The effects of caffeine on intermittent hypoxaemia in late preterm neonates

Elizabeth Anne Oliphant

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Paediatrics, the University of Auckland, 2024

Abstract

Aim

Late preterm infants have higher rates of intermittent hypoxaemia in the first weeks after birth and worse long-term neurodevelopment than term-born babies. Caffeine reduces hypoxaemia and improves outcomes in very preterm infants. We aimed to establish the most effective dose of caffeine to reduce intermittent hypoxaemia in late preterm infants and to evaluate and synthesise the evidence for the use of caffeine in preterm infants.

Method

We undertook a double-blind, five-arm, parallel, dose-finding randomised controlled trial to compare the effectiveness of oral caffeine citrate versus placebo in reducing intermittent hypoxaemia. Following the development of an appropriate oral formulation, we randomised 132 late preterm infants to 5, 10, 15 or 20mg.kg^{-1}.day^{-1} caffeine citrate or placebo daily until term equivalent age, with a primary outcome of intermittent hypoxaemia, two weeks post-randomisation. Finally, we formally evaluated the evidence for the use of caffeine for apnoea and prevention of neurodevelopmental impairment in preterm infants through a systematic review and meta-analysis.

Findings

At two weeks post-randomisation, caffeine citrate at doses of 10 and 20 mg.kg^{-1}.day^{-1} reduced intermittent hypoxaemia compared to placebo, increased mean oxygen saturation (SpO_2), and reduced time with SpO_2<90%, with 20 mg.kg^{-1}.day^{-1} being most effective. No adverse effects were observed on growth velocity or sleep at any dose, but tachycardia increased at two weeks with all doses, and persisted at term in the 5, 10 and 20 mg.kg^{-1}.day^{-1} groups.

The systematic review included 15 studies (3,530 infants). Caffeine possibly reduced apnoea (very low certainty evidence) and probably reduced bronchopulmonary dysplasia (moderate certainty evidence), with higher doses probably more effective. Only one trial reported neurodevelopmental outcomes beyond early childhood, with moderate-certainty evidence indicating a probable lack of effect on neurocognitive impairment in early childhood but possible benefit on motor function in middle childhood.

Conclusion

Caffeine citrate 20 $mg.kg^{-1}.day^{-1}$ was most effective in reducing intermittent hypoxaemia in late preterm infants and was well tolerated. Further research is needed to determine if this dose improves neurodevelopmental outcomes; if it does, then use in this population could be rapidly adopted to improve outcomes by means of a simple intervention suitable for delivery outside the hospital setting.

Dedication

This thesis is dedicated in memory of my father

Peter Bunning

10 April 1955 – 28 August 2021

England ~ Hong Kong ~ New Zealand

who taught me the value of hard work and dedication

Acknowledgements

'*No man is an island*', wrote John Donne, and likewise, no thesis is truly the work of a single individual. The work contained herein – and my ongoing sanity – owes its existence to an extensive cast of people who have encouraged, supported, guided, contributed, critiqued, and taught me over the last five years.

First and foremost, my sincere thanks to my supervisor Jane Alsweiler for her patient guidance throughout. Jane – I'm still not entirely sure how you convinced me this was a good idea, but you were right, and it has been an incredible journey. I have learnt so much along the way, found new and exciting challenges, and a new career direction. Thank you for your many explanations, endless reviews of pieces of writing (and your incredible ability to cull words!), and especially for your support and understanding keeping me on track as I have navigated the juggle of family, study, and other clinical and academic roles, alongside the madness of the world at large over the last few years. My thanks also to my co-supervisors, Sara Hanning and Chris McKinlay for your unending support, tireless proof-reading, and answers to many random questions.

My thanks also to the following people who have assisted with the work reported in this thesis:

- Members of the ON TRACK network for assistance in developing and refining the Latte Dosage study design – and now the Latte trial as well.
- Sara Hanning and Trusha Purohit for advice on development of the caffeine assay and laboratory methods, and to Trusha for spending hours in the lab teaching this clinical pharmacist laboratory skills never previously learnt or long since forgotten.
- Anthony Walters for independent assistance in conducting risk of bias assessment and data extraction for the Latte Dosage Trial in the systematic review included in this thesis.

- Alana Cavadino; biostatistician extraordinaire, for assistance with the statistical analysis of Latte Dosage results and prompt responses to my many queries.
- David McNamara, for teaching me to interpret oximetry traces and reports – a skill not part of the average pharmacist's stock-in-trade.

A special mention to Sarah Phillipsen, Sabine Huth, Lisa Mravicich and Florella Keen for their amazing work in setting up the Latte Dosage trial, recruiting mothers and babies – and keeping them in the trial - and the data collection involved. A particular thanks to Sabine, who held my hand as I learnt the art of trial recruitment and taught me many a nursing skill, including the tricks to getting a good oximetry trace!

A huge thank you to all the mothers, babies and families who participated in Latte Dosage, with the aim of improving the lives not of themselves and their babies, but of those who follow. After many years of working in tertiary hospitals, it was such a refreshing change to be welcomed into so many homes to visit babies outside the walls of the hospital and as life was settling into the new normal for these families.

Heartfelt thanks to my parents, Sue and Peter Bunning, parents-in-law, Rick and Margie Oliphant, and sister and sister-in-law Claire Chaffey and Georgie Barker, for their assistance over the years – especially with childcare - which have enabled me to undertake this work towards my PhD, and without whom it would have been an impossible task. A particular thanks to my late father, who helped design and build us "The Shed" in the garden as a work-from-home office space and writing bolt-hole during the pandemic, but who didn't get to see it finished. Many late nights and early mornings have since been spent in its quiet shelter, working on this thesis and other projects.

Finally, to Chris – my rock and support through it all. For knowing me better than I know myself, and for helping to hold our wee family together through these roller-coaster few years of work, study, family ups and downs, topped off with a global pandemic! And to our boys - now almost young men – James and Edward, who have got used to oximeters piled in the hallway, Mummy in the office at all hours, and who can now talk more knowledgeably about intermittent hypoxaemia and preterm babies than most! The last word goes to James, who concluded his talk on science for his primary school speech competition thus:

"So now you know.... Science isn't about mad scientists with crazy hair.

It's about trying your very best and never, ever, giving up".

~ James Oliphant (aged 10, 2019)

May you both follow your dreams, achieve your goals, try your best and never, ever give up.

Table of Contents

List of figures

List of tables

List of abbreviations

ANZNN	Australia and New Zealand Neonatal Network
BPD	Bronchopulmonary dysplasia
CAP	Caffeine for apnoea of prematurity trial
CI	Confidence interval
CNS	Central nervous system
CPAP	Continuous positive airway pressure
GA	Gestational age
GABA	Gama-aminobutyric acid
IH	Intermittent hypoxaemia
IV	Intravenous
IVH	Intraventricular haemorrhage
NEC	Necrotising enterocolitis
NG	Naso-gastric
NICU	Neonatal intensive care unit
NZ	New Zealand
O_2	Oxygen
OR	Odds ratio
PaO_2	Partial pressure of oxygen in arterial blood
PDA	Patent ductus arteriosus
PDE	Phosphodiesterase
PGE_2	Prostaglandin E_2

PMA	Post-menstrual age
PCO_2	Partial pressure of carbon dioxide
PO	Per oral
PO_2	Partial pressure of oxygen
RDS	Respiratory distress syndrome
RR	Risk ratio
SD	Standard deviation
TEA	Term equivalent age (40 weeks post-menstrual age)
UK	United Kingdom of Great Britian and Northern Ireland
USA	United States of America
WHO	World Health organisation

Co-authorship forms

Publication / copyright permissions

i. Chapter 3: Analytical methods and caffeine oral liquid formulation stability

Oliphant EA, Purohit TJ, Alsweiler JM, McKinlay CJD, Hanning SM. Validation and application of a simple and rapid stability-indicating liquid chromatographic assay for the quantification of caffeine from human saliva. Journal of Liquid Chromatography and Related Technologies 2022; doi: 10.1080/10826076.2022.2095402

ii. Chapter 4: The Latte Dosage Trial protocol

Oliphant EA, McKinlay CJD, McNamara DG, Alsweiler JM. Caffeine prophylaxis to improve intermittent hypoxaemia in infants born late preterm: a randomised controlled dosage trial (Latte Dosage Trial). BMJ Open 2020; 10:e038271. doi: 10.1136/bmjopen-2020-038271

iii. Chapter 5: The Latte Dosage Trial

Oliphant EA, McKinlay CJD, McNamara DG, Cavadino A, Alsweiler JM. Caffeine to prevent intermittent hypoxaemia in late preterm infants: randomised controlled dosage trial. Archives of Disease in Childhood: Fetal and Neonatal Edition 2022; doi:10.1136/archdischild-2022-324010

iv. Chapter 6: Systematic Review: Caffeine for apnoea and the prevention of neurodevelopmental impairment in preterm infants

Oliphant EA, Hanning SM, McKinlay CJD, Alsweiler JM. Caffeine for apnea and prevention of neurodevelopmental impairment in preterm infants: systematic review and meta-analysis. J Perinatol. 2024 Mar 29. doi: 10.1038/s41372-024-01939-x. Epub ahead of print. PMID: 38553606.

1. Introduction

1.1 Overview

The World Health Organization (WHO) defines preterm birth as that occurring at less than 37 completed weeks of gestation. This is then further subdivided into:

- Extremely preterm (<28 completed weeks of gestation)
- Very preterm (28-<32 weeks)
- Moderate to late preterm (32-<37 weeks)(March of Dimes et al., 2012)

This final category is then commonly further broken down into moderate preterm (32-<34 weeks) and late preterm (34 -<37 weeks) groups (Engle, Tomashek, & Wallman, 2007).

On a world-wide level, an estimated 11.1% of all live births in 2010 occurred preterm, equating to 14,936,700 babies (Blencowe et al., 2012). This rate has not changed significantly in the last decade, with an estimated 13.4 million preterm births in 2020 (Lawn et al., 2023). Preterm birth is more common in low and middle income countries, with the highest rates in Southern Asia, where preterm births are estimated to account for 13.3% of all live births, compared to 7.9% of births in higher income countries (Lawn et al., 2023).

Preterm birth may occur as a result of spontaneous preterm labour or following preterm premature rupture of membranes, or as a result of medically-induced delivery for maternal or fetal reasons (commonly due to pre-eclampsia or eclampsia, or intrauterine growth restriction)(Goldenberg et al., 2008). The reasons for spontaneous preterm birth are often unclear, but may include infection, inflammation, vascular disease or uterine overdistension or abnormalities. A large number of risk factors for

preterm birth have been identified, including previous preterm birth, multiple pregnancy, ethnicity, extremes of age, periodontal disease and other infectious diseases, exposure to air pollution, smoking and alcohol, physical workload or intimate partner violence, short inter-pregnancy interval, gestational diabetes and poor nutritional status (Goldenberg et al., 2008; Hunter et al., 2023).

Preterm birth is the leading cause of neonatal death, and the leading cause of death in children under the age of five worldwide (L. Liu et al., 2015). Infants born preterm have an increased risk of morbidity in the neonatal period and are more likely to suffer infections, intercranial bleeding, necrotising enterocolitis (NEC) and respiratory complications (Ashorn et al., 2023). In the longer term, they experience a wide array of consequences ranging from growth impairment, delays in development and cognitive impairment, to increased rates of disease in both childhood and adulthood, including asthma, cerebral palsy, epilepsy, hypertension, cardiovascular disease, metabolic syndromes, renal dysfunction and more (Ashorn et al., 2023).

A number of interventions – administered either to mothers antenatally when preterm birth appears imminent, or to infants in the postnatal period – have been shown to improve outcomes in the preterm population and have been incorporated into clinical guidelines (World Health Organization, 2022). The most notable of these are antenatal corticosteroids to improve neonatal outcomes, including mortality (McGoldrick et al., 2020), and exogenous surfactant for respiratory distress syndrome (RDS)(Seger & Soll, 2009). Antenatal/intrapartum magnesium sulfate is administered to prevent neurological complications in preterm infants (Doyle et al., 2009). Antibiotics are recommended for women with preterm premature rupture of membranes, for their effects in prolonging pregnancy and reducing maternal and neonatal infectious morbidity (Kenyon et al., 2013).

1.2 Late preterm infants

1.2.1 Incidence of late preterm birth: The size of the problem

Late preterm infants (those born between 34 and 36^{+6} weeks of gestation) form the largest group within the preterm population, accounting for 68% of all preterm births or 5.2% of all births in New Zealand (NZ) in 2017 (the most recent year for which this data is available) and equating to approximately 3,200 babies annually (Ministry of Health, 2019). This rate is similar to that seen in England and Wales (5.3% in 2020)(Bradford, 2022), while in the United States (USA), late preterm infants account for approximately 7% of all births (Osterman et al., 2022).

Of the 11.1% of live births that occur preterm globally, moderate and late preterm births (32-36 weeks) have been estimated to account for 84.3% (Blencowe et al., 2012). By comparison, very preterm births (28-31 weeks gestation) account for 10.4%, and extremely preterm births (<28 weeks gestation) 5.2%, of preterm births (Blencowe et al., 2012). Therefore, although medical and research attention is often focused on those infants born at the extremes of prematurity, late preterm infants account for the vast majority of babies born before term.

1.2.2 Complications of late preterm birth

Late preterm infants are physiologically and metabolically immature (Engle et al., 2007), and have a higher risk of morbidity and mortality in the neonatal period than full-term infants (McIntire & Leveno, 2008). They are more likely than full-term infants to have delayed establishment of oral feeding, temperature instability, hypoglycaemia, jaundice and respiratory distress, and undergo investigation for sepsis (Wang, Dorer, Fleming, & Catlin, 2004). Despite these risks, their size and weight mean they are often managed in a similar manner as full-term infants and cared for on postnatal wards rather than in neonatal units (Boyle et al., 2015).

In the longer term, late preterm infants are more likely to be diagnosed with cerebral palsy (Moster et al., 2008; Odd et al., 2013), developmental delay (Darlow et al., 2009; Woythaler et al., 2011), cognitive impairment (Heinonen et al., 2015; Quigley et al., 2012; Talge et al., 2010) and behavioural disorders (Huddy et al., 2001) compared to term infants.

While the individual morbidity is generally lower in late preterm infants than those born more prematurely, the much larger size of the late preterm population nevertheless means that they account for a significant portion of all neonatal morbidity. Late preterm birth thus has significant implications in terms of resource use both in the immediate care needed in the neonatal period, as well as in the longer term in both the health and education systems (Premji, 2019).

1.2.3 Respiratory effects of late preterm birth

Several factors place late preterm infants at higher risk of respiratory morbidity. Lung development is not yet complete, as alveolar development occurs during this period, with maturation of the gas exchange surfaces occurring along with capillary growth in the terminal sacs and an increase in surfactant (Engle et al., 2007). Correspondingly, rates of RDS are higher in late preterm infants than those born at term, with an incidence of 10.6% at 34 weeks to 2.7% at 36 weeks gestation, compared to 0.36% at term (Teune et al., 2011), and transient tachypnoea of the newborn affects approximately 10% of late preterm infants (Gyamfi-Bannerman et al., 2016). Developmental immaturity of the central nervous system (CNS) may also increase the risk of centrally mediated apnoea (Kinney, 2006), and while the rate of apnoea is lower than in more preterm infants and varies between studies (within a range of approximately 2-7%) it remains significantly above that of term infants (Gyamfi-Bannerman et al., 2016; Henderson-Smart, 1981; Ramanathan et al., 2001; Teune et al., 2011). Likewise, the need for continuous positive airway pressure therapy (CPAP), mechanical ventilation or surfactant treatment follows a similar pattern, with rates

decreasing across the late preterm gestational age window, but being significantly higher for all late preterm infants than for those born at term (Teune et al., 2011).

However, the greater maturity of the respiratory system in these infants compared to those born at earlier gestations means that significant and long-term respiratory conditions are rare, with rates of bronchopulmonary dysplasia (BPD) of 0.6% reported (Gyamfi-Bannerman et al., 2016).

1.2.4 Long term neurodevelopmental outcomes

Late preterm infants are at higher risk of adverse neurodevelopmental outcomes than their full-term peers. (Cheong et al., 2017; Kerstjens et al., 2012; Petrini et al., 2009; Teune et al., 2011; Woythaler, 2018). They have a 3-6-fold higher risk of being diagnosed with cerebral palsy (Hirvonen et al., 2014; Petrini et al., 2009), and higher rates of seizures and intraventricular haemorrhage (IVH)(Teune et al., 2011). A recent national data-linkage study from Sweden has demonstrated an increase in intellectual disability with decreasing gestational age, with the trend continuing throughout the late preterm window, and up until 40 weeks post-menstrual age (PMA)(Yin et al., 2022). Similar results have been reported in NZ, with those born at lower gestational ages having higher scores for hyperactivity and lower ones for prosocial behaviours in the Strengths and Difficulties parental and teacher questionnaires prior to school entry, and being more likely to need input from the Resource Teachers: Learning and Behaviour service, again with the trend continuing through the late preterm window (Berry et al., 2018).

In infancy and early childhood developmental delay is more common in late preterm infants, with odds ratios of 1.36 -1.52 reported (Petrini et al., 2009; Woythaler et al., 2011). Multiple studies have assessed measures of school readiness and academic achievement and have also found poorer outcomes at primary school age for children who had been born late preterm than those born at term. At 5-6 years of age,

significantly poorer results were found in assessments of reading, maths, expressive language and school readiness in late preterm infants in a cohort born in the USA in 2001 (Woythaler et al., 2015), while in a contemporary cohort in the United Kingdom (UK) the risk of not achieving a good level of overall achievement at the end of the first school year was 12% higher in late preterm children (Quigley et al., 2012). In a national cohort of school-aged children in Scotland in 2005, the odds of having special educational needs were 1.53 (95%CI 1.27-1.45) for infants born between 33 and 36 weeks gestation (Mackay et al., 2010). However, in New Zealand and at a later timepoint, children born between 33 and 36 weeks gestational age (GA) were no less likely to achieve Level 1 of the National Certificate in Educational Achievement than those born at 39-40 weeks GA (Level 1 achieved by 73% of those born late preterm vs 74% of those born at term; difference not statistically significant)(Berry et al., 2018).

This increased risk of neurodevelopmental impairment is likely multifactorial, and may be due to changes in development associated with maturation occurring outside the uterus, the effects of maternal or fetal causes of preterm delivery, and morbidity associated with prematurity (Kugelman & Colin, 2013).

The brain undergoes significant development in the last few weeks of pregnancy, and preterm birth during this critical period may alter the trajectory of this development (Walsh et al., 2014). Magnetic resonance imaging for newborns born prematurely and at term has established that at 34 weeks gestation brain volume is only 65% of that of the full-term brain, and gyri and sulci formation is far from complete (Hüppi et al., 1998). The cortex gains approximately half its volume in the last 6 weeks before term, and there is a five-fold increase in white matter between 35 and 41 weeks gestation (Hüppi et al., 1998). Moderate and late preterm infants (32 – 36^{+6} weeks gestation) have been shown to have smaller brains with larger extra-axial spaces when imaged at term-equivalent age, compared with babies born at full-term, and are more likely to show features consistent with brain immaturity, including delayed gyral maturation and

incomplete myelination of the posterior limb of the internal capsule (Walsh et al., 2014). Similar features in very premature infants have been linked to cognitive delay, motor delay, cerebral palsy and neurosensory impairment at two years of corrected age (Woodward et al., 2006).

Antenatal complications that contribute to or result in preterm birth, including congenital malformation, intra-uterine growth retardation and chorioamnionitis, and maternal conditions such preeclampsia, hypertension, diabetes and maternal smoking may also potentially be associated with poor neurodevelopmental outcomes or behavioural problems (Kugelman & Colin, 2013).

Physiological consequences of prematurity may also result in conditions that increase the risk of neurodevelopmental impairment. Hypoglycaemia is more common in late preterm babies than term-born infants, as a result of low body fuel stores, inadequate nutritional intake and an increased rate of conditions such as sepsis and hypothermia. The risk of hypoglycaemia is exacerbated by feeding difficulties and a lack of effective suck and swallow common in premature infants (Wang et al., 2004). Neonatal hypoglycaemia increases the risk of poor executive function and visual motor function (McKinlay et al., 2017) and developmental delay (Kerstjens et al., 2012) at preschool age. However, this effect appears to have diminished by the end of primary schooling, with children who experienced neonatal hypoglycaemia having similar rate of low educational achievements to those who did not experience hypoglycaemia at 9-10 years of age, although overall this group of children (who were all born with risk factors for hypoglycaemia) had high rates of low academic achievement (Shah et al., 2022).

Hyperbilirubinaemia results from production of bilirubin in excess of that which is able to be metabolised and hence excreted, and may result in acute bilirubin encephalopathy and kernicterus if untreated (Olusanya et al., 2018). Late preterm

infants have an increased risk of developing significant hyperbilirubinaemia compared to full-term infants (Newman et al., 2000; Sarici et al., 2004), due to physiological immaturity that includes a lower concentration of uridine diphosphoglucuronate glucronosyltransferase (the rate-limiting enzyme in the excretion pathway for bilirubin)(Kawade & Onishi, 1981), immature gastrointestinal function and feeding difficulties that can lead to increased enterohepatic recirculation, decreased stool frequency and dehydration, all of which predispose to hyperbilirubinaemia (Engle et al., 2007). In addition, late preterm infants have an higher risk of developing neurologic sequalae and neurotoxicity than their term-born peers with similar plasma bilirubin concentrations, especially during the early postnatal period (Bhutani & Johnson, 2006), as a result of developing neurons and astrocytes being more sensitive to neurotoxic insult in the late preterm brain (Adams-Chapman, 2006).

Finally, conditions such as IVH, which cause injury to the structure of the brain and are associated with cerebral palsy, cognitive and behavioural abnormalities, are inversely related to gestation at birth. While much less common in late preterm infants than in those born more prematurely, the rates of IVH are nonetheless higher in the late preterm group than in those born at full-term (Marrocchella et al., 2014).

1.2.5 Interventions to improve outcomes

Infants born prematurely may receive several interventions with the intention of improving outcomes in the neonatal period and beyond (World Health Organization, 2015). These interventions may be administered to mothers antenatally or the infant either at birth or in the post-natal period, and include antenatal corticosteroids to accelerate lung maturity (McGoldrick et al., 2020), antenatal/intrapartum magnesium sulfate to prevent neurological complications in preterm infants (Doyle et al., 2009), delayed cord clamping to allow for the transfusion of blood from the placenta to the newborn (Mcdonald et al., 2013), dextrose gel for the prevention or treatment of neonatal hypoglycaemia (Edwards et al., 2021; Edwards, Liu, et al., 2022) and iron

supplements for infants with or at risk of iron deficiency (Mills & Davies, 2012). These interventions all aim to improve outcomes for the infant in the short or long term or both, and many are offered prophylactically to all infants identified as being at risk of a specific condition, either because of their gestational age at birth, or the presence of other identified risk factors.

Antenatal corticosteroids have been used since the 1970s to prevent respiratory distress in the newborn following preterm birth. An early study by Liggins and Howie reported a reduction in early neonatal mortality from 15% to 3.2% with betamethasone treatment, and a reduction in the incidence of RDS from 25.8% to 9% in preterm infants of less than 32 weeks gestation whose mothers received corticosteroids at least 24 hours prior to delivery (Liggins & Howie, 1972), or from 14.4 % to 8.8% across all participants (Walters, Lin, et al., 2023). The most recent Cochrane review on the topic found that antenatal corticosteroids reduced the risk of perinatal death (Relative risk (RR) (95% Confidence interval (CI)) 0.85, 0.77 - 0.93), neonatal death (RR (95% CI) 0.78, 0.70-0.87), and RDS (RR (95% CI) 0.71, 0.65-0.78)(McGoldrick et al., 2020), without long term effects on cardiometabolic or respiratory disease in the infants (Walters, Crowther, et al., 2023). Though some questions remain about the optimum steroid, dose and regimens, use for caesarean sections near term, and in women with coexisting medical conditions (Shanks et al., 2019), the WHO recommend administration of antenatal corticosteroids to women at risk of preterm birth from 24-34 weeks gestation when preterm birth is considered imminent and there is no clinical evidence of maternal infection(World Health Organization, 2015). In late preterm infants, the ALPS trial found infants whose mothers were randomised to antenatal corticosteroids had a lower risk of requiring respiratory support in the 72 hours following birth (RR (95%CI) 0.8, 0.66-0.97; p=0.02), and of severe respiratory complications (RR (95%CI) 0.66 (0.52-0.82); p<0.001), with no difference in major neonatal morbidity (NEC, sepsis or IVH), but with an increase in neonatal hypoglycaemia (RR (95%CI) 1.60, 1.37-1.87; p<0.001), and increased likelihood of a

prolonged stay in a special care nursery (RR (95%CI) 0.89, 0.80-0.98; p=0.03) (Gyamfi-Bannerman et al., 2016). A more recent trial of antenatal corticosteroids for late preterm birth in a lower-resource country did not find a reduction in neonatal death or severe respiratory distress, but was closed early due to lower than expected prevalence of primary outcomes and slow recruitment after recruiting only 782 of the 22,589 women required to adequately power the trial (WHO ACTION Trials Collaborators, 2022).

Magnesium sulfate was initially used for the prevention of eclamptic seizures and as a tocolytic, before use was associated with a reduction in IVH and cerebral palsy (Kuban et al., 1992; Nelson & Grether, 1995). Maternally administered magnesium sulfate rapidly crosses the placenta to the fetus, where it crosses the blood brain barrier and is thought to act to prevent post-hypoxic brain injury by inhibiting the release of glutamine, reducing calcium influx (Elsayed et al., 2021; Hallak & Cotton, 1993). Systematic review has established that antenatal administration of magnesium sulfate to women at risk of preterm birth significantly reduces the risk of cerebral palsy (RR (95% CI) 0.68, 0.54-0.87), and motor dysfunction (RR (95% CI) 0.61, 0.44-0.85), without affecting paediatric mortality or other disabilities in early life (Doyle et al., 2009). However, though the Cochrane review considered all preterm births, the majority of participants in included trials were at gestations of less than 30-34 weeks, and clinical guidelines currently variously recommend use for women at risk of delivery at less than 30 (The Antenatal Magnesium Sulphate For Neuroprotection Guideline Development Panel, 2010), 32 weeks (World Health Organization, 2015) or 34 weeks (Magee et al., 2019) gestation. The recently-published MAGENTA trial investigated the benefit of magnesium sulfate in infants born 30-34 weeks gestation, and found no difference in death or cerebral palsy at 2 years corrected age, though a reduction in the rate of RDS (RR (95% CI) 0.85, 0.76-0.95) and BPD was observed (RR (95% CI) 0.69, 0.48-0.99) (Crowther et al., 2023).

At birth, the umbilical cord connecting the infants circulation to the placenta is cut, either immediately following birth, or after a short 'delay' (a minute or more after delivery, or after the cord has ceased pulsation)(Mcdonald et al., 2013). A delay in the cutting of the cord allows for the transfer of blood in the placenta to the newborn circulation, increasing the red blood cell volume by approximately 40% in full-term infants (Yao et al., 1969). Though concerns have been raised of potential harms, including polycythaemia, hyperviscosity, hyperbilirubinaemia and transient tachyphnea of the newborn, these have not been borne out in clinical trials or resulted in significant adverse effects (Mercer, 2001). Instead, systematic review has found increased birthweight and haemoglobin levels (Mean Difference (95% CI) -1.49 g/dL, -1.78 - -1.21) at 24-48 hours of age in term infants with delayed cord clamping and a reduction in jaundice requiring phototherapy (RR (95% CI) 0.62, 0.41-0.96). Furthermore, iron deficiency at three to six months of age is reduced, with infants whose cords were clamped early being over twice as likely to be iron deficient as those for whom clamping was delayed (RR (95% CI) 2.65, 1.04 - 6.73)(Mcdonald et al., 2013). This reduction in anaemia may be particularly important for infants in low-resource settings where poor dietary intake or iron can lead to anaemia, which negative effects on psychomotor development (World Health Organization, 2014). Benefits are even greater for preterm infants, with a reduction in hospital mortality in preterm infants who had delayed cord clamping (Fogarty et al., 2018; Rabe et al., 2019). In late preterm infants, a retrospective cohort study of placental transfusion (delayed cord clamping or umbilical cord milking) reported significantly higher haematocrit (47.6 ± 6.2% for immediate cord clamping vs 53.5 ± 6.6% for placental transfusion; $p<0.01$) and a lower incidence of neonatal intensive care unit (NICU) admission for respiratory distress (16.9% vs 4.5%; $p=0.03$) for infants who received placental transfusion, compared to those who received immediate cord clamping, with no effects on requirement for phototherapy, symptomatic polycythemia, length of hospital stay or NICU admissions (Chiruvolu et al., 2022). Furthermore, delayed cord clamping may reduce the incidence of neonatal hypoglycaemia in the first hour of life in late preterm infants exposed to antenatal corticosteroids (Hitchings et al., 2022).

Iron is required for normal growth and organ development, and deficiency in early life can result in long term alterations in brain structure and function (Georgieff, 2011). Infants born preterm have reduced iron stores as accretion of iron occurs across the third trimester of pregnancy (Lackmann et al., 1998), and supplementation with enteral iron in these infants improves haemoglobin levels, reducing the incidence of iron deficiency anaemia (Mills & Davies, 2012). Meta-analysis has found an improvement in linear growth with iron supplementation, and though beneficial effects on neurodevelopmental outcomes have not been observed, there is a lack of adverse effects at standard doses (Manapurath et al., 2022). However, the evidence was of low quality and significant heterogeneity has limited meta-analysis (Manapurath et al., 2022). Given the evidence for poorer outcomes in infants and children with iron deficiency anaemia, and a reduction in the incidence of anaemia with supplementation, guidelines recommend that preterm and low birthweight infants receive prophylactic enteral iron at a dose of 2-3 $mg.kg^{-1}day^{-1}$, starting from two weeks after birth (Agostoni et al., 2010; Domellöf et al., 2014; World Health Organization, 2022).

Finally, neonatal hypoglycaemia is associated with brain injury (Burns et al., 2008), neurodevelopmental problems (Kerstjens et al., 2012; McKinlay et al., 2017) and poor school performance (Kaiser et al., 2015), though how far-reaching these effects are is unclear (Shah et al., 2022; Tin et al., 2012). Oral dextrose gel, administered to the mucosal surfaces of the mouth, has been used for both the treatment of identified hypoglycaemia and the prevention of hypoglycaemic episodes in high-risk infants, such as those born to diabetic mothers, preterm, or small or large for gestational age (Edwards et al., 2021; Edwards, Liu, et al., 2022). When used prophylactically, oral dextrose gel reduces the risk of hypoglycaemia (RR (95% CI) 0.87, 0.79-0.95) without increasing adverse effects (Edwards et al., 2021), and results from an initial dose-finding study indicated dextrose probably reduced the risk of major neurological disability at two years corrected age (RR (95% CI) 0.21, 0.05-0.78)(Edwards et al., 2021; Griffith et

al., 2021). However, assessment of infants at two years corrected age in the largest trial to date found no difference in neurosensory impairment between those randomised to caffeine or placebo, but the dextrose group had a significantly higher risk of motor delay (RR (95%CI) 3.79, 1.27-11.32) and significantly lower scores for cognitive, language and motor performance (Edwards, Alsweiler, et al., 2022). Prophylactic use of dextrose gel has been found to be cost effective, reducing direct health-system costs and improving quality of life, as well as increasing exclusive breastfeeding rates (Glasgow et al., 2020; Makker et al., 2018).

It is thus clear that prophylactic interventions provided ante- or perinatally, or in the neonatal period can have significant effects on improving both short and long term outcomes for infants born preterm. Given that late preterm infants account for the large majority of preterm births, interventions that improve outcomes in this group may be expected to have the largest impact on public health in the long term (March of Dimes et al., 2012).

1.2.6 Pharmacokinetic changes

Numerous small studies investigating the pharmacokinetics of specific drugs in the preterm population have been published. Despite this, limited information on drug pharmacokinetics is available to support safe and effective prescribing in this population. This is due to the diversity of the preterm population in terms of gestational and postnatal age, comorbidities, diet, antenatal and postnatal environment and exposures, and genetic polymorphisms, combined with the challenges of conducting detailed pharmacokinetic research in this population (Allegaert et al., 2014; Ward, 2006). In recent years, attempts have been made to address the lack of information, and the limitations inherent in conducting individual studies for every drug, through the development of age-defined physiologically based pharmacokinetic models. Currently, these models have substantial assumption-based limitations and can only give an rough indication of likely concentrations in preterm infants, however

with refinement and further data input these models are likely to offer benefit in the future (van den Anker & Allegaert, 2021).

While some generalisations (such as decreased clearance resulting in prolonged half-lives in preterm infants) can be made, developmental changes can be unpredictable, non-linear, and occur at different rates for different processes. Consequently few developmental changes have been studied in sufficient detail in a large enough group to allow for a detailed description of the changes occurring at each week of gestation to be made (Ward, 2006). Further complicating pharmacokinetic analysis is the fact that developmental maturation is a function of both gestational and postnatal age, so that the level of functioning of organ systems, and subsequent effects on drug disposition, in a newborn infant of 32 weeks gestation may be significantly different to that in another infant with a corrected gestational age (CGA) also of 32 weeks, but who is 6-weeks old and was born at 26 weeks gestation (Salem et al., 2021; Völler et al., 2021; Y. Wu et al., 2022).

