6 (0.5 to 5.7)	0.45
Executive function scores						
Teacher reported	53.2 ± 9.9	52.4 ± 9.9	0.7 (-2.7 to 4.2)	0.67	-0.8 (-5.4 to 3.7)	0.71
Caregiver reported	54.7 ± 13.4	53.3 ± 11.0	1.4 (-3.2 to 5.9)	0.56	-3.8 (-8.8 to 1.2)	0.14
Global behavior *z*-score	-0.01 ± 0.92	-0.23 ± 1.21	0.22 (-0.18 to 0.62)	0.28	0.44 (0.02 to 0.86)	0.04
Total problem score	50.8 ± 11.8	52.7 ± 11.4	-1.86 (-6.18 to 2.47)	0.40	-6.70 (-11.91 to -1.49)	0.01

Data are n (%), mean ± SD, odds ratios (OR) or mean differences and 95% confidence intervals (95% CI)

† Adjusted for sex, gestational age, birthweight *z*-score, deprivation index, multiple birth and CRIB II score.

Due to failure to meet threshold for testing a: HYPER n = 52, CONTROL n = 49; b: HYPER n = 54, CONTROL n = 51; c: HYPER n = 47, CONTROL n = 47; d: HYPER n = 40, CONTROL n = 45; e: HYPER n = 54, CONTROL n = 51

Table 7-4: Growth, blood pressure, glucose metabolism and body composition outcomes in HYPER and CONTROL children at 7 years

	HYPER n = 57	**CONTROL n = 54**	**OR or mean difference (95% CI)**	***P* value**	**† Adjusted OR or mean difference (95% CI)**	**† Adjusted *P* value**
Growth						
Weight (kg)	25.3 ± 8.0	24.9 ± 5.6	0.45 (-2.28 to 3.19)	0.75	0.08 (-2.88 to 3.05)	0.96
Weight *z*-score	0.11 ± 1.60	0.15 ± 1.25	-0.04 (-0.59 to 0.50)	0.88	-0.10 (-0.71 to 0.51)	0.75
Height (cm)	123.2 ± 6.1	124.2 ± 5.9	-1.03 (-3.39 to 1.33)	0.39	-1.53 (-4.30 to 1.24)	0.28
Height *z*-score	0.12 ± 1.14	0.28 ± 1.11	-0.16 (-0.60 to 0.28)	0.48	-0.27 (-0.79 to 0.24)	0.30
Leg length (cm)	55.8 ± 3.5	56.2 ± 3.5	-0.36 (-1.77 to 1.05)	0.61	-0.65 (-2.46 to 1.15)	0.48
Leg length *z*-score	-0.23 ± 1.10	-0.18 ± 1.12	-0.06 (-0.50 to 0.38)	0.80	-0.20 (-0.76 to 0.36)	0.48
Head circumference (cm)	51.5 ± 1.9	51.9 ± 2.1	-0.32 (-1.09 to 0.45)	0.42	0.37 (-0.44 to 1.18)	0.37
Head circumference *z*-score	-1.18 ± 1.29	-0.99 ± 1.56	-0.18 (-0.73 to 0.36)	0.51	0.22 (-0.39 to 0.83)	0.48
Body mass index ($kg.m^{-2}$)	16.4 ± 3.7	16.0 ± 2.5	0.44 (-0.78 to 1.66)	0.48	0.32 (-1.01 to 1.64)	0.64
Body mass index *z*-score	0.06 ± 1.56	0.00 ± 1.24	0.06 (-0.48 to 0.60)	0.82	0.10 (-0.51 to 0.70)	0.76
Z-score change birth to 7 years						
Weight	0.24 ± 1.40	-0.17 ± 1.33	0.41 (-0.10 to 0.92)	0.11	-0.10 (-0.71 to 0.51)	0.75
Height	0.39 ± 1.21	-0.00 ± 1.35	0.39 (-0.11 to 0.90)	0.13	-0.22 (-0.79 to 0.35)	0.45
Head circumference	-1.26 ± 1.49	-1.40 ± 1.38	0.14 (-0.40 to 0.67)	0.62	0.09 (-0.59 to 0.76)	0.80

Table 7-4 continued…	**HYPER n = 57**	**CONTROL n = 54**	**OR or mean difference (95% CI)**	***P* value**	**† Adjusted OR or mean difference (95% CI)**	**† Adjusted *P* value**
Blood Pressure						
Systolic blood pressure (mmHg)	97 ± 10	99 ± 8	-1.44 (-4.86 to 1.98)	0.41	0.15 (-3.88 to 4.19)	0.94
Systolic blood pressure *z*-score	0.03 ± 0.90	0.12 ± 0.79	-0.08 (-0.41 to 0.24)	0.61	0.07 (-0.31 to 0.45)	0.71
Diastolic blood pressure (mmHg)	56 ± 6	55 ± 6	1.32 (-0.84 to 3.49)	0.23	1.23 (-1.46 to 3.92)	0.37
Diastolic blood pressure *z*-score	-0.17 ± 0.54	-0.31 ± 0.50	0.14 (-0.06 to 0.33)	0.16	0.14 (-0.10 to 0.39)	0.25
Mean blood pressure (mmHg)	70 ± 7	70 ± 6	0.73 (-1.73 to 3.18)	0.56	1.72 (-1.06 to 4.51)	0.23
Mean blood pressure *z*-score	0.02 ± 1.01	-0.09 ± 0.95	0.11 (-0.26 to 0.47)	0.57	0.25 (-0.16 to 0.67)	0.23
Abnormal blood pressure	7 (12%)	2 (4%)	3.64 (0.72 to 18.46)	0.12	3.11 (0.96 to 10.11)	0.06
Hypertension	5 (9%)	2 (4%)	-	-		
Prehypertension	2 (4%)	0	-	-		
Glucose metabolism	**n = 38**	**n = 34**				
Fasting blood glucose concentration (mM)	4.87 ± 0.35	4.92 ± 0.33	-0.05 (-0.20 to 0.10)	0.49	-0.05 (-0.24 to 0.15)	0.64
Fasting insulin concentration ($mIU.L^{-1}$)	6.78 ± 6.35	5.09 ± 2.05	1.69 (-0.44 to 3.82)	0.12	0.04 (-1.76 to 1.85)	0.96
Glucose effectiveness ($x10^{-2}.min^{-1}$)	3 ± 1	3 ± 1	-0.00 (-0.01 to 0.00)	0.34	0.00 (-0.01 to 0.01)	0.90

Table 7-4 continued…	**HYPER n = 57**	**CONTROL n = 54**	**OR or mean difference (95% CI)**	***P* value**	**† Adjusted OR or mean difference (95% CI)**	**† Adjusted *P* value**
Glucose disappearance constant	2.98 ± 1.20	3.11 ± 1.10	-0.13 (-0.64 to 0.37)	0.60	0.01 (-0.52 to 0.54)	0.98
Insulin sensitivity $((mU/L)^{-1}.min^{-1})$	7.93 ± 4.61	9.00 ± 3.91	-1.08 (-3.06 to 0.91)	0.29	-0.31 (-2.45 to 1.83)	0.78
Acute insulin response to glucose $(mU.L^{-1}.min)$	767 ± 1086	493 ± 290	274 (-86 to 635)	0.14	264 (-253 to 780)	0.32
Disposition index $(x10^3)$	3.75 ± 2.37	3.88 ± 1.99	- 0.12 (-1.09 to 0.85)	0.81	-0.07 (-1.18 to 1.05)	0.91
Body Composition	**n = 55**	**n = 52**				
Fat mass (kg)	5.61 ± 5.27	4.63 ± 3.39	0.97 (-0.87 to 2.82)	0.30	0.21 (-1.89 to 2.30)	0.85
Height adjusted fat mass	-	-	1.59 (0.23 to 2.95)	0.02	0.62 (-0.85 to 2.08)	0.41
Lean mass (kg)	18.45 ± 3.17	19.03 ± 2.64	-5.77 (-1.65 to 0.54)	0.31	-0.28 (-1.38 to 0.82)	0.62
Height adjusted lean mass	-	-	-0.23 (-0.88 to 0.42)	0.48	0.20 (-0.45 to 0.85)	0.54
Bone mineral density $(g.cm^{-3})$	0.68 ± 0.06	0.69 ± 0.05	-0.01 (-0.03 to 0.01)	0.51	-0.01 (-0.04 to 0.02)	0.44
Height adjusted bone mineral density	-	-	-0.00 (-0.02 to 0.01)	0.85	-0.00 (-0.02 to 0.02)	0.79

Data are n (%), mean ± SD, odds ratios (OR) or mean differences and 95% confidence intervals (CI)

† Adjusted for sex, gestational age, birthweight *z*-score, deprivation index, multiple birth and CRIB II score.

Chapter 8. Relationships between early neonatal nutrition and neurodevelopment at school age in children born very preterm

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8.1. Background

In the majority of infants born very preterm, immaturity of the gastrointestinal tract, non-availability of maternal breastmilk and critical illness necessitate a period of parenteral nutrition immediately after birth. However, the ideal composition of that nutrition is not yet clear. Deficient postnatal growth associated with iatrogenic malnutrition is a cause for concern, as failure to meet growth potential may be associated with adverse neurodevelopmental outcomes in later life (Ehrenkranz et al., 2006). The cessation of placental blood flow at birth is accompanied by a precipitous drop in protein supply to the infant, and protein supply does not match *in utero* intakes for many days after birth (Cormack & Bloomfield, 2006). Increasing early neonatal protein intakes may mitigate postnatal growth faltering (Cormack & Bloomfield, 2013; Morgan et al., 2014). Although there is clear evidence for the benefit of higher vs lower early enteral protein intake (Lucas et al., 1990), the results of contemporary studies of higher parenteral protein intakes in the very preterm population are mixed (Burattini et al., 2013; Morgan et al., 2014; Scattolin et al., 2013; Tan, Abernethy, & Cooke, 2008; Tan & Cooke, 2008), including reports of worsened growth and neurodevelopmental outcomes (Balasubramanian et al., 2013; Blanco et al., 2012). Furthermore, many studies of neonatal nutrition report only short-term outcomes and do not examine the impact of early nutrition on long-term neurodevelopment.

8.2. Aim

To determine whether a change in neonatal nutrition protocol intended to increase early protein intake was associated with altered neurodevelopmental outcomes in 7 year old children born very preterm and, if so, to determine which individual nutritional components contributed to this difference.

8.3. Methods

8.3.1 **Participants**

Eligible participants for the PIANO (Protein, Insulin And Neonatal Outcomes) study were identified from 3 sources: children who had been recruited as neonates to a randomized controlled trial (RCT) of tight glycemic control for neonatal hyperglycemia (Alsweiler et al., 2012b); children who had been matched to RCT participants as non-hyperglycemic preterm controls, and children included in a contemporaneous audit of the effect of the change in nutrition protocol on neurodevelopmental outcomes at 2 years' corrected age (Cormack et al., 2011). We excluded infants who did not survive to 7 years' corrected age, those exposed to both old and new nutrition protocols within the first 7 days, and those who were transferred in to NICU after 24 hours or transferred out prior to postnatal day 7.

The study received ethics approval from the Northern B ethics committee (NTY/12/05/035), and institutional approval from the Auckland District Health Board (ADHB 5486). Caregivers gave written informed consent, and children verbal assent, to participation.

8.3.2 **Assessment at 7 years' corrected age**

Eligible participants were traced and invited to take part in an assessment at the Liggins Institute, University of Auckland, New Zealand. Assessment procedures have been described previously (Tottman et al, Submitted for publication) and included: Wechsler Intelligence Scale for Children 4th Edition (Australian) (WISC IV); Movement Assessment Battery for Children 2nd Edition (MABC-2); Beery-Buktenica test of visual motor integration; Test of Everyday Attention in Children (TEA-Ch- Sky Search, Score!, Creature Counting and Sky Search DT subtests only) administered by a trained assessor (all Pearson, Texas, USA); neurological examination and functional motor assessment (Palisano et al., 2000) by a

Pediatrician; visual acuity assessment by an optometrist; growth measures using standard techniques with results averaged and converted to *z*-scores; body composition using dual x-ray absorptiometry (Lunar Prodigy utilising enCORE software, both GE Healthcare, USA); blood pressure using an oscillometric method and converted to *z*-scores (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004), and a modified frequently sampled glucose tolerance test, with fasting glucose and insulin concentrations taken as the average of three baseline samples and analyzed using minimal modelling software (MinMod Millennium software v. 6.02). In addition, caregivers were asked to complete the Behavioral Rating Inventory of Executive Function (BRIEF-PAR Inc. Florida, USA) and a demographic questionnaire. Teachers were also asked to complete the BRIEF. Although exposure to old and new nutritional protocols was birth-date dependent and thus not able to be fully concealed, assessments were performed blind to children's actual neonatal nutritional intakes.

8.3.3 **Neonatal data collection**

All actual enteral and parenteral fluid intakes (excluding blood products) for the first 28 days after birth were collected from the medical record, and macronutrient intakes per kilogram per day calculated for each infant using the reference data given in chapter 11 (appendix A), and the latest, highest weight. The calendar day of birth was excluded from further analysis due to its variable duration, and fluid, macronutrient and energy intakes were then averaged for days 1-7 (week 1), days 1-14 (fortnight 1) and days 1-28 (month 1). In addition, for the exploratory analyses, total fluid, macronutrient and energy intakes per kilogram were summed for days 1-7 and days 1-14.

Neonatal weight, length and head circumference measures were collected from the medical record and converted to *z*-scores (Fenton & Kim, 2013). Neonatal demographic data were

used to determine NZ deprivation index (Salmond et al., 2007) and the clinical risk index for babies (CRIB II) score (Parry et al., 2003), and details of the following neonatal morbidities were collected: intraventricular hemorrhage (grade III/IV) (Papile et al., 1978); necrotizing enterocolitis (Bell stage ≥ 2) (Bell et al., 1978); retinopathy of prematurity (stage III/IV) ("An international classification of retinopathy of prematurity. Prepared by an international committee.," 1984); chronic lung disease (the requirement for ventilatory support or supplemental oxygen at 36 weeks' postmenstrual age); major neonatal surgery (the opening of a body cavity) (Chow, 2013), and sepsis (a positive blood, urine or cerebrospinal fluid culture) (Chow, 2013).

8.3.4 **Outcomes**

The primary outcome was neurodevelopmental impairment at 7 years' corrected age, defined as any of: full scale IQ < 85 (-1 SD); MABC-2 score $\leq 5^{th}$ centile; cerebral palsy; blindness (presenting visual acuity of 6/60 or worse in the best eye), or deaf requiring aids. Because the original audit inclusion criteria selected for survivors, death was not included in the primary outcome. Secondary outcomes included individual components of the primary outcome, growth, blood pressure, glucose metabolism and body composition.

8.3.5 **Statistical methods**

Analyses were performed using SAS v.9.4 and JMP v.11.2.0. Significance level was set at 5% with no adjustment for multiple comparisons. Continuous variables are summarised using mean (standard deviation) or median (inter-quartile range), and compared between groups using two-sample t-test or Wilcoxon test for non-normal data. Categorical variables are summarised as frequencies and percentages, and compared between groups using Chi-square test or Fishers' exact test for cells with counts <5.

Prior to analysis, we considered potential confounders likely to be strongly associated with the outcome of neurodevelopmental impairment. Comparison of baseline characteristics between groups while blind to the results showed that sex (female/male) and birthweight *z*-score differed by >10% between groups and were thus included as covariates in adjusted analyses. Outcomes assessed at 7 years' corrected age were compared between groups using generalised linear regression models with an appropriate link function, both unadjusted and adjusted. Results are presented as mean differences for continuous variables, or odds ratios (OR) for categorical variables with 95% confidence intervals (CI). In the primary analysis, the presence of twins in the cohort was considered as a cluster effect using generalised estimating equations.

Exploratory analyses were also conducted to explore relationships between actual neonatal nutritional intakes and neurodevelopmental outcomes using generalised linear regression models, adjusted for multiple birth (yes/no), sex, gestational age (in weeks), birthweight *z*-score and PIANO study eligibility arm (RCT participants randomized to tight glycemic control vs. RCT participants randomized to standard glycemic control vs. others). The level of significance was taken as 0.05 throughout.

8.4. Results

Of 536 infants < 1,500 grams or < 30 weeks' gestation admitted to NICU from July 2005 – October 2008, 201 were eligible for inclusion in this study. Assessments could not be performed in 73 / 201 (36%) children (Figure 1), resulting in the primary outcome of neurodevelopmental impairment being available for 55 / 89 (62%) of children exposed to the old nutrition protocol and 73 / 112 (65%) of those exposed to the new nutrition protocol.

In infants exposed to the old nutrition protocol, those who were assessed at 7 years' corrected age (OldPro) were more likely to be from the least deprived socioeconomic decile, and to

have a smaller crown-heel length at birth than infants who were not assessed (Table 8-1). In infants exposed to the new nutrition protocol, those who were assessed (NewPro) had different maternal ethnic distribution and smaller head circumferences at birth than those who were not assessed. NewPro infants were similar to OldPro in gestational age, birth weight *z*-score, sex, CRIB-II score, size at birth and 36 weeks' post menstrual age, neonatal morbidities and receipt of parenteral nutrition. NewPro infants were around 25% less likely than OldPro to have neonatal hyperglycemia, but had similar rates of insulin treatment. In week 1, NewPro infants received a mean of 0.4 $g.kg^{-1}.d^{-1}$ more protein, 1.8 $g.kg^{-1}.d^{-1}$ less carbohydrate and 4 $kcal.kg^{-1}.d^{-1}$ less energy than OldPro infants. In month 1, NewPro received a similar amount of protein, but less fat, carbohydrate and energy than OldPro. Full enteral feeds were achieved at a median of 10 days in both groups (Table 8-1).

The primary outcome of neurodevelopmental impairment was found in 55/128 (43%) children assessed, and was not different between groups (Table8-2). NewPro children were almost half as likely as OldPro to have a WISC-IV score <-1 SD (adjusted OR (95% CI) 0.52 (0.23 - 1.17)), but this difference did not reach statistical significance (P = 0.12) (Table 2). Scores for FSIQ and all subdomains were higher in the NewPro group, but only that for working memory score reached statistical significance (adjusted mean difference (95% CI) 6.32 (1.15 - 11.50), P = 0.02).

Cerebral palsy tended to be more common in NewPro children (9/73 (12%)) than in OldPro children (1/55 (2%)) (AOR (95% CI) 7.36 (0.88 - 61.40) P = 0.07) (Table 8-2). Children with cerebral palsy had GMFCS ≤ 2 in all but 1 case (Table8-2). Despite the difference in the incidence of cerebral palsy, NewPro and OldPro groups had similarly high rates of motor impairment, with 55/128 (43%) of the cohort having MABC-2 total scores at or below the referent 15^{th} centile. More children in the NewPro group had an abnormal neurological

examination (31 (42%) vs. 13 (24%), P = 0.02), and motor dexterity scores were higher in the NewPro group without any other differences in MABC-2 scores (Table 8-2).

A majority of the cohort had attentional impairment on testing (109/128 (85%)), but a smaller proportion had impaired executive function reported by caregivers (25/128 (20%)) and teachers (18/128 (14%)). Measures of attention and executive function were similar in NewPro and OldPro groups (Table 8-2).

At 7 years' of age, the cohort had an average weight of 25.23 ± 6.89 kg and 29/128 (23%) were overweight or obese. Weight, length and head circumference, their respective *z*-scores, and the incidence of abnormal BMI were similar in NewPro and OldPro groups (Table 8-2). There were also no differences in measures of blood pressure. Frequently sampled intravenous glucose tolerance testing was performed in 82/128 (64%) children. Fasting glucose concentration was 0.2 (0.0 – 0.3) mM higher in the NewPro group, but no child had an abnormal fasting blood glucose concentration. There were no other differences in measures of glucose metabolism, nor were there any differences in body composition among the 124 (97%) children who underwent dual x-ray absorptiometry (Table 8-2).

Greater enteral protein, fat and carbohydrate intakes in the first 7 days, and volume of breastmilk intake in the first 14 days, tended to be associated with reduced OR of neurodevelopmental impairment (Table 8-3). There were no associations between total intake of any macronutrient and WISC FSIQ score < 85. In the first 14 days, increasing total fat, carbohydrate and energy intakes were associated with reduced odds of having an MABC-2 score ≤ 5th centile, but higher protein-energy ratio was associated with triple the odds of having an MABC-2 score ≤ 5th centile (Table 8-3). Parenteral protein intake in the first 14 days was also associated with an increased risk of MABC-2 score ≤ 5th centile, but enteral intakes of protein, fat and carbohydrate in days 1-7 and days 1-14 were all associated with

reduced odds of this outcome. This is reflected in the markedly reduced odds of MABC-2 score ≤ 5th centile associated with higher enteral:parenteral ratios of protein intake (AOR (95% CI) days 1-7: 0.15 (0.03 – 0.91), P = 0.04; days 1-14: 0.64 (0.43 – 0.96), P = 0.03). Greater total and parenteral protein intakes in the first 7 days (Figure 8-2) and higher protein-energy ratios in days 1-7 and 1-14 were also strongly associated with an increase in the odds of cerebral palsy. Intake of other macronutrients was not associated with cerebral palsy, and enteral feeding did not appear to have any protective effect (Table 8-3).

8.5. Discussion

The introduction of a new nutrition protocol which resulted in greater protein intake but reduced intake of carbohydrate and energy in the first week was not associated with change in the overall rate of neurodevelopmental impairment in this cohort of very preterm children. Children exposed to the new protocol were less likely to have a WISC FSIQ < 85, but tended to be more likely to have cerebral palsy than those exposed to the old protocol. Growth, blood pressure, glucose metabolism and body composition measures were unchanged. Further analysis of infants' actual nutrient intakes showed no associations between intake of any macronutrient and cognitive impairment, but a strong association between early protein intake and motor impairment and cerebral palsy. Increased enteral intakes were associated with a reduced likelihood of neurodevelopmental impairment, including motor impairment, but not cognitive impairment, or cerebral palsy.

Our finding of an association between early parenteral protein intake and adverse long term outcome is not without precedent. A study of 61 ELBW infants randomized to receive higher (starting at 2 $g.kg^{-1}.d^{-1}$) or standard (starting at 0.5 g. $kg^{-1}.d^{-1}$) parenteral protein intakes showed an increase in chronic lung disease, lower *z*-scores for weight, length and head circumference, and lower mental development scores at 18 months in the group receiving

higher early parenteral protein (Blanco et al., 2012). Other randomized trials of early parenteral protein intakes have shown short-term growth outcomes that are better (Morgan et al., 2014), unchanged (Uthaya et al., 2016) or worsened (Balasubramanian et al., 2013) in the groups receiving the higher early parenteral protein loads. We did not see any differences in weight, height or head circumference, nor measures of body composition, between OldPro and NewPro groups at 7 years' corrected age, suggesting that any potential changes to growth associated with early neonatal nutrition do not persist into childhood.

The rate of cognitive impairment almost halved in children exposed to the new protocol, although this difference did not reach statistical significance, probably due to our relatively small sample size. However, our finding of an increased rate of cerebral palsy in the NewPro group, although also not statistically significant, was unexpected. Of note, the difference in the rates of cerebral palsy between the groups did not so much reflect a notably high rate in the NewPro group (12%), but rather, a rate in the OldPro group (2%) that was lower than expected for such a preterm population (Blair & Watson, 2006). Nevertheless, these findings highlight the possibility of a trade-off between cognitive and motor outcomes in preterm children that may be obscured when using a composite neurodevelopmental outcome.