Pharmacokinetics are usually described in terms of the four constituent processes of absorption, distribution, metabolism and excretion. Absorption and distribution are relatively poorly studied in preterm infants, though differences from older children and adults may be expected based on known physiological changes and the physiochemical properties of drugs. Absorption of drugs administered orally (and hence onset of action) in the late preterm infant may be slower due to reduced gastrointestinal motility (Broussard, 1995)and decreased bile secretion (Strandvik et al., 1994). An increased gastric pH resulting from lower levels of gastric acid secretion (Hyman et al., 1985) may affect drugs differently depending on their nature as acids or bases. The near-constant presence of milk in the gastrointestinal tract may result in drug binding, preventing absorption, and the effects of this may differ depending on whether the infant is breastmilk or formula-fed (Ward, 2006). Thinner skin in preterm infants allows greater absorption of topical products (Oranges et al., 2015), while a lack of

muscle mass and higher muscle capillary density affects intramuscular drug administration (Tayman et al., 2011).

Distribution is affected by differences in body composition, with a higher water : fat ratio in preterm infants than older children and adults affecting drugs differently depending on their degree of water or lipid solubility (van den Anker & Allegaert, 2021). Distribution is also affected by differences in plasma protein concentrations in infants, as only free drug is available for both pharmacological effect and metabolism and excretion. Lower concentrations of plasma proteins (such as albumin) in late preterm infants result in a reduction in the proportion of drug bound to these carrier proteins, and hence an increase in the proportion of free drug at equivalent plasma concentrations, potentially leading to therapeutic or toxic effects at lower total drug levels than those seen in adults, as was demonstrated with theophylline (Aranda et al., 1976).

Compared to absorption and distribution, relatively more research has been devoted to metabolism and excretion, which together influence the clearance of the drug from the body. These functions are generally performed by the hepatic (metabolism) and renal (excretion) systems, in which different aspects mature at different rates, making generalisations difficult (Allegaert et al., 2014). Hepatic drug metabolising enzymes may be grouped into three classes based on developmental trajectory. Class 1 enzymes are most active in the fetus in the first trimester, Class 2 are expressed relatively consistently from fetal life to adulthood, and Class 3, represents the majority of enzymes, covering those that are expressed at negligible-to-low levels *in-utero* and increase over time (Hines, 2013). Within this final group, the onset and time to maximum expression varies considerably, with significant increases occurring within weeks to years after birth, but beginning before birth for some enzymes, and not reaching adult levels of functioning until after puberty for others (Hines, 2013). Dose adjustments for prematurity will thus depend on the metabolic pathway for a specific

drug, but are also affected by concomitant disease characteristics, treatment modalities, pharmacogenetics, environmental factors and drug interactions (van den Anker & Allegaert, 2021). Excretion, or elimination of the drug from the body, occurs primarily through the kidneys as a result of glomerular filtration, tubular excretion and tubular reabsorption. Glomerular filtration is low at birth, and increases rapidly in the postnatal period as cardiac output, renal blood flow and arterial blood pressure increase and renal vascular resistance decreases. As a result, renal clearance is typically higher in infants with a greater postnatal age, compared to infants with the same gestational age but born more recently (Salem et al., 2021), though the magnitude and clinical importance of this difference is disputed (Anderson & Holford, 2018; Iacobelli & Guignard, 2021).

1.3 Respiratory function

1.3.1 Fetal respiratory development

Development of the respiratory tract begins at approximately 4 weeks post-fertilisation (6 weeks GA), and continues throughout pregnancy, the peripartum period and childhood and into early adulthood. Lung growth is divided into five stages of development, starting with the formation of the trachea and bronchi during the embryonic stage (5-9 weeks GA) and progressing through pseudoglandular and canalicular stages, with progressive branching of the respiratory tract and cell differentiation at each stage (Schittny, 2017). Primitive alveoli begin to form during the canalicular stage (19-29 weeks GA), and pulmonary surfactant is excreted from approximately 26 weeks (Schittny, 2017). Further dilation of the of the saccules and thinning of the airway walls during the saccular stage (30-38 weeks GA) results in an increase surface area for gas exchange and, combined with increases in pulmonary surfactant, resulting in a lower incidence of respiratory distress with increasing gestation at birth (Joshi & Kotecha, 2007). The final stage of fetal lung development occurs from approximately 38 weeks GA, and continues throughout childhood and

adolescence, with the saccules subdividing though the formation of septa into a rapidly increasing number of alveoli (Narayanan et al., 2012).

Although the human fetus develops within a fluid-filled sac, relying on placental transfer rather than respiration for gas exchange, breathing movements have been observed from early in pregnancy. Along with fetal hiccups, fetal breathing movements closely follow the development of the diaphragm at 8-10 weeks, with fetal breathing being observed from 9 weeks onwards, and all fetuses in an early ultrasound study demonstrating fetal breathing by 12 weeks gestation (de Vries et al., 1982).

Pulmonary distension from both static stretch (due to the production and retention of lung fluid) and episodic stretch (cycles of fetal breathing) are important for lung development *in utero* (Kitterman, 1996). Animal studies involving the occlusion of the trachea or bronchi have demonstrated this results in hyperplasia of the lungs (Alcorn et al., 1977), and similar effects have been reported in infants born with congenital abnormalities that result in a laryngeal or tracheal obstruction (Scurry et al., 1989) or following pregnancy complicated by ogliohydramnios (C. S. Wu et al., 2017).

Fetal breathing movements provide mechanical stimulus to the developing lungs, stretching the developing tissue in an episodic manner as fluid moves in and out of the lungs. These breathing movements are thought to prime the infant for postnatal respiration by conditioning the respiratory muscles and enabling development of neural control of respiration, as well as contributing to differentiation and growth of the lung tissue (Kitterman, 1996; Koos & Rajaee, 2014). As with static stretch, animal research has demonstrated the importance of fetal breathing in normal pulmonary development, in that abolition of fetal breathing results in decreased lung growth (Wigglesworth & Desai, 1979), and in infants with congenital conditions resulting in

abnormal or absent fetal breathing movements, lung hypoplasia is common (Kitterman, 1988).

Fetal breathing follows an episodic pattern with periods of rapid breathing interspersed with periods of apnoea. The proportion of the time the fetus spends breathing increases over the course of the pregnancy, from approximately 2% at 10 weeks (de Vries et al., 1985) to approximately 35% at 30-34 weeks gestation, and approximately 50% at 35-39 weeks GA (Fox et al., 1979). This increase is most marked between 26 and 30 weeks GA, with the increase continuing at a lesser rate to 36 weeks gestation, though there is considerable inter-individual variation in both the incidence and duration of breathing episodes (Pillai & James, 1990).

Although fetal breathing is not due to a requirement for respiration, many of the conditions and triggers that affect postnatal respiration, such as hypo- or hypercapnia or the presence of CNS depressants, also exert similar effects on fetal breathing patterns. Initially, breathing movements appear random, but as pregnancy progresses the links between triggers and fetal breathing movements become more apparent, along with diurnal variation (Koos & Rajaee, 2014). Movements peak in the evening, and are at their least frequent and shallowest in the early hours of the morning (de Vries et al., 1987). Fetal breathing movements are responsive to changes in glycaemia, with movements occurring more frequently after maternal meals, and for longer periods in fetuses where the mother has gestational diabetes (Yeoshoua et al., 2012). Fetal breathing movements also respond to changes in maternal blood concentrations of respiratory gases, with hypercapnia increasing the incidence of fetal breathing movements and hypocapnia reducing them (J. Ritchie & Lakhani, 1980). These changes in $PaCO_2$ affect the incidence of fetal breathing movements without affecting more general fetal movements (K. Ritchie, 1980). Fetal hypoxia is associated with a biphasic response, with a brief initial increase in ventilation, followed by a profound decrease, often to apnoea (Walker et al., 2000). Mirroring post-natal reflexes, severe oxygen

deprivation results in deep gasping respiratory movements *in utereo*, and this may account for meconium aspiration and the presence of other amniotic fluid-debris in the lungs of stillborn and asphyxiated fetuses (Hooper & Harding, 1990).

Reduced fetal breathing movements may be observed following exposure to CNS depressants such as ethanol (McLeod et al., 1983), while respiratory stimulants such as doxapram and the methylxanthines are associated with increased fetal breathing movements (Hogg et al., 1977; Moss & Scarpelli, 1981). Nicotine from cigarette smoking has been shown to change the pattern of fetal breathing movements, increasing the rate of breathing, but also increasing the interval between episodes of breathing (Eriksen et al., 1983). With the onset of labour, fetal breathing movements are inhibited (P. Boylan & Lewis, 1980), likely due to the increase in circulating prostaglandins (Stojanovska et al., 2022).

1.3.2 Transition

The transition from fetal to neonatal life requires that the infant establishes continuous respiration to replace the placental oxygenation that has occurred during intrauterine life, as well as independent metabolic and nutritional capabilities and changes in cardiovascular circulation. This transition involves multiple, rapid and profound adaptations that must occur around the time of delivery, the most pressing of which are the changes to the pulmonary and cardiovascular systems (P. Reynolds, 2013). In particular, in relation to respiration, fluid which has filled the lungs must be absorbed or expelled and the lungs, and especially the alveoli, must expand and establish functional residual capacity (Jain & Eaton, 2006).

The mechanisms by which these changes occur are complex and multifactorial, with the onset of changes beginning prior to established labour (Swanson & Sinkin, 2015). Fetal lung fluid is secreted in increasing amounts throughout pregnancy, but this

secretion drops significantly prior to the onset of labour (Brown et al., 1983; Dickson et al., 1986; Kitterman, Ballard, et al., 1979). In addition, in late pregnancy the maturing fetal adrenal gland produces increasing amounts of cortisol which results in an increase in the number of epithelial sodium channels, leading to sodium resorption and the consequent movement of fetal lung fluid from the alveolar air spaces to the interstitial and intravascular spaces (Andersson et al., 2010; Jain et al., 2001). Animal studies have demonstrated that this process begins prior to labour, but increases significantly during labour, and it is likely that this mechanism is responsible for a significant portion of the lung fluid clearance which was previously attributed to the physical compression of the thorax as the fetus passes through the birth canal (Jain & Eaton, 2006). Cortisol also stimulates the production of surfactant, which reduces the surface tension at the gas-fluid interface within the alveoli and maintains alveolar expansion (Swanson & Sinkin, 2015).

The first extrauterine breaths result in an increase in the partial pressure of oxygen (PO_2) in the circulation over that seen in utero. This causes dilation of the pulmonary arteries, and hence a rapid reduction in pulmonary vascular resistance which results in increased pulmonary blood flow, further increasing gas exchange (P. Reynolds, 2013; Swanson & Sinkin, 2015). At the same time, the resistance of the systemic circulation rises as the low-resistance placental circulation is removed by the vasoconstriction of the umbilical artery as a result of the increase in oxygenation and/or clamping of the umbilical cord. The increase in systemic vascular resistance and decrease in pulmonary vascular resistance, combined with the increase in oxygen saturation triggers the closure of the ducts that have supported fetal circulation, resulting in transition to postnatal circulation (Hooper et al., 2015).

1.3.3 Initiation and maintenance of respiration

In addition to the physical changes to the respiratory and cardiovascular systems described above, at birth the breathing pattern must change from the intermittent

breathing movements that occur *in utero* to a continuous post-natal pattern to support gas exchange (Dekker et al., 2019). The relative contribution of various triggers for this change remains unclear, but may include activation of chemoreceptors responding to increased partial pressure of carbon dioxide (PCO_2), loss of inhibition of the respiratory centre due to a changes in prostaglandins, progesterone metabolites or adenosine and/or physical stimuli including cold, light and handling (Dekker et al., 2019).

Animal studies have found an increased breathing rate at birth in preterm rats when tactile stimulation is provided by the mother (Ronca & Alberts, 1995) and reported death from respiratory distress when this is not provided (Faridy, 1983), while in preterm infants, proprioceptive stimulation of the limbs reduced breathing pauses and bradycardia (Kesavan et al., 2016). It is thus assumed that physical stimulation increases respiratory effort, and as a result resuscitation guidelines advise drying and rubbing the back or soles of the feet at birth to stimulate the onset of respiration (Wyllie et al., 2015). In addition to stimulation provided by handling, cold stimulation of cutaneous thermoreceptors induces deep, regular respiratory movements in fetal lambs, and the change in temperature at birth thus probably also contributes to the onset of respiration (Gluckman et al., 1983).

During labour, contractions cause constriction of the umbilical vessels, reducing placental blood flow, increasing carbon dioxide levels and hence acidosis. This causes stimulation of the central chemoreceptors of the respiratory system, increasing breathing movements in both fetuses and adults (Adamson, 1991; Hohimer et al., 1983). Hypercapnic sensitivity increases with gestational age at birth, with a maturational effect unrelated to body or lung size (Krauss et al., 1975).

It has long been proposed that one or more placentally-produced mediators inhibits respiratory function *in utero*, and that the loss of placenta at birth results in the loss of

this inhibition, with corresponding onset of respiration (Kitterman, Liggins, et al., 1979). Prostaglandin E_2 (PGE_2) levels rise during labour, with increasing levels correlated with decreased fetal breathing activity, before falling over the following 24-48 hours, suggesting that PGE_2 may be important in maintaining continuous breathing (Adamson, 1991; Challis et al., 1976; Kitterman et al., 1983). Adenosine has similar neuromodulatory actions which inhibit respiration, with varying levels around the time of delivery, and a decrease in the levels of adenosine may likewise contribute to establishing post-natal breathing patterns (Irestedt et al., 1989; Sippell et al., 1978). More recently, lack of pituitary adenylate cyclase-activating polypeptide has been found to result in increased apnoea and respiratory dysfunction, and a surge in this peptide has been observed at birth, suggesting a role in establishing respiration (Shi et al., 2021).

While *in utero*, hypoxia (partial pressure of oxygen in arterial blood (PaO_2) < 25-30 mmHg) results in inhibition of fetal breathing movements, while hyperoxia stimulates breathing movements in the fetus. After birth, this pattern persists initially, transitioning gradually over a period of days to weeks to the standard situation of child and adulthood, where hypoxia stimulates the respiratory drive (Dekker et al., 2019). However, the immature pattern at birth means that if respiration is not established promptly, profound hypoxia inhibits respiration, making the establishment of breathing more difficult (Adamson, 1991).

Maintenance of respiration throughout the lifespan requires modulation of breathing patterns to match respiratory gas exchange with metabolic requirements, maintaining stable partial pressures of oxygen and carbon dioxide and hydrogen ion concentrations in the bloodstream (Jaryal & Singh, 2020). Changes in these parameters are detected by the peripheral chemoreceptors (which primarily detect hypoxaemia) and central chemoreceptors (which primarily detect alterations in CNS pH as a result of changes in carbon dioxide concentrations)(Edelman et al., 1973). The respiratory centres within

the pons and medulla integrate input from the central and peripheral chemoreceptors with that from peripheral mechanoreceptors (which provide information on lung volume, airway stretch and vascular congestion) to modulate the respiratory rhythm by means of neural messaging to the respiratory muscles (Adler & Janssens, 2019).

Hypoxia in adults triggers an increase in ventilation, primarily by means of an increase in tidal volume, over 1-2 minutes before decreasing over the following 5-20 minutes to a new, higher baseline (W. J. Reynolds & Milhorn, 1973). In neonates, a transient rise in ventilation is followed by a decline and a corresponding marked decrease in metabolism (Cross & Oppé, 1952). Hypercapnia triggers an even faster increase in ventilation, with rapid increase in both rate and depth of breathing (Edelman et al., 1973).

1.3.4 Assessment and monitoring of respiratory function

Two main tools are available to assess and monitor respiratory function in neonates. Polysomnography is considered the gold standard in the investigation and diagnosis of sleep-disordered breathing, and enables central and obstructive apnoea to be differentiated (Oliveira & Teng, 2016). A full polysomnography study includes measurement of oral and nasal airflow, continuous electroencephalogram, electrocardiogram and electro-oculogram and chin electromyography, monitoring of oxygen saturations and end-tidal carbon dioxide, plethysmography bands monitoring chest and abdominal movement and electromyography of genioglossus, diaphragm and abdominal muscles, accompanied by audio-visual monitoring of airway noises and sleep state, however modified versions which exclude elements of the full study are also used (T. Di Fiore, 2005; Oliveira & Teng, 2016; Roberts et al., 2017). Polysomnography is typically conducted overnight in a sleep laboratory in older infants, children and adults, but this is time consuming, disruptive to children and families and expensive, and little data is available on normative values for preterm infants and newborns (Kanack et al., 2022). Furthermore, in very small preterm infants, polysomnography is technically

challenging, given the impact the large quantity of equipment has on the ability to undertake normal cares in the preterm infant, and the fragility of preterm skin (Joosten et al., 2017). Due to the differences in sleep-wake cycles in young infants, nap polysomnography has also been used as an alternative to overnight studies (Kanack et al., 2022; Roberts et al., 2017).

Pulse oximetry uses two diodes, one light emitting and the other light receiving, opposite each other on either side of the palm, wrist or foot to measure pulsatile variations in optical density of tissues in red and infrared wavelengths, correlating these to determine the proportions of oxygenated and deoxygenated haemoglobin, and hence the oxygen saturation (S. Ali et al., 2021). Pulse oximetry is widely used in neonatal units as it allows for continuous, non-invasive determination of oxygen saturations, allowing for real-time monitoring and adjustment of care and treatments (Flint & Davies, 2018). In addition, oximetry recordings over longer periods allow for the assessment of trends in oxygen saturations, and is often used to asses babies with chronic cardiorespiratory conditions (Flint & Davies, 2018). Analysis of the recording allows assessment of mean oxygen saturation, desaturation events, and the amount of time spent with oxygen saturation above or below specific levels (Flint & Davies, 2018). However, results are distorted by motion artefact (though this is less of a problem with newer machines than with older technology), and may not be reliable in patients with hypotension, hypoperfusion, severe anaemia and haemoglobinopathy (S. Ali et al., 2021). Compared to polysomnography, pulse oximetry does not distinguish between central and obstructive apnoea, but as few apnoeic events in preterm infants are of solely obstructive nature (Eichenwald & Committee on Fetus and Newborn, 2016; Falconer et al., 2023) this may be of less importance than in older patients. Studies comparing the two methods of assessing cardiorespiratory status in preterm infants prior to discharge have found correlation between the rates of intermittent hypoxia on oximetry and apnoea on concomitant nap polysomnography (Roberts et al., 2017), and the widespread availability of pulse oximetry in neonatal units combined with the ease

of use and non-invasive nature of the devices make them a much more practical and acceptable way of assessing respiratory function in the preterm population.

1.3.5 Respiratory disorders in the neonate

1.3.5.1 Bronchopulmonary dysplasia

Bronchopulmonary dysplasia (BPD) is a chronic lung disease of prematurity which can cause lifelong impairment in respiratory function (Rite et al., 2022). Infants with BPD have increased rates of respiratory morbidity in the long term (Davidson & Berkelhamer, 2017), along with longer hospital stays (Klinger et al., 2006)and increased healthcare costs (Álvarez-Fuente et al., 2017).

1.3.5.1.1 Pathophysiology

Originally described in 1967, BPD was initially primarily reported in moderately preterm infants treated for respiratory distress who had received mechanical ventilation with high concentrations (80-100%) of heated, humidified oxygen for periods of at least 150 hours (Northway et al., 1967). The disorder was initially considered a 'prolonged healing phase' of respiratory distress, with pulmonary oxygen toxicity suggested as the primary cause (Northway et al., 1967). Subsequently, the association specifically with positive pressure ventilation via an endotracheal tube was identified and the disorder was considered a form of ventilator-induced lung injury, with the degree of pulmonary impairment being considered a function of the duration and pressure of ventilation used and the concentration of oxygen (Philip, 1975).

The characteristics of BPD have changed considerably over the years. When it was first described, BPD was seen predominantly in moderately to late preterm infants (those born around 34 weeks gestation), with those born more prematurely unlikely to survive (Northway et al., 1967). Improvements in neonatal care, and especially the use of antenatal corticosteroids, surfactant treatment, and changes in respiratory support,

have resulted in a decline in the BPD in more mature preterm infants, and improved survival of infants born very and extremely preterm, who often exhibit a milder form of the respiratory disorder (Bancalari & Jain, 2019). This milder form is characterised by a lack of alveolar development and a decreased number of capillaries, more of which are dysmorphic, in contrast to the originally described disorder in which inflammation, fibrosis and hypertrophy of the smooth muscle in the airways predominated. This more recent form of BPD is commonly termed "New BPD", and is thought to result primarily from an arrest in lung development in infants born during the late canalicular or early saccular stages of lung development (Jobe, 1999).

Though, in the past, infants who would go on to be diagnosed with BPD often presented early with significant respiratory failure, in the current era many infants initially exhibit mild or no respiratory disease and are managed with non-invasive respiratory support, or low ventilatory pressures and oxygen levels before a gradual deterioration over a period of days to weeks requires an escalation in respiratory support. A slow period of steady improvement in respiratory status usually follows, allowing weaning of respiratory support, though more serious cases may develop respiratory failure, pulmonary hypertension and right heart failure (Laughon et al., 2009).

As the understanding, characteristics and presentation of BPD have changed, so too has the definition. Early definitions combined clinical signs of respiratory disease, a history of positive pressure ventilation, prolonged requirement for oxygen and radiographic changes on chest x-rays indicating chronic lung pathology (Bancalari et al., 1979). With the increasing survival of more premature infants with milder disease and the associated inconsistency in radiographic evidence of disease, a simpler definition involving only ongoing oxygen requirement at 36 weeks PMA was adopted, based on its predictive value for abnormal pulmonary findings in the longer term (Shennan et al., 1988). A consensus definition for BPD adopted in 2001 built on the previous definition,

expanding it to include requiring supplementary oxygen for at least 28 days, and dividing the diagnosis into mild, moderate and severe classifications (Jobe & Bancalari, 2001). This was further refined in 2018 to "preterm infants born at ≤ 32 weeks gestation with persistent parenchymal lung disease, radiographic confirmation of parenchymal lung disease, who at 36 weeks PMA requires respiratory support for ≥ 3 days to maintain arterial oxygen saturation in the 90-95% range". This definition again includes grading of the disease (Grades I – III based on the level of respiratory support required), and also includes those infants who have died of lung disease before 36 weeks PMA (R. Higgins et al., 2018).

An ongoing need for supplementary oxygen at 36 weeks PMA remains the most commonly used definition in clinical trials, though as a clinical judgement, the use of oxygen at this timepoint may be subject to variation between clinicians, institutions and countries, potentially impacting the rates of diagnosis (Siffel et al., 2021).

1.3.5.1.2 Incidence

The incidence of BPD is inversely related to gestational age (Jensen & Schmidt, 2014; Lee et al., 2022). In extremely preterm infants, the rates of between 10 and 89 % have been reported, with variation depending on the definition used, infant birthweight, country and institution and survival rates (Siffel et al., 2021). Despite this variation, the rates of BPD appear to have remained stable over time (when the same population is examined and the same definition used)(Costeloe et al., 2012; Lee et al., 2022). This is thought to be as a result of increases in survival in the most premature infants, who are also those most at risk for BPD (Parker et al., 1992).

1.3.5.1.3 Treatment and pharmacotherapy

With any disease, prevention is better than treatment, and the use of antenatal corticosteroids, surfactant and non-invasive ventilation all substantially decrease the likelihood of a preterm infant developing BPD.

Caffeine was reported to reduce the incidence of BPD in the large CAP trial (Schmidt et al., 2006)(see section 1.4.8). The effects of caffeine in preventing BPD appear to be greatest when it is started soon after delivery, although questions remain about the optimal dose and timing of treatment (Alhersh et al., 2020; Pakvasa et al., 2018). Caffeine is thought to act primarily by decreasing the duration of mechanical ventilation, thereby reducing one of the major risk factors for BPD, though other mechanisms may also be involved, including antioxidant effects, the promotion of angiogenesis and improvements in tissue remodelling, and a reduction in treatment for PDA (Jensen, 2020; Tian et al., 2022).

Vitamin A is an essential fat-soluble nutrient integral to the development and function of many organ systems. Stores of vitamin A are laid down in the third trimester of pregnancy, so deficiency is common in preterm infants and has been associated with a high incidence of BPD (Verma et al., 1996). A small but statistically significant reduction in the incidence of BPD has been reported in extremely preterm infants treated with intramuscular Vitamin A (Darlow et al., 2016), but the inappropriateness of intramuscular administration in preterm infants with extremely limited muscle mass and the cost of the product limited clinical use (Jensen et al., 2015). More recently trials that have used oral vitamin A and systematic reviews of all routes of administration have not found an benefit of vitamin A on BPD incidence (Rakshasbhuvankar et al., 2021; Ye et al., 2022).

Azithromycin, a macrolide antibiotic, has both antibiotic and anti-inflammatory properties, and has been suggested as a preventative therapy for BPD following its use in inflammatory lung diseases in older patients. Meta-analysis of the available trials found a reduction in the incidence of BPD in extremely preterm infants treated with azithromycin (Nair et al., 2014), but data on safety and quality are lacking, and along with antimicrobial stewardship concerns, this precludes widespread use (Jensen et al., 2015).

Postnatal corticosteroids, with their potent anti-inflammatory action, have been widely used in different regimens for both the prevention and treatment of BPD. Dexamethasone and hydrocortisone are most commonly prescribed, but the optimal dose and timing of treatment remains unknown (Hay et al., 2023). Early treatment (within the first week after birth), especially with dexamethasone, has been found to reduce the incidence of BPD, but is associated with significant risks of negative effects (including cerebral palsy, gastrointestinal perforation, and growth failure) in both the short and long term (Doyle, Cheong, Hay, Manley, Halliday, et al., 2021). Later treatment is reported to decrease the risk of BPD without evidence of increased cerebral palsy, though the data on long term outcomes is limited (Doyle, Cheong, Hay, Manley, & Halliday, 2021). Given the significant potential side effects of treatment, use of corticosteroids is generally restricted to infants who cannot be weaned from mechanical ventilation without their use (Doyle, Cheong, Hay, Manley, & Halliday, 2021).

1.3.5.2 Apnoea

The term 'apnoea' describes an absence of respiratory airflow, which may be due to either a failure of the central respiratory drive (central apnoea), obstruction of the upper airway (obstructive apnoea), or a combination of the two (mixed apnoea)(Atik et al., 2017; R. Martin, 2017; Morton & Smith, 2016). In premature infants, the majority of

apnoeic events are mixed apnoea, where obstruction leads to central apnoea or vice versa (Eichenwald & Committee on Fetus and Newborn, 2016).

The American Academy of Paediatrics defines apnoea of prematurity as "*a pause in breathing of greater than 20 seconds, or one of less than 20 seconds and associated with bradycardia or oxygen saturation (cyanosis)*" where the onset of these events occurs prior to 37 weeks GA (American Academy of Paediatrics, 2003). However, the definition of apnoea and the method of diagnosing it has differed significantly between studies, with the duration being variously defined as a minimum of 10 (Jones, 1982), 15 (Barrington & Finer, 1990), 20 (American Academy of Paediatrics, 2003; Henderson-Smart, 1981) or 30 (Lagercrantz et al., 1980) seconds, with (Barrington & Finer, 1990; Jones, 1982) or without (Henderson-Smart, 1981) the presence of physiologic changes such as bradycardia or hypoxaemia, or repeated events within a defined timeframe (Jones, 1982). Differing methods of monitoring and identification of apnoea between studies further limits the ability to compare data from different trials, and a lack of consensus about the clinical significance of apnoea of prematurity leads to significant variation in clinical practice (Eichenwald & Committee on Fetus and Newborn, 2016).

1.3.5.2.1 Pathophysiology

Obstructive apnoea is caused by closure of the upper airway, often due to insufficient airway tone, inhibiting airflow into the lungs. Most commonly this occurs within the pharynx, though obstruction of the airway within the larynx is also possible. Neck flexion can cause obstructive apnoea in premature infants, though apnoea can occur without such flexion (R. Martin, 2017; Morton & Smith, 2016). Obstructive apnoea is characterised by respiratory effort with movement of the chest wall but with no resulting nasal airflow, and thus may not be detected by routine cardiorespiratory monitoring (Morton & Smith, 2016).

Central apnoea results from a cessation of respiratory effort, due to the immaturity of the respiratory control system in the CNS of the preterm infant, with decreased synaptic connections and dendritic arborization and poor myelination compared to the mature brain (Mathew, 2011). The ventilatory response to hypercapnia, which in term neonates (as in adults) results in increased tidal volume and breathing frequency is reduced in preterm neonates, and increases with increasing gestational age (Mathew, 2011).

Though distinction is often made between these two forms of apnoea, either form may lead to the other, and mixed apnoea is the most common form of apnoea in premature infants, accounting for 50-75 % off all apnoeic episodes (Poets, 2010)(M. Miller et al., 1985; Poets, 2010).

1.3.5.2.2 Incidence

Apnoea is common in premature neonates, with the incidence and severity of apnoea being inversely correlated with gestational age (Atik et al., 2017; Henderson-Smart, 1981; Morton & Smith, 2016). A large observational study published in 1981 reported that the incidence of apnoea was highest in infants born at 30-31 weeks' gestation at 54% and lowest in those born at 34-35 weeks' gestation at 7% (Henderson-Smart, 1981). However, as this study covered the period 1974-1979 survival at gestations less than 30 weeks was rare, especially in the early part of the study period. When the latter years of the study (1978-1979) are considered alone, the incidence of apnoea in infants born at 26-27 weeks was reported as 78%, and at 28-29 weeks was 75%, supporting the authors conclusions that "*the incidence of recurrent apnoea increased with decreasing gestational age at delivery*". It should be noted that incidences of apnoea of prematurity reported in this study are likely an underestimate due to the low rates of survival at low gestations in the early years of the study (Henderson-Smart, 1981), and the use of impedance apnoea monitoring for identifying apnoea which may not identify all cases of obstructive apnoea (Brouillette et al., 1987). In another smaller observational study,

all 20 otherwise-well infants born at less than 34 weeks gestation experienced episodes of apnoea within the first 24 hours of life (Barrington & Finer, 1991). More recent data on the incidence of apnoea at different gestational ages is lacking, but it is widely accepted that the lower the gestational age and the smaller a baby is at birth, the higher the risk of apnoea (Atik et al., 2017), with almost all babies born at less than 28 weeks gestation or weighing less than 1000g having apnoea (Morton & Smith, 2016). Apnoea is rare in full-term babies without other causes such as intracranial haemorrhage, asphyxia or seizures (Henderson-Smart, 1981; Morton & Smith, 2016).

1.3.5.2.3 Treatment and pharmacotherapy

Methylxanthines have been used since the 1970's for the treatment of apnoea of prematurity (Henderson-Smart & Steer, 2010), with aminophylline and theophylline initially being used, followed by caffeine use becoming more widespread. However, the choice of treatments has remained limited by the availability of suitable formulations in different regions (Nabwera et al., 2021). Methylxanthines are considered a mainstay in both the treatment and prevention of apnoea of prematurity and are used to reduce the frequency of apnoea, and hence hypoxaemia and bradycardia, in preterm neonates (Schoen et al., 2014). They act as antagonists at adenosine A_1 and A_{2A} receptors to activate the medullary respiratory centre, increase oxygen sensitivity and bronchodilation and improve the functioning of the diaphragm (Dobson & Hunt, 2018).

Structural similarities between aminophylline, theophylline and caffeine (Figure 1.1) mean they act in a very similar manner, but there are slight variations in both therapeutic activity and incidence of adverse events that affect the choice of treatment (Schoen et al., 2014). Caffeine has been shown in multiple studies and meta-analyses to have a wider therapeutic window (reducing the incidence of toxicity and hence the need for therapeutic drug monitoring) and a longer half-life than theophylline (resulting in a less frequent dosing regimen), and so is widely regarded as the first-line treatment (Schoen et al., 2014)(Eichenwald & Committee on Fetus and Newborn, 2016).

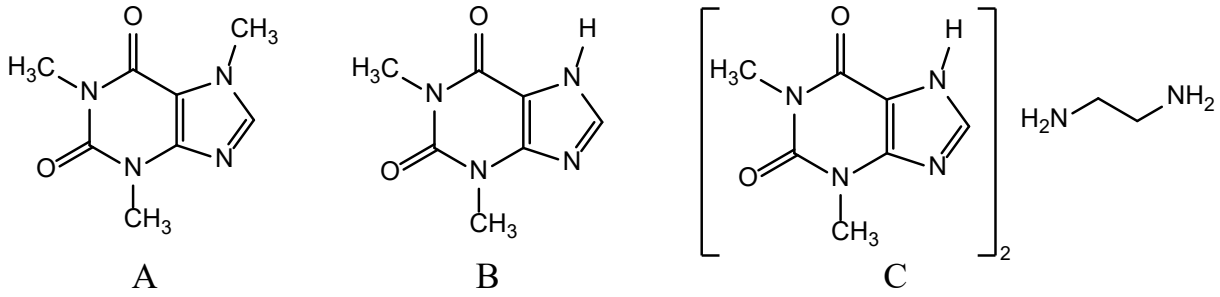


Figure 1.1: Structural diagram of the methylxanthines caffeine (A), theophylline (B) and aminophylline (C)

1.3.5.2.3.1 Aminophylline and Theophylline

Structurally, aminophylline is a complex of theophylline and ethylenediamine in a 2:1 ratio (Figure 1.1). The addition of the ethylenediamine moiety increases the water solubility of aminophylline 20-fold over that of theophylline, and thus formulations for intravenous (IV) injection tend to contain aminophylline, while oral preparations contain theophylline (Aslaksen et al., 1981). In biological fluids aminophylline dissociates rapidly, releasing theophylline, which is responsible for the pharmacological effect and subsequent pharmacokinetics are thus as for theophylline. Cross-over studies of both IV and oral theophylline and aminophylline have found near-identical time-concentration curves for both drugs with no differences between the two in terms of pharmacokinetics or protein binding (Aslaksen et al., 1981).

A 2010 meta-analysis of five trials involving a total of 108 infants found that there was no difference in the mean apnoea rate between groups treated with caffeine and those treated with theophylline, however, the rate of adverse events (tachycardia or feed intolerance that lead to a change in dosing) was lower in those treated with caffeine compared to those treated with theophylline (Henderson-Smart & Steer, 2010).