Our finding that early parenteral protein intake may be specifically associated with impaired motor function and cerebral palsy at 7 years' corrected age is in keeping with the findings of a small randomized control trial of parenteral versus enteral nutrition in preterm piglets, where piglets randomized to parenteral nutrition showed a significant decrement in motor function after 3 days, and reduced white matter myelination and smaller cerebellar weights at day 10 (Choudhri, Sable, Chizhikov, Buddington, & Buddington, 2014). There are a number of possible reasons for the association between early parenteral protein intake and impaired motor function seen in our study. Firstly, the strong association between protein-energy ratio and cerebral palsy may suggest that utilization of parenteral protein is altered in states of

relative energy deficiency, and that without adequate calories, amino acids are preferentially catabolized, resulting in increased concentrations of ammonia or other potentially neurotoxic metabolites during a critical window of white matter pathway development (Back & Rosenberg, 2014) and resulting in long term motor deficits. However, the tendency towards improved cognitive scores in the new protocol group makes it less likely that higher parenteral protein intake is associated with a generalised neurotoxic effect. Rather our observation of increased motor impairment and cerebral palsy in association with higher parenteral protein intakes may reflect a potential imbalance in individual amino acids and not total protein intake per se. The ideal composition of amino acids used in neonatal parenteral nutrition is not known. We used a commercial amino acid preparation based on TrophAmine (B.Braun, Pennsylvania, USA), containing relatively high arginine concentrations. Arginine stimulates pancreatic insulin secretion, has previously been associated with blood glucose control in preterm infants receiving parenteral amino acid solutions (Burgess et al., 2014), and may be responsible for the decrease in hyperglycemia we have previously reported in a larger neonatal cohort exposed to this change in nutrition protocol (Tottman et al, submitted for publication). However, in excess, arginine concentrations are associated with progressive spasticity and motor impairment (Crombez & Cederbaum, 2005). Although measurements are not available for our cohort, in a previous study of early amino acid supplementation (Blanco et al., 2012) increased neonatal phenylalanine, isoleucine, valine and leucine concentrations were associated with decreased mental development index at 2 years. There is very little literature on the normal amino acid profiles of well preterm infants, and formulations of parenteral amino-acid solutions developed to match amino acid profiles from term infants (Heird et al., 1987) may not be appropriate for the very preterm population. Further, trials of parenteral nutrition using short-term growth outcomes as a primary endpoint may miss important long-term neurological effects. Recent recommendations advocating the

use of hypocaloric, high protein nutrition in critically unwell adults (Hoffer, 2017) should not be translated into neonatal practice without robust analysis of the potential effects on this nutritionally sensitive, developmentally vulnerable group.

It is possible that our finding of an apparently protective effect of enteral nutrition for motor outcomes may merely reflect that well babies are easier to feed. However, the strong association between enteral intakes of all macronutrients on days 1-7 and a reduced risk of neurodevelopmental impairment at 7 years suggests that enteral feeding is not merely a marker for a less sick infant, as unit policy was to give only maternal breastmilk during this period, and thus very early enteral intakes are much more likely to reflect maternal supply than infant feed tolerance. Enteral feeding is associated with production of incretins and maturation of the enteroinsular axis (Lucas et al., 1985; Stoll et al., 2012) resulting in stabilisation of blood glucose concentrations (Tottman et al, submitted for publication), and enhanced integrity of the intestinal mucosa (Mochizuki et al., 1984), an important component of the immune system in preterm infants (Neu, 2007). It is not clear which, if any, of these effects, may be associated with improved motor outcomes.

Despite the imbalance in the incidence of cerebral palsy between groups, the overall percentage of children with motor impairment was not different in children exposed to the different nutritional protocols. Children with mild cerebral palsy had similar MABC-2 motor scores to those without cerebral palsy but with motor impairment, highlighting the challenge of categorizing motor difficulties in children born very preterm and suggesting that motor outcomes lie on a continuum. Whilst clinically detectable signs of neurological abnormality were used to define cerebral palsy, it is possible that those children with mild cerebral palsy do not have functional outcomes different from children without neurological signs but with motor impairment. Conversely, it is clear that there is a substantial group of children born very preterm who do not have a diagnosis of cerebral palsy but who are experiencing

significant motor difficulties in childhood, a finding that has also been reported in a large international cohort of 11 year olds born at very low birth weights (Schmidt, Roberts, Anderson, & et al., 2017).

The major limitation of this study is its relatively small sample size, restricted by the original numbers recruited into the RCT and nutritional audits, and hence limited power to detect small differences in neurodevelopmental outcomes. The retrospective, non-contemporaneous nature of the cohorts increases the possibility that there were other changes to neonatal care during the study recruitment period which may have affected the study outcome, although we are not aware of any significant differences in practice during this period. Nevertheless, this study represents one of the largest cohorts reported in a neonatal nutrition study, and is one of very few where assessments have been made in mid-childhood to allow determination of long-term outcomes. Our findings are also made more robust by the collection and interrogation of actual nutritional intakes, regardless of nutritional protocol exposure.

In summary, a change in nutritional protocol which resulted in higher protein and lower carbohydrate and energy intakes in early life did not change the overall rate of neurodevelopmental impairment at 7 years. Fewer children exposed to the new protocol had low IQ, but more had motor impairment and cerebral palsy. Specifically, higher early parenteral protein intakes were strongly associated with cerebral palsy, whereas higher early enteral intakes appeared protective against motor but not cognitive impairment.

8.6. Conclusions

Higher early protein intakes may be detrimental to long term motor development. There is an urgent need for randomized controlled trials of different early neonatal protein intakes with growth and neurodevelopmental outcomes assessed at least into mid-childhood.

8.7. Figures and tables

Figure 8-1: Flow diagram of eligible participants

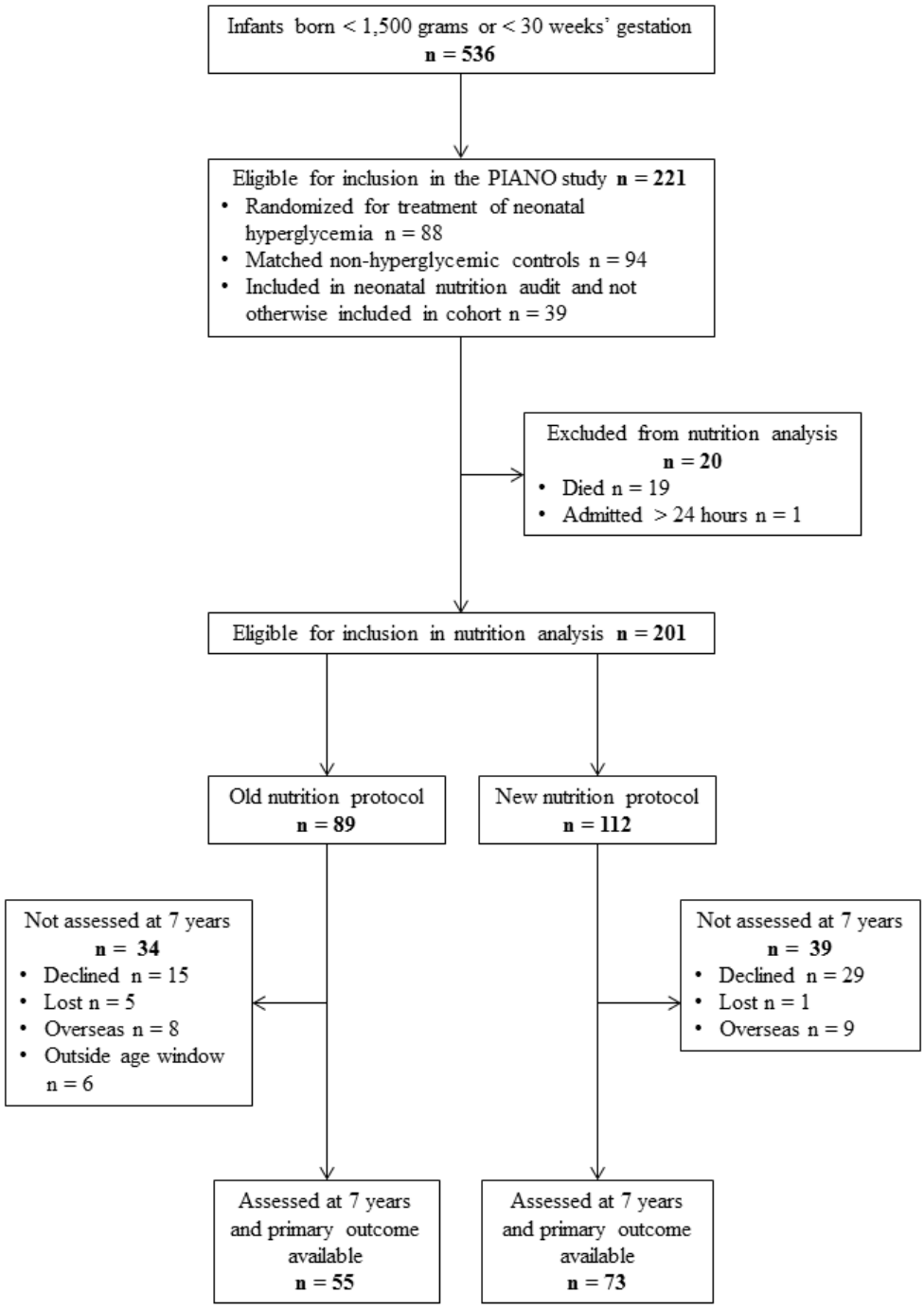
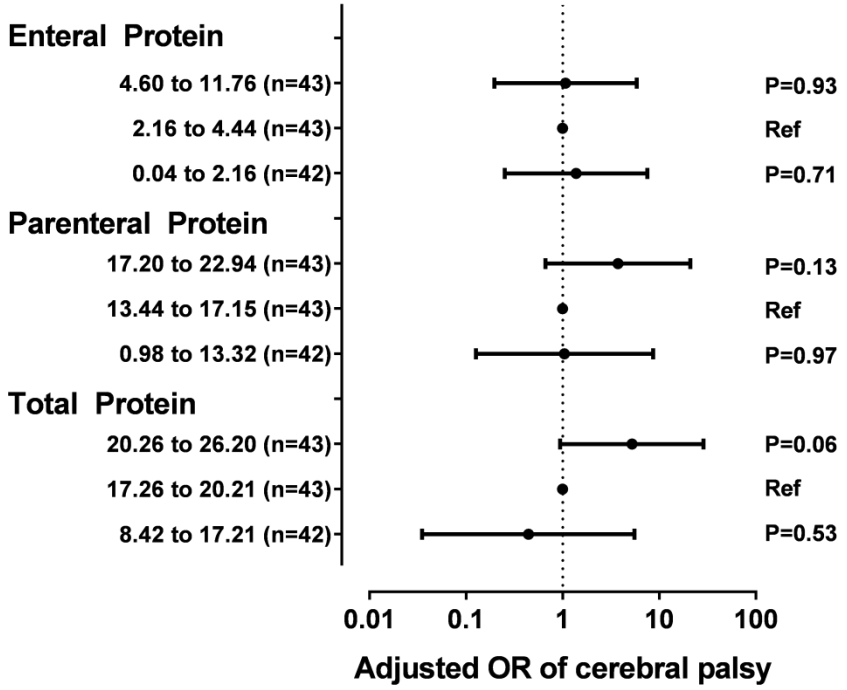


Figure 8-2: Adjusted odds ratios of cerebral palsy and MABC-2 score ≤ 5th centile in children exposed to different tertiles of total enteral, parenteral and total protein intakes (g/kg) in postnatal days 1-7

Odds ratios are adjusted for gestational age, birth weight *z*-score, sex, multiple birth and PIANO study inclusion arm.



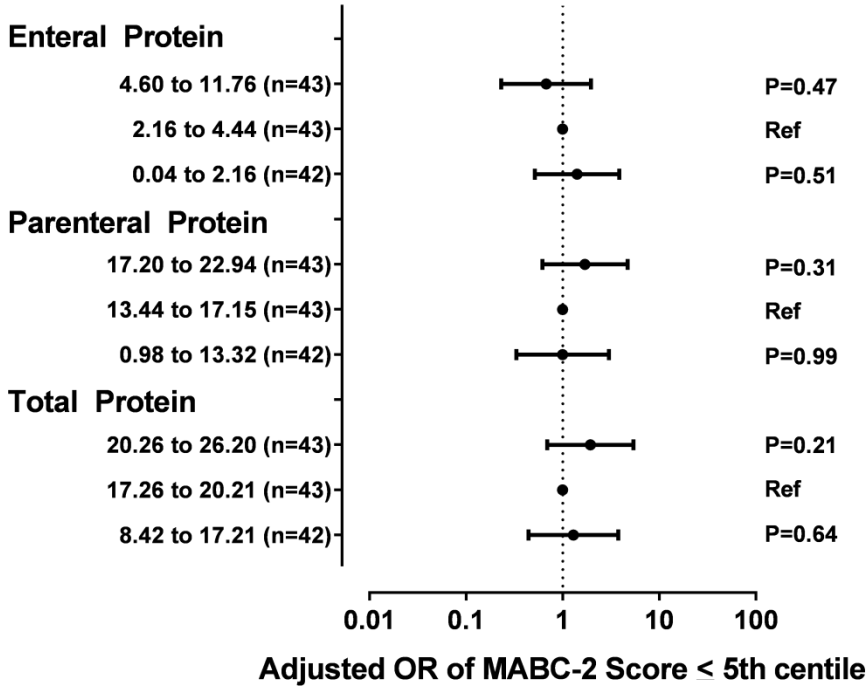


Table 8-1: Perinatal characteristics of eligible children exposed to old or new nutrition protocols who were and were not assessed at 7 years' corrected age

	Exposed to old protocol n = 89		**Exposed to new protocol n = 112**		**Comparison between old and new protocol cohorts assessed**
	Assessed n = 55	**Not assessed n = 34**	**Assessed n = 73**	**Not assessed n = 39**	
Maternal characteristics					
Maternal diabetes	3 (5%)	0 (0%)	3 (4%)	4 (10%)	>0.99
Multiple pregnancy	13 (24%)	8 (24%)	22 (30%)	9 (23%)	0.41
Prioritised ethnicity	-	-	-*	-	0.26
Māori	13 (24%)	8 (24%)	19 (26%)	7 (18%)	
Pacific Island	4 (7%)	8 (24%)	12 (16%)	5 (13%)	
Asian	7 (13%)	6 (17%)	12 (16%)	17 (43%)	
NZ European / Other	31 (56%)	12 (35%)	30 (41%)	10 (26%)	
Deprivation index					
Most deprived	10 (18%)	5 (15%)	14 (19%)	8 (21%)	0.86
Least deprived	7 (13%)*	0 (0%)	4 (5%)	0 (0%)	0.15
Infant characteristics					
Inborn	53 (96%)	29 (85%)	67 (92%)	38 (97%)	0.46
Male sex	26 (47%)	18 (53%)	42 (58%)	24 (62%)	0.29
CRIB II score	10 (7 – 13)	9 (6 – 12)	10 (8 – 12)	9 (8 – 11)	0.77

Table 8-1 continued…	**Exposed to old protocol n = 89**		**Exposed to new protocol n = 112**		**Comparison between old and new protocol cohorts assessed**
	Assessed n = 55	**Not assessed n = 34**	**Assessed n = 73**	**Not assessed n = 39**	
Apgar score					
1 minute	6 (3 – 8)	6 (5 – 8)	6 (4 – 7)	6 (4 – 7)	>0.99
5 minute	8 (7 – 9)	9 (8 – 10)	8 (7 – 9)	8 (7 – 9)	0.98
Gestational age (weeks)	26 (25 – 29)	27.5 (26 – 29)	26 (25 – 27)	27 (26 – 28)	0.52
Birth measurements					
Weight (g)	935 ± 269	1034 ± 280	908 ± 189	982 ± 227	0.80
Weight *z*-score	-0.02 ± 0.93	0.17 ± 1.05	0.06 ± 0.95	0.06 ± 0.85	0.31
Crown-heel length (cm)	34.5 ± 3.1 *	36.0 ± 3.3	34.7 ± 2.8	35.4 ± 2.7	0.65
Length *z*-score	-0.11 ± 1.00	0.14 ± 1.18	0.01 ± 1.17	0.13 ± 0.94	0.46
Head circumference (cm)	24.8 ± 2.2	25.5 ± 2.1	24.5 ± 1.9*	25.4 ± 2.1	0.52
Head circumference *z*-score	0.33 ± 1.02	0.27 ± 1.27	0.09 ± 1.11	0.46 ± 1.01	0.28
Small for gestational age	5 (9%)	4 (12%)	9 (12%)	3 (8%)	0.78
Intraventricular hemorrhage	1 (2%)	1 (3%)	6 (8%)	2 (5%)	0.24
Necrotizing enterocolitis	2 (4%)	2 (6%)	3 (4%)	1 (3%)	>0.99
Retinopathy of prematurity	5 (9%)	2 (6%)	10 (14%)	3 (8%)	0.58
Early onset sepsis	0 (0%)	1 (3%)	1 (1%)	3 (8%)	>0.99

Table 8-1 continued…	**Exposed to old protocol n = 89**		**Exposed to new protocol n = 112**		**Comparison between old and new protocol cohorts assessed**
	Assessed n = 55	**Not assessed n = 34**	**Assessed n = 73**	**Not assessed n = 39**	
Late onset sepsis	9 (16%)	6 (18%)	14 (19%)	8 (21%)	0.82
Major neonatal surgery	3 (5%)	2 (6%)	9 (12%)	2 (5%)	0.23
Chronic lung disease	13 (24%)	10 (29%)	26 (36%)	12 (31%)	0.18
Received insulin	19 (35%)	8 (24%)	28 (38%)	9 (23%)	0.71
Hypoglycemia (<2.6 mM)	25 (45%)	14 (41%)	31 (42%)	10 (26%)	0.74
Hyperglycemia (>8.5 mM)	44 (80%)	21 (62%)	47 (64%)	20 (51%)	0.05
Received postnatal steroids	10 (18%)	7 (21%)	17 (23%)	8 (21%)	>0.99
Any parenteral nutrition	55 (100%)	34 (100%)	73 (100%)	39 (100%)	-
Day enteral feeds established [a]	10 (8 – 12)	11 (7 – 15)	10 (9 – 14)	10 (9 – 13)	0.14
Protein intake ($g.kg^{-1}.d^{-1}$)					
Days 1-7	2.3 ± 0.3	2.4 ± 0.4	2.9 ± 0.4	2.9 ± 0.4	<0.001
Days 1-14	2.9 ± 0.4	3.0 ± 0.5	3.2 ± 0.3	3.3 ± 0.3	<0.001
Days 1-28	3.3 ± 0.3	3.3 ± 0.3	3.4 ± 0.3	3.5 ± 0.3	0.11
Carbohydrate intake ($g.kg^{-1}.d^{-1}$)					
Days 1-7	11.7 ± 1.4	11.7 ± 1.6	10.1 ± 1.4	10.1 ± 1.3	<0.001
Days 1-14	13.4 ± 1.3	13.8 ± 1.4	12.3 ± 1.3	12.5 ± 1.0	<.0001
Days 1-28	15.1 ± 1.5	15.3 ± 1.3	14.2 ± 1.3	14.3 ± 1.5	0.001

Table 8-1 continued…	**Exposed to old protocol n = 89**		**Exposed to new protocol n = 112**		**Comparison between old and new protocol cohorts assessed**
	Assessed n = 55	**Not assessed n = 34**	**Assessed n = 73**	**Not assessed n = 39**	
Fat intake ($g.kg^{-1}.d^{-1}$)					
Days 1-7	3.6 ± 0.8	3.5 ± 1.0	3.5 ± 0.8	3.4 ± 0.6	0.51
Days 1-14	5.0 ± 0.8	4.9 ± 1.1	4.8 ± 0.9	4.9 ± 0.8	0.56
Days 1-28	6.1 ± 0.8	6.1 ± 0.7	5.8 ± 0.9	6.0 ± 0.9	0.04
Energy intake ($Kcal.kg^{-1}.d^{-1}$)					
Days 1-7	84 ± 10	84 ± 15	80 ± 10	79 ± 9	0.01
Days 1-14	107 ± 14	108 ± 17	103 ± 13	104 ± 11	0.09
Days 1-28	127 ± 14	128 ± 12	121 ± 14	123 ± 14	0.01
Enteral feed volume ($ml.kg^{-1}.d^{-1}$)					
Days 1-7	40 ± 26	44 ± 35	31 ± 22	29 ± 21	0.04
Days 1-14	89 ± 31	87 ± 44	76 ± 35	79 ± 36	0.08
Days 1-28	128 ± 29	128 ± 29	114 ± 35	118 ± 36	0.01
Total fluid intake ($ml.kg^{-1}.d^{-1}$)					
Days 1-7	145 ± 15	143 ± 16	123 ± 13	123 ± 14	<0.001
Days 1-14	157 ± 10	158 ± 12	145 ± 11	146 ± 8	<0.001
Days 1-28	167 ± 9	167 ± 9	158 ± 14	157 ± 18	<0.001

Table 8-1 continued…	**Exposed to old protocol n = 89**		**Exposed to new protocol n = 112**		**Comparison between old and new protocol cohorts assessed**
	Assessed n = 55	**Not assessed n = 34**	**Assessed n = 73**	**Not assessed n = 39**	
28 day measurements					
Weight (g)	1268 ± 364	1385 ± 400	1241± 261	1361 ± 331	0.74
Weight *z*-score	-0.85 ± 0.70	-0.78 ± 0.92	-0.81 ± 0.74	-0.72 ± 0.67	0.70
Length (cm)	37.2 ±3.2	38.9 ± 3.8	37.5 ± 2.6	38.5 ± 2.5	0.36
Length *z*-score	-0.96 ± 0.84	-0.71 ± 1.15	-0.79 ± 0.90	-0.68 ± 0.65	0.19
Head circumference (cm)	25.9 ± 2.5	27.1 ± 2.7	26.1 ± 1.9	26.7 ± 2.3	0.33
Head circumference *z*-score	-1.3 ± 0.9	-1.0 ± 1.2	-1.17 ± 0.85	-1.02 ± 0.77	0.53
36 week measurements					
Weight (g)	2281 ± 444	2337 ± 439	2268 ±363	2387 ± 342	0.86
Weight *z*-score	-1.00 ± 1.03	-0.84 ± 1.00	-1.00 ± 0.92	-0.73 ± 0.83	0.91
Length (cm)	43.8 ±2.4	43.4 ± 2.6	43.9 ± 2.2	44.7 ± 1.9	0.91
Length *z*-score	-1.27 ± 0.89	-1.37 ± 1.00	-1.25 ± 0.92	-1.00 ± 0.79	0.99
Head circumference (cm)	31.3 ± 1.5	31.1 ± 1.7	31.1 ± 1.3 **	32.0 ± 1.3	0.51
Head circumference *z*-score	-0.86 ± 1.01	-0.96 ± 1.20	-0.99 ± 0.86 **	-0.44 ± 0.81	0.37

Table 8-1 continued…	**Exposed to old protocol n = 89**		**Exposed to new protocol n = 112**		**Comparison between old and new protocol cohorts assessed**
	Assessed n = 55	**Not assessed n = 34**	**Assessed n = 73**	**Not assessed n = 39**	
Length of neonatal stay (days)	84 (66 – 98)	78 (58 – 107)	93 (76 – 112)	80 (59 – 99)	0.06
Discharged with home oxygen	14 (25%)	5 (15%)	20 (27%)	8 (21%)	0.84

Data are n (%), median (IQR) or mean ± SD.