1.3.5.2.3.2 Caffeine

Aranda and colleagues published the first reports on the use of caffeine for the treatment of apnoea in newborn infants in 1977, treating 18 preterm infants with caffeine citrate and reporting a significant reduction in the incidence and frequency of apnoea following treatment, as well as spontaneous recovery from those apnoeas that did occur. They also reported an increased response to tactile stimulation akin to the effect observed after administering theophylline to similar infants (Aranda et al., 1977). Initially, infants enrolled in the trial were treated with doses of caffeine citrate of 10-20mg/kg one to three times per day. However, monitoring of caffeine plasma concentrations lead to the realisation that the half-life was significantly prolonged in premature infants, and the dosing schedule was amended to a loading dose of 20mg/kg caffeine citrate, followed within 2-3 days by 5-10mg/kg caffeine citrate once or twice a day (Aranda et al., 1977); a regimen that bears remarkable similarity to those widely used today (Lista et al., 2016).

Following this initial use report by Aranda et al, caffeine use for apnoea of prematurity became more widespread. A number of small-scale trials were published, which indicated that caffeine had similar short-term effects to theophylline (Henderson-Smart & Steer, 2010), though concern remained, based on animal studies, that caffeine and methylxanthine treatment may have unintended long-term adverse effects, or be of little value (Schmidt, 1999). In response to these unanswered questions the Caffeine for Apnea of Prematurity (CAP) trial was developed, and this landmark study is summarised separately later in this chapter. Many small-scale studies have been undertaken since in an attempt to answer questions relating to the optimal dose, time of initiation, and duration of treatment with caffeine. The use of caffeine in neonatal medicine is considered in more detail in section 1.4 below and is systematically reviewed in Chapter 6.

1.3.5.2.3.3 Doxapram

Like the methylxanthines, doxapram is a respiratory stimulant. Its mechanism of action is dose-dependent, acting via peripheral carotid chemoreceptors at lower doses, with additional stimulation of central respiratory centres as doses increase (Barrington et al., 1986; Scott et al., 1977). Stimulation of these receptors results in an increased tidal volume, accompanied by an increase in respiratory rate. Doxapram also causes catecholamine release resulting in increased cardiac output (Yost, 2006).

Doxapram has been used to treat apnoea of prematurity and a number of randomised controlled trials have reported comparable efficacy for doxapram when compared with aminophylline or theophylline (Eyal et al., 1985; Möller et al., 1999; Peliowski & Finer, 1990; Romeo et al., 1991), though they have involved only a very small number of patients, and are thus underpowered to detect a significant difference between the two treatments (Henderson-Smart & Steer, 2000). In addition, there have been reports of serious adverse effects with the use of doxapram, including developmental delay (Lando et al., 2005; Sreenan et al., 2001) seizures (Barrington et al., 1986; Czaba-Hnizdo et al., 2014), prolongation of the QT interval (Maillard et al., 2001), hypokalaemia (Fischer et al., 2013), gastrointestinal effects including NEC (Lando et al., 2005), and hypertension at doses greater than 1.5mg/kg/hr (Barrington et al., 1987), though these are generally from case reports or series, and it is thus not known if the rate of these effects is significantly higher than the background rate in this population (Vliegenthart et al., 2017). In some countries, doxapram for injection is formulated using benzyl alcohol, a preservative known to accumulate in neonates and to cause neurologic damage at high concentrations, and it is thus possible that some of the adverse effects may be due to this excipient rather than doxapram itself (G. D. Jordan et al., 1986; Yost, 2006).

Given the evidence now available for positive long-term outcomes for caffeine treatment of apnoea of prematurity, doxapram is now generally only used as an

alternative or adjunctive treatment in cases where caffeine alone is proving ineffective. Evidence is very limited, and a large, well designed randomised controlled trial with long term follow-up is required to categorically define the place of doxapram in the treatment of apnoea of prematurity (Vliegenthart et al., 2017).

1.3.5.2.3.4 Positive-pressure ventilation

Continuous positive airway pressure ventilation (CPAP) at pressures of four to six centimetres of water reduces both the incidence and severity of apnoea (Eichenwald & Committee on Fetus and Newborn, 2016). The positive pressure splints the airway open, distending the pharynx and larynx and preventing upper airway obstruction and thus reducing the incidence of obstructive apnoea (Balain & Oddie, 2014), while the increase in end expiratory volume may reduce the depth and duration of hypoxaemia in central apnoea (Eichenwald & Committee on Fetus and Newborn, 2016).

1.3.5.2.3.5 Blood transfusion

Blood transfusions have been suggested as a possible treatment for apnoea of prematurity though the studies of the effect of transfusion on incidence and severity of apnoea are somewhat conflicting (Eichenwald & Committee on Fetus and Newborn, 2016). A systematic review comparing restrictive and permissive transfusion thresholds in preterm babies found no effect of changes to the transfusion threshold within the studied range on the incidence of infants requiring intervention for apnoea (RR 1.01; 95% CI 0.95 - 1.08)(Whyte & Kirpalani, 2011), though the definition of apnoea varied significantly between studies from requiring bag and mask ventilation or intubation (Kirpalani et al., 2006) to treatment with methylxanthines (Bell et al., 2005) and any presence of apnoea (Y. C. Wang et al., 2017).

1.3.5.3 Intermittent Hypoxaemia

Intermittent hypoxaemia refers to a brief, transient decrease in oxygen saturation from baseline. These events are often clinically inapparent, as the duration and magnitude of the drop in saturation is generally insufficient to cause cyanosis (Wellington et al., 2018). Unlike apnoea, there is currently no widely accepted definition for intermittent hypoxaemia, which may be defined either based on a decrease in oxygen saturation below a specific level (often 80 or 85%), or as a drop of a specified percentage below baseline (typically 3, 4 or 10%)(J. Di Fiore et al., 2019; Wellington et al., 2019).

Early pulse oximeters used a longer averaging time and were prone to motion artefact. Changes to oximeters over the last two decades have meant newer devices employ shorter averaging times (typically 2 seconds), which has increased the detection of brief desaturations. Newer devices also include artefact rejection algorithms, which remove much of the motion artefact that was previously automatically included, resulting in higher mean oxygen saturations. These changes mean historical data may not be directly comparable to that obtained more recently (Falconer et al., 2023).

1.3.5.3.1 Pathophysiology

The mechanism behind intermittent hypoxaemic events is not well understood. Central apnoea have been reported to rise and fall over the same timeframes as desaturation in early life, and it has thus been suggested that immature respiratory control causing apnoea and periodic breathing may have a role in causing desaturations, though the higher incidence of intermittent hypoxaemia than apnoea indicates other mechanisms are also involved (J. Di Fiore et al., 2019; Falconer et al., 2023). Metabolic demand increases in the post-natal period and haemoglobin concentration decreases, and it is possible that these factors may contribute to the increased rate of desaturations over the first few weeks after birth (Falconer et al., 2023).

As a result of their small size and immature lung structure and function, preterm infants are susceptible to ventilation-perfusion mismatch and intrapulmonary shunting of deoxygenated blood (Poets et al., 1992), which may explain the existence of desaturations despite continued breathing. This may be due to end-expiratory volumes lower than closing volumes causing collapse of the distal airways and constriction of the distal airway and pulmonary vascular bed as a result of airway hypoxia resulting in accelerated blood flow through the lungs and a reduction in diffusion of oxygen from the intrapulmonary space into the blood (J. Di Fiore et al., 2019; Poets et al., 1992)

Episodes of hypoxaemia are followed by reoxygenation, causing ATP depletion, xanthine oxidase activation and the generation of free radicals without a compensatory increase in antioxidant activity (R. J. Martin et al., 2011). Repeated cycles of hypoxia/reoxygenation may result in activation of a pro-inflammatory cascade in which periods of hypoxia are thought to result in activation of pro-inflammatory transcription factors, (particularly nuclear factor-κB) which in turn activate inflammatory cells (lymphocytes and monocytes), resulting in the expression of proinflammatory mediators causing inflammation and cell apoptosis (He et al., 2014).

1.3.5.3.2 Incidence

Observational studies using pulse oximetry in preterm infants have demonstrated a non-linear correlation with post-natal age (rather than CGA), with marked increase in intermittent hypoxaemia over the first 2-3 weeks after birth, peaking at 2-4 weeks postnatally, before gradually declining again (J. Di Fiore et al., 2010; Falconer et al., 2023; Williams et al., 2018). While the same pattern of a rapid rise, followed by a slower decrease in event has been observed across multiple studies, the number of events reported has varied depending on the definition of a desaturation, but is considerably higher than the rate of apnoea (Falconer et al., 2023). The age at which desaturations peak appears to be related to gestational age at birth, with studies in the most immature infants reporting a later peak than those in infants born at later gestational

ages (4 weeks postnatally in a cohort of infants born at 24-28 weeks GA, 3 weeks in a cohort of 32-35 week infants and 2 weeks in a cohort of 34-37 week infants)(J. Di Fiore et al., 2010; Falconer et al., 2023; Williams et al., 2018). Mean oxygen saturations mirror these timeframes, with a nadir corresponding with the peak of desaturations (Falconer et al., 2023).

1.3.5.3.3 Treatment and pharmacotherapy

Intermittent hypoxaemia exists on a spectrum with apnoea as its more severe form. Currently, intermittent hypoxaemia is lacking a single widely-accepted definition, and normative ranges for different gestational and post-natal ages are not well defined. While intermittent hypoxaemia is associated with adverse outcomes (Bass et al., 2004; Poets et al., 2015), there is currently no consensus on a threshold for treatment, or its modality, in the absence of apnoea, though clinical trials have reported that treatments used for apnoea are also successful at reducing episodes of clinically inapparent intermittent hypoxaemia (Dobson et al., 2017; Rhein et al., 2014; Seppä-Moilanen et al., 2022).

Supplemental oxygen has been shown to reduce periodic breathing and oxygen desaturations in late preterm infants, with 25% oxygen reducing the number of desaturations of at least 3% from baseline from 38 to 10 events per hour (Seppä-Moilanen et al., 2022).

Caffeine is widely used for apnoea of prematurity, and the frequency of intermittent hypoxaemia after ceasing caffeine treatment for apnoea, and the effects of extending caffeine treatment beyond when it was usually discontinued was assessed in a study in 2010-2011. Infants born between 25 and 32 weeks gestation who were at least 33 weeks PMA when caffeine was discontinued by the clinical team were randomised to restart caffeine citrate (20 $mg.kg^{-1}$ load followed by 6$mg.kg^{-1}.day^{-1}$) 5 days after its

discontinuation, or to receive no further treatment. Continuous oximetry recording was conducted (for as much of the time as was clinically feasible or reasonably practical for parents) until the infant was at least 40 weeks PMA and had been home for at least one week. Intermittent hypoxaemia (defined as a decrease in SaO2 by at least 5% from baseline to less than 90% that lasted at least 5 seconds) was significantly lower in the caffeine group than the control group at 35 weeks (-52%; 95%CI -70 to -22%) and 36 weeks (-46%; 95%CI -65 to -11%), and time with oxygen saturation <90%, 85% and 80% was likewise reduced in the caffeine group at these timepoints. However, there was no significant difference between the two groups at 37, 38 or 39 weeks, which the authors attribute to the increasing metabolism of caffeine as infants mature rendering the dose subtherapeutic (Rhein et al., 2014). A subsequent study using increased doses of 14 or 20 $mg.kg^{-1}.day^{-1}$ from 36 weeks PMA found these doses were sufficient to maintain a therapeutic level of caffeine and reduced intermittent hypoxaemia (SaO2 decrease of ⩾10% from baseline and lasting for ⩾5 seconds) at 37 (-68%; 95%CI -77 to -54%) and 38 (-63%; 95%CI -79 to -38%) weeks gestation (Dobson et al., 2017).

1.4 Caffeine

1.4.1 Background

Caffeine is a methylxanthine known chemically as 1,3,7 trimethylxanthine (Faudone et al., 2021). It occurs naturally in several plant species, including *Camellia, Coffea* and *Cola* species, and is present in many common beverages and foods including tea, coffee, cola drinks and chocolate, giving the substance its reputation as the world's most widely consumed drug (El-Yazigi et al., 1999; Newton et al., 1981). Caffeine has been extensively used for hundreds of years for its CNS stimulant properties in increasing wakefulness (Kreutzer & Bassler, 2014). It is also commonly used in combination with other mild analgesics such as paracetamol, on the basis that adjuvant use increases the analgesic effectiveness. Numerous studies have reported widely differing results on the effectiveness of this approach, with a meta-analysis of 20 studies covering various pain

conditions calculating that the addition of caffeine increased the number of participants obtaining effective relief by 5-10% over analgesics alone (Derry et al., 2014).

Caffeine is used less commonly or in experimental settings for a wide range of other ailments including oral use for orthostatic hypertension (Onrot et al., 1985) and preventing neurological decline in the elderly (K. Ritchie et al., 2007). In high intravenous doses caffeine has been used as an adjunct to lengthen seizure duration in electroconvulsive therapy (Coffey et al., 1990). Caffeine is also used topically for psoriasis (Vali et al., 2005). Small studies have shown that caffeine significantly decreases the number of participants with ongoing post dural puncture headache, and correspondingly decreases the rate of other supplementary procedures required (Basurto Ona et al., 2015).

Caffeine has been used in the treatment of asthma, though other methylxanthines are more effective at bronchodilation and so are generally preferred (Tilley, 2011; Welsh et al., 2010). Tolerance to the stimulant effects of caffeine occurs rapidly and abrupt cessation can precipitate physical withdrawal symptoms including headache, irritability, restlessness and lethargy (Evans & Griffiths, 1992).

In addition to therapeutic use, caffeine has also been used as a probe by those investigating liver function in both adult and paediatric patients (El-Yazigi et al., 1999). Caffeine undergoes oxidative demethylation and hydroxylation in the liver to form a wide range of metabolites. The relative formation of these metabolites following administration of a caffeine dose has been used to quantify the activity of cytochrome P450-containing mixed-function mono-oxygenases (particularly CYP 1A2), polymorphic N-acetyltransferase (NAT) and xanthine oxidase (XO) during hepatic function assessment. Caffeine appears well-suited to this application as single oral doses are non-toxic in humans, absorption is rapid and complete, and saliva and urine

concentrations correlate directly with plasma concentrations, allowing non-invasive collection of samples for quantification (El-Yazigi et al., 1999).

1.4.2 Comparison to other methylxanthines

Theophylline is a methylxanthine with structural similarities to caffeine, and a very similar pharmacokinetic profiles in adult patients (Ginsberg et al., 2004). However, in neonates theophylline is metabolised and eliminated significantly faster than caffeine (Aranda et al., 1976; Aranda, Cook, et al., 1979). Following the initial use of theophylline for the treatment of apnoea of prematurity in the 1970's (Henderson-Smart & Steer, 2010), caffeine has become widely used as an alternative due to its significantly longer half-life in neonates (which allows for longer dosing intervals) and the wider therapeutic index with its corresponding lower risk of toxic effects (Aranda, Cook, et al., 1979; Atik et al., 2017; Morton & Smith, 2016). In addition, caffeine is more reliably absorbed from the gastrointestinal tract than theophylline and aminophylline (Miao et al., 2022).

A Cochrane review in 2010 reported no difference in the short-term effectiveness of caffeine and theophylline when used for the treatment of apnoea of prematurity (Henderson-Smart & Steer, 2010). In more recent years, the publication of trials reporting long term outcomes after caffeine therapy for apnoea of prematurity has further strengthened the evidence for caffeine as the methylxanthine of first choice for apnoea of prematurity (Schmidt et al., 2017).

1.4.3 Caffeine base vs citrate

Caffeine is available primarily as two different forms: an unionised or 'base' form ($C_8H_{10}N_4O_2$), and a citrate salt ($C_8H_{10}N_4O_2$,$C_6H_8O_7$), both of which have been used therapeutically (Buckingham, 2018). The addition of the citrate moiety increases the molecular weight of the compound approximately two-fold from 194.2 g/mol for

caffeine base to 386.3 g/mol for caffeine citrate. Correspondingly, the therapeutic dose of caffeine when expressed in mg/kg depends on the salt being used, with a dose of 5mg/kg of caffeine base being equivalent to 9.95 mg/kg of caffeine citrate; more commonly quoted as 5mg caffeine base being considered equivalent to 10mg caffeine citrate (Ainsworth, 2014; Buckingham, 2018). This difference in dosing has given rise to confusion and drug dosing errors, especially in paediatrics where liquid formulations are often used and a wide range of doses are easily obtained from the one formulation (Health Quality and Safety Commission New Zealand, 2011).

The landmark Caffeine for Apnoea of Prematurity (CAP) trial used caffeine citrate (Schmidt et al., 2006), as did other trials subsequently, and it has become common practice to prescribe and document the dose of caffeine as 'caffeine citrate' when used for apnoea of prematurity and other paediatric indications (Ainsworth, 2014; New Zealand Medicines Formulary Limited Partnership, 2018). This is in contrast to the studies of the late 1970's and early 1980's, which tended to report doses in terms of caffeine base equivalents, even if caffeine citrate was used in the research (Aranda, Cook, et al., 1979).

Throughout this thesis, doses for caffeine are given in terms of caffeine citrate.

1.4.4 Pharmacokinetics

1.4.4.1 Absorption

Caffeine is rapidly and completely absorbed from the gastrointestinal tract in healthy adults at doses between 50 and 750 mg. This was demonstrated in a study conducted that compared the area under the curve of a time / plasma concentration plot following identical doses of caffeine given orally or by intravenous injection (Newton et al., 1981).

Aranda et al. measured the absorption of caffeine citrate following administration of a single oral dose of 20mg/kg in three premature infants (GA 26-32 weeks; postnatal age 1-44 days). In keeping with studies conducted in adults, they found rapid and complete absorption of oral caffeine, with peak plasma caffeine concentrations of 6-10 mg/L occurring 30 minutes to two hours after drug administration. The concentrations obtained were comparable to those following intravenous caffeine administration, indicating near-complete bioavailability (Aranda, Cook, et al., 1979).

In their population-based pharmacokinetic modelling study of 110 premature infants of less than 30 weeks GA, Charles et al. reported complete absorption of orally administered caffeine citrate, though with an absorption half-life of approximately twice that observed in adults. This was attributed to slower gastric emptying and reduced intestinal motility in these infants (Charles et al., 2008).

1.4.4.2 Distribution

Caffeine rapidly enters the intracellular fluid space and is distributed throughout all body fluids (including plasma, saliva, cerebrospinal fluid, bile and breastmilk) and organs (Arnaud, 2011). In pregnancy, caffeine crosses the placenta by passive diffusion (Mose et al., 2008). Plasma protein binding levels are low, and the volume of distribution for caffeine has thus been assumed to be equivalent to total body water when developing pharmacokinetic models of caffeine for apnoea of prematurity in newborn infants (Micallef et al., 2007).

1.4.4.3 Metabolism

In adults, children and infants over the age of four months, caffeine is almost completely metabolised prior to excretion, primarily by cytochrome P450 1A2 (CYP1A2) in the liver. However, in young infants, including those born preterm, CYP 1A2 activity is almost entirely absent, and as a result almost all administered caffeine is excreted

unchanged in the urine (Pons et al., 1988). Given the central role of CYP1A2 in the metabolism of caffeine, caffeine is widely used as a probe in establishing the functioning of CYP1A2 where this information is necessary to inform dosing decisions for other drugs metabolised by the same enzyme (Perera et al., 2010).

There is significant genetic variation in the expression of CYP1A2, with urinary caffeine metabolite ratios indicating a difference in CYP1A2 activity of up to 70-fold within populations (Butler et al., 1992). The distribution of CYP1A2 phenotypes in non-smokers is trimodal, allowing categorisation of individuals as slow, intermediate and rapid metabolisers, but with the relative distribution of the general population between these three groups varying in different geographical locations (Butler et al., 1992). However, the trimodal distribution is lost when smokers are considered, presumably due to enzymatic induction by components of cigarette smoke (Butler et al., 1992).

A number of other factors are known affect the metabolism of caffeine, primarily due to their influence on CYP 1A2. Pregnancy is associated with a significant decrease in CYP 1A2 activity across the course of the pregnancy (Tracy et al., 2005) decreasing the metabolism and subsequent elimination of caffeine, while smoking induces CYP1A2 resulting in increased metabolism of caffeine and hence a shorter half-life (Bock et al., 1994; Butler et al., 1992; Parsons & Neims, 1978). Liver disease results in impaired functioning of CYP 1A2 (Wahlländer et al., 1990), and use of oral contraceptives inhibits CYP 1A2, both of which decrease the metabolism of caffeine (Arnaud, 2011).

There is no diurnal variation in the metabolism of caffeine (Brice & Smith, 2001; Hashiguchi et al., 1992), but dietary choices may affect caffeine levels, including reduction in caffeine clearance and prolongation of half-life with the consumption of grapefruit juice (half-life prolonged by 31%)(Fuhr et al., 1993) or alcohol (half-life prolonged by 72%)(George et al., 1986). In infants, caffeine elimination has been found

to be increased in those fed using cow-milk-based formula compared to breast-fed infants, due to the induction of CYP1A1 and CYP1A2 enzymes in formula-fed infants (Xu et al., 2005).

1.4.4.4 Excretion

The vast majority of caffeine consumed is metabolised prior to excretion, with one pharmacokinetic study conducted in healthy adults reporting a mean of 1.83% caffeine excreted unchanged in urine over a 48-hour period, though interindividual variation was significant (range 0.26% – 13.12%)(Newton et al., 1981). Accumulation of caffeine or its metabolites during long-term use has not been observed in adult studies (Arnaud, 2011).

1.4.4.5 Effects of prematurity

As with many drugs, the half-life of caffeine is significantly prolonged in the newborn infant relative to adults. This is probably due to the reduced hepatic metabolism (particularly CYP P450-mediated metabolism) in neonates (Aranda, Collinge, et al., 1979). However, the decrease in clearance and resulting increase in half-life is more pronounced than might be expected based on comparable drugs such as theophylline, phenytoin and phenobarbitone. While phenytoin and phenobarbitone exhibit a 2- to 6-fold increase in half-life which decreases to adult values by approximately 1 month of age, for caffeine the half-life at birth has been reported as 17-times that of an adult, and the elimination rates only approaches adult values at 3-4 ½ months of age (Aranda, Cook, et al., 1979; Aranda, Collinge, et al., 1979). The early pharmacokinetic study in premature newborns by Aranda et al. found that, in contrast to other drugs, there did not appear to be a change in caffeine elimination during the neonatal period (the first month after birth). The authors reported no correlation between half-life and indices of maturity such as birth-weight, gestational age and post-natal age within this period (Aranda, Cook, et al., 1979), though this study included only 32 infants across both single- and multiple-dose groups, and later larger studies have reported different

findings (Falcão et al., 1997; Thomson et al., 1996). This first pharmacokinetic study of caffeine in premature neonates found a mean half-life of 102.9 hours, with no infant demonstrating a half-life of caffeine of less than 40 hours. This supports a once-daily dosing schedule for caffeine in this age group (Aranda, Cook, et al., 1979).

The available information on pharmacokinetics is more limited in preterm neonates than for the population as a whole as traditional pharmacokinetic studies involve intensive blood sampling following dosing with the medication of interest, so are ethically and logistically more challenging in this population (Falcão et al., 1997; Thomson et al., 1996). As a result, with the exception of the early studies by Arana et al (Aranda, Cook, et al., 1979; Aranda, Collinge, et al., 1979), much of the available pharmacokinetic information is based on studies that have used statistical modelling to pool data obtained during clinical care from a large number of individuals and thus investigate variables of significance (Falcão et al., 1997; Thomson et al., 1996).

Thomson et al used 77 serum caffeine concentrations obtained during clinical care for 60 infants to establish a model for the calculation of caffeine clearance. This model was then tested against the results of a further 20 infants for validation. The factors found to be of most significance and included in the initial clearance model included weight, postnatal age, and the presence of either dexamethasone or BPD. Validation testing found the parameters for dexamethasone and/or BPD failed to reach statistical significance, leading to it being discarded, and the final formula thus included consideration of weight and postnatal age only (Thomson et al., 1996).

Falcão et al. also utilised mixed effects modelling to develop a pharmacostatistical model for caffeine clearance in preterm neonates. In their study, the most important factor was found to be current weight, followed by postnatal age, which essentially acts as a proxy for renal clearance given the inherent difficulties in estimating renal function

in preterm neonates (Abitbol et al., 2014; Bhongsatiern et al., 2016; Falcão et al., 1997). Likewise, low gestational age was found to correlate better with caffeine clearance than low birth weight (Falcão et al., 1997). In addition, concurrent treatment with parenteral nutrition appeared to decrease caffeine clearance by 16.5% (Falcão et al., 1997). In a third study, Charles et al measured caffeine serum concentrations in 100 premature infants born at less than 30 weeks gestation who were prescribed caffeine in the periextubation period. Their pharmacostatistical modelling discounted various other covariates, and used only postnatal age and weight in the final calculations (Charles et al., 2008).

A clinical trial of extended use of caffeine citrate at 6 $mg.kg^{-1}.day^{-1}$ to 40 weeks PMA in infants born at <32 weeks GA and prescribed caffeine clinically found the rate of intermittent hypoxaemia was decreased at PMAs of 35 and 36 weeks, but not from 37 weeks onwards. This is likely due to increasing metabolic clearance with increasing gestation (Rhein et al., 2014).

1.4.5 Transfer to breastmilk

Caffeine, along with various metabolites including theophylline, paraxanthine and theobroma, is known to pass from maternal plasma into breastmilk. Pharmacokinetic studies are small, but have found peak concentrations in breastmilk approximately one hour after oral intake, with concentrations in breastmilk lower than in maternal serum (Stavchansky et al., 1988; Tyrala & Dodson, 1979). Infant intake via breastmilk has been estimated at 7-10% of the maternal weight-adjusted dose (Calvaresi et al., 2016; Oo et al., 1995; Stavchansky et al., 1988), with studies reporting infant caffeine concentrations levels ranging from undetectable to 1 $mg.L^{-1}$ following maternal caffeine ingestion (Berlin et al., 1984; Hildebrandt & Gundert-Remy, 1983; Vohra & Marraffa, 2019). Adverse effects including jitteriness, poor sleep, restlessness and irritability resulting from maternal use of caffeine have been reported in breastfed infants, but these have been in case reports where maternal consumption has included 10-20 cups of tea

and/or coffee or bottles of cola per day (Clement, 1989; Martín et al., 2007; Rustin, 1989).

1.4.6 Therapeutic drug monitoring

Therapeutic drug monitoring was initially widely used to monitor caffeine therapy with a target therapeutic range of 5-20 mg^{-1}. This was based on early work that assumed equivalence of activity between caffeine and theophylline (Aranda et al., 1977). However, multiple studies have demonstrated that the use of standard doses (20 $mg.kg^{-1}$ loading dose and 5-10$mg.kg^{-1}.day^{-1}$) result in levels within the accepted range for the vast majority of preterm infants, and that even in infants with levels above this range, adverse events were rare (Bosma et al., 2012; Leon et al., 2007). Furthermore, studies have found poor correlation between episodes of apnoea and caffeine serum concentrations (Yu et al., 2016). It is thus now generally accepted that therapeutic drug monitoring for caffeine is of little clinical value, except in cases where there are concerns of toxicity or ineffectiveness of therapy, or where the infant has co-existing conditions that may result in alternations to pharmacokinetics likely to lead to accumulation (Schoen et al., 2014).

1.4.7 Pharmacodynamics

Methylxanthines act as respiratory stimulants, improving minute ventilation through increasing chemoreceptor responsiveness to carbon dioxide as well as improving respiratory muscle functioning, though the precise mechanism through which it exerts these effects has not been conclusively demonstrated (Atik et al., 2017; Henderson-Smart & Steer, 2010).

The most widely accepted mechanism is that methylxanthines competitively antagonise the adenosine receptors A_1 and A_{2A} (Atik et al., 2017). Adenosine, a neurotransmitter which controls arousal, sleep and cerebrovascular homeostasis, acts

by binding to receptors, causing inhibition of inspiratory neurons and resulting in respiratory depression (Tian et al., 2022). Through competitive antagonism of the A_1 and A_{2A} receptors, caffeine indirectly stimulates the respiratory centre, increasing sensitivity to carbon dioxide, enhancing diaphragmatic contractility and improving respiratory rate and tidal volume (Tian et al., 2022). It has also been proposed that the respiratory stimulant effects of caffeine may be medicated by their effects on gamma-aminobutyric acid (GABA) receptors, inhibition of phosphodiesterase (PDE) or calcium release, though these are considered less likely pathways, as the levels of methylxanthines required to exert these effects is likely to cause toxicity *in vivo* (Atik et al., 2017).

Furthermore, caffeine is an antioxidant, and beneficial actions in reducing BPD may also result in part from its ability to reduce levels of reactive oxygen species (which are elevated in mechanically ventilated infants and are associated with lung damage), alleviating hyperoxic pulmonary injury and promoting pulmonary vascular development (Tian et al., 2022). Caffeine also inhibits secretion of pro-inflammatory cytokines, reducing inflammation in the lungs and so providing another potential route for benefit in preventing BPD (Köro1lu et al., 2014).

1.4.8 Caffeine for Apnoea of Prematurity trial summary

1.4.8.1.1 Background

The CAP trial began recruitment in 1999, with the aim of establishing the short and long-term efficacy and safety of methylxanthine therapy in infants with very low birth weight (Schmidt et al., 2006). It is considered the landmark study in the field (Stevenson, 2007), and demonstrated the safety of caffeine use in the treatment and prevention of apnoea in very low birth weight infants, and its effectiveness in reducing BPD, more than 30 years after methylxanthines were first used for apnoea in preterm infants (Kuzemko & Paala, 1973).

Over a five-year period between 1999 and 2004, 2006 infants with birthweights between 500 and 1250g, whose clinicians considered them eligible for methylxanthine treatment in the first 10 days of life, were recruited in neonatal units in Canada, the United States of America, Australia, Europe and Israel. Infants whose clinicians considered methylxanthine therapy was indicated for the prevention or treatment of apnoea, or to facilitate endotracheal tube removal were randomised to treatment with caffeine citrate (loading dose of 20mg/kg followed by a daily dose of 5 mg/kg/day, increased to 10mg/kg/day if deemed necessary by the treating clinician) or placebo (Schmidt et al., 2006).

1.4.8.1.2 Results

The primary outcome of the trial was a composite of death, cerebral palsy, cognitive delay, hearing loss requiring amplification or bilateral blindness at 18-21 months corrected age (Schmidt et al., 2007). Treatment with caffeine resulted in a significant increase in the rate of survival without neurodevelopmental disability at a corrected age of 18-21 months (OR (95% CI); 0.77, 0.64-0.93; P=0.008) with a number needed to treat of 16 (95% CI; 9-56) (Schmidt et al., 2007). The incidence of both cerebral palsy (OR (95% CI); 0.58, 0.39-0.87; P=0.009) and cognitive delay (OR (95% CI) 0.81, 0.66-0.99; P=0.04) were significantly lower in the caffeine treatment group. (Schmidt et al., 2007). The rates of death, deafness and blindness were low and did not show a significant difference between the treatment and placebo groups, as shown in Table 1.1 below. A post-hoc stepwise logistic-regression analysis which included six variables (positive airway pressure through an endotracheal tube, any positive airway pressure, oxygen therapy, postnatal corticosteroids, surgery to close patent ductus arteriosus and BPD), showed all the variables were reduced by caffeine therapy, and all six variables together explained 55% of the observed benefit of caffeine on the primary outcome at age 18-21 months (Schmidt et al., 2007).

There was a significant reduction in rates of BPD with caffeine treatment, (control group 46.9% v caffeine 36.3%, adjusted OR (95% CI); 0.63 (0.52-0.76), P<0.001) (Schmidt et al., 2006). Other secondary outcomes, including death prior to initial discharge home, brain injury (identified on ultrasound) and NEC showed no significant difference between the intervention group and controls (Schmidt et al., 2006). The weight and head circumference of infants participating in the study was recorded weekly. Infants in the caffeine treatment group gained significantly less weight than those in the placebo group for the first three weeks following randomisation but no significant differences in weight gain were observed between weeks four and six, nor was there any difference in head circumference throughout the course of treatment (Schmidt et al., 2006). Follow-up at 18-21 months of age did not find any significant difference in height, weight or head circumference between the two groups (Schmidt et al., 2007).

While not initially examined for, on *post-hoc* analysis babies treated with caffeine had lower rates of intervention (drug or surgical treatment) for patent ductus arteriosus (Schmidt et al., 2006) and lower rates of severe retinopathy of prematurity (but not any retinopathy of prematurity) (Schmidt et al., 2007).

1.4.8.1.3 Subgroup analysis

In addition to the primary and secondary outcomes reported above, *post-hoc* analysis was conducted of several subgroups within the CAP trial. These analyses considered the impact of the indication for use, the level of respiratory support at study entry and the timing of study commencement (P. Davis et al., 2010). For each subgroup a logistic regression model determined the heterogeneity, defined as the treatment x subgroup interaction, and each analysis was then adjusted for significant variables: gestational age; sex; level of maternal education; antenatal steroid treatment, and multiple births. However, it is important to note that the *post-hoc* nature of this analysis meant the trial may not have been adequately powered to detect differences between the groups.

1.4.8.1.3.1 Indication

Analysis was conducted on the basis of the clinician-reported indication for treatment with caffeine, with the groups being defined as treatment of apnoea, prevention of apnoea or facilitation of removal of endotracheal tube as mutually exclusive indications. There was no evidence that any of the outcomes assessed varied by indication for initiation of caffeine (P. Davis et al., 2010).

1.4.8.1.3.2 Positive pressure ventilation

When analysis considered the level of respiratory support at study entry (none, non-invasive respiratory support, or ventilation via endotracheal tube) there was significant heterogeneity observed between groups two outcomes - death or major disability (P=0.03) and cognitive delay (P = 0.02), with the benefits of caffeine being greater in those infants receiving respiratory support at the time of study entry. There was no statistically significant difference in heterogeneity between groups for other outcomes (P. Davis et al., 2010).

1.4.8.1.3.3 Timing of Commencement

The effect of caffeine on death or major disability was not affected by the timing of the commencement of the drug (<3 days v ≥ 3 days). Babies who received caffeine before 3 days of age had a greater reduction in BPD (p=0.02) but this was no longer significant after adjustment for baseline variables), the only outcomes demonstrating a statistically significant improvement with the early use of caffeine (<3 days of age) were PMA at last endotracheal intubation (P = 0.04) and PMA at last positive pressure ventilation (P = 0.03) (P. Davis et al., 2010)

1.4.8.1.4 5-year follow-up

The CAP Trial group followed up of 82% of the original cohort at 5 years of age and assessed them for a primary outcome of death or survival with 1 or more of: motor impairment, cognitive impairment, behaviour problems, poor general health, severe hearing loss or bilateral blindness. There was not a statistically significantly difference in the primary outcome, or the individual components of the composite, between groups, though there was a non-significant trend of improvement in the primary outcome with caffeine citrate treatment (Schmidt et al., 2012). The rate of cognitive impairment reported was significantly lower at 5 years of age than at 18-21 months, which may reflect the reported cognitive delay at 18-21 months being due to developmental delay, rather than lasting cognitive impairment (Schmidt et al., 2012). The rate of dyspraxia (developmental coordination disorder) as assessed by the Movement Assessment Battery for Children at 5 years of age was lower in the caffeine-treated group than in the control group (odds ratio (95% CI) 0.71 (0.52-0.97); P=0.032)(Doyle et al., 2014).

1.4.8.1.5 Sleep assessment

A subgroup of 201 children who participated in the CAP study underwent assessment of sleep at 5-12 years of age. The dual primary outcomes of total sleep time (measured using actigraphy) (control group 493.3 minutes v caffeine 488.3 minutes, mean difference adjusted for covariates (95% CI); -6.7 minutes (15.3, 2.0), P = 0.13) and obstructive apnoea-hypopnoea (based on polysomnography) (control group 0.3 events·hr^{-1} v caffeine 0.3 events·hr^{-1}; rate ratio caffeine:placebo (95% CI); 0.89 (0.55, 1.43), P = 0.63) did not differ significantly between those that had received caffeine as a neonate and those who had received placebo, indicating no long-term effects on sleep quality attributable to treatment with caffeine during the neonatal period (Marcus et al., 2014).

1.4.8.1.6 11-year follow-up

A further follow-up study was conducted when the original trial participants were 11 years of age to assess academic performance, motor skills and behaviour. A subset comprising approximately half of the original participants were assessed. There was not a statistically significantly difference in the primary outcome (a composite of poor academic performance, motor impairment and behavioural problems) between the caffeine and placebo groups (Schmidt et al., 2017). The incidence of motor impairment (mainly due to dyspraxia) was significantly reduced in the caffeine group (adjusted odds ratio (95% CI); 0.66 (0.48-0.90), p=0.009), with 13 infants requiring caffeine treatment to prevent one case of motor impairment at 11 years of age (Schmidt et al., 2017). There was no difference in academic performance or behaviour problems between the two groups. Analysis of secondary outcomes, including general intelligence, attention, executive function, visuomotor integration and perception, found no significant difference between groups on tests of general intelligence, attention, executive function and behaviour, but identified beneficial effects of neonatal caffeine treatment on visuomotor integration (OR = 0.74; 95% CI 0.55-0.99; P=0.04), visual perception (OR=0.63; 95% CI 0.43-0.92; P=0.02) and fine motor skills (OR=0.69; 95% CI 0.52-0.92; P=0.01), in keeping with the beneficial effect on motor impairment (Mürner-Lavanchy et al., 2018). The authors concluded that the beneficial effects of caffeine on cognitive function seen at 18 months of age were outweighed by social and environmental influences on the child as they grow up (Schmidt et al., 2017). Caffeine was not associated with significant differences in self-reported quality of life across most domains assessed, with the sole exception of perceptions of peer support, where the effect was small (mean difference in T-score on Kidscreen-52 questionnaire, adjusted for centre = –1.9; 95% CI –3.3 to –0.4; P=0.01)(Schmidt et al., 2019).

1.4.8.1.6.1 Lung function testing

In addition to the assessments above, at 11 years of age, children who were assessed by the team at the Royal Women's Hospital in Melbourne underwent lung function

testing. Expiratory flow rates were better in participants who had been treated with caffeine in the neonatal period than those treated with placebo, with *z*-scores for FEV_1being lower in the caffeine-treated group (mean difference = 0.49; 95% CI, 0.07 to 0.91; P=0.023). However, when BPD was taken into account as a covariate in regression modelling this effect was reduced and became statistically insignificant (mean difference in FEV_1 z-score = 0.24; 95% CI, -0.15 to 0.62; P=0.24)(Doyle et al., 2017). This indicates the improvements in respiratory function is due to the effect of caffeine in reducing ventilator and oxygen dependency and hence reducing lung injury (Doyle et al., 2017).

Table 1.1: Summary of key outcomes from the CAP trial

Outcome	Caffeine group n/N (%)	Placebo group n/N (%)	Odds Ratio*	95% confidence interval	P-value
Bronchopulmonary dysplasia N= 2006 (Schmidt et al., 2006)	350/963 (36.3)	447/954 (46.9)	0.63	0.52-0.76	<0.001
Composite death / neurodevelopmental disability at 18-21 months N= 1869 (Schmidt et al., 2007)	377/937 (40.2)	431/932 (46.2)	0.77	0.64-0.93	0.008
Death before 18 months	62/974 (6.4)	63/970 (6.5)	0.97	0.67-1.40	0.87
Cerebral palsy	40/909 (4.4)	63/970 (7.3)	0.58	0.39-0.87	0.009
Cognitive delay	293/867 (33.8)	329/858 (38.3)	0.81	0.66-0.99	0.04
Severe hearing loss	17/909 (1.9)	22/905 (2.4)	0.77	0.4-1.45	0.41
Bilateral blindness	6/911 (0.7)	8/905 (0.9)	0.74	0.26-2.15	0.58
Composite death / neurodevelopmental disability at 5 year N=1640 (Schmidt et al., 2012)	176/833 (21.1)	200/807 (24.8)	0.82	0.65-1.03	0.09
Death before 5 years	59/867 (6.8)	58/837 (6.9)	0.98	0.70-1.43	0.92
Motor impairment	13/803 (1.6)	21/773 (2.7)	0.59	0.29-1.18	0.20
Cognitive impairment	38/768 (4.9)	38/750 (5.1)	0.97	0.61-1.55	0.89
Behaviour problem	42/773 (5.4)	53/748 (7.1)	0.75	0.49-1.15	0.18
Poor general health	32/805 (4.0)	33/775 (4.3)	0.92	0.56-1.52	0.75
Severe hearing loss	22/798 (2.8)	25/773 (3.2)	0.85	0.47-1.52	0.58
Bilateral blindness	7/792 (0.9)	7/763 (0.9)	0.96	0.34-2.75	0.94
Composite functional impairment at 11 years N= 920 (Schmidt et al., 2017)	145/457 (31.7)	174/463 (37.6)	0.78	0.59-1.02	0.07
Poor academic performance	66/458 (14.4)	61/462 (13.2)	1.11	0.77-1.61	0.58
Motor impairment	90/457	130/473	0.66	0.48-0.9	0.009

	(19.7)	(27.5)			
Behaviour problems	52/476 (10.9)	40/481 (8.3)	1.32	0.85-2.07	0.22

*Adjusted for centre

1.4.8.1.7 Economic Analysis

A retrospective economic evaluation of the cost effectiveness of treatment with caffeine for apnoea of prematurity was undertaken using data from the CAP trial. Direct medical costs per survivor without neurodevelopmental impairment were calculated based on Canadian costs for all trial participants (regardless of country of birth)(Dukhovny et al., 2011). The mean cost per infant was (in 2008 Canadian dollars) $124,466 for those in the caffeine group versus $133,505 for those in the placebo group, giving a cost saving of $9039 (95% CI -14,749—3375; adjusted P = 0.014) for treatment with caffeine (Dukhovny et al., 2011). Although the main cost analysis was based on Canadian prices, calculations showed that even allowing for caffeine prices varying through a 100-fold range and other medical costs varying from 50-1000% of the values used in the main calculations, caffeine remained a cost-effective treatment. This indicates a strong economic benefit from caffeine use in this population, with caffeine being found to be one of the most economically beneficial treatments in neonatal intensive care (Dukhovny et al., 2011).

1.4.8.1.8 Summary

Overall, the CAP Trial has found no evidence of long-term harm from caffeine use in premature infants weighing 500 -1250g at delivery. Infants treated with caffeine had significantly reduced rates of BPD, and a lower risk of death or neurodevelopmental disability at 18-21 months of age (Schmidt et al., 2007). At 18-21 months of age, children treated with caffeine as neonates had less developmental delay (Schmidt et al., 2007), but there was no effect on cognitive impairment by the age of 5 years (Schmidt et al., 2012). The beneficial effects of caffeine on motor function are maintained to at least the age of 11 years (Schmidt et al., 2017), with beneficial effects on lung function and no

long-term adverse effects on sleep (Marcus et al., 2014). The use of caffeine in this population is highly cost-effective (Dukhovny et al., 2011).

1.4.9 Current usage in neonatology

The use of caffeine for the prevention and treatment of apnoea, and to facilitate extubation is well established, with caffeine citrate consistently being reported among the most frequently used medications in neonatal units (Al-Turkait et al., 2022; Clark et al., 2006; Hsieh et al., 2014; Stark et al., 2022) and almost all infants born at less than 28 weeks GA receiving the drug (Puia-Dumitrescu et al., 2019). Generally, clinical practice guidelines recommend a loading dose of 20 $mg.kg^{-1}$ and an initial maintenance dose of 5 $mg.kg^{-1}.day^{-1}$, increased if necessary to 10 $mg.kg^{-1}.day^{-1}$, in keeping with the doses used in the CAP trial (Schmidt, 2023). However, in recent years there has been upwards creep in the doses used, with some guidelines recommending doses of up to 80 $mg.kg^{-1}$ as a loading dose, and up to 20 $mg.kg^{-1}.day^{-1}$ or higher as maintenance therapy (Schmidt, 2023). There is also considerable variation in practice between neonatal units and neonatal clinicians (Siddhi & Foster, 2023a).

Recent surveys of neonatal units and staff in the United Kingdom, Australia and New Zealand have found near-universal use of caffeine for the most premature infants, but considerable variation in practice for parameters including how soon after birth caffeine is started, gestational ages at which caffeine use is routine, whether caffeine is continued during periods of mechanical ventilation, and the timing of discontinuation, in addition to variations in the dose used for both loading and maintenance doses (Grainge et al., 2022; Gray & Chauhan, 2016; Siddhi & Foster, 2023b).

In 2016, a survey of neonatologists within the Australia and New Zealand Neonatal Network (ANZNN) found that almost all (98.9%) used caffeine to treat apnoea of prematurity, with fewer using caffeine to prevent apnoea or facilitate extubation (77.0

and 70.1% respectively)(Gray & Chauhan, 2016). While the 20 $mg.kg^{-1}$ load / 5 $mg.kg^{-1}.day^{-1}$ regimen was most common, doses reported in Australia ranged between 10-80 $mg.kg^{-1}$ for the loading dose and 4-20 $mg.kg^{-1}.day^{-1}$ maintenance (Gray & Chauhan, 2016); variation which is broadly similar to that reported in overseas surveys (Grainge et al., 2022; Siddhi & Foster, 2023b).

Use of caffeine and its early initiation has increased over the last decade, with 82.6% of infants in the UK receiving caffeine within the first two days of life in 2020 (Szatkowski et al., 2023), and approximately 50% of NICUs reporting initiation within the first hour of life (Grainge et al., 2022). The majority of units continue caffeine during periods of ventilation (Grainge et al., 2022).

Parameters around the discontinuation of caffeine are similarly variable. In the ANZNN survey, neonatologists reported discontinuing caffeine at PMAs of between 32 and 36 weeks gestation, and monitoring infants post-cessation for periods of between 24 hours and 2 weeks, with 5-7 days being most common (Gray & Chauhan, 2016). In a similar survey of British neonatal units in 2021 timing of discontinuation was similarly varied, with the majority of units indicating discontinuation was a patient-specific decision, but a significant number discontinuing treatment at 34 weeks PMA irrespective of respiratory support (Grainge et al., 2022). Retrospective cohort data from the USA reports PMA at caffeine discontinuation of between 32 and 37 weeks gestation, and again, considerable variation in the period of observation between discontinuation and discharge (Ji et al., 2020).

1.5 Summary

Late preterm infants are physiologically immature and have poorer neurodevelopmental outcomes than infants born at full-term. They also experience higher rates of intermittent hypoxaemia, and the potential for organ hypoxia as a result

of these episodes provides a possible mechanism by which intermittent hypoxaemia may cause neurodevelopmental impairment.

Caffeine is used for the prevention and treatment of apnoea in very and extremely low birthweight infants, and one large, randomised trial has shown it may reduce the rates of BPD and improve long term neurodevelopmental outcomes for these infants. Use of caffeine in late preterm infants may be expected to reduce intermittent hypoxaemia, and hence improve neurodevelopmental outcomes in these infants. However, maturation of the renal and hepatic systems in late preterm neonates means these infants may require higher doses for therapeutic effect than infants born more prematurely.

2. Overview, rationale and aims

2.1 Summary of chapter contents

This thesis reports as its central theme the conduct and results of the Latte Dosage Trial. It includes the results of laboratory-based testing necessary to support the performance of this trial, and a systematic review of the wider use of caffeine in preterm infants. This chapter explains the rationale for the research undertaken, an explanation of how the research described in the subsequent chapters fits together and the aims of the projects.

This thesis is written and formatted in accordance with the University of Auckland 2020 PhD statute guideline. Chapters 3 – 6 of this thesis have been published in peer-reviewed journals. Chapters that are already published papers have been included without any changes to content. Formatting, including table and figure numbers and citations have been adjusted to maintain consistency throughout the thesis. The published papers have been edited and included with necessary permission from the publishers (see page 62).

2.2 Project significance

Approximately 8% of all live births in New Zealand occur at gestations of less than 37 weeks (Ministry of Health, 2021), and late preterm infants account for approximately 70% of these preterm births, equating to 5.2% of all births, or approximately 3,300 babies per year (Ministry of Health, 2019). These figures are similar to those in other comparable countries, such as England and Wales (where late preterm babies account for 5.3% of all births)(Bradford, 2022) and the USA (7% of all births)(Osterman et al., 2022). These late-preterm infants are at higher risk of experiencing morbidity and mortality in the neonatal period than full-term infants (McIntire & Leveno, 2008), and in later childhood are more likely to receive a neurodevelopmental diagnosis, including

cerebral palsy (Moster et al., 2008; Odd et al., 2013), developmental delay (Darlow et al., 2009; Woythaler et al., 2011), cognitive impairment (Heinonen et al., 2015; Quigley et al., 2012; Talge et al., 2010) and behavioural disorders (Huddy et al., 2001), compared to term infants.

The significant size of the late preterm population and the long-term nature of many of the issues they face mean that overall, these infants account for significant resource use in both the health and education systems (Premji, 2019). Thus, any intervention that reduces neurodevelopmental impairments in late preterm infants – especially if inexpensive, easily administered and well-tolerated – may be expected to significantly reduce the overall cost burden of preterm birth, as well as improving quality of life for those affected. Caffeine has the potential to meet these requirements, given its effect in reducing intermittent hypoxaemia (Doyle et al., 2010) and improving long term outcomes (Schmidt et al., 2017), and its cost-effectiveness (Dukhovny et al., 2011) in more preterm infants.

2.3 Rationale for studies

2.3.1 Analytical methods and caffeine oral liquid formulation stability

2.3.1.1 Caffeine citrate oral liquid formulation design and stability study

The design of the Latte Dosage Trial as a double-blind, placebo-controlled dose-finding randomised controlled trial required both a means of administering the different doses of caffeine citrate to late preterm infants in a manner that maintained blinding, and a matching placebo. This necessitated the trial medication being available at a range of different concentrations to allow the administration of a standard volume of trial medication to every baby in the trial, regardless of the dose group they were assigned to. As caffeine citrate is only available commercially in NZ in a single strength, the required range of concentrations had to be prepared extemporaneously. Many commonly used excipients are known to be potentially harmful to neonates and

infants, where physiological immaturity of organ systems and metabolic pathways may result in accumulation and an increased incidence of adverse reactions compared to older patients (Cuzzolin, 2017). As a result, and in accordance with recommendations (Nahata, 2009), a conscious decision was made to reduce the excipients used in the formulation as far as possible, and consequently a simple solution of caffeine citrate (with water as a placebo) was investigated as a preferred option. Four different concentrations were prepared, the most concentrated of which, at 20mg.mL^{-1}, had similar properties to the product currently used in clinical practice in New Zealand.

In order to comply with legislative requirements surrounding pharmaceutical manufacture and wholesaling in NZ (Medicines Act, 1981), it was necessary for each site to independently compound medication for the infants enrolled at that site, rather than supplying all medication from one hospital. This required a formulation that was quick and simple to manufacture using standard extemporaneous compounding techniques.

Clinical use of the formulations required confirmation the products would remain chemically and physically stable throughout the intended duration of use. As there was no published data available on suitable formulations at the requisite range of concentrations, laboratory work to develop formulations and confirm their stability during storage was required prior to the trial commencing. This work is reported in Chapter 3, alongside other laboratory-based methods. The developed formulations were then prepared by the pharmacies at the two hospitals from which patients were recruited, in accordance with legal requirements and standard extemporaneous compounding practices in New Zealand.

2.3.1.2 Salivary caffeine analysis

The Latte Dosage Trial required infants to be given differing doses of caffeine to identify which dose was most effective in reducing intermittent hypoxaemia without

significant side effects. Caffeine ingested by breastfeeding mothers is transferred to the breastmilk (Berlin et al., 1984; Ryu, 1985), and may be consumed by breastfeed infants, potentially adding to their daily intake of caffeine. To assess if this incidental exposure was likely to have any impact on infant caffeine exposure across the dose groups we were using, a survey of maternal caffeine intake was included at each of the three timepoints (baseline, two weeks and term). However, assessment of maternal intake using surveys relies on accurate recall, and there is considerable variation in caffeine content of foods and drink by brand and method of preparation (S. Boylan et al., 2008). Infant caffeine intake via breastmilk is then subject to further variation due to interindividual variation in maternal pharmacokinetics and the timing of feeds (Berlin et al., 1984). We thus included analysis of caffeine concentrations in both mother and infant as part of the trial.

Salivary samples were used in preference to plasma as salivary sampling is simple and non-invasive, avoiding both a painful procedure for participants and the need for staff trained in phlebotomy. Salivary caffeine concentrations have been shown to be highly correlated with plasma concentrations in preterm infants at $33\text{-}36^{+6}$ PMA (Dobson et al., 2016) and in lactating mothers (Berlin et al., 1984). However, analysis of the samples collected during the trial required the development and validation of a method for analysing the caffeine concentration of saliva, using reagents and equipment available in a standard laboratory setting.

Maternal salivary caffeine concentrations were measured to allow us to assess if maternal caffeine and hence neonatal exposure, differed between groups within the trial. Neonatal salivary concentrations were ascertained to also allow us to confirm that babies in the higher dose groups did in fact receive higher doses of caffeine.

As the half-life of caffeine is significantly shorter in adults, at approximately 3-6 hours, than it is in neonates (Axelrod & Reichenthal, 1953; Gorodischer & Karplus, 1982;

Newton et al., 1981), mothers participating in the trial were asked to collect three saliva samples over a 24-hour period (at 10am, 2pm and 6pm) to allow concentrations to be averaged, giving a picture of total daily intake. The researcher conducting the visit then collected a saliva sample from the infant during the two-week visit, and transported that, along with the maternal samples, to the laboratory where they were stored at -20°C until analysis. The practicalities of this aspect of the trial made it necessary to undertake stability studies to confirm that caffeine was stable in saliva, and concentrations would not be affected by the planned collection timeframes, and in particular by any delay in getting samples into the laboratory freezer.

Consideration was also given to collecting expressed breast milk and assessing the caffeine content of this, to give a more direct assessment of infant caffeine intake via maternal sources. However, collection of expressed breast milk for analysis requires consideration of ethical and practical issues. Collection of milk for research has been described as "*taking a primary foodstuff from an infant*" (E. Miller et al., 2013) and so is not a preferable option where the relevant information can be obtained by other means, especially for preterm infants where the establishment of feeding is already challenging (Maastrup et al., 2014). On a practical level, analysis of breastmilk requires expressing milk, either by hand or by pump, which mothers in our study were not necessarily doing routinely. This potentially requires extra equipment and a more complex collection process than saliva samples collected by direct expectoration into a tube. In light of these considerations, and the fact that full pharmacokinetic evaluation was not the primary aim of this trial, assessment of salivary caffeine concentrations in both infant and mother was felt to be sufficient.

The methods and results of both the stability testing of the caffeine citrate oral liquid formulations (physical, chemical and microbiological) and the validation of the methods of analysis of caffeine in saliva are presented together in Chapter 3.

2.3.2 The Latte Dosage Trial

Late preterm infants are known to have higher rates of neurodevelopmental impairment than babies born at term, including a higher risk of being diagnosed with cerebral palsy (Moster et al., 2008; Odd et al., 2013), developmental delay (Darlow et al., 2009; Woythaler et al., 2011), cognitive impairment (Heinonen et al., 2015; Quigley et al., 2012; Talge et al., 2010) and behavioural disorders (Huddy et al., 2001).

Recent research has demonstrated that infants born late preterm experience more frequent episodes of intermittent hypoxaemia than infants born at term (Williams et al., 2018). These episodes peak in frequency at two weeks of age, returning to baseline levels at around term corrected age (Williams et al., 2018).

Exposure to hypoxic environments may have negative long-term cognitive effects in adults (Bahrke & Shukitt-Hale, 1993; Weaver et al., 2002), and even small decreases in neonatal oxygen saturations have been shown to negatively affect survival and neurodevelopment of very preterm infants (Carlo et al., 2010; Saugstad & Aune, 2013; Stenson et al., 2011). Transient intermittent hypoxaemic events are associated with poor neurodevelopmental outcomes in extremely preterm infants (Poets et al., 2015) and in children with sleep disordered breathing (N. Ali et al., 1996) and congenital heart disease (Bass et al., 2004). We thus postulated that the increased incidence of neurodevelopmental impairment in late preterm infants may be due, at least in part, to the higher rates of intermittent hypoxaemia observed in this population.

Caffeine is already widely used in neonatal medicine as a respiratory stimulant in very preterm infants to prevent and treat apnoea of prematurity and intermittent hypoxaemia. It reduces the incidence of BPD, cerebral palsy, and cognitive delay in these infants, and is well tolerated (McNamara et al., 2004; Schmidt et al., 2006, 2007).

As late preterm infants have an increase in hypoxaemic events compared to term infants, and hypoxaemic events are associated with poor neurodevelopmental outcomes, it is possible that caffeine - an intervention that reduces hypoxaemic events and has already been shown to improve long-term outcomes in extremely and very preterm infants - may be effective at improving outcomes in late preterm infants. This possible benefit of caffeine on long-term neurodevelopmental outcomes is the ultimate hypothesis of the Latte trials.

The pharmacokinetics of caffeine are known to vary considerably with age. The usual hepatic route for metabolism of caffeine in adults (via cytochrome P450 1A2)(Anderson et al., 1999) is almost entirely absent in newborn preterm infants, and instead the majority of caffeine administered is eliminated via the kidneys, which are also functionally immature. Caffeine elimination is thus slow in extremely preterm infants, prolonging the half-life. With increasing postconceptial age the elimination of caffeine increases (Falcão et al., 1997; Le Guennec et al., 1985), and larger doses may be needed to maintain a therapeutic effect. In one trial in very preterm infants, 6 $mg.kg^{-1}$ of caffeine citrate reduced intermittent hypoxaemia at 35 and 36 weeks' PMA, but not after 36 weeks' PMA. The authors hypothesised that this may be due to increasing drug clearance resulting in an insufficient dose as infants matured (Rhein et al., 2014).

Although caffeine is usually well tolerated by very preterm infants, there have been reports of tachycardia, feed intolerance, and a reduction in weight gain (Schmidt et al., 2006; Steer et al., 2004). There are concerns that side effects of caffeine reported by adults, such as irritability, sleeplessness and gastrointestinal disturbance may also occur in infants. For caffeine to be used as a prophylactic medication in a large number of late preterm infants, it would need to be prescribed at a dose that has a low risk of significant side effects.

Thus, before a Phase III trial investigating the effect of caffeine on neurodevelopmental outcomes could occur, further work was needed to determine an appropriate dose for the reduction of intermittent hypoxaemia in the late preterm population. As a result, the Latte Dosage Trial was developed as a randomised, placebo-controlled dose-finding and feasibility study. The aim was to determine the most effective and best tolerated dose of oral caffeine citrate to reduce intermittent hypoxaemia in late preterm infants, with the intent that this would in turn inform the development of a larger and longer-term trial of caffeine with neurodevelopment as the primary outcome (The Latte Trial). The protocol for the Latte Dosage Trial is described in Chapter 4, and the results in Chapter 5.

2.3.3 Systematic Review: Caffeine for apnoea and the prevention of neurodevelopmental impairment in preterm infants

At the time of writing the protocol for this review, systematic reviews on caffeine use in preterm infants were outdated, with available Cochrane reviews being published approximately 10 years prior, and not updated, and in many cases including other methylxanthines, such as theophylline, no longer routinely used in clinical practice (Henderson-Smart & Davis, 2010; Henderson-Smart & De Paoli, 2010a, 2010b; Henderson-Smart & Steer, 2010). Since publication of these Cochrane reviews, follow-up data (at 5 and 11 years of age) from the CAP trial (Schmidt et al., 2012, 2017), and outcomes of a dosage trial in Queensland had been published (Gray et al., 2011), providing much-needed data on the long-term effects of caffeine in this vulnerable population, along with several other more recent randomised trials of caffeine use in preterm infants that needed to be incorporated into this body of evidence (Katheria et al., 2015; McPherson et al., 2015; Mohammed et al., 2015; Rhein et al., 2014).

A systematic review was thus designed to identify and analyse the relevant data on the effectiveness and optimal dose of caffeine in preterm infants, encompassing both short and long-term outcomes. This review was designed to include two comparisons:

1. Caffeine vs placebo

2. High vs low dose caffeine

with the following subgroup analyses performed by study type or individual trial subgroup data, as available:

1. Primary indication:
    a. early/prophylaxis
    b. apnoea or peri-extubation
    c. late hypoxaemia/established lung disease
2. Gestation:
    a. <28 weeks
    b. 28 ≤ 32 weeks
    c. 33 ≤ 36 weeks

The review was carried out with a final search performed on 11 July 2022, and is reported in Chapter 6. However, insufficient information on the indication for treatment and the gestation of the participants was reported in included trials to allow the planned subgroup analyses to be undertaken.

2.4 Aims

The aims of the research projects described above are as follows:

2.4.1 Analytical methods and caffeine oral liquid formulation stability

To develop and validate an extraction protocol and liquid chromatography assay for the extraction and quantification of caffeine from human saliva.

To evaluate the chemical, physical and microbial stability of caffeine solutions at 5, 10, 15 and 20 $mg.mL^{-1}$.

2.4.2 The Latte Dosage Trial

To determine the most effective and best tolerated dose of caffeine citrate to reduce transient intermittent hypoxaemia events in late preterm infants.

2.4.3 Systematic Review: Caffeine for apnoea and the prevention of neurodevelopmental impairment in preterm infants

To identify, evaluate, and summarize the evidence for the effectiveness of caffeine in reducing the rate or occurrence of apnoea and reducing long-term neurodevelopmental impairment in preterm infants (<37 weeks' PMA).

To assess if there is any difference in these outcomes between caffeine given at standard doses (≤10 $mg.kg^{-1}$ caffeine citrate equivalent) and high doses (>10 $mg.kg^{-1}$ caffeine citrate equivalent).

3. Analytical methods for caffeine in solution and saliva and caffeine oral liquid formulation stability

3.1 Abstract

A clinical trial is currently underway to examine the efficacy of using caffeine citrate to prevent intermittent hypoxemia in late preterm neonates. Determining caffeine concentration using saliva in this population would be preferable as it is less invasive than plasma sampling, but a suitable method of analysis is required. This paper presents the development and validation of a rapid, efficient and reproducible stability-indicating high-performance liquid chromatography (HPLC) method and extraction protocol for the quantification of caffeine present in saliva. The stability of extemporaneously prepared caffeine citrate solutions (at 20–25 °C) was determined, along with the stability of caffeine spiked saliva samples (at 20–25 and 2–8 °C), to ensure the suitability of the developed method in the analysis of clinical trial samples. Protein precipitation using acetonitrile ensured the complete removal of salivary proteins and resulted in extraction recovery of ≥95% for all samples. The HPLC assay following extraction was linear (R^2>0.99) over the range 0.3–50 µg/mL (lower limit of quantification 0.3 µg/mL). The accuracy of the quality control samples was 94–100% and the relative standard deviation (RSD) was <7% for all samples. Caffeine-spiked saliva samples were stable for three freeze-and-thaw cycles pre-extraction and up to 72 hr post-extraction. The extraction and HPLC methods described were thus suitable for the analysis of clinical trial samples from the Latte Dosage Trial. All caffeine solutions were physically and chemically stable, with concentrations at the end of the three-month test period being within 4% of the baseline concentrations, indicating appropriateness for use as clinical trial medications.

3.2 Introduction

Caffeine is a methylxanthine drug widely used in neonatal intensive care units for the prevention and treatment of apnoea of prematurity, and is one of the most cost-effective interventions in this population(Dukhovny et al., 2011; Eichenwald & Committee on Fetus and Newborn, 2016). It has been used in neonatal units in both IV and oral formulations since the 1970s, and various commercial formulations are available in different countries. The Latte Dosage Trial, which is currently being conducted to examine the efficacy of using caffeine citrate to prevent intermittent hypoxemia in late preterm neonates, requires the use of caffeine citrate oral liquid at four different strengths to allow for trial blinding alongside a placebo(Oliphant et al., 2020). In New Zealand (NZ), caffeine citrate is available commercially in a single 20 mg/mL strength, so extemporaneous compounding by pharmacists at participating sites is required to obtain the appropriate strengths (5, 10, 15 and 20 mg/mL) for the trial to maintain blinding. The formulation of caffeine produced for the Latte Dosage Trial consists solely of caffeine citrate dissolved in water to avoid exposing preterm infants to unnecessary excipients. Before being used in a clinical setting, the storage stability of preservative-free caffeine solutions needs to be determined.

While plasma samples are most commonly used for analysis of drug concentrations in pharmacokinetic studies, saliva has many advantages over plasma. Saliva is easily obtained by non-invasive techniques, increasing patient acceptability (especially where a large number of samples or children are involved) and decreasing the technical skills required for collection (Gorodischer et al., 1994). These factors make it more suitable than plasma for use in neonatal clinical trial participants. Furthermore, the concentration in saliva reflects free or unbound drug concentrations in plasma, and as this represents the pharmacologically active drug concentration it may be more meaningful for some drugs than plasma concentrations, which often reflect both bound and unbound drug (Horning et al., 1977). In both preterm neonates and adults, salivary

caffeine concentrations have been shown to be comparable to plasma concentrations (Alkaysi et al., 1988; Carrillo et al., 2000; Dobson et al., 2016).

Previously reported methods for the quantification of caffeine from biological samples have used phase separation techniques for the extraction of caffeine (Jordan et al., 2015; Newton et al., 1981; Perera et al., 2010). This technique is difficult with saliva, as the clear and colorless nature of both phases makes it difficult to find the phase boundary during the extraction process. Saliva also contains a variety of endogenous substances such as proteins, which need to be removed before chromatographic drug analysis (McDowall et al., 1989). Incomplete removal of proteins from samples for high performance liquid chromatography (HPLC) analysis impacts negatively on both column performance and the sensitivity of the analytical method (McDowall et al., 1989).

The aim of this paper was to develop and validate a liquid chromatography assay for *in vitro* and *in vivo* quantification of caffeine and use this to determine the stability of caffeine citrate solution. The primary objectives were to a) develop and validate an extraction protocol and liquid chromatography assay for the extraction and quantification of caffeine from human saliva, and b) evaluate the chemical, physical and microbial stability of caffeine solutions..

3.3 Experimental methods

3.3.1 Materials

Caffeine citrate used in the formulation of the oral liquid preparations and standards for analysis was purchased from ACROS Organics, Fisher Scientific (Belgium). Water for irrigation was used in the extemporaneous formulation of the oral liquids, in keeping with usual compounding practice at the local hospital, and was purchased from Baxter Healthcare (Australia). Acetonitrile (Merck, Germany) was analytical grade

and Milli-Q water for HPLC was obtained from Millipak (Millipore, 0.22 µm). Blank saliva samples for method validation and stability evaluation were obtained by direct expectoration from six healthy adult volunteers who had abstained from caffeine-containing food and beverages for a period of at least 24 hr before morning collection. Each volunteer provided a single sample of at least 4 mL for use in this research, and all samples were combined to give a standardized saliva mix for validation experiments [Health and Disability Ethics Committee (Northern A), New Zealand; reference number 18/NTA129].

3.3.2 Chromatographic conditions

An Agilent 1260 HPLC machine (Agilent Corporation, Germany) was used for sample analysis. A Kinetex C18 column (5 µm, 4.6 × 150 mm) was used with a mobile phase comprising acetonitrile-water (1:9 v/v) and the flow rate set at 0.9 mL/min. An injection volume of 20 µL was used, and the UV absorbance was measured at 273 nm using a photodiode array (PDA) detector. This chromatographic method was validated for both caffeine solutions (see Supplementary Data) and human saliva samples and was applied to determine the stability of caffeine in solution for use as a trial medication, as well as to analyze saliva samples from trial participants.