* $P < 0.05$ and ** $P < 0.01$ for the comparison with children not assessed.

a: Where enteral intake ≥ 150 $ml.kg^{-1}.d^{-1}$ and no further intravenous nutrition given.

Table 8-2: Neurodevelopmental impairment, cognitive, motor and executive function, growth, blood pressure, glucose metabolism and body composition in children assessed at 7 years of age

	Exposed to old protocol n = 55	**Exposed to new protocol n = 73**	**OR (95% CI)**	***P* value**	***Adjusted OR (95% CI)**	** P* value**
Neurodevelopmental impairment	**25 (45%)**	**30 (41%)**	**0.84 (0.39 – 1.79)**	**0.65**	**0.78 (0.35 – 1.70)**	**0.55**
WISC FSIQ < 85 (-1 SD)	22 (40%)	20 (27%)	0.57 (0.25 – 1.26)	0.16	0.52 (0.23 – 1.17)	0.12
MABC-2 score ≤ 5th centile	13 (24%)	22 (30%)	1.39 (0.60 – 3.22)	0.44	1.36 (0.58 – 3.19)	0.48
Cerebral palsy	1 (2%)	9 (12%)	7.59 (0.93 – 62.09)	0.06	7.36 (0.88 – 61.40)	0.07
Deaf	1 (2%)	1 (1%)	-		-	
Blind	0 (0%)	0 (0%)	-		-	
Cognitive function						
Cognitive impairment	22 (40%)	20 (27%)	0.57 (0.25 – 1.26)	0.16	0.52 (0.23 – 1.17)	0.12
Mild (FSIQ <-1 to -2 SD)	16 (29%)	16 (22%)	-		-	
Moderate (<-2 to -3 SD)	5 (9%)	1 (1%)	-		-	
Severe (<-3 SD)	1 (2%)	3 (4%)	-		-	
Full scale IQ score	88 ± 17	91 ± 16	3.00 (-2.96 – 8.95)	0.32	3.60 (-2.30 – 9.50)	0.23
Verbal comprehension score	95 ± 16	96 ± 13	1.40 (-3.95 – 6.74)	0.61	1.68 (-3.62 – 6.98)	0.53
Perceptual reasoning score	88 ± 15	90 ± 14	1.58 (-3.77 – 6.92)	0.56	2.01 (-3.39 – 7.41)	0.47
Working memory score	87 ± 16	93 ± 13	5.78 (0.41 – 11.15)	0.03	6.32 (1.15 – 11.50)	0.02
Processing speed score	92 ± 14	95 ± 14	3.71 (-1.79 – 9.22)	0.19	4.38 (-1.06 – 9.82)	0.11

Table 8-2 continued…	**Exposed to old protocol n = 55**	**Exposed to new protocol n = 73**	**OR (95% CI)**	***P* value**	***Adjusted OR (95% CI)**	** P* value**
Motor function						
Motor impairment	23 (42%)	32 (44%)	1.09 (0.51 – 2.33)	0.83	1.00 (0.44 – 2.30)	0.99
Likely (≤ 15th centile)	10 (18%)	10 (14%)	-		-	
Definite (≤ 5th centile)	13 (24%)	22 (30%)	-		-	
Motor ABC-2 total score	8 ± 3	8 ± 4	0.04 (-1.27 – 1.34)	0.96	0.19 (-1.07 – 1.45)	0.77
Balance and coordination score [a]	9 ± 3	9 ± 4	0.07 (-1.17 – 1.32)	0.91	0.36 (-0.80 – 1.51)	0.54
Motor dexterity score [b]	7 ± 3	8 ± 3	1.37 (0.05 – 2.69)	0.04	1.57 (0.32 – 2.82)	0.01
Aiming and catching score [c]	10 ± 3	10 ± 3	0.33 (-0.69 – 1.35)	0.52	0.26 (-0.76 – 1.29)	0.61
Abnormal neurological examination	13 (24%)	31 (42%)	2.38 (1.08 – 5.27)	0.03	2.57 (1.14 – 5.81)	0.02
Impaired visual motor integration	10 (18%)	14 (19%)	1.11 (0.46 – 2.68)	0.82	1.08 (0.44 – 2.68)	0.87
Visual motor integration score	92 ± 10	91 ± 8	-0.92 (-4.16 – 2.31)	0.58	-0.57 (-3.93 – 2.79)	0.74
Executive function						
Sky search score	6 ± 3	7 ± 3	0.67 (-0.49 – 1.83)	0.26	0.63 (-0.52 – 1.79)	0.28
Score! score	7 ± 4	7 ± 3	0.11 (-1.19 – 1.41)	0.87	0.11 (-1.19 – 1.41)	0.87
Creature counting score	6 ± 3	6 ± 3	-0.11 (-1.35 – 1.12)	0.86	-0.18 (-1.38 – 1.01)	0.76
Sky search DT score	5 ± 4	4 ± 5	-0.71 (-2.54 – 1.12)	0.45	-0.78 (-2.60 – 1.05)	0.40

Table 8-2 continued…	**Exposed to old protocol n = 55**	**Exposed to new protocol n = 73**	**OR (95% CI)**	***P* value**	***Adjusted OR (95% CI)**	** P* value**
Impaired attention	49 (89%)	60 (82%)	0.61 (0.17 – 2.25)	0.46	0.52 (0.14 – 1.94)	0.37
Selective	24 (43%)	23 (32%)	-		-	
Sustained	27 (49%)	33 (45%)	-		-	
Shifting	27 (49%)	36 (49%)	-		-	
Dual task	27 (49%)	41 (56%)	-		-	
Impaired executive function						
Home	8 (15%)	17 (23%)	1.82 (0.73 – 4.55)	0.20	1.77 (0.70 – 4.43)	0.23
Classroom	9 (16%)	9 (12%)	0.75 (0.26 – 2.16)	0.59	0.76 (0.26 – 2.20)	0.61
Executive function scores						
Caregiver report	53 ± 12	55 ± 12	2.27 (-2.00 – 6.53)	0.30	2.31 (-1.99 – 6.60)	0.29
Teacher report	54 ± 10	53 ± 9	-1.06 (-4.74 – 2.62)	0.57	-1.13 (-4.78 – 2.53)	0.54
Growth						
Weight (kg)	24.26 ± 6.68	25.97 ± 6.99	1.71 (-0.81 – 4.24)	0.18	1.46 (-0.94 – 3.86)	0.23
Weight *z*-score	-0.11 ± 1.47	0.37 ± 1.38	0.48 (-0.04 – 1.00)	0.07	0.41 (-0.08 – 0.90)	0.10
Height (cm)	122.7 ± 5.8	124.4 ± 6.1	1.7 (-0.5 – 3.9)	0.13	1.5 (-0.7 – 3.6)	0.19
Height *z*-score	0.02 ± 1.02	0.34 ± 1.16	0.33 (-0.08 – 0.73)	0.11	0.29 (-0.11 – 0.69)	0.15
Leg length (cm)	55.5 ± 3.3	56.3 ± 3.5	0.8 (-0.5 – 2.1)	0.22	0.6 (-0.6 – 1.9)	0.32
Leg length *z*-score	-0.35 ± 0.99	-0.11 ± 1.14	0.24 (-0.16 – 0.64)	0.24	0.21 (-0.18 – 0.61)	0.29

Table 8-2 continued…	**Exposed to old protocol n = 55**	**Exposed to new protocol n = 73**	**OR (95% CI)**	***P* value**	***Adjusted OR (95% CI)**	*** *P* value**
Sitting height (cm)	67.2 ± 3.4	68.0 ± 3.1	0.8 (-0.4 – 2.0)	0.20	0.7 (-0.5 – 1.9)	0.27
Sitting height *z*-score	0.40 ± 1.07	0.63 ± 1.03	0.23 (-0.16 – 0.62)	0.25	0.23 (-0.17 – 0.62)	0.26
Head circumference (cm)	51.7 ± 1.7	51.9 ± 2.1	0.2 (-0.5 – 0.8)	0.59	0.0 (-0.6 – 0.7)	0.92
Head circumference *z*-score	-1.10 ± 1.13	-0.98 ± 1.57	0.13 (-0.34 – 0.59)	0.60	0.06 (-0.41 – 0.52)	0.81
Body Mass Index (BMI) ($kg.m^{-2}$)	15.9 ± 3.1	16.6 ± 3.2	0.7 (-0.5 – 1.9)	0.23	0.6 (-0.5 – 1.7)	0.29
BMI *z*-score	-0.18 ± 1.49	0.25 ± 1.37	0.43 (-0.09 – 0.95)	0.10	0.36 (-0.12 – 0.85)	0.14
Abnormal BMI	15 (27%)	19 (26%)	0.94 (0.42 – 2.11)	0.88	0.93 (0.42 – 2.07)	0.85
Obese	3 (5%)	7 (10%)	-		-	
Overweight	8 (15%)	11 (15%)	-		-	
Thin	4 (7%)	1 (1%)	-		-	
Abdominal circumference (cm)	56.8 ± 8.0	58.5 ± 9.1	1.7 (-1.5 – 4.9)	0.31	1.5 (-1.6 – 4.5)	0.34
Abdominal circumference *z*-score	0.53 ± 1.49	0.86 ± 1.39	0.33 (-0.20 – 0.85)	0.22	0.29 (-0.20 – 0.78)	0.25
Waist: height ratio	0.46 ± 0.05	0.47 ± 0.06	0.01 (-0.01 – 0.03)	0.48	0.01(-0.01 – 0.03)	0.51

Table 8-2 continued…	**Exposed to old protocol n = 55**	**Exposed to new protocol n = 73**	**OR (95% CI)**	***P* value**	***Adjusted OR (95% CI)**	** P* value**
Change in *z*-scores (birth to age 7)						
Weight	-0.21 (-0.98–0.80)	0.16 (-0.65 – 1.01)	0.40 (-0.12 – 0.93)	0.14	0.41 (-0.08 – 0.90)	0.10
Height	0.11 ± 1.31	0.30 ± 1.37	0.19 (-0.30 – 0.68)	0.45	0.21 (-0.25 – 0.67)	0.37
Head circumference	-1.41 ± 1.33	-1.14 ± 1.56	0.28 (-0.24 – 0.79)	0.29	0.24 (-0.23 – 0.72)	0.32
Blood pressure (BP)						
Systolic BP (mmHg)	99 ± 8	98 ± 10	-1 (-4 – 3)	0.76	-1 (-4 – 3)	0.71
Systolic BP *z*-score	0.14 ± 0.76	0.02 ± 0.94	-0.12 (-0.42 – 0.19)	0.45	-0.11 (-0.42 – 0.2)	0.47
Diastolic BP (mmHg)	55 ± 5	55 ± 6	-0 (-2 – 2)	0.98	0 (-2 – 2)	0.95
Diastolic BP *z*-score	-0.20 ± 0.47	-0.24 ± 0.59	-0.04 (-0.23 – 0.15)	0.68	-0.02 (-0.22 – 0.17)	0.80
Mean BP (mmHg)	71 ± 6	70 ± 7	-1 (-3 – 2)	0.64	-1 (-3 – 2)	0.62
Mean BP *z*-score	0.03 ± 0.96	-0.05 ± 1.02	-0.08 (-0.42 – 0.26)	0.64	-0.09 (-0.43 – 0.26)	0.63
Abnormal BP	5 (9%)	8 (11%)	1.18 (0.36 – 3.87)	0.78	1.30 (0.40 – 4.20)	0.66
Hypertension	1 (2%)	6 (8%)	-		-	
Prehypertension	4 (7%)	2 (3%)	-		-	