3.3.3 Preparation of calibration and quality control (QC) standards in saliva

Ten-fold concentrated caffeine citrate solutions ranging from 3 to 500 µg/mL were prepared from a 1 mg/mL stock solution of caffeine citrate in water. Blank saliva was spiked with the appropriate volume of respective spiking solution to prepare triplicates of seven calibration standards at 0.3, 0.6, 2, 5, 10, 25, and 50 µg/mL. Similarly, three different QC standards: 1, 15, and 30 µg/mL, were spiked in triplicate as standards to determine the accuracy and precision of the method.

3.3.4 Extraction of caffeine from saliva

Samples were prepared as follows: 50 µL of saliva was mixed with 50 µL of Milli-Q water and 900 µL acetonitrile. The mixture was vortex mixed at 2000 rpm for 3 min at room temperature (22 °C) then centrifuged at 19,100 rcf at 4 °C for 10 min. The supernatant (975 µL) was transferred to a new Eppendorf tube and evaporated under a gentle stream of nitrogen at 42 °C. The residue was then reconstituted using 50 µL water followed by vortex mixing at 2000 rpm for 3 min at room temperature (22 °C), then centrifuged at 19,100 rcf at 4 °C for 10 min. A 20 µL aliquot of the supernatant was injected for HPLC analysis.

The extraction recoveries of spiked quality control (QC) saliva samples prepared at three different concentration levels (1, 15, and 30 µg/mL) were evaluated by injecting nine samples of each concentration. The extraction recoveries were calculated using Equation (1) below (US Food and Drug Administration et al., 2018).

$$Recovery\ (\%) = \frac{Peak\ area\ of\ caffeine\ extracted\ from\ saliva \times 100}{Peak\ area\ of\ caffeine\ in\ standard\ solution} \quad \text{Eq. 1}$$

3.3.5 Method validation

Following extraction, the method for saliva sample analysis was validated according to the Food and Drug Administration (FDA) guidelines for validation of bioanalytical methods (US Food and Drug Administration et al., 2018). The following tests were repeated daily for three consecutive days.

3.3.5.1 Sensitivity

The sensitivity of the method was determined by calculating the limit of quantitation (LOQ) based on the standard deviation (SD) of the lowest non-zero calibrator and

slope of the linearity curve as described in Eq 2. The signal to noise ratio of the lowest concentration (0.3 µg/mL) was also determined.

$$LOQ = \frac{10 \times \text{standard deviation of the lowest concentration}}{\text{Slope of the calibration curve}} \quad \text{Eq. 2}$$

3.3.5.2 Linearity

Linearity demonstrates that the sample solutions are within a concentration range where analyte response is directly proportional to concentration. This was assessed by analyzing freshly prepared spiked saliva samples (n =7) ranging from 0.3–50 µg/mL daily for three consecutive days. A linearity standard curve was obtained by plotting the peak area against concentration.

3.3.5.3 Selectivity and specificity

Blank saliva samples (n = 3) were compared and evaluated for interference of endogenous compounds during analysis to establish the selectivity of the method. Specificity was evaluated based on the selectivity of the method and ability to detect caffeine citrate (± 20% LLOQ) from the sample matrix. The chromatograms of blank samples and spiked samples (0.3 µg/mL; n = 3) were analyzed and compared to determine specificity of the method.

3.3.5.4 Accuracy and precision

The accuracy (% A) of the method is the degree of closeness of the measured concentration of the analyte to the true concentration of the analyte in QC samples (US Food and Drug Administration et al., 2018). Accuracy was determined by analyzing nine QC samples at three different concentration levels (1, 15 and 30 µg/mL), then calculated using Eq. 3 below.

$$\% A = [Measured\ concentration/Nominal\ concentration] \times 100 \qquad \text{Eq. 3}$$

Precision is the degree of closeness between the results. Intra-day and inter-day precision were determined by analysis of three separate QC samples at each of the three different concentrations (1, 15 and 30 µg/mL), both over a single day and for three consecutive days, respectively, and calculating the relative standard deviations (RSD). According to the FDA guidelines accuracy and precision of a method are considered acceptable provided the RSD values remain below 15% (US Food and Drug Administration et al., 2018).

3.3.5.5 Sample storage and post-extraction stability

The stability of caffeine stored in an auto sampler (without temperature control) was determined by reinjecting the QC caffeine samples (n =3 for each concentration) over a period of 72 h. Freeze-and-thaw stability of spiked saliva samples was also determined at three QC concentrations in triplicate to determine the stability of caffeine over three freeze-and-thaw cycles (–20 °C to ambient temperature). Stability was considered adequate provided the accuracy at each concentration level remained within ± 15% (US Food and Drug Administration et al., 2018).

Longer-term storage of spiked saliva samples was assessed at two concentrations (0.5 µg/mL and 3 µg/mL; n =3 each) using blank saliva samples as negative controls. Samples were stored at room temperature (20-25 °C) and under refrigeration (2-8 °C). A portion of each sample was withdrawn immediately following preparation (time 0), day 1, 2 and 7 and analyzed with the developed method to determine the quantity of caffeine remaining following storage at each of the conditions.

3.3.6 Stability analysis of extemporaneously prepared caffeine citrate formulation

3.3.6.1 Sample preparation

Samples were prepared using standard hospital extemporaneous compounding protocols. First, a stock solution of caffeine citrate 20 mg/mL was prepared using water for irrigation. The stock solution was further diluted with water for irrigation as required to give 5, 10, 15 and 20 mg/mL solutions. Six separate samples were prepared at each strength and stored individually in amber polypropylene medicine bottles. From these, three samples at each strength were labelled and used for HPLC stability testing, and the remaining three samples were labelled and used for pH and organoleptic analysis. An additional five samples of the 20 mg/mL formulation were prepared for microbial analysis. All samples were stored at room temperature (20-25 °C) under florescent lighting in the local hospital pharmacy to mimic in-use conditions.

3.3.6.2 HPLC assessment

Evaluation of chemical stability was performed at pre-determined intervals (weekly for one month, then fortnightly for one month, then at three months from the date of preparation). At each timepoint, samples were withdrawn from the three storage bottles, and analyzed directly using the HPLC parameters and methods described in section 2.2 above. Details of the validation of this method are provided as a supplement to this paper.

3.3.6.3 Organoleptic and pH assessment

At each time point, 2-3 mL of sample was decanted into a test tube for analysis. Samples were visually examined and photographed against both light and dark backgrounds for clarity, color, precipitation or other visual change, and assessed for any olfactory change. In addition, the pH of each sample was measured using a pH meter (SevenEasy pH meter, Mettler-Toledo Inc., Columbus, OH). To ensure accuracy of the measurement, the pH meter was calibrated using standard buffer solutions at pH 4, 7 and 10 on the day of stability assessment.

3.3.6.4 Microbial analysis

To allow for comparison with the commercially available formulation, a sample of the 20 mg/mL caffeine citrate formulation was submitted to an accredited contracting laboratory at each of 0, 7, 14, 21 and 28 days after compounding. These samples were tested for the presence of *Cronobacter* spp., *Listeria* spp., *Salmonella* spp., *Bacillus cereus*, Coagulase-positive Staphylococci, Coliforms, *Escherichia coli* and yeasts and moulds . This includes pathogens specified by the European Pharmacopoeia in the section on microbiological quality of aqueous preparations for oral use, plus others to known to be pathogenic in neonates, and/or to have been identified as contaminants in medications or neonatal food products (Council of Europe, 2019; Dong & Speer, 2015; New Zealand Ministry of Health, 2012).

3.4 Results and discussion

3.4.1 Development and selection of extraction protocol

Biological matrices, such as saliva, contain many proteins which, unless removed in a pre-treatment step, may precipitate on contact with the mobile phase within a HPLC system, degrading the performance of the HPLC column (Li et al., 2019). The extraction method for caffeine in saliva was adapted from that used by Perera et al (2010). Ethyl acetate has been used widely for extraction of polar analytes from biological samples by liquid-liquid extraction technique as it is effective in extracting compounds with a broad range of polarity (Bogialli et al., 2007). However, preliminary trials for extraction of caffeine from saliva using ethyl acetate as described by Perera *et al*. (2010) were complicated by difficulties establishing solvent boundaries between the saliva solution and ethyl acetate, and correspondingly challenges in removing only the organic phase for further processing, due to the colorless nature of both phases. Therefore, an alternative method was developed for the extraction of caffeine by protein precipitation using acetonitrile. Acetonitrile has the highest efficiency in protein removal (Polson et al., 2003) and is, hence, the most commonly used organic solvent for protein

precipitation in bioanalysis (Li et al., 2019). Acetonitrile is water-miscible, allowing it to mix freely with saliva, where it displaces water from the solvation layer of the proteins causing aggregation and precipitation. At a ratio of 2:1 acetonitrile: aqueous phase, the resulting supernatant is essentially protein-free (Li et al., 2019). Precipitation of salivary proteins using acetonitrile was found to be simple, uniform and repeatable. A small amount of water (50 µL) was added to increase the solubility of caffeine during the extraction process, with the organic to inorganic solvent ratio being maintained at 1:9 to ensure maximum removal of proteins (Li et al., 2019).

3.4.1.1 Extraction recovery

The extraction recovery of QC samples was found to be 95-111% at 1, 15 and 30 µg/mL (Table 3.1).

Table 3.1 Accuracy, precision and extraction recovery data for caffeine citrate at concentrations of 1, 15 and 30 µg/mL.

Concentration (µg/mL)	Intra-day (*n* = 3)		Inter-day (*n* = 9)		Extraction recovery (*n* = 9)	
	Accuracy (% Nominal)	Precision (% RSD)	Accuracy (% Nominal)	Precision (% RSD)	Mean (%)	RSD (%)
1	97.8	3.1	94.7	4.0	110.6	3.5
15	98.8	0.9	96.9	6.9	95.0	6.8
30	97.7	1.4	99.7	5.2	95.1	5.2

All values are expressed as a mean percent (%). RSD – Relative standard deviation.

The final extraction method described above was simple, rapid and accurate while not requiring particularly specialized equipment, thus making it suitable for the analysis of a large number of samples from a clinical trial in a standard laboratory.

3.4.2 Method validation

The developed method was found to be simple, rapid, sensitive and reproducible with caffeine eluting at 6.5 ± 0.2 minutes. The results of various validation parameters are discussed below.

3.4.2.1 Sensitivity

Using Eq. 2 described above, the developed method was found to be sensitive in quantifying caffeine concentration to 0.3 µg/mL This was further confirmed by calculating the signal to noise ratio at this concentration, which was found to be above 10, indicating adequate sensitivity at this concentration. The LOQ of the developed method was thus considered to be 0.3 µg/mL.

3.4.2.2 Linearity

The plasma concentration vs. peak area plot was found to be linear for caffeine (n = 3) within the concentration range of 0.3 to 50 µg/mL in saliva with an R^2 value of 0.9975. The regression equation is shown below (Eq. 4).

$$y = 28.516\,x + 4.073 \qquad \text{Eq. 4}$$

This concentration range is expected to cover the range of observed concentrations of caffeine in the saliva of mothers and babies participating in a clinical trial of caffeine citrate in late preterm babies, based on previous reports of salivary caffeine levels equivalent to 2.48-18.44 µg/mL in the saliva of lactating women, following ingestion of a variety of caffeine-containing beverages (Berlin et al., 1984), and neonatal salivary concentrations of 0.4-36.8. µg/mL in a previously reported clinical trial (Chaabanea et al., 2017).

3.4.2.3 Selectivity and specificity

The developed method was found selective for caffeine in the presence of other endogenous compounds. Analysis of multiple blank samples showed that there was no interference from the endogenous compounds in saliva (Figure 3.1).

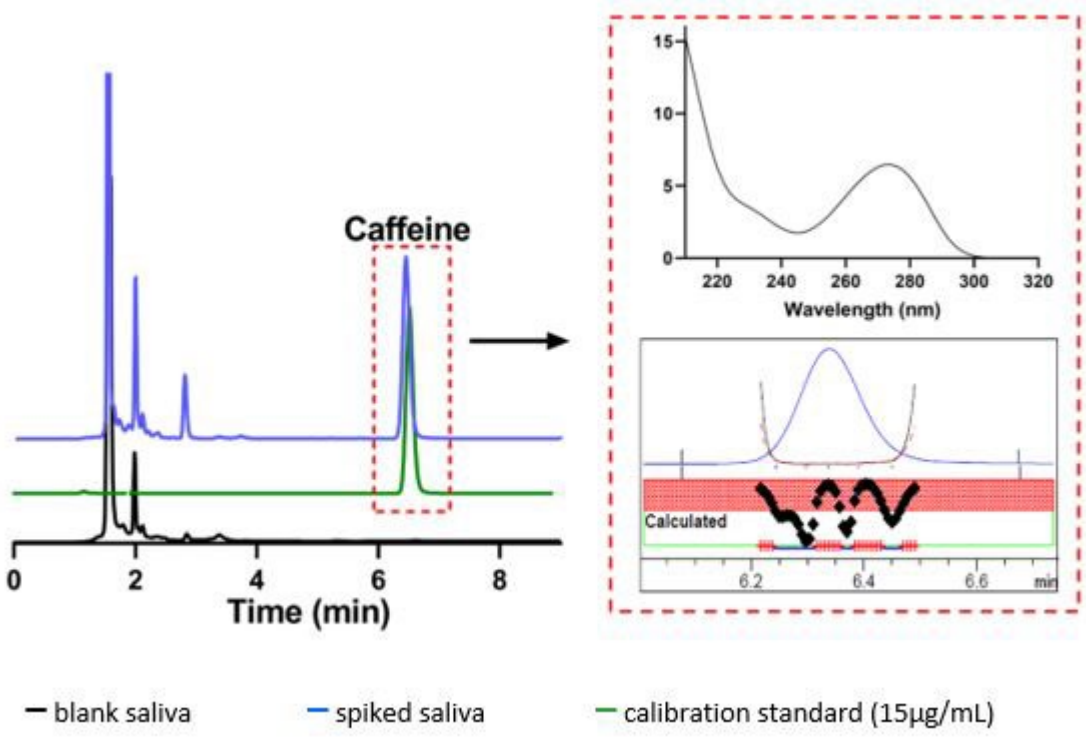


Figure 3.1 Stacked chromatograms of blank and spiked saliva samples (15 µg/mL) and calibration standard (15 µg/mL) at 273 nm, with caffeine peak eluting at 6.35 minutes. Graphs in the dotted box show caffeine spectra and representative peak purity of the samples.

3.4.2.4 Accuracy and precision

The method was accurate and precise at three different concentrations (Table 3.1). The accuracy was between 94–100%. The precision (percentage bias) was below 7, less than the ± 15% provided for in the FDA guidelines (US Food and Drug Administration et al., 2018) for both inter- and intra-day samples, indicating that the method is repeatable and reproducible.

3.4.2.5 Sample storage and post-extraction stability

Stability of caffeine-spiked saliva samples were determined both before and after extraction to mimic conditions during collection and transportation periods as well as sample analysis. The concentration of caffeine in saliva samples was found to remain stable with storage at both room temperature and under refrigeration for a period of seven days (Table 3.2).

Table 3.2 Stability of caffeine in saliva under refrigeration (2-8 °C), at room temperature (20-25 °C) and frozen (-20°C).

Concentration (µg/mL)	**Percentage of caffeine remaining**								
	24 hours			***48 hours***			***7 days***		
	20-25 °C	*2-8 °C*	*-20 °C*	*20-25 °C*	*2-8 °C*	*-20 °C*	*20-25 °C*	*2-8 °C*	*-20 °C*
0.5	96.8 (13.3)	98.0 (12.0)	76.3 (20.0)	100.5 (18.7)	106.7 (32.2)	80.1 (7.5)	107.1 (19.9)	97.1 (30.7)	72.5 (1.2)
3	97.0 (2.9)	104.7 (1.42)	92.1 (11.9)	101.1 (3.1)	100.3 (2.7)	95.3 (6.6)	97.2 (1.74)	101.2 (1.24)	106.9 (7.8)

Data presented as mean (SD); n = 3.

Caffeine in saliva was stable for three freeze-and-thaw cycles (–20 °C to ambient temperature), indicating that freezing and thawing of clinical trial samples for analysis will not affect the results. In addition, caffeine was stable at all three concentrations (1, 15 and 30 µg/mL) for at least two days post-extraction when kept in the auto sampler (

Table 3.3). This indicates post-extraction stability of caffeine in all the samples until analysis of the final sample in the sequence, despite the temperature not being controlled in the auto sampler.

Table 3.3 Stability evaluation of extracted caffeine samples from saliva kept in auto sampler for 72 hours and for three freeze-and-thaw cycles (from -20 °C to room temperature)

Concentration (µg/mL)	Auto sampler, 48 h		Three cycles, freeze-and-thaw	
	Drug remaining (%)	RSD (%)	Drug remaining (%)	RSD (%)
1	106.7	1.2	102.0	5.4
15	103.5	2.7	92.6	7.8
30	103.7	2.9	102.0	8.5

Data are mean values (n=3). RSD - relative standard deviation.

3.4.2.6 Summary of method validation for determination of caffeine in saliva

The validation testing described above provides data to support the developed method for the quantification of caffeine in human saliva. This method will be used to quantify salivary caffeine levels in infants participating in a randomized controlled trial of caffeine for the prevention of intermittent hypoxemia, and their mothers (Oliphant et al., 2020). While it would have been preferable to perform this validation using neonatal saliva as well as adult saliva, ethical and practical issues preclude this, and any differences between the two matrices are likely to be minor.

The stability testing of saliva samples spiked with caffeine citrate confirms that the caffeine concentration in saliva is not significantly affected by storage at room

temperature or under refrigeration, so delays in the transport of clinical trial samples to the laboratory for analysis will not significantly alter the results obtained from analysis. Stability through freeze-thaw cycles indicates that freezing of samples until the end of the trial before analyzing them (as is required to avoid breaking the trial blinding) will not alter the results obtained.

3.4.3 Stability analysis of extemporaneously prepared caffeine citrate formulation

3.4.3.1 Organoleptic assessment

Throughout the three months of storage, all solutions remained clear and colorless with no visible precipitation. There was no noticeable odor from any of the tested solutions at any point during the study. This is in keeping with reported results from stability studies on preserved caffeine citrate solution (Barnes et al., 1994).

3.4.3.2 pH assessment

During the three-month period of stability testing, no significant changes in pH were observed in any of the four concentrations of caffeine citrate solution (

Table 3.4). Our results are similar to those reported by Barnes et al. in their stability testing of a 10 mg/mL preserved caffeine citrate solution (Barnes et al., 1994).

Table 3.4 pH of caffeine citrate samples (stored at room temperature for up to 3 months.

Timepoint	pH of solution			
	Concentration (mg/mL)			
	5	10	15	20
Baseline	2.43 (0.16)	2.15 (0.01)	2.06 (0.03)	1.90 (0.06)
7 days	2.53 (0.02)	2.29 (0.03)	2.20 (0.02)	2.16 (0.02)
14 days	2.44 (0.05)	2.22 (0.08)	2.13 (0.03)	2.03 (0.02)

21 days	2.46 (0.03)	2.25(0.00)	2.15 (0.01)	2.07 (0.05)
28 days	2.49 (0.05)	2.33 (0.02)	2.23 (0.02)	2.09 (0.02)
6 weeks	2.44 (0.02)	2.21 (0.04)	2.09 (0.01)	1.95 (0.05)
2 months	2.52 (0.04)	2.35 (0.01)	2.20 (0.03)	2.16 (0.01)
3 months	2.31 (0.03)	2.09 (0.01)	1.91 (0.02)	1.85 (0.04)

Data presented are mean (SD); n = 3

3.4.3.3 HPLC analysis

Calibration curve construction demonstrated that detection of caffeine citrate in solution was linear across the range tested, from 1 – 25 µg/mL. Throughout the trial period, all strengths of the caffeine citrate solution retained adequate potency (Figure 3.2), defined as deviating by not more than 10% of the initial concentration(Mehta, 1993) with no new peaks observed on the chromatogram. After three months, the 5, 10, 15 and 20 mg/mL solutions were found to have mean (SD) caffeine concentrations of 5.0 (0.1), 9.9 (0.2), 15.0 (0.1) and 20.3 (0.5) mg/mL remaining, respectively, which was within 4% of corresponding baseline concentrations.

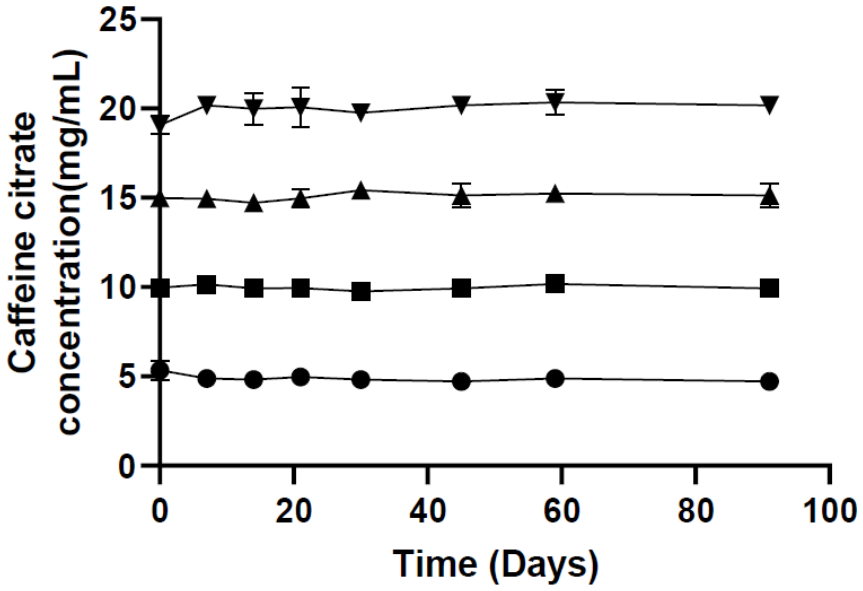


Figure 3.2 Concentration of 5 (●), 10 (■), 15 (▲) and 20 (▼) mg/mL caffeine citrate solutions over a three-month period. Data presented as mean ± SD; n = 3.

3.4.3.4 Microbial analysis

None of the 20 mg/mL samples, prepared under standard extemporaneous compounding conditions at the local hospital pharmacy, showed any growth for *Cronobacter* spp., *Listeria* spp., *Salmonella* spp., *Bacillus cereus*, Coagulase-positive Staphylococci, Coliforms, *Escherichia coli* or yeasts and moulds when stored in amber polypropylene medicine bottles under florescent lighting at room temperature for time periods of up to 28-days post-manufacture. As solutions were extemporaneously compounded and did not contain any preservative, use beyond 28 days post-manufacture would not be considered in clinical practice.

3.4.3.5 Summary of stability analysis of extemporaneously prepared caffeine citrate formulation

As blinding requirements for the Latte Dosage Trial require the use of caffeine citrate oral liquid at four different strengths alongside a water placebo, and such products are not available commercially, we required a stable formulation of caffeine citrate that could be easily extemporaneously compounded by pharmacists at participating sites. As caffeine is both highly water soluble and known to be stable (Barnes et al., 1994; Sigma-Aldrich., 2014), we formulated caffeine citrate as a simple aqueous solution in order to minimize the exposure of neonates to unnecessary pharmaceutical excipients. At 20 mg/mL this gave a formulation with comparable appearance and pH as that available commercially in NZ.

In keeping with the commercially available formulation, the prepared solutions were stored at room temperature. Refrigerated testing was considered unnecessary, as the commercial product was known to be stable at room temperature, and storage is easier both within the hospital and for patients at home if refrigeration is not required. The

findings of the stability testing reported above provide reassurance that caffeine citrate, when compounded extemporaneously as an aqueous solution, is stable at the concentrations tested and is suitable for use in the Latte Dosage Trial.

3.5 Conclusion

The method presented above for the extraction and quantification of caffeine in human saliva was found to be simple, rapid, accurate and reproducible, and hence suitable for the analysis of clinical trial samples. Salivary caffeine is stable for at least three freeze-and-thaw cycles and for at least seven days at both room temperature and under refrigeration, meaning delays in transport of samples from the point of collection to the laboratory, and freezing before analysis at the end of the trial will not affect salivary caffeine concentrations. Extemporaneously compounded caffeine citrate aqueous solutions are chemically and physically stable at room temperature for at least three months, with no growth of organisms of interest on microbial testing for at least one month after manufacture.

4. The Latte Dosage Trial Protocol

4.1 Abstract

Introduction

Infants born late preterm (34^{+0} – 36^{+6} weeks' gestational age) have frequent episodes of intermittent hypoxaemia compared to term infants. Caffeine citrate reduces apnoea and intermittent hypoxaemia and improves long-term neurodevelopmental outcomes in infants born very preterm and may have similar effects in late preterm infants. Clearance of caffeine citrate increases with gestational age and late preterm infants are likely to need a higher dose than very preterm infants. Our aim is to determine the most effective and best tolerated dose of caffeine citrate to reduce transient intermittent hypoxaemia events in late preterm infants.

Methods and analysis

A phase IIB, double-blind, five-arm, parallel, randomised controlled trial to compare the effect of four doses of oral caffeine citrate versus placebo on the frequency of intermittent hypoxaemia. Late preterm infants will be enrolled within 72 hours of birth and randomised to receive 5, 10, 15 or 20 mg/kg/day caffeine citrate or matching placebo daily until term corrected age. The frequency of intermittent hypoxaemia (events/hour where oxygen saturation concentration is ≥10% below baseline for ≤two minutes), will be assessed with overnight oximetry at baseline, two weeks after randomisation (primary outcome) and at term corrected age. Growth will be measured at these timepoints, and effects on feeding and sleeping will be assessed by parental report. Data will be analysed using generalised linear mixed models.

Ethics and dissemination

This trial has been approved by the Health and Disability Ethics Committees of New Zealand (reference 18/NTA/129) and the local institutional research review committees. Findings will be disseminated to peer-reviewed journals, to clinicians and researchers at local and international conferences and to the public. The findings of the trial will inform the design of a large multi-centre trial of prophylactic caffeine in late preterm infants, by indicating the most appropriate dose to use and providing information on feasibility. Trial Registration: ACTRN12618001745235.

4.2 Strengths and limitations of this study

- This study seeks to address the rates of neurodevelopmental impairment among late preterm infants by investigating the optimal dose of caffeine, as a potential primary neuroprotective strategy.
- This is the first randomised placebo-controlled trial of four different doses of caffeine for the prevention of intermittent hypoxaemia in late preterm infants.
- A strength of the trial is that both clinicians and parents will be blinded to treatment allocation with all groups receiving the same volume of an identical-appearing masked suspension.
- The success of the trial depends on high compliance of administration of study medication by caregivers, which will be monitored by intermittent measurement of study bottle contents and infant salivary caffeine concentrations.
- Future studies will be required to determine if caffeine reduces neurodevelopmental impairment in late preterm infants, based on the optimal dose determined by this trial.

4.3 Introduction

Late preterm infants are those born between 34 weeks and 36 weeks and 6 days' gestation (Engle et al., 2007). They form the largest group within the preterm population, accounting for 68% of all preterm births or 5% of all births in New Zealand (Ministry of Health, 2019) and 7% of all births in the United States of America (J. Martin et al., 2017). Late preterm infants are physiologically and metabolically

immature (Engle et al., 2007) and have a higher risk of morbidity and mortality in the neonatal period than full-term infants(McIntire & Leveno, 2008). They are more likely than full-term infants to have delayed establishment of oral feeding, temperature instability, hypoglycaemia, jaundice and respiratory distress, and to undergo investigation for sepsis (M. Wang et al., 2004). Despite these risks, their size and weight mean they are often managed in a similar manner to full-term infants and cared for on postnatal wards rather than in neonatal units (Boyle et al., 2015), and are not treated with the routine prophylactic interventions such as caffeine, nutritional supplements and probiotics that are common practice in very and extremely preterm infants.

Apnoea of prematurity are prolonged pauses in breathing, of 20 seconds or more, which may cause a reduction in the oxygen saturation and bradycardia, and are associated with an increased incidence of brain injury (Butcher-Puech et al., 1985) and neurodevelopmental impairment(Janvier et al., 2004). Late preterm infants experience apnoea of prematurity, although less frequently than in very preterm infants(Henderson-Smart, 1981). Recently, we have demonstrated that late preterm infants have frequent episodes of intermittent hypoxaemia,(Williams et al., 2018) transient repetitive decreases in oxygen saturation not associated with apnoea, but potentially causing similar organ hypoxia. In late preterm infants, the frequency of intermittent hypoxaemia peaks at two weeks' postnatal age, before reducing to baseline levels at term corrected age (Figure 4.1)(Williams et al., 2018).

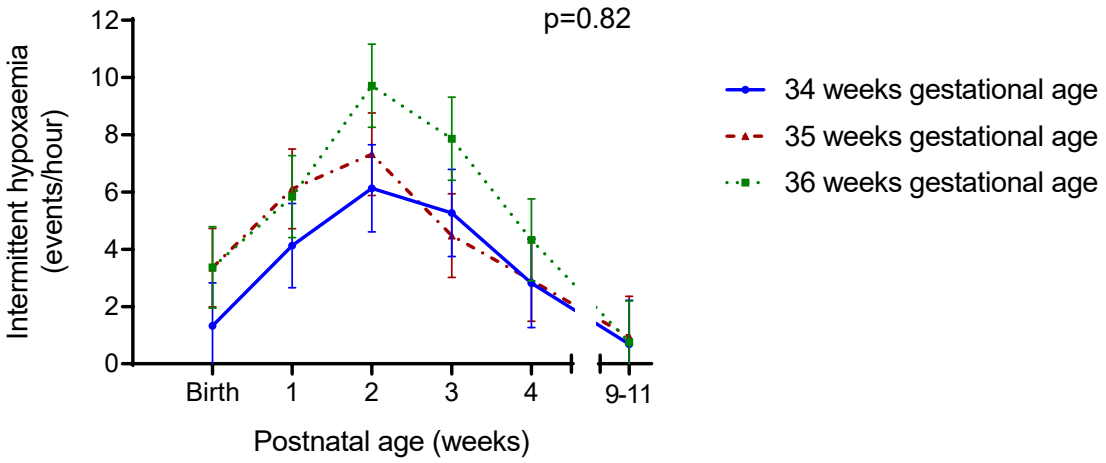


Figure 4.1: Rate of Intermittent Hypoxaemia in Late Preterm Infants in the 9-11 Weeks Following Birth (adapted from Williams et al, 2018)

Studies in adults have consistently shown that even brief exposure to hypoxia, whether from high altitude (Bahrke & Shukitt-Hale, 1993) or carbon monoxide poisoning (Weaver et al., 2002), can have long-term adverse effects on cognition. Even small changes in oxygen saturations in the neonatal period have been shown to significantly affect survival and neurodevelopment of very preterm infants (Carlo et al., 2010; Saugstad & Aune, 2013; Stenson et al., 2011). Intermittent hypoxaemia is associated with altered brain development in neonatal mice (Kanaan et al., 2006) and reduced cognition and behavioural deficits in neonatal rats (Row et al., 2002). In humans, transient intermittent hypoxaemic events are associated with poor neurodevelopmental outcomes in extremely preterm infants(Poets et al., 2015) and in children with sleep disordered breathing (N. Ali et al., 1996) and congenital heart disease (Bass et al., 2004).

Caffeine is a respiratory stimulant which is effective in the prevention and treatment of apnoea of prematurity and intermittent hypoxaemia, and also reduces the incidence of chronic lung disease, cerebral palsy, and cognitive delay in very preterm infants (McNamara et al., 2004; Schmidt et al., 2006, 2007). Follow-up to 11 years of age has

recently shown that caffeine treatment reduces the risk of motor dysfunction by a third in infants born very preterm(Doyle et al., 2017; Schmidt et al., 2017). While some of the long term beneficial effects of caffeine may be due to its effect in reducing the incidence of bronchopulmonary dysplasia (P. Davis et al., 2010), there is also benefit from reducing the amount of time that infants are hypoxic, independent of the effect on bronchopulmonary dysplasia (Doyle et al., 2010). Thus, caffeine has become the standard of care for very preterm infants and is in widespread use in neonatal units around the world as one of the few neonatal treatments that has been proven to have long term neurodevelopmental benefit, and to also be very well tolerated.

In the longer term, late preterm infants are more likely to be diagnosed with cerebral palsy (Moster et al., 2008; Odd et al., 2013), developmental delay (Darlow et al., 2009; Woythaler et al., 2011), cognitive impairment (Heinonen et al., 2015; Quigley et al., 2012; Talge et al., 2010) and behavioural disorders (Huddy et al., 2001) compared to their term-born peers. However, few studies have investigated interventions to improve the neurodevelopmental outcomes of late preterm infants. As late preterm infants have an increase in hypoxaemic events compared to term infants, and hypoxaemic events are associated with poor neurodevelopmental outcomes, it is possible that caffeine, an intervention that reduces hypoxaemic events and has already been shown to improve long-term outcomes in extremely and very preterm infants, may be effective at improving outcomes in late preterm infants.

In adults, most caffeine metabolism is via cytochrome P450 1A2 in the liver (Anderson et al., 1999). However, in newborn preterm infants, hepatic metabolism of caffeine is almost absent, and most caffeine is eliminated via the kidneys, which are also immature. Therefore, caffeine elimination is slow in extremely preterm infants, and the half-life of caffeine is long. With increasing postconceptial agethe elimination of of caffeine increases (Falcão et al., 1997; Le Guennec et al., 1985) , and larger doses may be needed to maintain a therapeutic effect. However, the pharmacokinetic studies of

caffeine in preterm infants to date have been done to treat apnoea in very preterm infants, rather than to treat intermittent hypoxaemia in late preterm infants(Thomson et al., 1996).

There is a wide range in the dose of caffeine citrate given to extremely preterm infants, from daily doses of 5 mg/kg (Schmidt et al., 2006) to 20 mg/kg (Steer et al., 2004). The Caffeine for Apnoea of Prematurity (CAP) trial used a dose of 5 mg/kg, which could be increased to 10 mg/kg if necessary to control apnoea of prematurity (Schmidt et al., 2006). The trial by Rhein et al. found that in very preterm infants, 6 mg/kg of caffeine citrate reduced intermittent hypoxaemia at 35 and 36 weeks' post-menstrual age, but not after 36 weeks' post-menstrual age (Rhein et al., 2014). The authors hypothesised that this may have been due to an insufficient dose as the infants matured. Therefore, the most effective dose of caffeine to treat intermittent hypoxaemia in late preterm infants is unknown.

In very preterm infants, caffeine is usually well tolerated, but occasionally infants on caffeine develop tachycardia and feed intolerance (Steer et al., 2004). Caffeine also causes reduced neonatal weight gain compared to placebo (Schmidt et al., 2006), and in ventilated preterm infants a higher dose of caffeine citrate (20 mg/kg) leads to reduced weight gain compared with a low dose (5 mg/kg)(Steer et al., 2004). As in adults, infants on caffeine can develop irritability, sleeplessness and gastrointestinal disturbance. For caffeine to be used as a prophylactic medication in a large number of late preterm infants, it will need to be prescribed at a dose that has a low risk of significant side effects.

We are therefore undertaking the Latte Dosage Trial, a randomised, placebo-controlled dosage trial, to determine the most effective and best tolerated dose of oral caffeine citrate to reduce intermittent hypoxaemia in late preterm infants.

4.4 Aim

To determine the most effective and best tolerated dose of caffeine citrate to reduce intermittent hypoxaemia in late preterm infants.

4.5 Hypothesis

Caffeine citrate will reduce the frequency of intermittent hypoxaemia in late preterm infants in a dose dependant manner.

4.6 Methods and analysis

4.6.1 Study Design

The Latte Dosage Trial is a phase IIB, double-blind, five-arm, parallel, randomised controlled trial to compare the effect of four different doses of oral caffeine citrate versus placebo on the frequency of intermittent hypoxaemia in late preterm infants.

4.6.1.1 Recruitment and randomisation

Participants will be recruited by trial investigators or study nurses / midwives within 72 hours of birth from the neonatal unit and postnatal wards at Auckland City and Middlemore Hospitals in Auckland, New Zealand. Following written informed consent and enrolment, trial participation may occur in hospital, at a primary maternity unit or at home, as the patient's clinical care dictates. Eligible participants are those infants born between 34 weeks and 36 weeks' and six days gestation without contradiction to caffeine treatment, with the following exclusions:

- Major congenital abnormality
- Minor congenital abnormality likely to affect respiration, growth or development
- Previous caffeine treatment

- Renal or hepatic impairment
- Tachyarrhythmia
- Seizures
- Hypoxic ischaemic encephalopathy
- Residing outside of the Auckland region

Infants will be assigned randomly via an internet randomisation service (Clinical Data Research Hub, Liggins Institute, University of Auckland) to receive either daily caffeine citrate 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg or placebo. The allocation sequence will be generated by the study statistician, with 1:1:1:1:1 allocation stratified by study site and gestational age at birth (34, 35 or 36 weeks)(Figure 4.2) using variable block sizes, with infants from multiple births being randomised to the same treatment group.

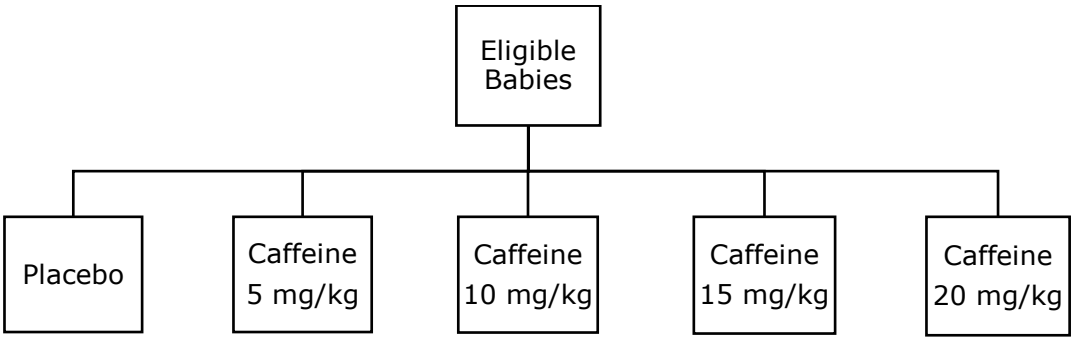


Figure 4.2: Flow diagram of randomisation schedule

Different concentrations of the caffeine citrate (5 mg/ml, 10 mg/ml, 15 mg/ml and 20 mg/ml) will be prepared in identical bottles to the placebo so that all infants receive the same dose volume (2 ml/kg loading dose followed by 1 ml/kg, once daily). Bottles will be labelled in randomisation blocks using a lettering system which will change halfway through the study in order to maintain concealment from study personnel.

4.6.2 Study Intervention

The infant will be given an enteral loading dose of the study drug (10 mg/kg, 20 mg/kg, 30 mg/kg or 40 mg/kg of caffeine citrate or water) in the morning after baseline oximetry (i.e. prior to the infant reaching 96 hours of age), followed by a daily dose each morning (5 mg/kg, 10mg/kg, 15 mg/kg or 20 mg/kg of caffeine citrate or placebo) until term equivalent age (40 weeks' post-menstrual age). The dose will be recalculated weekly for weight after the infant has regained birth weight using the weight recorded by study staff at two weeks after randomisation and those recorded by usual care providers between two weeks' and term corrected age. The study drug will be given via a nasogastric tube for infants with a tube *in situ*, and orally for infants who do not require a nasogastric tube. Infants who are not able to tolerate enteral medications will have the study drug withheld until they are able to tolerate enteral intake**.**

Compliance will be assessed by measurement of study medication remaining in the bottle. At the two-week visit, study staff will collect the initial bottle(s) issued at the start of the study and replace it with a new bottle(s), which will in turn be collected at the final visit. Liquid remaining in the bottle on each occasion will be measured and compared with the expected volume to assess compliance. Good compliance will be defined as ≥80% of the expected volume having been removed from the bottle. At the final visit, parents will be asked which treatment they think their infant received to assess the adequacy of blinding.

Apart from the study intervention and associated assessments, all other clinical care, including the decision on when to discharge participants from the hospital and/or primary birthing units, will continue to be provided by the local clinical team, in accordance with usual guidelines and practices. Should an infant participating in the study require treatment for apnoea or intermittent hypoxaemia, clinicians will be encouraged to use oxygen or positive pressure ventilation as first line treatments. If necessary, a loading dose of caffeine citrate may be given. If ongoing treatment with

caffeine is necessary in the opinion of the treating clinician, they can discuss the option of partially unblinding the infant (caffeine or placebo) with the Site Principal Investigator. If unblinding is required, information on the allocation of the participant will be communicated by the data management team to the treating clinician or pharmacist (who may inform the parents if requested), with the research team remaining blinded. Clinical open-label use of caffeine will be recorded. Unblinded infants will remain in the study unless parents request withdrawal, with infants analysed on an intention-to-treat basis.

4.6.3 Outcomes

The primary outcome for this study is the frequency of intermittent hypoxaemia (events/hour), defined as a brief transient fall in oxygen saturation concentration ≥10% below baseline on overnight oximetry, two weeks after randomisation. Events longer than 2 minutes are considered a change in baseline rather than a transient desaturation event. Transient intermittent hypoxaemic events, if frequent or severe, are thought to have neurocognitive effects as significant as prolonged hypoxaemia (Bass et al., 2004; Blunden & Beebe, 2006). Although a 3% threshold is used in polysomnography to define desaturation events, a definition of 10% is commonly used in the neonatal literature. In addition, due to the variability of events we considered a 10% threshold more repeatable and reliable than a 3% threshold for defining events.

Secondary outcomes include:

- Respiratory: frequency of intermittent hypoxaemia on overnight oximetry at term equivalent age; mean overnight oxygen saturation at 2 weeks and term equivalent age; use of respiratory support, including oxygen, until term equivalent age)
- Growth: growth velocity from birth to term equivalent age for weight gain, length and head circumference; failure to regain birthweight by 2 weeks of age

- Side effects: Feed intolerance as reported by parents (Kleinman et al., 2006); duration of tube feeding; sleep and arousal as reported by parents (measured by subscale nine on the Infant Behaviour Questionnaire-Revised, modified for neonates (Gartstein & Rothbart, 2003)); tachycardia; study drug stopped due to presumed side effects; neonatal seizures requiring anticonvulsant treatment before 44 weeks postmenstrual age; neonatal or infant death
- Maternal and infant salivary caffeine concentration at two weeks after randomisation (Dobson et al., 2016)
- Readmission to hospital until 44 weeks post-menstrual age or open label caffeine use
- Maternal caffeine intake at birth, two weeks and term corrected age and mental health (Edinburgh postnatal depression score) at birth and term corrected age (Cox et al., 1987)

The timing of the study intervention and assessments is summarised in Table 4.1 below.

Table 4.1: Study intervention and assessment

	Baseline	Morning following baseline oximetry	1 week	2 weeks	3-5 weeks	Term equivalent age
Pulse oximetry	X			X		X
Randomisation	X					
Loading dose		X				
Demographics, contacts	X					
Dose adjustment for weight				X	X	
Neonatal salivary caffeine concentration				X		
Maternal salivary caffeine concentration				X		
Drug Diary		X	X	X	X	X
Compliance assessment				X		X
Parental Questionnaires:						
Maternal smoking in pregnancy & household smoke exposure questionnaire	X					
Sleep questionnaire				X		X
Feed tolerance questionnaire				X		X
Maternal caffeine intake questionnaire	X			X		X
Edinburgh Postnatal Depression Scale	X					X

4.6.4 Data collection methods

Online data management services will be provided by the Clinical Data Research Hub (Liggins Institute, University of Auckland). Data collection will utilise the REDCap platform (Vanderbilt University) for clinical report forms, with password-protected secure servers used to store data.

Pulse oximetry: Overnight pulse oximetry (Rad 8, Masimo Corp., Irvine, CA) will be recorded for a period of 12 hours from either foot at baseline, two weeks after randomisation (range 12-21 days) and at term corrected age (range 40 to 41 weeks

postmenstrual age) using a 2-second averaging time and 2-second resolution. Recordings will be conducted at home, in primary birthing units or at home as dictated by the clinical care requirements of the participants. Where recordings are conducted at home, parents will be visited the day that recording starts by a member of the research team. The oximeter will be set up, and the parents will receive instruction in attaching the probe to the baby's foot and be instructed to do this when placing the baby down to sleep in the evening. If necessary, the research team member may visit late in the day to apply the probe or provide support via a video call to ensure this is done correctly by parents. Unless clinically required, oximeters will be operated in sleep mode, with no displays or alarms. The oximetry recording will be downloaded with PROFOX oximetry software (version Masimo 2011.27D, PROFOX Associates Inc, Esconditso, CA) and edited to remove readings with poor signal or aberrant data. Only recordings with more than six hours will be included in the analysis, recordings with less than six hours of edited data will be repeated the following night.

Anthropometry: Weight, length and head circumference will be measured at study entry and at the two-week and term visits, with birthweight and neonatal centiles calculated using Fenton-WHO growth charts for preterm infants (Fenton & Kim, 2013), and growth velocity calculated between birth and term equivalent using an exponential model (Patel et al., 2009).

Salivary caffeine concentrations: Two weeks after randomisation, a saliva sample will be collected from infants for assessment of caffeine concentration. Samples will be taken using a mouth swab prior to administration of the morning dose of trial medication. In the 24 hours preceding this, mothers will be asked to collect three saliva samples by spitting into collection tubes at three pre-determined timepoints during the day, with the mean of these three samples used to determine average daytime maternal salivary caffeine concentration. Collection of these samples will allow us to compare maternal and infant salivary caffeine concentrations to establish if maternal caffeine intake

contributes significantly to infant caffeine levels via breastfeeding or not, and to help assess compliance with the study intervention.

Questionnaires: Mothers will complete questionnaires to provide demographic and contact details at enrolment, and to assess smoking, infant feeding and sleeping, maternal caffeine intake and maternal mental health as detailed in Table 1.

Neonatal morbidity: Information on neonatal morbidity, including supplemental oxygen, respiratory support and apnoea requiring stimulation, will be recorded from the neonatal record. Exposure to antenatal corticosteroids will be recorded.

4.6.5 Discontinuation of intervention / withdrawal

The allocated treatment may be stopped at any time by the parents or the clinician caring for the infant if they feel that this is in the best interests of the infant, without formally withdrawing, in which case data collection will continue and results will be analysed on an intention-to-treat basis.

Should a parent wish to withdraw from the study, they will have the option of:

- Discontinuation of study drug, with continuation of collection of minimum outcome data
- Withdrawal from the study and discontinuation of further data collection, with data collected prior to withdrawal used
- Complete withdrawal from the study, with removal of previously collected data

4.6.6 Patient and Public Involvement

The Latte Dosage Study methodology was discussed, developed and refined as part of the 2017 On-Track Network clinical trial development workshop which included consumer and Maori cultural advisor input. Perinatal consumer representatives provided advice and input into the development of the clinical trial protocol.

4.6.7 Sample size

Based on our previous study (Williams et al., 2018) we estimate a background mean (SD) frequency of 6.9 (3.4) episodes of intermittent hypoxaemia per hour at two weeks' post randomisation. To detect a 50% reduction of 3.5 episodes per hour with 90% power, allowing for a 10% drop out rate and clustering of multiples with an ICC of 0.05, we will require 24 infants in each of the five arms (total 120 infants), with two-sided alpha of 0.05. Recruitment to the study started in February 2019 and is scheduled to conclude in December 2020.

4.6.8 Data analysis

The primary analysis will compare primary and secondary outcomes between groups using generalised linear mixed models with adjustment for gestational age at birth and site (fixed effects), non-independence of multiples (random effect) and pairwise comparisons between the different caffeine groups and between the caffeine groups and the placebo group using Dunnett's multiple comparison test. The selection of the optimal dose will be based on a combination of the dose with the greatest reduction of intermittent hypoxaemia with a minimum number of side effects and a pragmatic consideration of the ease of administration. Linear trends, such as growth, will be tested using orthogonal contrasts. In keeping with CONSORT recommendations (Moher et al., 2010), baseline imbalance between babies in the randomised groups will not be formally tested. Edinburgh Postnatal Depression Scale scores will be adjusted for baseline values. Categorical data will be presented as number and percent, and continuous data as mean and standard deviation or median and inter-quartile range, as

appropriate. Denominators will be given for all outcomes. Treatment effects will be presented as odds ratio, count ratio, mean difference or ratio of geometric means (positively skewed data), as appropriate, with 95% confidence intervals. All tests will be two-tailed, with $P<0.05$ considered significant. The data will be analysed on an intention-to-treat basis.

The following secondary analyses will be performed:

- *Compliance*: A per-protocol analysis will be performed for the primary outcome that includes only those infants who were compliant with the study drug.
- *Open-label caffeine treatment:* A sensitivity analysis will be performed for the primary outcome that includes only those infants who did not receive additional open-label caffeine treatment.
- *Maternal caffeine:* An exploratory analysis will be performed on the effect of maternal caffeine intake on the primary outcome by performing additional adjustments for maternal caffeine intake from the questionnaire and maternal salivary caffeine concentration. For infants that are fully formula fed, infant caffeine exposure to maternal caffeine intake will be assumed to be zero.

An independent data monitoring committee will review trial data after enrolment of 60 infants to the trial. The data monitoring committee provide advice to the trial steering group on any modifications that may be required. There are no formal stopping guidelines.

4.7 Ethics and dissemination:

Ethical approval has been obtained from the Health and Disability Ethics Committees of New Zealand (reference 18/NTA/129) and by the local institutional research review

committees for each centre. The trial is registered with the Australian New Zealand Clinical Trials Registry (Registration number: ACTRN12618001745235) from 24 October 2018.

The results of the trial will be published in an international peer-reviewed journal and disseminated via presentations at local and international conferences to researchers and clinicians. A lay summary of the research findings will be made available to those parents who indicated a wish to receive these on their consent forms.

4.8 Discussion

Late preterm infants experience higher rates of intermittent hypoxaemia than their term-born peers, and have poorer long-term neurodevelopmental outcomes (Darlow et al., 2009; Heinonen et al., 2015; Huddy et al., 2001; Moster et al., 2008; Odd et al., 2013; Quigley et al., 2012; Talge et al., 2010; Woythaler et al., 2011). Caffeine is well established as a treatment for apnoea of prematurity in very and extremely preterm infants, and improves long term neurodevelopmental outcomes in these infants (Mürner-Lavanchy et al., 2018; Schmidt et al., 2007, 2012). Caffeine use in late preterm infants may also reduce episodes of intermittent hypoxaemia and improve long term outcomes for these infants. As late preterm infants make up the majority of preterm infants, interventions that improve long term outcomes in this population are likely to have the greatest public health impact in terms of interventions for preterm infants (March of Dimes et al., 2012).

The Latte Dosage Trial seeks to establish the most effective and best tolerated dose of caffeine citrate for the prevention of intermittent hypoxaemia in late preterm infants. It is the first trial to investigate the use of caffeine, an inexpensive medication already widely used in neonatal care, for this indication. Data from the Latte Dosage Trial will be used to inform the development of a large-scale, multicentre trial investigating the

efficacy of caffeine treatment in late preterm infants in preventing neurodevelopmental impairment by indicating the most appropriate dose to use and providing information on feasibility.

5. Results: The Latte Dosage Trial

5.1 Abstract

Objective

To establish the most effective and best tolerated dose of caffeine citrate for the prevention of intermittent hypoxaemia (IH) in late preterm infants.

Design

Phase IIB, double-blind, five-arm, parallel, randomised controlled trial.

Setting

Neonatal units and postnatal wards of two tertiary maternity hospitals in New Zealand.

Participants

Late preterm infants born at 34^{+0} – 36^{+6} weeks' gestation, recruited within 72 hours of birth.

Intervention

Infants were randomly assigned to receive a loading dose (10, 20, 30 or 40 $mg.kg^{-1}$) followed by 5, 10, 15 or 20 $mg.kg^{-1}.day^{-1}$ equivolume enteral caffeine citrate or placebo daily until term corrected age.

Primary Outcome

Intermittent hypoxaemia (events/hour with oxygen saturation concentration ≥10% below baseline for ≤2 minutes), 2 weeks post-randomisation.

Results

132 infants with mean (SD) birthweight 2561 (481) g and gestational age 35.7 (0.8) weeks were randomised (24-28 per group). Caffeine reduced the rate of IH at 2 weeks post-randomisation (geometric mean (GM): 4.6, 4.6, 2.0, 3.8 and 1.7 events/hour for placebo, 5, 10, 15 and 20 $mg.kg^{-1}.day^{-1}$, respectively), with differences statistically significant for 10 $mg.kg^{-1}.day^{-1}$ (GM ratio [95% CI] 0.39 [0.20, 0.76]; p=0.006) and 20 $mg.kg^{-1}.day^{-1}$ (GM ratio [95% CI] 0.33 [0.17, 0.68]; p=0.003) compared to placebo. The 20 $mg.kg^{-1}.day^{-1}$ dose increased mean (SD) SpO2 (97.2 (1.0) v placebo 96.0 (0.8); p<0.001), and reduced median (IQR) percentage of time SpO2 <90% (0.5 (0.2-0.8) v 1.1 (0.6-2.4); p<0.001) at 2 weeks, without significant adverse effects on growth velocity or sleeping.

Conclusion

Caffeine reduces IH in late preterm infants at 2 weeks of age, with 20 $mg.kg^{-1}.day^{-1}$ being the most effective dose.

5.2 Relevance of this paper

What is already known on this topic

- Hypoxaemia is associated with negative effects on cognition and neurodevelopmental outcomes in preterm infants and episodes of intermittent hypoxaemia are more common in late preterm infants than their term-born peers.
- Caffeine reduces episodes of apnoea of prematurity and intermittent hypoxaemia and improves neurodevelopmental outcomes in very preterm infants.

What this study adds

- Doses of 10 or 20 mg.kg^{-1}.day^{-1} of caffeine citrate are effective at reducing intermittent hypoxaemia in late preterm infants, without adverse effects on gastrointestinal reflux or sleep, but with an increase in tachycardia.

How this study might affect research, practice or policy

- If caffeine is proven to improve neurodevelopmental outcomes in late preterm infants, widespread use could provide long term benefits for brain development in this important patient group.
- Establishing an effective dose that is associated with minimal side effects is a necessary step towards this goal and allows the development of a larger and longer-term trial of effectiveness.

5.3 Introduction

Late preterm infants (34^{+0}–36^{+6} weeks gestation) comprise the majority of preterm births (Ministry of Health, 2021; United States Department of Health and Human Services et al., 2021), and are physiologically and metabolically immature (Engle et al., 2007), with a higher risk of morbidity and mortality in the neonatal period than term infants (McIntire & Leveno, 2008). Late preterm infants are more likely to be diagnosed with cerebral palsy (Moster et al., 2008; Odd et al., 2013), developmental delay (Cheong et al., 2017; Darlow et al., 2009; Woythaler et al., 2011) and cognitive impairment (Berry et al., 2018; Cheong et al., 2017; Heinonen et al., 2015; Quigley et al., 2012; Talge et al., 2010) compared to term infants. Late preterm infants also experience frequent episodes of intermittent hypoxaemia (IH)(Williams et al., 2018); transient repetitive decreases in oxygen saturation not associated with apnoea but potentially causing similar organ hypoxia. The frequency of these episodes peaks at 2 weeks' postnatal age, before reducing to near-birth levels at term corrected age (Williams et al., 2018). During the neonatal period, even small changes in pulse oximetry oxygen saturations (SpO_2)

significantly affect survival and neurodevelopment of very preterm infants (Askie et al., 2018; Carlo et al., 2010; Stenson et al., 2011) and transient intermittent hypoxaemic events are associated with poor neurodevelopmental outcomes in extremely preterm infants (Poets et al., 2015).

Caffeine is effective in the prevention and treatment of apnoea of prematurity and IH, and reduces the incidence of chronic lung disease, cerebral palsy, and cognitive delay in very preterm infants (McNamara et al., 2004; Schmidt et al., 2006, 2007). Due to hepatic immaturity, caffeine elimination is slow in extremely preterm infants (Le Guennec et al., 1985). With increasing gestational age the elimination of caffeine increases (Falcão et al., 1997; Le Guennec et al., 1985) , requiring larger doses to maintain a therapeutic effect (Rhein et al., 2014). In very preterm infants caffeine is usually well tolerated, but can reduce neonatal weight gain and occasionally infants on caffeine develop tachycardia and feed intolerance (Steer et al., 2004)(Schmidt et al., 2006). The most effective dose of caffeine to treat IH in late preterm infants remains unknown.

Aim: To determine the most effective and best tolerated dose of caffeine citrate to reduce IH in late preterm infants.

5.4 Methods

The study protocol of the Latte Dosage Trial has been reported previously (Oliphant et al., 2020). Briefly, late preterm infants delivered at two maternity hospitals in Auckland, New Zealand were eligible if born between 34^{+0}–36^{+6} weeks gestation, without relevant

exclusions (major congenital abnormality, minor congenital abnormality likely to affect respiration, growth or development, previous caffeine treatment or contraindications to caffeine). Following parental consent, participating infants were randomised by a member of the trial team to one of five parallel groups (5, 10, 15 or 20 $mg.kg^{-1}.day^{-1}$ of caffeine citrate or placebo) within 72 hours of birth using an internet randomisation service with varying block sizes and 1:1:1:1:1 allocation stratified by study site and gestational age at birth (34, 35 or 36 weeks). Twins were allocated to the same group. Participating infants received an enteral loading dose of study drug (10, 20, 30 or 40 $mg.kg^{-1}$ of caffeine citrate or placebo (water)) followed by a daily dose each morning (5, 10, 15 or 20 $mg.kg^{-1}$ of caffeine citrate or placebo) until term equivalent age (TEA; 40 weeks' post-menstrual age), with the dose recalculated weekly for weight gain. Trial medication was prepared at various strengths, so each infant received the same volume (2 $mL.kg^{-1}$ loading dose; 1 $mL.kg^{-1}.day^{-1}$ thereafter) of identical-appearing trial medication. Parents, clinical staff and those assessing outcomes were all blinded to treatment group, and all other care decisions, including discharge, were made by the clinical team. Post-discharge, babies were cared for at home by parents, who continued to give the trial medication until the final visit at TEA.

Participating infants, whether in hospital or at home, underwent overnight oximetry using a motion-resistant oximeter (Masimo Rad-8, Masimo Corporation, Irvine, CA, USA) prior to administration of the loading dose, at 2 weeks post-randomisation and TEA. Oximetry recordings had a two-second averaging time and were edited by a single investigator using Profox software (Profox Associates Inc, Coral Springs, FL, USA) to automatically remove low confidence and aberrant data, followed by a final manual review (Wellington et al., 2018). A minimum of 6 hours of edited data was required. At the same timepoints, data were collected on maternal caffeine intake (Bühler et al., 2014) and infant feeding (Kleinman et al., 2006), sleeping (Bosquet Enlow et al., 2016; Gartstein & Rothbart, 2003), and anthropometry. Saliva samples were collected from mothers (three samples across an 8-hour daytime period) and infants (prior to the

study drug) at the two-week timepoint and analysed to determine caffeine concentrations (Oliphant et al., 2022).

The primary outcome was the rate of IH (events/hour, Spo_2fall ≥10% below baseline for >2 s and <2 minutes) on overnight oximetry, 2 weeks post-randomisation. Pre-specified secondary outcomes are available in the protocol (Oliphant et al., 2020), and included neonatal growth, tachycardia, and salivary caffeine concentrations.

Based on our previous study (Williams et al., 2018), we estimated a mean (SD) rate of 6.9 (3.4) IH episodes per hour at 2 weeks' post-randomisation. To detect a 50% reduction (3.5 episodes per hour) in any group vs placebo with 90% power, allowing for a 10% drop out and clustering of multiples (intraclass correlation coefficient 0.05) would require 24 infants in each group (total 120 infants), with two-sided α=0.05. The trial was not powered to conduct comparisons between caffeine doses.

Statistical analysis was performed using Stata (Stata, version 16). Caffeine groups were compared with the placebo group for outcomes using generalised linear mixed models (Kenward & Roger, 1997) with adjustment for gestational age at birth, site, and non-independence of multiples. Analysis was intention-to-treat, with separate models for each timepoint. Distributions of outcome variables and model residuals were visually assessed for deviations from normality; where data were highly skewed, a log transformation was used to improve model fit. Treatment effects are expressed as mean difference, geometric mean ratio (RGM) or odds ratio, with 95% confidence intervals (CI).

Pre-specified secondary analyses for the primary outcome included a comparison of infants allocated to placebo with those allocated to any dose of caffeine citrate (i.e., all

caffeine groups combined), a per-protocol analysis of infants who received the correct intervention and were compliant with the protocol (Oliphant et al., 2020) (80% of study drug administered at 2 weeks), a sensitivity analysis excluding multiples, and exploratory analyses adjusting separately for baseline oximetry, and maternal caffeine intake and salivary caffeine concentrations at 2 weeks. Wilcoxon rank-sum tests were used to compare maternal caffeine intake and salivary concentrations, due to highly skewed distributions. A two-tailed $P<0.05$ was considered statistically significant. Kenward-Roger correction was applied to mixed models to maintain nominal error rate. Additional adjustment for testing of multiple secondary outcomes was not performed and these results are interpreted cautiously, cognisant of the risk of type-I error.

The trial was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12618001745235) and approved by the Health and Disability Ethics Committees of New Zealand (18/NTA/129).

5.5 Results

Between February 2019 and December 2020, 131 infants were randomly allocated to placebo or one of four caffeine citrate groups, with primary outcome data available for 107 infants (Figure 5.1). Baseline characteristics were similar across groups (Table 5.1). The mean (SD) duration of overnight oximetry recordings after editing was 10.6 (1.9) hours.

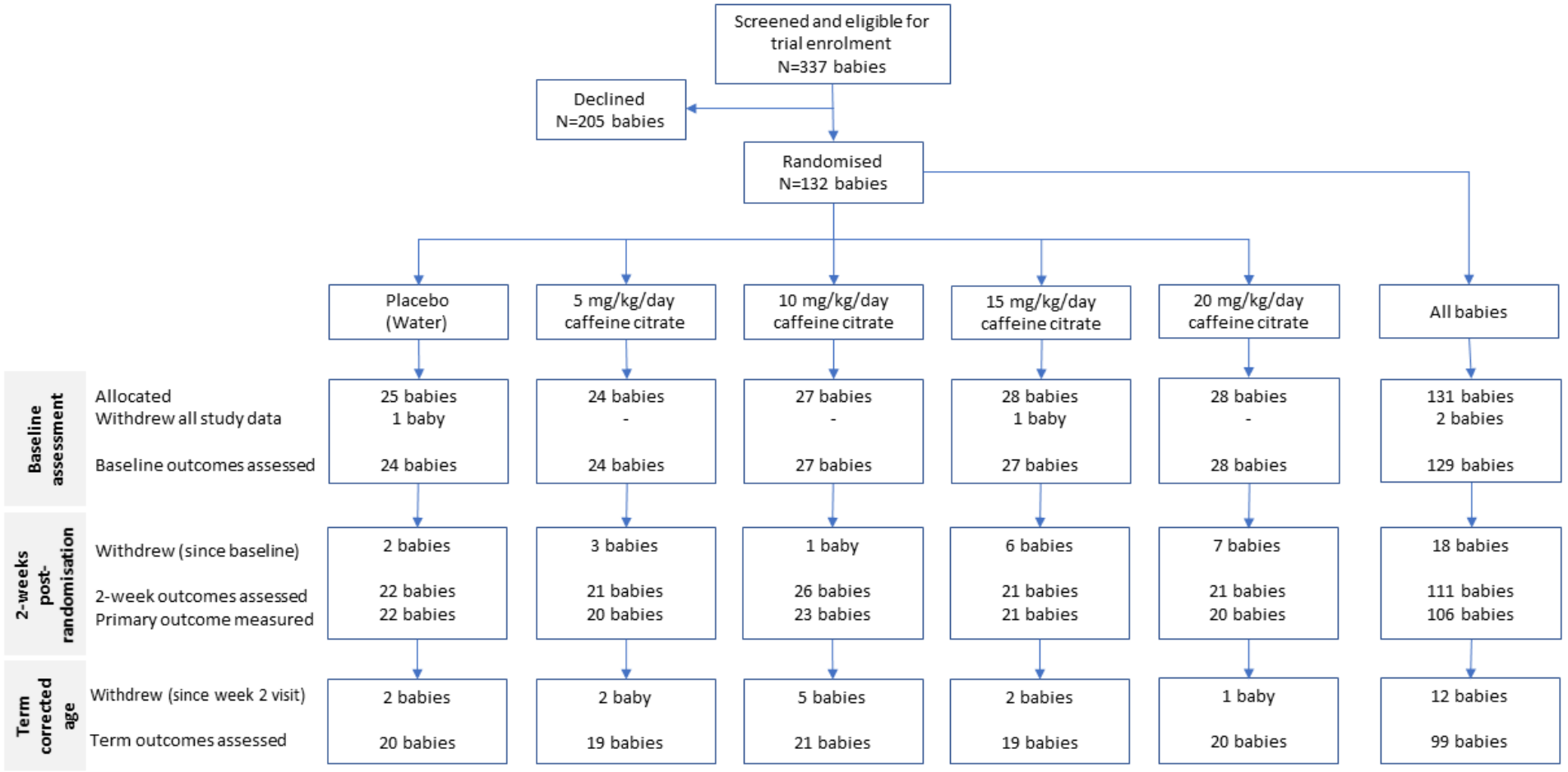

Screened and eligible for trial enrolment N=337 babies
Declined N=205 babies
Randomised N=132 babies
Placebo (Water)
5 mg/kg/day caffeine citrate
10 mg/kg/day caffeine citrate
15 mg/kg/day caffeine citrate
20 mg/kg/day caffeine citrate
All babies
Baseline assessment
Allocated
Withdrew all study data
Baseline outcomes assessed
25 babies
1 baby
24 babies
24 babies
-
24 babies
27 babies
-
27 babies
28 babies
1 baby
27 babies
28 babies
-
28 babies
131 babies
2 babies
129 babies
2-weeks post-randomisation
Withdrew (since baseline)
2-week outcomes assessed
Primary outcome measured
2 babies
22 babies
22 babies
3 babies
21 babies
20 babies
1 baby
26 babies
23 babies
6 babies
21 babies
21 babies
7 babies
21 babies
20 babies
18 babies
111 babies
106 babies
Term corrected age
Withdrew (since week 2 visit)
Term outcomes assessed
2 babies
20 babies
2 baby
19 babies
5 babies
21 babies
2 babies
19 babies
1 baby
20 babies
12 babies
99 babies

Figure 5.1 Flow diagram of participants

Table 5.1 Baseline characteristics of 121 mothers and 129 infants participating in the Latte Dosage Trial