Table 8-2 continued…	**Exposed to old protocol n = 55**	**Exposed to new protocol n = 73**	**OR (95% CI)**	***P* value**	***Adjusted OR (95% CI)**	*** *P* value**
<u>Glucose metabolism</u>	n = 33	n = 49				
Fasting blood glucose concentration (mmol/l)	4.8 ± 0.3	5.0 ± 0.3	0.2 (0.1 – 0.3)	0.01	0.2 (0.0 – 0.3)	0.02
Fasting insulin concentration (mu/l)	5.80 ± 5.39	5.85 ± 4.13	0.06 (-2.09 – 2.20)	0.96	-0.04 (-2.09 – 2.02)	0.97
Glucose effectiveness (min^{-1})	0.03 ± 0.01	0.03 ± 0.01	-0.00 (-0.01 – 0.00)	0.26	-0.00 (-0.01 – 0.00)	0.32
Glucose disappearance constant ($x10^{-2}$)	2.97 ± 1.17	3.15 ± 1.14	0.18 (-0.36 – 0.72)	0.52	0.24 (-0.29 – 0.76)	0.38
Insulin sensitivity ($((mu/l)^{-1}.min^{-1})$)	9.03 ± 4.69	7.90 ± 3.76	-1.14 (-3.06 – 0.79)	0.25	-0.88 (-2.69 – 0.94)	0.34
Acute insulin response to glucose ($mu.l^{-1}.min$)	544 ± 530	706 ± 926	162 (-166 – 490)	0.33	143 (-151 – 437)	0.34
Disposition index ($x10^{3}$)	3.78 ± 2.09	3.92 ± 2.13	0.14 (-0.85 – 1.13)	0.78	0.29 (-0.67 – 1.25)	0.56

Table 8-2 continued…	**Exposed to old protocol n = 55**	**Exposed to new protocol n = 73**	**OR (95% CI)**	***P* value**	***Adjusted OR (95% CI)**	** P* value**
<u>Body composition</u>	n = 53	n = 71				
Fat mass (kg)	4.78 ± 4.14	5.48 ± 4.72	0.70 (-0.98 – 2.37)	0.42	0.65 (-0.95 – 2.24)	0.43
Fat mass, adjusted for height and lean mass[#]	5.26 ± 0.43	5.12 ± 0.38	-0.14 (-1.29 – 1.01)	0.82	0.13 (-0.90 – 1.16)	0.81
Android/gynoid fat ratio	0.30 ± 0.11	0.32 ± 0.11	0.02 (-0.02 – 0.06)	0.26	0.01(-0.01 – 0.06)	0.21
Lean mass (kg)	18.42 ± 2.92	19.09 ± 2.87	0.67 (-0.39 – 1.73)	0.22	0.37 (-0.59 – 1.32)	0.45
Height adjusted lean mass[#]	18.84 ± 0.24	18.78 ± 0.21	-0.06 (-0.73 – 0.61)	0.86	-0.19 (-0.79 – 0.41)	0.53
Bone mineral density ($g.cm^{-3}$)	0.67 ± 0.06	0.69 ± 0.05	0.02 (-0.00 – 0.04)	0.06	0.02(-0.00 – 0.04)	0.10
Height adjusted bone mineral density[#]	0.68 ± 0.01	0.69 ± 0.01	0.01(-0.01 – 0.03)	0.27	0.01(-0.01 – 0.02)	0.30
Central fatness (android fat mass, adjusted for height, lean mass and gynoid fat mass)[#]	412 ± 13	437 ± 11	25.13 (-9.25 – 59.51)	0.15	26.76 (-8.22 – 61.74)	0.13

Data are n (%), mean ± SD, median (IQR), odds ratio or mean difference (95% CI), or [#] Standard regression-adjusted mean ± (SE). Children exposed to the old protocol is the reference group.

* Adjusted for sex and birthweight *z*-score

a: New protocol n = 67 (6 assigned minimum score, all with cerebral palsy) b: New protocol n = 66 (7 assigned minimum score, 6 with cerebral palsy) c: New protocol n = 70 (3 assigned minimum score, all with cerebral palsy).

Table 8-3: Relationships between early nutritional intakes and outcomes at 7 years of age

	Neurodevelopmental impairment		**WISC FSIQ < 85**		**MABC-2 score ≤ 5th centile**		**Cerebral palsy**	
Nutritional intakes	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**
Total protein ($g.kg^{-1}$)								
Days 1-7	0.96 (0.85 – 1.08)	0.49	0.95 (0.84 – 1.08)	0.43	1.03 (0.91 – 1.18)	0.63	1.49 (1.12 – 1.98)	0.006
Days 1-14	0.97 (0.90 – 1.05)	0.45	0.98 (0.91 – 1.07)	0.68	0.98 (0.90 – 1.07)	0.66	1.17 (0.98 – 1.39)	0.08
Total fat ($g.kg^{-1}$)								
Days 1-7	0.93 (0.86 – 1.01)	0.07	0.98 (0.91 – 1.06)	0.69	0.91 (0.83 – 1.00)	0.04	1.01 (0.88 – 1.14)	0.94
Days 1-14	0.98 (0.94 – 1.01)	0.16	1.00 (0.96 – 1.03)	0.77	0.96 (0.93 – 1.00)	0.04	0.98 (0.93 – 1.04)	0.54
Total carbohydrate ($g.kg^{-1}$)								
Days 1-7	1.00 (0.96 – 1.03)	0.86	1.01 (0.98 – 1.05)	0.47	0.99 (0.95 – 1.03)	0.56	1.00 (0.94 – 1.05)	0.72
Days 1-14	0.99 (0.97 – 1.01)	0.41	1.01 (0.99 – 1.03)	0.34	0.97 (0.95 – 1.00)	0.03	0.98 (0.94 – 1.01)	0.21
Total energy ($kcal.kg^{-1}$)								
Days 1-7	1.00 (0.99 – 1.00)	0.10	1.00 (0.99 - 1.01)	0.97	0.99 (0.99 – 1.00)	0.07	1.00 (0.99 – 1.01)	0.71
Days 1-14	1.00 (1.00 – 1.00)	0.14	1.00 (1.00 – 1.00)	0.94	1.00 (0.99 – 1.00)	0.02	1.00 (1.00 – 1.00)	0.48

Table 8-3 continued…	**Neurodevelopmental impairment**		**WISC FSIQ < 85**		**MABC-2 score ≤ 5th centile**		**Cerebral palsy**	
Nutritional intakes	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**
Protein:energy ratio ($g.100\ kcals^{-1}$)								
Days 1-7	1.19 (0.61 – 2.29)	0.61	0.77 (0.39 – 1.54)	0.46	1.90 (0.92 – 3.94)	0.08	5.62 (1.43 – 22.18)	0.01
Days 1-14	1.39 (0.49 – 3.96)	0.54	0.74 (0.25 – 2.19)	0.59	3.04 (1.00 – 9.27)	0.05	7.21 (1.4 – 37.13)	0.02
Parenteral protein ($g.kg^{-1}$)								
Days 1-7	1.03 (0.95 – 1.12)	0.52	0.99 (0.91 – 1.08)	0.86	1.08 (0.98 – 1.19)	0.11	1.27 (1.03 – 1.57)	0.03
Days 1-14	1.03 (0.99 – 1.07)	0.20	1.00 (0.96 – 1.04)	0.93	1.05 (1.01 – 1.10)	0.03	1.06 (0.99 – 1.14)	0.07
Enteral protein ($g.kg^{-1}$)								
Days 1-7	0.85 (0.73 – 1.00)	0.05	0.95 (0.81 – 1.11)	0.51	0.81 (0.69 – 0.98)	0.03	0.93 (0.70 – 1.23)	0.60
Days1-14	0.97 (0.93 – 1.01)	0.10	1.00 (0.96 – 1.04)	0.90	0.95 (0.91 – 0.99)	0.02	0.97 (0.91 – 1.03)	0.33

Table 8-3 continued…	**Neurodevelopmental impairment**		**WISC FSIQ < 85**		**MABC-2 score ≤ 5th centile**		**Cerebral palsy**	
Nutritional intakes	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**
Enteral:parenteral protein ratio								
Days 1-7	0.38 (0.11 – 1.29)	0.12	0.68 (0.24 – 1.96)	0.48	0.15 (0.03 – 0.91)	0.04	0.16 (0.01 – 3.76)	0.26
Days 1-14	0.78 (0.57 – 1.06)	0.11	0.92 (0.72 – 1.18)	0.53	0.64 (0.43 – 0.96)	0.03	0.59 (0.28 – 1.25)	0.17
Parenteral fat ($g.kg^{-1}$)								
Days 1-7	1.06 (0.94 – 1.20)	0.37	1.05 (0.92 – 1.20)	0.49	1.07 (0.93 – 1.23)	0.35	1.17 (0.89 – 1.55)	0.26
Days 1-14	1.03 (0.99 – 1.08)	0.18	1.01 (0.96 – 1.06)	0.72	1.04 (0.99 – 1.09)	0.16	1.02 (0.95 – 1.11)	0.55
Enteral fat ($g.kg^{-1}$)								
Days 1-7	0.94 (0.88 – 1.00)	0.05	0.98 (0.92 – 1.04)	0.50	0.92 (0.85 – 0.99)	0.03	0.97 (0.86 – 1.09)	0.63
Days 1-14	0.98 (0.96 – 1.01)	0.12	1.00 (0.97 – 1.02)	0.72	0.98 (0.95 – 1.00)	0.05	0.99 (0.95 – 1.02)	0.50

Table 8-3 continued…	**Neurodevelopmental impairment**		**WISC FSIQ < 85**		**MABC-2 score ≤ 5th centile**		**Cerebral palsy**	
Nutritional intakes	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**	***Adjusted OR (95% CI)**	***_P_ value**
Parenteral carbohydrate ($g.kg^{-1}$)								
Days 1-7	1.02 (0.99 – 1.04)	0.21	1.01 (0.99 – 1.04)	0.32	1.02 (0.99 – 1.04)	0.26	1.00 (0.96 – 1.05)	0.93
Days 1-14	1.01 (1.00 – 1.02)	0.11	1.01 (0.99 – 1.02)	0.41	1.01 (1.00 – 1.02)	0.09	1.00 (0.99 – 1.02)	0.80
Enteral carbohydrate ($g.kg^{-1}$)								
Days 1-7	0.96 (0.93 – 1.00)	0.05	0.99 (0.95 – 1.02)	0.47	0.95 (0.91 – 1.00)	0.03	0.98 (0.92 – 1.05)	0.61
Days 1-14	0.99 (0.98 – 1.00)	0.08	1.00 (0.99 – 1.01)	0.76	0.99 (0.98 – 1.00)	0.02	0.99 (0.98 – 1.01)	0.45
Total breastmilk ($ml.kg^{-1}$)								
Days 1-7	1.00 (1.00 – 1.00)	0.09	1.00 (1.00 – 1.00)	0.71	1.00 (0.99 – 1.00)	0.05	1.00 (1.00 – 1.00)	0.73
Days 1-14	1.00 (1.00 – 1.00)	0.05	1.00 (1.00 – 1.00)	0.43	1.00 (1.00 – 1.00)	0.009	1.00 (1.00 – 1.00)	0.91

* Adjusted for multiple birth, sex, gestational age, birthweight *z*-score and PIANO study arm.