	Placebo	Caffeine citrate 5 $mg.kg^{-1}.day^{-1}$	Caffeine citrate 10 $mg.kg^{-1}.day^{-1}$	Caffeine citrate 15 $mg.kg^{-1}.day^{-1}$	Caffeine citrate 20 $mg.kg^{-1}.day^{-1}$	Any dose of caffeine
Number of mothers (% of total)	24 (19.8)	23 (19.0)	24 (19.8)	25 (20.7)	25 (20.7)	97 (80.2)
Age (years)	31.1 (6.0)	31.6 (5.3)	30.6 (5.5)	32.1 (5.8)	31.3 (6.3)	31.4 (5.7)
Primiparous	9 (37.5)	11 (47.8)	16 (66.7)	15 (60.0)	13 (52.0)	55 (56.7)
Body mass index (BMI)(kg/m^2)	26.1 (23.5, 30.7)	27.9 (24.2, 31.5)	26.3 (23.3, 30.6)	24.9 (21.9, 28.4)	28.6 (23.4, 32.5)	26.5 (23.2, 30.1)
Multiple pregnancy	0 (0.0)	1 (4.3)	3 (12.5)	2 (8.0)	2 (8.0)	8 (8.2)
Antenatal events						
Maternal diabetes	5 (20.8)	3 (13.0)	9 (37.5)	2 (8.0)	7 (28.0)	21 (21.6)
Preterm pre-labour rupture of membranes	12 (50.0)	13 (56.5)	8 (33.3)	15 (60.0)	8 (32.0)	44 (45.4)
Preterm labour	20 (83.3)	18 (78.3)	13 (54.2)	19 (76.0)	17 (68.0)	67 (69.1)
Hypertension in pregnancy	3 (12.5)	1 (4.3)	4 (16.7)	2 (8.0)	5 (20.0)	12 (12.4)
Antepartum haemorrhage	1 (4.2)	6 (26.1)	1 (4.2)	5 (20.0)	2 (8.0)	14 (14.4)
Suspected fetal growth restriction	3 (12.5)	3 (13.0)	6 (25.0)	4 (16.0)	6 (24.0)	19 (19.6)
Antenatal glucocorticosteroids	5 (20.8)	4 (17.4) [a]	8 (33.3)	8 (32.0) [a]	4 (16.0)	24 (24.7)

	Placebo	Caffeine citrate 5 $mg.kg^{-1}.day^{-1}$	Caffeine citrate 10 $mg.kg^{-1}.day^{-1}$	Caffeine citrate 15 $mg.kg^{-1}.day^{-1}$	Caffeine citrate 20 $mg.kg^{-1}.day^{-1}$	Any dose of caffeine
Number of infants (% of total)	24 (18.6)	24 (18.6)	27 (20.9)	27 (20.9)	27 (20.9)	105 (81.5)
Gestational Age						
34 weeks	6 (25.0)	5 (20.8)	6 (22.2)	5 (18.5)	6 (22.2)	22 (21.0)
35 weeks	7 (29.2)	8 (33.3)	9 (33.3)	10 (37.0)	9 (33.3)	36 (34.3)
36 weeks	11 (45.8)	11 (45.8)	12 (44.4)	12 (44.4)	12 (44.4)	47 (44.8)
Sex (male)	14 (58.3)	17 (70.8)	12 (44.4)	18 (66.7)	16 (59.3)	63 (60.0)
Singleton [b]	24 (100.0)	22 (91.7)	21 (77.8)	23 (85.2)	23 (85.2)	89 (84.8)
Ethnicity (Prioritised)						
Māori	2 (8.3)	6 (25.0)	1 (3.7)	2 (7.4)	7 (25.9)	16 (15.2)
Pacific Islander	7 (29.2)	5 (20.8)	2 (7.4)	5 (18.5)	5 (18.5)	17 (16.2)
Asian	7 (29.2)	5 (20.8)	13 (48.1)	11 (40.7)	7 (25.9)	36 (34.3)
Other	1 (4.2)	1 (4.2)	1 (3.7)	1 (3.7)	1 (3.7)	4 (3.8)
NZ European	7 (29.2)	7 (29.2)	10 (37.0)	8 (29.6)	7 (25.9)	32 (30.5)
Birth Weight (g)	2566.5 (272.2)	2674.6 (480.6)	2523.9 (603.7)	2641.9 (432.5)	2393.3 (515.1)	2555.1 (517.3)
Z-score [c]	-0.0 (0.7)	0.2 (1.1)	-0.1 (1.3)	0.1 (1.0)	-0.5 (1.1)	-0.1 (1.1)
Length (cm)	47.8 (2.0)	48.9 (2.5)	47.2 (3.4)	47.8 (2.1)	46.8 (3.2)	47.6 (2.9)
Z-score [c]	0.5 (0.6)	1.0 (1.0)	0.3 (1.3)	0.5 (0.9)	0.1 (1.1)	0.5 (1.1)
Head Circumference (cm)	32.4 (1.2)	33.8 (1.5)	32.5 (1.8)	33.1 (1.5)	32.3 (1.7)	32.9 (1.7)
Z-score [c]	0.2 (0.8)	1.0 (1.0)	0.2 (1.2)	0.6 (1.1)	-0.0 (1.0)	0.4 (1.1)
Caesarean delivery	8 (33.3)	10 (41.7)	15 (55.6)	12 (44.4)	10 (37.0)	47 (44.8)
Apgar score (5 minutes)	9.0 (9.0, 10.0)	9.0 (8.0, 10.0)	10.0 (9.0, 10.0)	9.0 (9.0, 10.0)	9.0 (9.0, 10.0)	9.0 (9.0, 10.0)
Admitted to neonatal intensive care (NICU)	12 (50.0)	12 (50.0)	13 (48.1)	13 (48.1)	17 (63.0)	55 (52.4)
Positive pressure respiratory support prior to enrolment	8 (33.3)	7 (29.2)	6 (22.2)	9 (33.3)	6 (22.2)	28 (26.7)
Oxygen prior to enrolment	2 (8.3)	3 (12.5)	4 (14.8)	4 (14.8)	3 (11.1)	14 (13.3)

Data are mean (SD), median (IQR) or n (%). [a] *n=1* with missing data in this group.[b] In some cases, only one infant was eligible for the trial or a twin pregnancy resulted in a single live birth. [c] Z-scores were calculated from the revised Fenton growth charts for preterm infants (Fenton & Kim, 2013)

The rate of IH at 2 weeks post-randomisation was significantly reduced among infants allocated to caffeine citrate 10 or 20 mg.kg^{-1}.day^{-1} compared to placebo (RGM [95%CI] 0.39 [0.20,0.76] and 0.33 [0.17,0.68], respectively), but not for the 5 or 15 mg.kg^{-1}.day^{-1} groups (Table 5.2). The rate of IH was significantly reduced for infants allocated to any dose of caffeine overall compared to placebo (Table 5.2). All secondary, sensitivity and exploratory analysis for the primary outcome gave similar results.

At 2 weeks post-randomisation, infants allocated to caffeine citrate 10 or 20 mg.kg^{-1}.day^{-1}, compared with placebo, had significantly higher mean SpO_2 and less time with SpO_2<90%, while the 15 mg.kg^{-1}.day^{-1} group had higher mean heart rate. Compared with placebo, all caffeine groups spent significantly more time with tachycardia (heart rate >180 beats per minute) at 2 weeks, which persisted at TEA in the 5, 10 and 20 mg.kg^{-1}.day^{-1} groups (Table 5.2). At TEA, there were no significant differences between placebo and caffeine groups in the rate of IH, mean Spo_2 or time with SpO_2<90% (Table 5.2).

Table 5.2 Primary outcome and cardiorespiratory secondary outcomes

	Placebo **N = 24** [a]	**Caffeine citrate** **5 mg.kg^{-1}.day^{-1}** **N = 24** [a]		**Caffeine citrate** **10 mg.kg^{-1}.day^{-1}** **N = 27** [a]		**Caffeine citrate** **15 mg.kg^{-1}.day^{-1}** **N = 27** [a]		**Caffeine citrate** **20 mg.kg^{-1}.day^{-1}** **N = 27** [a]		**Any dose of caffeine** **N = 105** [a]	
	Summary Data	Summary Data	RGM or mean difference (95% CI); p-value [b]	Summary Data	RGM or mean difference (95% CI); p-value [b]	Summary Data	RGM or mean difference (95% CI); p-value [b]	Summary Data	RGM or mean difference (95% CI); p-value [b]	Summary Data	RGM or mean difference (95% CI); p-value [b]
Primary Outcome											
Rate of intermittent hypoxaemia at 2 weeks; median (IQR) [geometric mean]	4.0 (1.8, 9.8) [4.6]	5.9 (2.8, 7.6) [4.6]	*0.97 (0.49,1.95) p=0.94*	2.5 (0.6, 5.7) [2.0]	*0.39 (0.20,0.76)* ***p=0.006***	3.3 (2.1, 8.8) [3.8]	*0.79 (0.40,1.56) p=0.49*	1.8 (0.9, 4.2) [1.7]	*0.33 (0.17,0.68)* ***p=0.003***	3.0 (1.3, 6.1) [2.7]	*0.56 (0.32,0.98)* ***p=0.043***
Secondary Outcomes											
Rate of intermittent hypoxaemia; median (IQR)[geometric mean]											
Baseline	0.9 (0.6, 1.4)	2.0 (0.9, 3.3)		1.1 (0.7, 2.0)		1.9 (1.1, 2.6)		1.5 (0.8, 4.4)		1.5 (0.9, 2.8)	
Term	3.0 (1.9, 6.2) [3.3]	4.0 (1.9, 6.9) [3.4]	*1.02 (0.50,2.07) p=0.96*	2.5 (1.0, 6.1) [2.4]	*0.65 (0.33,1.32) p=0.23*	3.3 (1.5, 8.2) [3.1]	*0.82 (0.40,1.69) p=0.59*	2.2 (1.0, 4.7) [1.9]	*0.54 (0.26,1.11) p=0.09*	2.9 (1.3, 6.7) [2.7]	*0.75 (0.43,1.30) p=0.30*

Mean SpO2; mean (SD)											
Baseline	96.4 (1.3)	96.4 (1.5)		95.6 (1.8)		96.6 (1.4)		95.5 (2.0)		96.0 (1.7)	
Two weeks	96.0 (0.8)	96.4 (1.4)	*0.39 (-0.28,1.07) p=0.25*	96.7 (1.0)	*0.68 (0.04,1.33)* ***p=0.039***	96.7 (1.3)	*0.66 (-0.01,1.33) p=0.06*	97.2 (1.0)	*1.31 (0.62,2.00)* ***p<0.001***	96.8 (1.2)	*0.74 (0.21,1.28)* ***p=0.007***
Term	97.3 (1.0)	97.5 (0.9)	*0.13 (-0.61,0.87) p=0.72*	97.2 (0.8)	*0.09 (-0.64,0.82) p=0.81*	97.2 (1.2)	*0.05 (-0.70,0.81) p=0.89*	97.4 (1.7)	*0.30 (-0.46,1.06) p=0.44*	97.3 (1.2)	*0.14 (-0.43,0.71) p=0.62*
Percentage of time Spo2 < 90%; median (IQR)											
Baseline	1.1 (0.3, 1.6)	1.2 (0.5, 2.4)		1.2 (0.3, 1.9)		1.0 (0.6, 1.4)		1.5 (0.5, 3.1)		1.2 (0.5, 2.3)	
Two weeks	1.1 (0.6, 2.4) [1.3]	1.0 (0.7, 2.0) [1.1]	*0.83 (0.43,1.60) p=0.58*	0.9 (0.3, 1.6) [0.6]	*0.40 (0.21,0.75)* ***p=0.005***	0.7 (0.4, 1.6) [0.8]	*0.63 (0.33,1.22) p=0.17*	0.5 (0.2, 0.8) [0.4]	*0.29 (0.14,0.56)* ***p<0.001***	0.7 (0.3, 1.6) [0.7]	*0.50 (0.29,0.85)* ***p=0.011***
Term	0.6 (0.3, 1.1) [0.6]	0.5 (0.3, 1.3) [0.6]	*0.89 (0.43,1.84) p=0.76*	0.4 (0.3, 1.1) [0.5]	*0.75 (0.37,1.53) p=0.43*	0.6 (0.4, 1.3) [0.6]	*0.84 (0.40,1.77) p=0.65*	0.3 (0.2, 1.7) [0.4]	*0.59 (0.28,1.22) p=0.15*	0.5 (0.2, 1.3) [0.5]	*0.76 (0.43,1.34) p=0.34*
Mean heart rate; mean (SD)											
Baseline	130.1 (9.0)	130.0 (6.8)		134.0 (10.2)		132.1 (10.4)		130.8 (9.3)		131.7 (9.3)	
Two weeks	147.7 (6.8)	150.3 (8.2)	*3.07 (-5.32,4.63) p=0.89*	150.8 (7.4)	*3.43 (-2.22,9.08) p=0.23*	156.0 (12.7)	*8.44 (2.59,14.30)* ***p=0.005***	152.4 (12.4)	*3.75 (-2.28,9.79) p=0.22*	152.3 (10.4)	*4.63 (0.04,9.21)* ***p=0.048***
Term	150.8 (8.6)	150.3 (6.9)	*-0.35 (-5.25,4.55)*	152.3 (5.7)	*1.26 (-3.66,6.19)*	155.5 (8.9)	*4.37 (-0.69,9.42)*	151.1 (8.5)	*0.07 (-5.05,5.19)*	152.3 (7.6)	*1.28 (-2.62,5.17)*

			p=0.89		*p=0.61*		*p=0.09*		*p=0.98*		*p=0.52*

Percentage of time HR>180; median (IQR)											
Baseline	0.0 (0.0, 0.0)	0.0 (0.0, 0.4)		0.1 (0.0, 0.3)		0.0 (0.0, 0.3)		0.0 (0.0, 0.2)		0.0 (0.0, 0.3)	
Two weeks	0.9 (0.2, 5.2) [0.9]	3.1 (2.0, 8.9) [3.8]	*4.34 (1.87,10.10)* ***p=0.001***	4.3 (2.4, 9.0) [3.8]	*4.03 (1.79,9.08)* ***p=0.001***	7.0 (4.0, 10.1) [5.5]	*5.94 (2.56,13.77)* ***p<0.001***	7.4 (4.4, 13.9) [6.1]	*5.71 (2.40,13.57)* ***p<0.001***	6.0 (2.9, 10.0) [4.7]	*4.87 (2.55,9.29)* ***p<0.001***
Term	2.0 (0.4, 5.6) [1.6]	4.7 (3.0, 8.0) [3.9]	*2.50 (1.07,5.86)* ***p=0.035***	5.5 (2.4, 9.4) [4.6]	*2.86 (1.23,6.63)* ***p=0.015***	5.6 (2.7, 9.9) [3.6]	*2.22 (0.93,5.27)* *p=0.07*	6.7 (4.4, 10.7) [5.2]	*3.19 (1.33,7.65)* ***p=0.010***	5.7 (2.7, 9.0) [4.3]	*2.66 (1.39,5.11)* ***p=0.004***
Compliant [c] with administration schedule at 2 weeks	21 (87.5%)	18 (78.5%)	0.33 (0.06,1.91) p=0.22	23 (85.2%)	0.54 (0.09,3.41) p=0.51	19 (70.4%)	0.20 (0.04,1.14) p=0.07	18 (66.7%)	0.21 (0.04,1.24) p=0.09	78 (73.6%)	0.28 (0.06,1.33) p=0.11
Study drug stopped due to presumed side effects[d]	2 (9.1%)	4 (16.7%)	2.07 (0.32,13.18) p=0.44	5 (19.2%)	2.49 (0.37,16.75) p=0.35	7 (28.0%)	4.21 (0.68,26.13) p=0.12	6 (22.2%)	2.96 (0.49,17.75) p=0.23	22 (21.6%)	*2.88 (0.58,14.31) p=0.20*

[a] Number of infants with oximetry traces of usable quality (% of total remaining in study in that group) at baseline, 2 weeks and term, respectively, are: 20 (83.3%),22 (100%), 20 (100%) in placebo group; 23 (95.8%), 20 (100%), 18 (94.7%) in 5 mg.kg^{-1}.day^{-1} group; 26 (96.3%), 24 (96.0%), 20 (95.2%) in 10 mg.kg^{-1}.day^{-1} group; 27 (100%), 21 (100%), 18 (94.7%) in 15 mg.kg^{-1}.day^{-1} group and 25 (89.3%), 20 (100%), 17 (85.0%) in 20 mg.kg^{-1}.day^{-1} group. [b] Where the mean (SD) is presented the exposure effect is a mean difference; where median (IQR) and geometric mean are presented the exposure effect is the geometric mean ratio (RGM). For all comparisons the reference category is the placebo group. [c] Compliant is defined as <20% of the expected study drug volume (as calculated for that child based on birthweight) remaining in the bottle when measured by the research team at the two-week visit (i.e. >80% of the study drug has been removed from the bottle). Information on compliance at 2 weeks is missing for n=4 (1 in each group except 10 mg.kg^{-1}). [d] Further breakdown of reasons for withdrawals is provided in the supplementary tables

Table 5.3 Secondary Outcomes

	Placebo N = 24[a]	**Caffeine citrate 5 mg.kg^{-1}.day^{-1} N = 24**		**Caffeine citrate 10 mg.kg^{-1}.day^{-1} N = 27**		**Caffeine citrate 15 mg.kg^{-1}.day^{-1} N = 27**		**Caffeine citrate 20 mg.kg^{-1}.day^{-1} N = 27**		**Any dose of caffeine N = 105**	
	Summary data	Summary data	Mean diff (95%CI), p-value *vs placebo*	Summary data	Mean diff (95%CI), p-value *vs placebo*	Summary data	Mean diff (95%CI), p-value *vs placebo*	Summary data	Mean diff (95%CI), p-value *vs placebo*	Summary data	Mean diff (95%CI), p-value *vs placebo*
Weight growth velocity (birth to term equivalent) (g.kg^{-1}day^{-1})[a, b]; mean (SD)	8.8 (3.1)	8.4 (3.4)	*-0.45 (-2.55,1.65) p=0.67*	7.5 (3.4)	*-1.51 (-3.59,0.56) p=0.15*	9.1 (3.4)	*0.02 (-2.10,2.15) p=0.98*	9.1 (3.5)	*-0.32 (-2.51,1.88) p=0.78*	8.5 (3.4)	*-0.62 (-2.27,1.04) p=0.46*
Length growth velocity (birth to term equivalent) (cm.week^{-1}) [a, b]; *mean (SD)*	0.7 (0.6)	0.7 (0.4)	*-0.08 (-0.39,0.23) p=0.61*	0.7 (0.6)	*0.02 (-0.28,0.33) p=0.89*	1.0 (0.4)	*0.20 (-0.11,0.52) p=0.20*	0.6 (0.4)	*-0.08 (-0.41,0.25) p=0.62*	0.8 (0.5)	*0.02 (-0.23,0.26) p=0.89*
Head circumference growth velocity (birth to term equivalent) (cm.week^{-1}) [a, b]; *mean (SD)*	0.6 (0.3)	0.4 (0.2)	*-0.23 (-0.40,-0.07)* ***p=0.006***	0.5 (0.3)	*-0.12 (-0.28,0.04) p=0.15*	0.5 (0.2)	*-0.16 (-0.33,0.00) p=0.05*	0.5 (0.2)	*-0.16 (-0.34,0.01) p=0.07*	0.5 (0.2)	*-0.17 (-0.30,-0.04)* ***p=0.010***
Failure to regain birth weight by 2 weeks postnatal age; N (%)[c]	2 (8.3%)	2 (8.3%)	*1.03 (0.13,8.21) p=0.98*	4 (14.8%)	*1.91 (0.28,13.29) p=0.51*	7 (25.9%)	*4.33 (0.72,26.16) p=0.11*	6 (21.4%)	*3.50 (0.55,22.30) p=0.18*	19 (17.9%)	*2.58 (0.51,13.11) p=0.25*

Sleep score; mean (SD) [d, e]											
Two weeks	4.2 (0.6)	4.3 (0.3)	*0.11 (-0.17,0.38) p=0.45*	4.3 (0.5)	*0.02 (-0.25,0.29) p=0.87*	4.2 (0.3)	*0.01 (-0.27,0.29) p=0.94*	4.1 (0.5)	*-0.09 (-0.38,0.20) p=0.53*	4.2 (0.4)	*0.02 (-0.20,0.23) p=0.87*
Term	4.4 (0.5)	4.4 (0.3)	*0.05 (-0.21,0.31) p=0.70*	4.5 (0.4)	*0.16 (-0.09,0.42) p=0.21*	4.3 (0.4)	*-0.03 (-0.30,0.23) p=0.80*	4.2 (0.4)	*-0.18 (-0.44,0.09) p=0.19*	4.4 (0.4)	*0.01 (-0.20,0.22) p=0.94*
Gastroesophageal symptoms; I-GERQ-R mean (SD)[d]											
Two weeks	29.7 (4.3)	27.6 (4.2)	*-2.35 (-5.56,0.85) p=0.15*	25.3 (6.1)	*-3.80 (-6.96,-0.64)* ***p=0.019***	26.1 (5.1)	*-3.27 (-6.46,-0.08)* ***p=0.045***	27.5 (6.3)	*-2.75 (-6.13,0.63) p=0.11*	26.6 (5.5)	*-3.07 (-5.54,-0.60)* ***p=0.015***
Term	27.5 (5.6)	25.2 (4.7)	*-2.38 (-5.60,0.84) p=0.15*	25.4 (4.9)	*-2.27 (-5.41,0.86) p=0.15*	25.8 (5.7)	*-2.16 (-5.36,1.05) p=0.18*	25.9 (4.1)	*-2.76 (-5.99,0.46) p=0.09*	25.6 (4.8)	*-2.39 (-4.85,0.08) p=0.06*
Duration of tube feeding (days); median (IQR) [Geometric Mean]	7.0 (2.0, 13.0) [2.6]	6.0 (3.5, 14.5) [5.6]	*1.67 (0.48,5.89) p=0.42*	13.0 (4.0, 15.0) [6.2]	*1.34 (0.39,4.68) p=0.64*	6.0 (1.0, 12.0) [2.5]	*0.77 (0.22,2.65) p=0.68*	13.5 (1.5, 17.5) [6.1]	*0.79 (0.23,2.70) p=0.71*	9.0 (2.0, 15.0) [3.4]	*1.07 (0.40,2.87) p=0.89*
Length of stay (number of days); median (IQR) [geometric mean]	9.0 (5.0, 15.0) [8.2]	7.5 (6.5, 20.0) [8.6]	*1.06 (0.76,1.48) p=0.73*	15.0 (8.0, 18.0) [10.8]	*1.31 (0.94,1.83) p=0.11*	11.0 (6.0, 16.0) [10.5]	*1.29 (0.95,1.75) p=0.10*	14.0 (6.0, 20.0) [11.1]	*1.36 (0.98,1.87) p=0.06*	12.0 (6.0, 18.0) [10.3]	*1.26 (0.99,1.59) p=0.06*

[a] Number of infants with at least one anthropometric measurement at 2 weeks and term, respectively, are: 22 and 20 in placebo group; 21 and 19 in 5 mg.kg-1.day-1 group; 26 and 21 in 10 mg.kg^{-1}.day^{-1} group; 21 and 19 in 15 mg.kg^{-1}.day^{-1} group and 22 and 18 in 20 mg.kg^{-1}.day^{-1} group. [b] Growth velocity for weight was calculated using an exponential model (Patel et al., 2009) for weight and linear models for length, and head circumference. [c] Estimated group comparisons for failure to regain birthweight are odds ratios. [d] Number of infants with feeding and sleeping data at 2 weeks

and term, respectively, are: 22 and 20 in placebo group; 21 and 19 in 5 $mg.kg^{-1}.day^{-1}$ group; 25 and 21 in 10 $mg.kg^{-1}.day^{-1}$ group; 21 and 19 in 15 $mg.kg^{-1}.day^{-1}$ group and 21 and 18 in 20 $mg.kg^{-1}.day^{-1}$ group. [e] Sleep score was calculated using subscale nine on the Infant Behaviour Questionnaire-Revised, modified for neonates(Gartstein & Rothbart, 2003)

There was no difference between placebo and caffeine groups in the proportion of infants not regaining birthweight by 2 weeks, or in growth velocity for weight or length at any timepoint (Table 5.3). Head circumference velocity was significantly lower in the 5 $mg.kg^{-1}.day^{-1}$ group compared with placebo (Table 5.3). Infants in the 20 $mg.kg^{-1}.day^{-1}$ group, compared with placebo, had significantly lower length z-scores at 2 weeks and TEA (Table 5.4, Figures 5.2 and 5.3). Infants in the 10 and 15 $mg.kg^{-1}.day^{-1}$ groups, compared with placebo, had significantly lower reflux symptom scores (I-GERQ-R) at 2 weeks (Table 5.3). No infant required caffeine outside of the trial protocol. Eight infants (6%) received ongoing positive pressure support beyond randomisation, with no difference in rates between placebo and caffeine groups, and only one (15 $mg.kg^{-1}.day^{-1}$ group) required respiratory support after enrolment (prior to administration of the study drug). There were no episodes of apnoea requiring stimulation after randomisation.

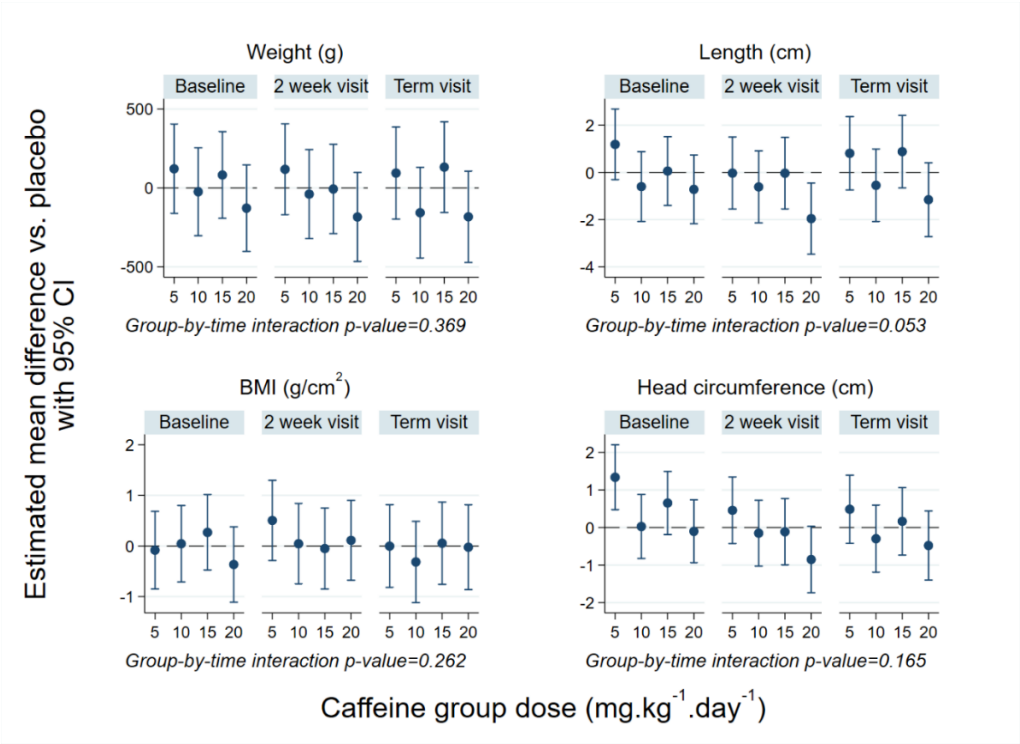


Figure 5.2 Estimated marginal mean differences between caffeine groups and placebo in untransformed anthropometry measures at each study visit, from linear mixed models with adjustment for gestational age at birth and study site

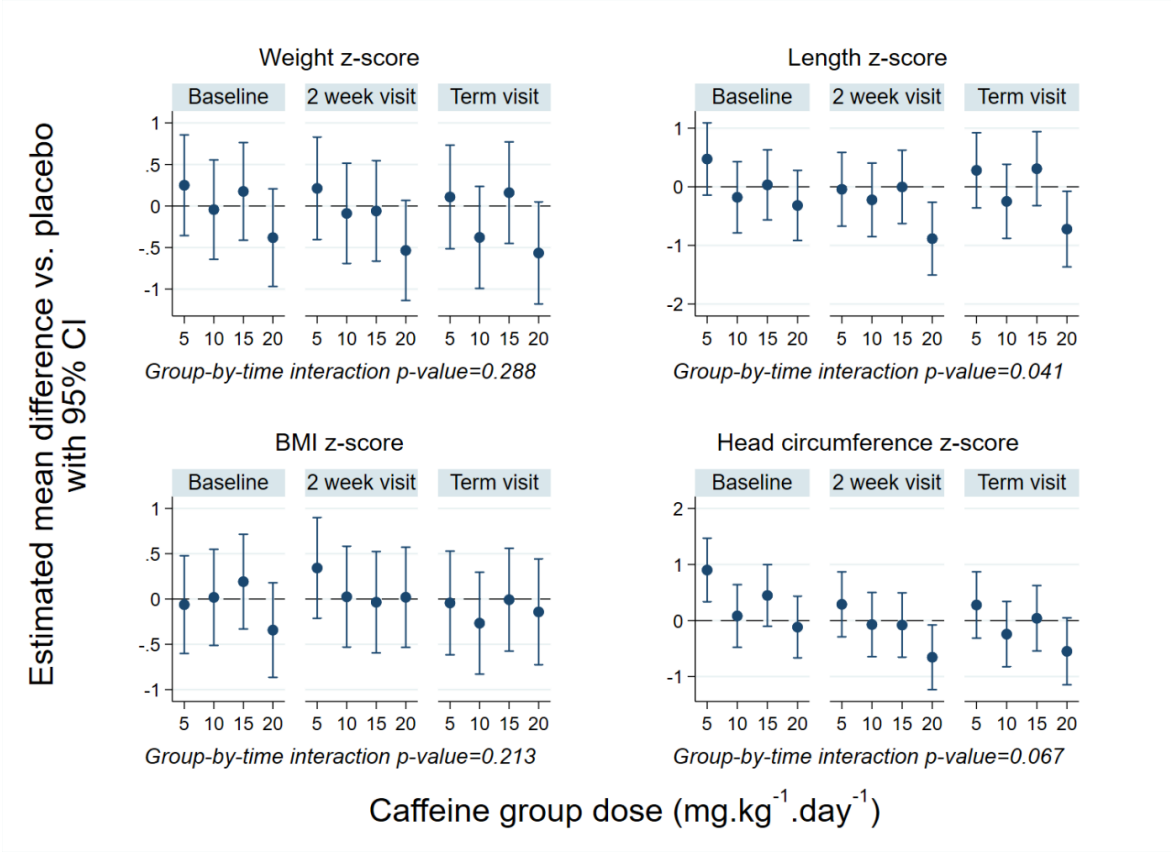


Figure 5.3 Estimated marginal mean differences between caffeine groups and placebo in z-scores for anthropometry measures at each study visit, from linear mixed models with adjustment for study site

One infant (15 mg.kg^{-1}.day^{-1} group), was readmitted to hospital prior to 44 weeks' post-menstrual age due to an upper respiratory tract infection. There were no seizures or episodes of sepsis, nor neonatal or infant deaths. One infant (15 mg.kg^{-1}.day^{-1} group) had study drug stopped due to tachycardia at 2 weeks.

Infant salivary caffeine concentrations were higher in infants receiving caffeine, with highest concentrations in the 20 mg.kg^{-1}.day^{-1} group (Table 5.5).

Table 5.4 Anthropometry outcome measures over the study period

	Placebo **N = 24[a]** Mean (SD)	**Caffeine citrate 5 mg.kg^{-1}.day^{-1}** **N = 24[a]** Mean (SD)	**Caffeine citrate 10 mg.kg^{-1}.day^{-1}** **N = 27[a]** Mean (SD)	**Caffeine citrate 15 mg.kg^{-1}.day^{-1}** **N = 27[a]** Mean (SD)	**Caffeine citrate 20 mg.kg^{-1}.day^{-1}** **N = 27[a]** Mean (SD)	**Any dose of caffeine** **N = 105[a]** Mean (SD)
Weight						
Two weeks (g)	2726.4 (352.0)	2826.9 (494.0)	2677.1 (600.7)	2708.1 (412.8)	2480.3 (492.7)	2671.2 (516.5)
Z score	-0.8 (0.7)	-0.6 (1.0)	-1.0 (1.2)	-0.9 (1.0)	-1.4 (1.1)	-1.0 (1.1)
Term (g)	3329.3 (519.4)	3384.7 (526.6)	3210.7 (592.5)	3431.3 (666.7)	3023.8 (594.3)	3264.4 (606.2)
Z score	-0.4 (1.0)	-0.4 (1.1)	-0.7 (1.3)	-0.3 (1.4)	-1.1 (1.3)	-0.6 (1.3)
Length						
Two weeks (cm)	49.1 (2.3)	49.0 (2.4)	48.2 (3.2)	49.0 (2.4)	46.8 (3.7)	48.2 (3.1)
Z score	0.1 (0.9)	0.0 (0.9)	-0.3 (1.2)	0.1 (0.8)	-0.9 (1.5)	-0.3 (1.2)
Term (cm)	50.9 (2.6)	51.5 (2.0)	50.3 (2.9)	51.6 (2.2)	49.2 (2.9)	50.7 (2.7)
Z score	0.0 (1.1)	0.2 (0.9)	-0.3 (1.2)	0.3 (0.9)	-0.8 (1.3)	-0.1 (1.2)
Head Circumference						
Two weeks (cm)	33.6 (1.4)	34.0 (1.7)	33.5 (1.7)	33.4 (1.5)	32.5 (1.8)	33.3 (1.7)
Z score	-0.1 (0.8)	0.2 (1.0)	-0.2 (1.0)	-0.2 (1.0)	-0.8 (1.1)	-0.3 (1.1)
Term (cm)	35.1 (1.5)	35.6 (1.5)	34.9 (1.4)	35.2 (1.5)	34.4 (1.3)	35.0 (1.5)
Z score	0.2 (1.0)	0.4 (1.1)	-0.1 (0.9)	0.1 (1.0)	-0.4 (0.9)	0.0 (1.0)

Body mass index (BMI)						
Two weeks (g/cm²)	11.3 (0.9)	11.7 (1.3)	11.3 (1.5)	11.1 (1.1)	11.3 (1.5)	11.4 (1.3)
Z score	-1.0 (0.7)	-0.7 (0.8)	-1.0 (1.0)	-1.1 (0.8)	-1.0 (1.0)	-1.0 (0.9)
Term (g/cm²)	12.8 (1.3)	12.7 (1.4)	12.6 (1.6)	12.8 (1.8)	12.6 (1.5)	12.7 (1.6)
Z score	-0.4 (0.9)	-0.5 (1.1)	-0.6 (1.2)	-0.5 (1.3)	-0.6 (1.1)	-0.5 (1.1)

Data presented are mean (SD). Analysis of changes in anthropometric measures over time were performed using linear mixed models with a group-time interaction term.
[a] Number of infants with at least one anthropometric measurement at 2 weeks and term, respectively, are: 22 and 20 in placebo group; 21 and 19 in 5 mg.kg-1.day-1 group; 26 and 21 in 10 mg.kg^{-1}.day^{-1} group; 21 and 19 in 15 mg.kg^{-1}.day^{-1} group and 22 and 18 in 20 mg.kg^{-1}.day^{-1} group. [b] *Z-scores for weight, length and head circumference were calculated from the revised Fenton growth charts for preterm infants* (Fenton & Kim, 2013)*, and Z-scores for BMI from the Olsen growth curves for preterm infants* (Olsen et al., 2015).