Chapter 9. Presence and pattern of scarring in children born very preterm

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9.1. Background

Fetal skin trauma may resolve without scar development due to the presence of epidermal growth factors, fluid immersion and sterility of the uterine environment (Leung, Crombleholme, & Keswani, 2012). Skin injury is common in neonatal intensive care, but the long-term consequences of injury to very preterm skin are not well studied. The authors of the only longitudinal study of neonatal scarring concluded that iatrogenic neonatal skin damage was common but that scarring burden was greatly reduced by the age of 9 years (Fox & Rutter, 1998)[2].

9.2. Aims

We aimed to determine the incidence of scarring in a multi-ethnic cohort of 7 year old children born very preterm in the era of modern neonatal intensive care, to describe the number, size and distribution of scars on the body, and to determine any relationships between indicators of neonatal illness severity and scarring at 7 years.

9.3. Methods

Participating children were born at < 30 weeks' gestation or < 1,500g birth weight, admitted to the neonatal intensive care unit at National Women's Hospital, Auckland from 2005-2008 and recruited to a follow-up research study. At 7 years' corrected age their skin was examined by a paediatrician to determine the presence of scarring in each of 9 body areas (head/neck, face, chest, abdomen, back, arms, legs, hands, and feet). Scars were further classified by size (large defined as ≥ 2 cm in any direction) and whether single or multiple scars were visible within each body area. Information regarding the neonatal course was obtained from the electronic medical record, including a standardised score of neonatal illness (Clinical risk index for

babies (CRIB)-II score), the occurrence of culture-positive neonatal sepsis and major neonatal surgery (involving the opening of a body cavity). Information about hospital readmissions and ethnicity were obtained from caregiver questionnaires.

Statistical analyses were performed using JMP 10.0.0 (SAS Institute Inc, N.C., USA). Categorical data were compared using Chi-square, with Fisher's Exact Test where appropriate. Continuous data were compared using ANOVA with Dunnett's *post hoc* testing, or Wilcoxon's rank test as appropriate. Neonatal factors associated with scarring at 7 years were explored using logistic regression. The level of significance was taken as 0.05. Data are presented as median (range), number (%) or odds ratios (OR), and 95% confidence intervals (CI).

Ethical approval for this study was obtained from the Northern B ethics committee (NTY/12/05/035). Caregivers gave written informed consent and children gave verbal assent to the study.

9.4. Results

A total of 129 children were assessed, of whom 90% (116/129) had scarring present (Table 9-1). Boys were more likely to have scarring present than girls (67/69 (97%) vs. 49/60 (82%), Odds Ratio (OR) (95% confidence interval) 7.52 (1.91–50.02), p=0.01). Scarring was also more common in children of non-European ethnicity (72/76 (95%) vs. 44/53 (83%), OR 3.68 (1.12–14.24), p=0.03). However, the incidence of scarring was not associated with gestational age, birth weight, birth weight *z*-score, neonatal sepsis, duration of respiratory support, CRIB II score or post-neonatal hospital re-admission (data not shown).

Large scars were seen in 77/129 children (60%), including all of the 12 (9%) who had undergone major neonatal surgery. Large scars most frequently affected the abdomen and arms, and were least likely to occur on the hands and head / neck (Table 9-2). The presence of

large scars was not associated with gestational age, birth weight or ethnicity, but was more common in boys than girls (OR 2.13 (1.05–4.41), p=0.04), and was more common in children who had been readmitted to hospital following discharge from the neonatal unit (OR 2.64 (1.19–6.00), p=0.02).

Multiple scars were seen in 104/129 (81%) children (Table 9-1), most frequently affecting the hands and feet (Table 9-2). Gestational age and ethnicity were not associated with the presence of multiple scars, but infants in the lowest quartile of birth weight were more likely to have multiple scars than those in the heaviest quartile (OR 4.03 (1.09–19.50), p=0.04). Multiple scars were more common in boys than in girls (OR 3.80 (1.51–10.50), p=0.01) and the likelihood of having multiple scars increased with each additional day requiring respiratory support (OR 1.02 (1.01–1.04), p=0.01).

Scarring affecting more than one area of the body was present in 97/129 (65%) children, with a median of 4 body areas affected (Table 9-1). One child had scarring in all 9 body areas examined. Boys were more likely than girls to have scarring on multiple body areas (OR 2.38(1.06–5.53), p=0.04), as were children of non-European ethnicity (OR 2.28(1.02–5.21), p=0.05). The likelihood of having scarring on multiple body areas increased with each additional day requiring respiratory support (OR 1.02(1.00–1.03), p=0.01). The number of body areas scarred also had a significant but weak association with decreasing gestational age (β = -0.25 (0.09) body areas/week, p=0.01).

9.5. Discussion and conclusions

We found that 90% of children born very preterm were scarred at 7 years of age, comparable to the 87% of adults who display scarring following intensive care admission (Badia, Trujillano, Serviá, March, & Rodriguez-Pozo, 2008). Further, most children had multiple scars and multiple body areas affected.

We found that boys were more frequently and more heavily scarred than girls. Children of non-European ethnicity were also more likely to be scarred and to have multiple body areas scarred than those of European ethnicity, but ethnicity was not associated with large or multiple scars. The mechanisms behind the differences in scarring between pigmented and non-pigmented skin are not clear. However, post-inflammatory hyper- and hypo-pigmentation are known to complicate injury to pigmented skin, with the incidence increasing with darker skin pigmentation. The phenomenon of dyspigmentation has been observed in the newborn and is associated with increased visibility of scarring (Chadwick, Heath, & Shah, 2012), which may explain the increased incidence of scarring in children of non-European ethnicity.

There were no relationships between the presence of scarring and birth weight or gestational age in this cohort, possibly because the overall incidence of scarring was so high. However, the pattern of scarring did vary with neonatal characteristics; lower birthweight was associated with multiple scars, and increased duration of respiratory support was associated with multiple scars and multiple body areas affected. Our findings contrast with those of a previous study of scarring in school aged children exposed to neonatal intensive care(Fox & Rutter, 1998), which reported much lower scarring rates, and might be explained by the lower median gestational age (26 vs. 32 weeks) and longer median duration of neonatal intensive care (88 vs. 9 days) of infants in our study.

Many more children had large scars than had undergone major surgery in the neonatal period. Large scars were predominantly found on the abdomen, but the arms were also frequently affected, perhaps reflecting complications of intravenous line insertion and peripheral venous drug and fluid delivery. It is also possible that children born very preterm were more likely to require surgery after discharge from the neonatal unit and we have not captured this in our data. The hands and feet were the body areas most frequently affected by scarring and

although the majority of this scarring was small in size, multiple scars were common in these areas, as reported previously (Fox & Rutter, 1998).

Strengths of this study are the inclusion of only very preterm children and the inclusion of Polynesian and Asian ethnicities. We did not formally classify skin colour and have used ethnicity as a proxy for skin pigmentation, a crude measure given that ethnic identification encompasses many factors not reliant upon skin colour.

We did not differentiate between scars resulting directly from iatrogenic skin injury, neonatal care or post-neonatal insults. This was because there were no reliable records of non-surgical skin damage to which a scar could be attributed, nor were parental memories sufficiently detailed to reliably determine whether an individual scar was related to neonatal care or not. In addition, preterm birth alters developmental trajectories of many organ systems, and it is possible that the skin of very preterm babies may be more susceptible to injury beyond the neonatal period.

The major limitation of our study is a lack of data on the scarring of 7-year-old children born at term with which to compare our findings. A control group in a large study of skin complications following childhood cancer reported 8-10% incidence of scarring. However, the controls were predominantly white and only 30% were 5-20 years of age(Kinahan et al., 2012) and, thus, comparison with our cohort is unlikely to be meaningful.

Children born very preterm and subject to modern neonatal intensive care are frequently and extensively scarred at 7 years of age. Practitioners should be mindful that skin injury in the neonatal period results in scars that persist into childhood.

9.6. Tables

Table 9-1: Neonatal characteristics and scarring visible at 7 years in 129 7-year-old children born very preterm

Gestational age (weeks)	26 (23 – 32)
Male sex	69 (53)
Birth weight (g)	870 (520 – 1560)
Birth weight *z*-score	0.16 (-2.09 – 2.28)
CRIB II Score [a]	10 (3 – 16)
Ethnicity	
Māori	39 (30)
Pacific Island	19 (15)
Asian	18 (14)
European/ Other	53 (41)
Post-menstrual age at neonatal discharge (weeks)	38 (33 – 50)
Major neonatal surgery	12 (9)
Days of respiratory support	51 (0 – 112)
Sepsis	24 (19)
Ever readmitted to hospital [b]	81 (69)
Any scar	116 (90)
At least one large scar	77 (60)
Multiple scars	104 (81)
Scarring in ≥ 2 body areas	97 (75)
Number of body areas affected	4 (2 – 9)

Data are number (%) of children or median (range).

a: n = 127

b: n = 117

Table 9-2: Distribution of scars in 7-year-old children born very preterm

	Head/ neck	Face	Chest	Back	Abdomen	Arms	Legs	Hands	Feet
Any scarring	8 (6)	24 (19)	36 (28)	22 (17)	39 (30)	56 (43)	50 (39)	97 (75)	70 (54)
Large scars	2 (25)	9 (38)	14 (39)	9 (41)	34 (87)	25 (45)	24 (48)	6 (6)	17 (24)
Multiple scars	4 (50)	10 (42)	19 (53)	14 (64)	32 (82)	40 (71)	38 (76)	86 (89)	60 (86)

Data are number (%) of children

Chapter 10. Discussion and conclusion

10.1. Discussion

Neonatal hyperglycaemia is commonly diagnosed and treated in very preterm infants, despite the ongoing lack of consensus as to how neonatal hyperglycaemia should be defined, when and how it should be treated, and what (if any) effects both hyperglycaemia and its treatment may have on infants' short- and long-term outcomes. The studies we have undertaken reveal that neonatal hyperglycaemia is most common in the smallest, sickest infants, and that it is these underlying perinatal characteristics, rather than high blood glucose concentrations themselves, that are associated with short- and long-term adverse outcomes.

Our finding that hyperglycaemia is a marker for a small, sick infant calls into question the utility of treating hyperglycaemia in the neonatal period at all. However, all hyperglycaemic infants within the PIANO cohort did have their blood glucose concentrations managed, either with standard or, within the smaller randomised control trial cohort, tight glycaemic control. The use of tight glycaemic control did not confer any benefits over standard management, but neither strategy succeeded in keeping more than 50% of infants' blood glucose concentrations within the range of 4-6 mM; a range associated with improved long-term outcomes in our exploratory analysis. It is possible that if we were able to improve our management of neonatal blood glucose concentrations such that blood glucose concentrations were more commonly within this range, long-term outcomes may be improved.

In the randomised controlled trial, treatment decisions were made based on intermittent blood glucose sampling, and changes to insulin infusions were made based on written protocols and clinical judgement. The use of continuous glucose monitoring with real-time display in very low birth weight infants has been recently shown to be effective in reducing abnormal blood glucose excursions and increasing the proportion of time spent within a target range (Galderisi et al., 2017). Thus, real-time continuous glucose monitoring may be a useful tool to allow better control of neonatal glycaemia, and could have the potential to improve outcomes. In

addition, individualised insulin dosing may be facilitated by new software such as STAR-GRYPHON (Le Compte et al., 2010), which uses stochastic modelling to predict insulin sensitivity with the aim of suggesting an insulin dose responsive to an individual infant's needs, thus reducing the incidence of iatrogenic hypoglycaemia and further improving neonatal glycaemic control. This strategy is currently being tested in the setting of a randomised control trial (Alsweiler, Williamson, Bloomfield, Chase, & Harding, 2017).

Although insulin is commonly used to treat high blood glucose concentrations, we have shown that nutritional manipulation may also be an important strategy for both prevention and treatment of neonatal hyperglycaemia. Our findings about the characteristics of infants who develop neonatal hyperglycaemia could assist with prospective identification of infants at the greatest risk of developing high blood glucose concentrations, and potentially allow intervention with nutritional strategies prior to hyperglycaemia developing. More rapid achievement of small, early enteral feed volumes was associated with more stable blood glucose concentrations in our study, reducing the incidence of both hypo and hyperglycaemia. We also saw a reduction in hyperglycaemia in association with higher early protein intakes (potentially due to parenteral delivery of amino acids that promote insulin secretion) and higher early fat intakes. This suggests that, in at least some cases, neonatal hyperglycaemia is a modifiable response to current nutritional strategies, and alterations in nutritional supply targeted to infants at high risk of hyperglycaemia may assist with glycaemic control and thus potentially improve long-term outcomes.

A move towards individualised, targeted nutritional management of infants based on gestational age, birth weight, degree of intrauterine growth restriction and infant sex may also be a future strategy for improving neonatal outcomes, especially for boys who remain at a disadvantage relative to girls born preterm for both short- and long-term outcomes. We found that preterm girls exposed to the same nutrition as boys had better survival free from

neurodevelopmental impairment at 2 years. Few neonatal nutrition studies have reported outcomes separately by sex, and there remains a gap in our knowledge as to how the nutritional requirements of neonatal girls and boys may differ. Our findings suggest that higher volume early enteral feeds and greater early fat intakes are associated with benefit in girls but not boys, but it is not clear whether a different nutritional strategy would benefit boys, whose vulnerability may be driven in part by an increased susceptibility to oxidative stress (Diaz-Castro et al., 2016; Minghetti, Greco, Zanardo, & Suppiej, 2013). Recruitment to future studies of neonatal nutrition should be stratified by sex. If boys and girls have different relationships between nutritional intakes and outcomes, these will be lost in a combined analysis, and may result in recommendations that are inadequate for both sexes. Analysing outcomes separately by sex would require a significant increase in the numbers of infants recruited to trials, and thus needs to be considered at the earliest stages of trial design.

That nutrition of preterm infants is associated with both short- and long-term outcomes is clear, but one of the difficulties in making recommendations regarding neonatal nutritional management is our lack of knowledge about what the normal nutritional requirements of a very preterm infant might be, and whether recommendations for well term infants can be extrapolated to the very preterm population. For example, the ideal composition of parenteral nutrition remains unknown, and although only an observational finding, the increase in cerebral palsy rates we found in children exposed to higher early parenteral protein intakes suggests that this nutritional strategy may have unintended later consequences. Nutrition may be manipulated to induce alterations in brain function in later life, as is seen in the use of ketotic diets to reduce seizure burden in childhood epilepsy (Martin, Jackson, Levy, & Cooper, 2016), but the impact of nutrition on the developing preterm brain and the different nutritional vulnerabilities that may occur in different developmental windows are not yet fully understood. There is an urgent need to establish the blood amino-acid profiles of preterm babies with good vs poor short- and long-term outcomes. This may be done prospectively but

could also (with appropriate consents) involve retrospective analysis of stored blood-spots collected for routine metabolic screening, ideally stratified by sex, gestational age, birth weight and birth weight *z*-score. An appropriate reference range might then be used to guide the composition of intravenous amino-acid solutions supplied to very preterm infants.

A common theme throughout our studies is the association between greater enteral feeds and better outcomes, both short- and long-term. After being exposed to relatively large swallowed volumes of protein-rich amniotic fluid in the fetal period (Mann, Nijland, & Ross, 1996; Sase et al.), neonates born preterm are often supplied with only minimal enteral feeds for a number of days after birth. This is largely because of slow establishment of maternal milk supply in a potentially unwell new mother, and concerns about increased rates of necrotising enterocolitis in preterm infants who receive early enteral feeds. However, necrotising enterocolitis rates are decreasing in association with increasing use of antenatal steroids, breastmilk, probiotics, and generalised improvements in neonatal care (Patel, Panagos, & Silvestri, 2017). If enteral feeds could be introduced earlier and at higher volumes in all infants, we might be able to improve neonatal and long-term neurodevelopmental outcomes further. However, this would necessitate further research into whether the benefits of waiting for mother's own milk are outweighed by the benefits of having an early enteral nutrient supply of an alternative feed in very preterm infants. The increasing availability of donor breastmilk for neonates born very preterm may provide a more acceptable alternative to formula milk with which to test the benefits of early enteral feeds.

Overall, the cohort we studied had a high rate of neurodevelopmental impairment at 7 years' corrected age. Although few children were severely disabled, the high rates of motor impairment, attentional and cognitive difficulties seen at school age are likely to affect children's ability to participate in the classroom, and may contribute to the poor educational attainment described in teenagers and young adults born very preterm (Buck et al., 2000;

Doyle & Anderson, 2010; Jaekel et al., 2013). As seen in previous studies of very preterm children at school age (Schmidt et al., 2017), the prevalence of developmental coordination disorder / motor impairment without cerebral palsy is high in our cohort. This is not a difficulty that is effectively screened for in the current models of neonatal follow-up in New Zealand, where routine developmental surveillance is focussed on early diagnosis of cerebral palsy or significant cognitive impairment and is often discontinued at 2 years if neither diagnosis is apparent (Burakevych et al., 2016; Doyle et al., 2014). Minor motor difficulties in extremely preterm children at 2 years may be predictive of attention difficulties in later childhood (Jeyaseelan, O'Callaghan, Neulinger, Shum, & Burns, 2006) and there is increasing evidence that maturational differences in the brains of infants born preterm persist beyond the infant and preschool period, with evidence of changes in white matter pathways relating to gestational age at birth reported in school-aged children (Murray et al., 2016; Travis, Adams, Ben-Shachar, & Feldman, 2015). A move towards recognising all very preterm children as being at risk of school-age motor, cognitive and executive function difficulties might result in increased developmental and educational support for this vulnerable group.

10.2. Future research directions

In contrast to our hypotheses that neonatal hyperglycaemia and protein intakes would be associated with altered metabolic function in childhood, the only difference in glucose-insulin metabolism we found was a lower fasting blood glucose concentration in children randomised to tight glycaemic control compared to children randomised to standard treatment for neonatal hyperglycaemia. This difference in might be mediated by the greater height-adjusted lean mass in the tight glycaemic control group, as we did not find any other differences in glucose-insulin metabolism measures between randomisation groups.

We did not see differences in glucose-insulin metabolism between hyperglycaemic infants and non-hyperglycaemic preterm controls, nor between infants who received old and new nutrition

protocols. This may be because the effect of preterm birth on pancreatic function is itself so large (Hofman et al., 2004b), that any additional contribution of neonatal hyperglycaemia to later glucose-insulin metabolism cannot be detected. Comparison of our intravenous glucose tolerance test results with those derived from a control cohort of 7-year olds born at term will allow us to further describe the contribution of very preterm birth to pancreatic function in childhood, and work to recruit this local reference cohort has recently been completed.

A re-assessment of the PIANO cohort after puberty may allow us to determine the significance of our finding of decreased standing height and leg length at 7 years' corrected age in children randomised to tight glycaemic control for neonatal hyperglycaemia. Shorter leg lengths are associated with adverse metabolic outcomes in adulthood (Mueller & Pereira, 2015) but not childhood (Haugaard et al., 2016), and final adult height is affected by the timing of pubertal take-off, which may be hastened by accelerated growth in mid-childhood (Karlberg, 2002). Another benefit of an assessment performed post-puberty would be the opportunity to ask directly about quality of life rather than using a parental proxy (Wallander, Schmitt, & Koot, 2001). The high burden of scarring we have shown within the cohort may begin to impact on self-esteem and quality of life measures during the adolescent period, as is seen in other non-preterm cohorts who suffered childhood scarring (Kinahan et al., 2012; Robert et al., 1999).

The studies we have performed in the PIANO cohort have highlighted a number of research questions, the answers to which have the potential to further improve neonatal care and neonatal outcomes. The association we saw between early enteral feeds and improved neonatal outcomes requires testing in the setting of a randomised trial. Infants of birth weight < 1,500g or < 30 weeks' gestation could be randomised to either the intervention group, who receive a minimum of 10% of their daily fluid totals as enteral feed (maternal breastmilk, donor breastmilk or formula) from birth onwards; or standard treatment, where infants receive maternal breastmilk as it becomes available. The primary outcome of survival without

neurodevelopmental impairment at 2 years' corrected age (to include a measure of executive function) (McKinlay et al., 2015), and secondary outcomes of neonatal mortality and morbidity (necrotising enterocolitis, retinopathy of prematurity, chronic lung disease and sepsis), weight, length and head circumference measures at term-equivalent and at 2 years' corrected age should be analysed separately for each sex to ensure that, if the relationships between early enteral nutrition and outcomes are different for girls and boys, outcomes are optimally assessed for each sex.

The association between higher early parenteral protein intakes and motor impairment in childhood that we observed is of concern, and whether this relationship is in fact causal also needs to be tested in a randomised trial. An international, multicentre randomised controlled trial of early additional intravenous amino acid supplementation for extremely preterm infants is currently under way (the PROVIDE study), with a primary outcome of survival free from neurodevelopmental disability at 2 years' corrected age. (Bloomfield et al., 2015).

10.3. Conclusion

Neonatal blood glucose concentrations are markers for neonatal characteristics and neonatal illness. Hyperglycaemia affects the smallest, sickest infants, and treating hyperglycaemia using current strategies to achieve tight or standard glycaemic control does not appear to change infants' school-age neurodevelopmental outcome. The association we observed between higher early protein and fat intakes and greater volumes of early enteral feeds, and a reduced incidence of high blood glucose concentrations, suggests that hyperglycaemia may potentially be ameliorated by modifying neonatal nutrition. However, increasing early protein (especially parenteral protein) intakes were, in this cohort, associated with motor impairment and an increased risk of cerebral palsy at school age. As these were observational findings, randomised control trials are required to test nutritional strategies to manage neonatal

hyperglycaemia, and to determine relationships between early life protein intakes and motor impairment in preterm infants.

Chapter 11. Appendix A: Macronutrient reference values

Table 11-1: Macronutrient values for parenteral nutrition

Fluid	Protein ($g.100ml^{-1}$)	Fat ($g.100ml^{-1}$)	Carbohydrate ($g.100ml^{-1}$)	Energy ($kCal.100ml^{-1}$)
Dextrose (Baxter, Auckland, NZ)				
5% Dextrose	0	0	5	17
7.5% Dextrose	0	0	7.5	25.5
10% Dextrose	0	0	10	34
12.5% Dextrose	0	0	12.5	42.5
15% Dextrose	0	0	15	51
Intravenous amino acid solutions (Cormack et al., 2011)				
Old protocol intravenous nutrition (IVN)	2	0	10	42.2
New protocol IVN	3.8	0	10	49.7
New protocol IVN (<1,000 grams, <72 hours' postnatal age, central access)	6.79	0	15	78.1
Intralipid (Fresenius Kabi AB, Uppsala, Sweden)				
20% Intralipid	0	20	2.2	200

Table 11-2: Macronutrient values for enteral nutrition

Fluid	Protein ($g.100ml^{-1}$)	Fat ($g.100ml^{-1}$)	Carbohydrate ($g.100ml^{-1}$)	Energy ($kCal.100ml^{-1}$)
Expressed breastmilk ("Foodworks," 2007)				
Transitional (< day 14)	1.566	3.86	6.89	68
Mature (≥ day 14)	1.357	4.28	7.2	72
Fortifier (Nutriprem, Nutricia, NZ)	0.8	0	3	16
Formula milk				
Term infant formula (Karicare, Nutricia, NZ)	1.5	3.8	7	68
Preterm formula (Nutriprem, Nutricia, NZ)	2.4	4.4	7.8	80
Hydrolysed formula (Pepti-Junior, Nutricia, NZ)	1.9	3.8	7	67

Chapter 12. Reference list

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