Table 5.5 Caffeine intake and salivary concentrations

	Placebo N = 24	**Caffeine citrate 5 mg.kg^{-1}.day^{-1} N = 24**	**Caffeine citrate 10 mg.kg^{-1}.day^{-1} N = 27**	**Caffeine citrate 15 mg.kg^{-1}.day^{-1} N = 27**	**Caffeine citrate 20 mg.kg^{-1}.day^{-1} N = 28**	**Any dose of caffeine N = 106**
Maternal caffeine intake in preceding 24 hours (mg)[b]						
Baseline	36.3 (13.9, 83.2)	63.3 (30.0, 115.6)	65.2 (10.6, 116.4)	42.3 (13.9, 67.7)	41.6 (10.6, 142.0)	51.5 (16.6, 105.0)
Two weeks	60.5 (10.6, 87.5)	84.1 (36.3, 110.4) p=0.064	39.5 (10.6, 144.8) p=0.675	36.3 (7.8, 78.8) p=0.752	88.2 (21.2, 175.0) p=0.099	63.5 (19.9, 115.6) p=0.252
Term	77.5 (41.8, 118.4)	84.6 (60.5, 139.1) p=0.529	98.1 (15.5, 121.0) p=0.873	60.5 (36.3, 105.0) p=0.396	43.2 (10.6, 63.5) p=0.075	63.5 (21.2, 112.5) p=0.559
Maternal salivary caffeine concentration at 2 weeks (μg.ml^{-1})[c]	1.6 (0.5, 2.6)	1.2 (0.6, 3.1) p=0.919	0.2 (0.0, 1.8) p=0.029	0.2 (0.0, 2.4) p=0.113	1.0 (0.2, 2.9) p=0.523	1.1 (0.1, 2.6) p=0.124
Infant salivary caffeine concentration at 2 weeks (μg.ml^{-1})[d]	0.6 (0.3, 0.9)	17.6 (14.2, 23.8) p<0.001	26.1 (11.3, 36.3) p<0.001	33.7 (20.2, 51.0) p<0.001	71.0 (52.3, 86.5) p<0.001	28.3 (18.2, 52.3) P<0.001

Data presented are median (IQR) [a] P-values are from a Wilcoxon rank-sum test, due to highly skewed distributions. [b] Number of mothers with completed surveys at baseline, 2 weeks and term, respectively, are: 23, 22 and 20 in placebo group; 23, 20 and 18 in 5 mg.kg^{-1}.day^{-1} group; 23, 22 and 19 in 10 mg.kg^{-1}.day^{-1} group; 25, 19 and 18 in 15 mg.kg^{-1}.day^{-1} group and 26, 18 and 18 in 20 mg.kg^{-1}.day^{-1} group. [c] Number of mothers with salivary samples is 19 in placebo group; 16 in 5 mg.kg^{-1}.day^{-1} group; 21 in 10 mg.kg^{-1}.day^{-1} group; 17 in 15 mg.kg^{-1}.day^{-1} group and 14 in 20 mg.kg^{-1}.day^{-1} group. [d] Number of infants with salivary samples is 21 in placebo group; 18 in 5 mg.kg^{-1}.day^{-1} group; 20 in 10 mg.kg^{-1}.day^{-1} group; 18 in 15 mg.kg^{-1}.day^{-1} group and 16 in 20 mg.kg^{-1}.day^{-1} group

Table 5.6 Maternal Edinburgh Postnatal Depression Scores

	Placebo	**Caffeine citrate 5 mg/kg/day**		**Caffeine citrate 10 mg/kg/day**		**Caffeine citrate 15 mg/kg/day**		**Caffeine citrate 20 mg/kg/day**		**Any dose of caffeine**	
	Summary data	Summary Data	Ratio of GMs (95% CI), p-value *vs placebo*	Summary Data	Ratio of GMs (95% CI), p-value *vs placebo*	Summary Data	Ratio of GMs (95% CI), p-value *vs placebo*	Summary Data	Ratio of GMs (95% CI), p-value *vs placebo*	Summary Data	Ratio of GMs (95% CI), p-value *vs placebo*
Baseline EPDS Score [a]											
N (% non-missing, of those with a baseline visit)	*22 (91.7%)*	*22 (95.7%)*		*23 (95.8%)*		*25 (100.0%)*		*26 (100.0%)*		*96 (98.0%)*	
Median (IQR)	6.5 (2.0, 9.0)	4.5 (2.0, 9.0)		5.0 (3.0, 8.0)		5.0 (2.0, 8.0)		5.5 (3.5, 9.0)		5.0 (2.5, 8.0)	
TEA EPDS Score [a]											
N (% non-missing, of those with a term visit)	*20 (100.0%)*	*18 (100.0%)*		*19 (100.0%)*		*18 (100.0%)*		*18 (100.0%)*		*73 (100.0%)*	
Median (IQR) [geometric mean; GM]	7.0 (2.0, 9.0) [2.6]	3.0 (1.0, 6.0) [1.6]	*0.65 (0.20,2.08) p=0.46*	6.0 (4.0, 9.0) [4.0]	*1.64 (0.52,5.22) p=0.40*	6.0 (2.0, 10.0) [5.6]	*1.73 (0.53,5.63) p=0.36*	4.0 (3.0, 6.0) [3.0]	*1.24 (0.38,4.06) p=0.72*	5.0 (3.0, 7.0) [5.6]	*1.21 (0.48,3.03) p=0.68*
Term score adjusted for baseline			*0.78 (0.26,2.34) p=0.65*		*2.16 (0.74,6.33) p=0.16*		*2.27 (0.75,6.83) p=0.14*		*1.30 (0.43,3.94) p=0.63*		*1.49 (0.62,3.53) p=0.38*

[a] EPDS data was available in a total of 118 (96.7% of 122) mothers at baseline and 93 (93.9% of 99 mothers remaining) at TEA visit

Fifteen infants across the four caffeine groups, but none in the placebo group, were withdrawn due to difficulties administering the study drug, the infant not tolerating the drug (spilling) or parental or investigator concerns about side effects (Table 5.7). The rate of stopping medication due to presumed side effects was not significantly different between the placebo and caffeine groups (Table 5.2).

Table 5.7 Side effects and withdrawals

	Placebo N = 24	**Caffeine citrate 5 mg.kg^{-1}.day^{-1} N = 24**	**Caffeine citrate 10 mg.kg^{-1}.day^{-1} N = 27**	**Caffeine citrate 15 mg.kg^{-1}.day^{-1} N = 27**	**Caffeine citrate 20 mg.kg^{-1}.day^{-1} N = 27**	**Any dose of caffeine N = 105**
Number withdrew (%)	4 (16.7%)	5 (20.8%)	6 (22.2%)	8 (29.6%)	7 (25.9%)	26 (24.7%)
Withdrawal reason; N						
Administration difficulties	0	0	2	3	2	7
Parents changed mind	4	1	1	3	3	8
Side effects or drug not tolerated	0	3	3	1	1	8
Other [a]	0	1	0	1	1	3

[a] Including: investigators discretion (n=1), moved outside study follow-up area (n=1), inability to follow up due to the COVID-19 lockdown (n=1)

5.6 Discussion

In this randomised placebo-controlled dosage trial, caffeine citrate at 10 or 20 mg.kg^{-1}.day^{-1} reduced the mean rate of IH by 61% and 67%, respectively. Overall, caffeine did not have adverse effects on sleep, gastro-oesophageal reflux or feeding, although the percentage of time that infants had tachycardia increased, in keeping with previous reports (Chen et al., 2018; Mohammed et al., 2015).

Currently, there is a lack of consensus definition for IH in preterm babies. We defined IH as Spo_2 fall ≥10% below baseline for <2 minutes, which previously we have shown to be increased in late preterm babies compared with term babies (Williams et al., 2018). Although a 3% threshold is used in polysomnography to define desaturation events, a definition of 10% is commonly used in the neonatal literature (Rhein et al., 2012), and we considered this higher threshold more repeatable and reliable. We chose to include even short episodes as these are believed to be as important as sustained hypoxaemia as a cause of subsequent neurocognitive deficits in children (Almendros et al., 2014; Bass et al., 2004).

The reason for a significant effect of caffeine citrate on the primary outcome at a dose of 10 and 20 but not 15 $mg.kg^{-1}.day^{-1}$ is unclear. There were no differences in baseline characteristics to suggest confounding, and compliance with study medication was not worse in this group. Moreover, salivary caffeine concentration in the 15 $mg.kg^{-1}.day^{-1}$ group was intermediate to that of the 10 and 20 $mg.kg^{-1}.day^{-1}$ groups, and the percentage of the time these infants experienced tachycardia was comparable to the 20 $mg.kg^{-1}.day^{-1}$ group, all of which indicate they received the study drug. Although the baseline rate of IH was higher in the 15 $mg.kg^{-1}.day^{-1}$ group than in the 10 and 20 $mg.kg^{-1}.day^{-1}$ groups, adjustment for this in secondary analysis did not alter results. It is possible that the lack of statistically significant reduction in IH in this group is due to type-II error.

Both the 10 and 20 $mg.kg^{-1}.day^{-1}$ doses were effective in late preterm infants as they reduced the rate of IH at 2 weeks, mean SpO_2 and time with SpO_2 less than 90%. This trial was powered to compare each caffeine citrate dose with placebo, rather than compare caffeine doses directly. However, the effect size in all respiratory measures was larger for the 20 $mg.kg^{-1}.day^{-1}$ dose, with similar effects on drug tolerability to the 10 $mg.kg^{-1}.day^{-1}$ dose. In addition, the 15 $mg.kg^{-1}.day^{-1}$ dose was not effective, which would be expected if the 10 $mg.kg^{-1}.day^{-1}$ dose was effective. Therefore, future trials in late preterm infants should consider using 20 $mg.kg^{-1}.day^{-1}$ of caffeine citrate.

In the Caffeine for Apnea of Prematurity (CAP) trial, very preterm infants receiving caffeine gained less weight than those in the placebo group during the first three weeks after randomisation, but there was no difference in weight by four weeks of age and no difference in head circumference (Kreutzer & Bassler, 2014). In contrast, in our trial the only growth parameters affected by caffeine treatment were the length z-score, which was lower in the 20 $mg.kg^{-1}.day^{-1}$ group at 2 weeks and TEA, and head circumference growth velocity, which was lower in the 5 $mg.kg^{-1}.day^{-1}$ group. In both cases, a statistically significant difference occurred only in a single dose group and for a single parameter, and other related parameters failed to show the same changes; it thus appears unlikely that caffeine has a significant impact on overall neonatal growth.

A small observational study in low-birthweight infants determined that the half-life of caffeine citrate is 86 hours at 34 weeks, reducing to 73 hours at 37 weeks and 6 hours at 60 weeks post-menstrual age(Le Guennec et al., 1985). In two other studies, caffeine citrate 6 $mg.kg^{-1}.day^{-1}$ reduced IH at 35 and 36 weeks' gestational age(Rhein et al., 2014), but at 37 and 38 weeks' gestational age higher doses of 14 or 20 $mg.kg^{-1}.day^{-1}$ were required to maintain caffeine salivary concentrations in the therapeutic range and reduce IH(Dobson et al., 2017). Our study supports the finding that higher doses of caffeine are required at later postmenstrual ages.

A limitation of this study was the higher rate of withdrawals in higher dose caffeine groups, mainly due to administration difficulties and poor tolerability. To maintain blinding, the trial drug was formulated at four different strengths, but at higher concentrations this resulted in a bitter solution, albeit one that is comparable to that used clinically. Unlike clinical use where very preterm infants receive caffeine citrate via a nasogastric tube, participating late preterm infants generally received the medication orally, meaning taste was important, and the volume was challenging to

administer in some infants. Further trials on the use of caffeine citrate in the late preterm population should use a more palatable formulation. In addition, primary outcome data was not available for infants who were withdrawn prior to 2 weeks post-randomisation, and it is possible that attrition bias may have affected the outcome However, it is unlikely that withdrawal from the study due to administration difficulties was linked to the primary outcome, so estimates of effectiveness should not have been affected by these withdrawals. A second limitation is that concurrent use of other medications was not formally recorded in this study. However, there are few clinically relevant drug interactions with caffeine citrate (Preston, 2022) so it is unlikely that any participating infant received any medication that significantly affected plasma caffeine concentrations.

5.7 Conclusion

Caffeine citrate reduces IH in late preterm infants, with doses of 10 and 20 $mg.kg^{-1}.day^{-1}$ being effective, although difficult to administer to some babies in the current formulation, possibly due to the taste. Side effects at these doses include tachycardia, and possibly growth. A longer, larger trial with neurodevelopmental impairment as the primary outcome is required to establish if the reduction in IH will result in clinically significant improvements in neurodevelopment.

6. Systematic Review: Caffeine for apnoea and the prevention of neurodevelopmental impairment in preterm infants

6.1 Abstract

This systematic review and meta-analysis evaluated the evidence for dose and effectiveness of caffeine in preterm infants. MEDLINE, EMBASE, CINHAL Plus, CENTRAL, and trial databases were searched to July 2022 for trials randomizing preterm infants to caffeine vs. placebo/no treatment, or low (≤10mg.kg-1) vs. high dose (>10mg.kg-1 caffeine citrate equivalent). Two researchers extracted data and assessed risk of bias using RoB; GRADE evaluation was completed by all authors. Meta-analysis of 15 studies (3530 infants) was performed in REVMAN across four epochs: neonatal/infant (birth-1 year), early childhood (1-5 years), middle childhood (6-11 years) and adolescence (12-19 years).

Caffeine reduced apnoea (RR 0.59; 95%CI 0.46,0.75; very-low certainty) and bronchopulmonary dysplasia (0.77; 0.69,0.86; moderate certainty), with higher doses more effective. Caffeine had no effect on neurocognitive impairment in early childhood but possible benefit on motor function in middle childhood (0.72; 0.57,0.91; moderate certainty). The optimal dose remains unknown; further long-term studies are needed.

6.2 Introduction

Infants born preterm are physiologically and metabolically immature and have higher rates of morbidity and mortality, and poorer long-term neurodevelopmental outcomes than those born at term (Moster et al., 2008). Amongst other issues, they are at risk of apnoea of prematurity (Henderson-Smart, 1981) and intermittent hypoxaemia (Williams et al., 2018), which result in a decrease in oxygen saturation and bradycardia and have been associated with increased risk of neurodevelopmental impairment

(Janvier et al., 2004; Poets et al., 2015). Rates of apnoea are correlated with the degree of prematurity, occurring most frequently in extremely preterm infants, though late preterm infants are also affected (Henderson-Smart, 1981). Late preterm infants also experience frequent episodes of intermittent hypoxaemia (Williams et al., 2018) and poorer neurodevelopmental outcomes than term-born infants (Woythaler et al., 2011).

Methylxanthines are respiratory stimulants that have been used in preterm neonates for decades to both prevent and treat apnoea of prematurity and to facilitate extubation (Muehlbacher et al., 2020). Caffeine is a naturally-occurring methylxanthine used extensively worldwide for hundreds of years for its CNS stimulant properties (Muehlbacher et al., 2020). Caffeine and other methylxanthines, such as theophylline, have been used in the treatment of apnoea in newborn infants since the 1970s (Aranda et al., 1977). The precise mechanism by which methylxanthines improve respiratory function continues to be debated, but caffeine is known to stimulate the respiratory centre in the medulla by antagonizing adenosine A1 and A2A receptors, increasing sensitivity and response to carbon dioxide and PO_2 and enhancing diaphragmatic function (Yuan et al., 2022). Caffeine is now used in preference to other methylxanthines due to its wider therapeutic window and longer duration of action in neonates, which allow for daily dosing and remove the need for therapeutic drug monitoring(Henderson-Smart & De Paoli, 2010a; Schoen et al., 2014).

Despite this longstanding clinical use there remain several evidence gaps, including indications for treatment, dosing regimen, the most appropriate patient population and the short- and long-term effects of caffeine therapy (Erickson et al., 2021). The aim of this systematic review was to assess the effectiveness of caffeine in reducing the rate or occurrence of apnoea and reducing long-term neurodevelopmental impairment in preterm infants (<37 weeks' post-menstrual age [PMA]). A secondary aim was to assess if there is any difference in these outcomes between caffeine given at standard doses

(≤10 mg.kg^{-1} caffeine citrate equivalent) and high doses (>10 mg.kg^{-1} caffeine citrate equivalent).

6.3 Methods

This systematic review was guided by the Cochrane Handbook for Systematic Reviews of Interventions (Thomas et al., 2022) and is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page et al., 2021). Prior to the literature search being conducted, the protocol was registered with the Prospective Register of Systematic Reviews (PROSPERO, CRD42020154678).

We included all randomized controlled trials (RCTs) in preterm infants (<37 weeks' PMA) of caffeine (at any dose and for any reason) vs. placebo or no treatment (comparison one), or high-dose caffeine (>10 mg.kg^{-1} citrate equivalent) vs. low-dose caffeine (≤10 mg.kg^{-1} caffeine citrate equivalent)(comparison two), which reported one or more prespecified outcomes. We included published studies and those published in abstract if they included sufficient information to confirm eligibility and allow Grading of Recommendations Assessment, Development and Evaluation (GRADE)(Schünemann et al., 2013). We did not include observational or non-randomized studies. No limit was placed on year of publication, and studies in any language were included and translated if an English abstract was available for the initial screening stage.

We reported outcomes across four developmental epochs: neonatal/infancy (<1 year of age), early childhood (ages 1-5 years), middle childhood (ages 6-11 years) and adolescence (ages 12-19 years). If longitudinal studies reported multiple assessments of an outcome within the epoch, the last assessment in each epoch was included in the analysis.

The primary outcome for the neonatal/infant epoch was apnoea, defined as a pause in breathing of ≥20 s, or <20 s with bradycardia (heart rate <100 beats per minute [bpm]), cyanosis or pallor (American Academy of Paediatrics, 2003), or as per author definitions. For all other epochs, the primary outcome was neurocognitive impairment, defined by authors, using standardized tests appropriate for age.

Secondary outcomes for the neonatal/infant epoch included bronchopulmonary dysplasia (BPD), defined as ongoing requirement for oxygen or respiratory support at 36 weeks' PMA; intermittent hypoxaemia, expressed as events per hour and defined as a fall in oxygen saturation (SpO_2) of 10% or more from baseline, or as defined by authors; retinopathy of prematurity (ROP) Stage III or worse (Garner et al., 1984); intraventricular haemorrhage (IVH) grade III or IV (Papile et al., 1978); patent ductus arteriosus (PDA), defined as use of medical or surgical treatment for ductal closure; tachycardia, defined as mean heart rate ≥160 bpm or as per authors; duration of mechanical ventilation; duration of positive pressure support; growth velocity, including weight gain ($g.kg^{-1}.day^{-}$), linear growth ($cm.week^{-1}$) and head growth ($cm.week^{-1}$) to 36 weeks' PMA (or as defined by authors); death; survival without neurosensory impairment (including, but not limited to deafness, blindness and cerebral palsy); and time to establish full enteral feeds (as defined by authors).

For all other epochs, secondary outcomes included: motor impairment, defined by authors using standardized tests appropriate for age; hearing impairment, defined as requiring one or more hearing aids or worse, or as per authors; visual acuity less than 1 LogMAR, or as per authors; death; survival without neurosensory impairment, including, but not limited to, deafness, blindness, death and cerebral palsy; emotional behavioural difficulties, as defined by authors; cerebral palsy; chronic lung disease,

defined as physician-diagnosed asthma or ≥2 episodes of parent-reported wheeze, or as per authors; and height and weight expressed as Z-scores.

6.3.1 Search strategy

We searched Pubmed, Medline, Embase, the Cumulative Index to Nursing and Allied Health Literature (CINHAL Plus) and the Cochrane Central Register of Controlled Trials (CENTRAL) databases from inception to 11 July 2022 using relevant MeSH terms and key words (caffeine and premature/ prematurity/ preterm/ low birthweight and variations). The search was limited to studies involving humans, with no limit on year of publication or language. No limits on study type were applied at the initial search stage. We also searched The World Health Organization International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/search/en/), the US National Library of Medicine Clinical Trials Registry (clinicaltrials.gov), and Australia and New Zealand Clinical Trials Registry (ANZCTR)(http://www.anzctr.org.au), for any additional trials meeting the inclusion criteria not located through the above searches. Where results of trials were not available in the public domain, we contacted the authors listed in the trial registration to confirm the status of the trial, and whether any results were available for inclusion. We hand-searched bibliographies of included studies, review papers and conference abstracts to identify any additional studies. Covidence (Covidence Systematic Review Software, Veritas Health Innovation, 2020) was used to manage search results and screen studies for inclusion.

6.3.2 Study selection

Two review authors independently screened all retrieved titles and abstracts to assess eligibility for inclusion. The full text of all potentially relevant studies was retrieved and assessed independently by two authors to determine eligibility. Any disagreements were resolved by mutual discussion and consultation with a third author, if required. Summary characteristics of each study were extracted and tabulated.

6.3.3 Data extraction, bias, and quality assessment

Two authors independently extracted data from all included studies using a pre-specified data form. Any discrepancies were resolved by mutual discussion and consulting a third author if required. Additional information was sought from study corresponding authors if information was unclear or not published.

Two review authors independently assessed the risk of bias (RoB) of all included trials using the Cochrane RoB tool (J. Higgins et al., 2011) for the following domains: sequence generation (selection bias); allocation concealment (selection bias); blinding of participants and personnel (performance bias); blinding of outcome assessment (detection bias); incomplete outcome data (attrition bias); selective reporting (reporting bias); any other bias. Any disagreements were resolved by mutual discussion or consulting a third author if necessary. For one study, where EO, JA and CM were investigators, an alternative independent colleague (AW) with no association to the study conducted the data extraction and RoB assessment in conjunction with SH.

Review Manager (RevMan version 5.4.1. The Cochrane Collaboration, 2020) was used to summarize and analyse the data. Meta-analysis using fixed effects was performed if data from $\geq$2 RCTs were available. Apnoea was reported using different measures that precluded a single meta-analysis; therefore, apnoea was analysed both as a dichotomous and continuous variable. We calculated the risk ratio (RR) for dichotomous outcomes and mean difference (MD) for continuous outcomes, with confidence intervals (CI) of 95%. If data were reported as median and interquartile range, means and standard deviations were estimated (Wan et al., 2014). Planned secondary analyses included subgroup analysis by indication for caffeine and gestation length. Statistical heterogeneity was defined as an I^2 >50% and low p value for the Chi Square test and categorized according to GRADE guidelines (Schünemann et al., 2013). Methodological causes of heterogeneity were explored via subgroup analysis and sensitivity analysis, excluding studies at high risk of bias.

Outcomes were classified by all authors according to their importance for decision-making using GRADE classifications (7-9 critical, 4-6 important but not critical, 1-3 less important)(Schünemann et al., 2013). Certainty of the evidence was assessed using the GRADE framework (Schünemann et al., 2013) and agreed by all authors. Imprecision was assessed using optimal information size (OIS) assuming alpha 0.05 and beta 0.2 (Guyatt et al., 2011), and considered serious if the total number of participants was less than the OIS for the outcome, or very serious if total participants numbered less than half the OIS. For continuous outcomes we assumed alpha 0.05 and beta 0.2, and delta 0.33.

Study characteristics and results were tabulated, and forest plots generated for all comparisons where data was available.

6.4 Results

6.4.1 Literature search and study selection

Our search identified 6,509 records (Figure 6.1). Following the removal of 3,542 duplicates, 2,968 studies were screened and 2,801 excluded. The full text of 159 papers were reviewed, resulting in the inclusion of 15 studies in the final review.

6.4.2 Study characteristics

We identified 15 eligible RCTs enrolling a total of 3,530 premature infants. Most trials enrolled infants born at <32 weeks' PMA (Bucher & Duc, 1988; Erenberg et al., 2000; Fakoor et al., 2019; Kori et al., 2021; S. Liu et al., 2020; Mohammed et al., 2015; Scanlon et al., 1992; Steer et al., 2003; Zhao et al., 2016), although some included infants up to 35 (Murat et al., 1981) or 36 (Oliphant et al., 2023) weeks, or defined eligibility based on birthweight (Armanian et al., 2016; Fakoor et al., 2019; Iranpour et al., 2022; Schmidt et

al., 2006) or clinical decision to treat with caffeine (Steer et al., 2004). Eight trials compared caffeine to placebo or no treatment (Armanian et al., 2016; Bucher & Duc, 1988; Erenberg et al., 2000; Fakoor et al., 2019; Iranpour et al., 2022; S. Liu et al., 2020; Murat et al., 1981; Schmidt et al., 2006). Seven trials compared different doses of caffeine (Kori et al., 2021; Mohammed et al., 2015; Oliphant et al., 2023; Scanlon et al., 1992; Steer et al., 2003, 2004; Zhao et al., 2016), including one (Oliphant et al., 2023) with four different dosing arms and a placebo arm, which contributed to both comparisons. Trials were widely geographically located and all except one (Oliphant et al., 2023) enrolled only infants in neonatal units. Most trials were small, with only one enrolling more than 300 infants (Schmidt et al., 2006) (Table 6.1). Eight of the included trials had high RoB in one or more domains (Armanian et al., 2016; Fakoor et al., 2019; Iranpour et al., 2022; S. Liu et al., 2020; Murat et al., 1981; Oliphant et al., 2023; Scanlon et al., 1992; Zhao et al., 2016), especially for 'incomplete outcome data' (Table 6.2). All included studies reported at least one outcome for the neonatal/infant epoch. Two studies (Schmidt et al., 2006; Steer et al., 2004) reported outcomes in early childhood, and only one study (Schmidt et al., 2006) reported outcomes in middle childhood. No studies reported results in adolescence.

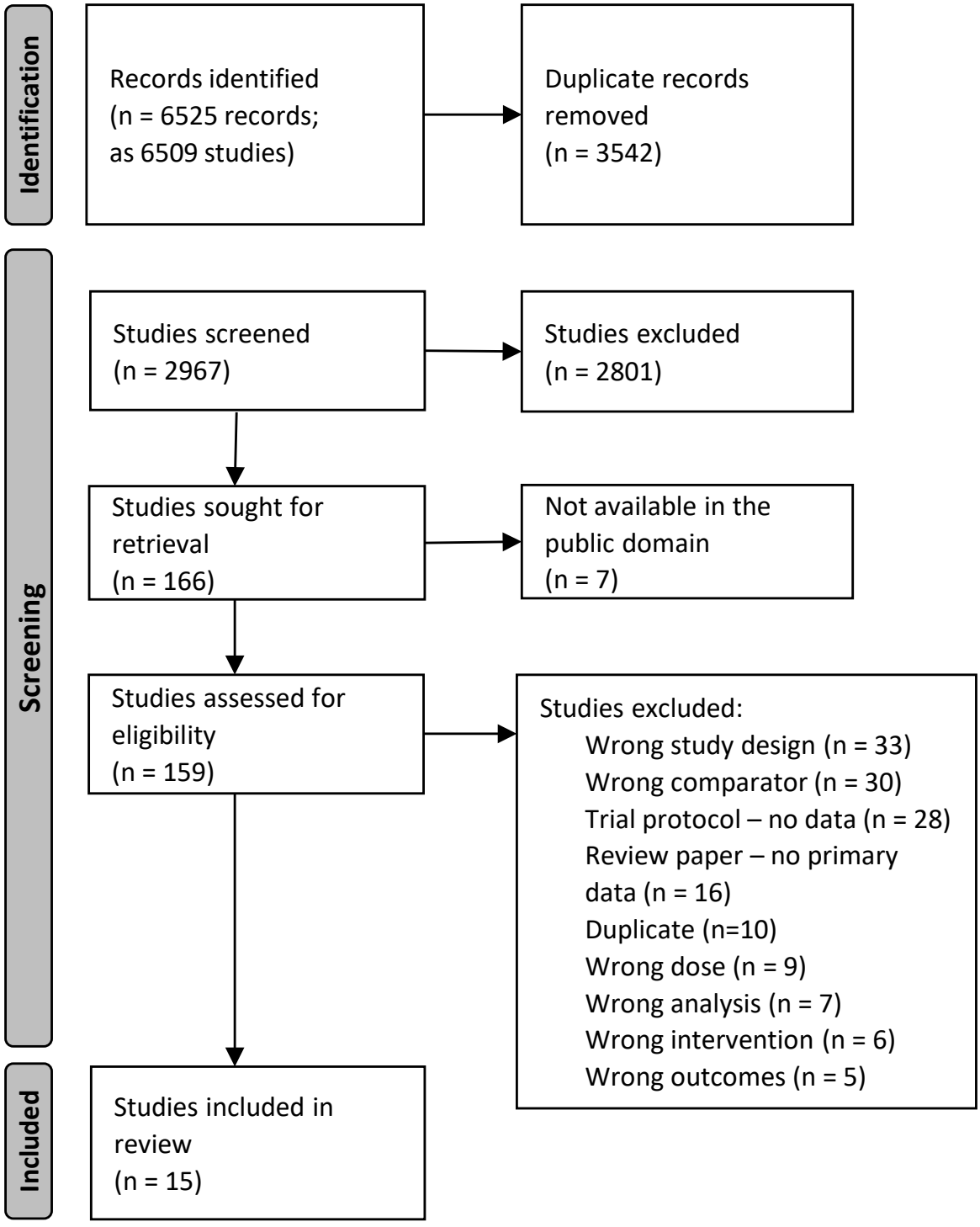


Figure 6.1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection

Table 6.1 Study characteristics

Study	Country	Eligibility	Enrolled (n)	Intervention	Comparator	Primary Outcome(s)[b]	Notes
Comparison 1: Caffeine vs Placebo / no treatment							
Armanian 2016 (Armanian et al., 2016)	Iran	Premature infants BW ≤1200 g Spontaneous breathing at 24 hours of life.	52	*Loading:* IV "caffeine" (salt not specified) 20 mg/kg loading dose on first day of life *Maintenance:* 5mg/kg/day for 1st 10 days of life.	*Loading & Maintenance:* Equivolume doses of IV saline 0.9%	Apnoea Bradycardia Cyanosis	If infants in the control group demonstrated apnoea, caffeine was administered
Bucher 1988 (Bucher & Duc, 1988)	Switzerland	≤ 32 weeks' GA, with spontaneous respiration for ≥24h at 48 h of age	50	*Loading:* 20 mg/kg (2mL/kg) caffeine citrate IV at 48 h of age *Maintenance:* 10 mg/kg (1mL/kg) caffeine citrate IV given at 72 and 96 hours of age	*Loading & Maintenance:* Equivolume doses of IV saline 0.9%	Intermittent hypoxaemia	
Erenberg 2000 (Erenberg et al., 1998, 2000)	USA	28–32 weeks' GA; >24 h old; ≥ 6 apnoea episodes within a 24-hour period	82	*Loading:* 20 mg/kg (1mL/kg) caffeine citrate IV *Maintenance:* Caffeine citrate 5 mg/kg (0.25ml/kg) IV/OG/NG Q24h, starting 24 hours after loading dose	*Loading & Maintenance:* Equivolume matching citric salt placebo solution	Apnoea	
Fakoor 2019 (Fakoor et al., 2019)	Iran	GA ≤ 32weeks'; BW ≤ 1500 g	100	*Loading:* 20 mg/kg "venous caffeine" *Maintenance:* 5 mg/kg/day "venous caffeine"	No treatment	Apnoea	Retrospective trial registration also states infants had to be at least 24 hours of age and have "self-contained breathing" in the first 24

							hours of life.
Iranpour 2022 (Iranpour et al., 2022)	Iran	GA ≤ 37 weeks'; BW 1250-2000 g; weight appropriate for age. Spontaneous respiration and clinical signs of respiratory distress requiring nasal CPAP	90	*Loading:* 20 mg/kg IV caffeine citrate *Maintenance:* 10mg/kg/day IV or PO (when enteral feeding) caffeine citrate, until respiratory support not required	No treatment	Duration of positive pressure support	
Liu 2020 (S. Liu et al., 2020)	China	GA ≤ 32 weeks'; BW < 1500g	194	*Loading:* 20 mg/kg IV caffeine citrates within 72 hours of birth *Maintenance:* 5 mg/kg/day IV caffeine citrate	*Loading & Maintenance:* Equivolume doses of IV saline 0.9%	White matter abnormality on cerebral magnetic resonance imaging (MRI)	Study was primarily looking at cranial MRI changes, but also included outcomes relating to respiration and short-term complications of caffeine treatment
Murat 1981 (Murat et al., 1981)	France	GA 29-35 weeks'; ≥3 episodes of idiopathic apnoea on cardiorespiratory monitoring within a 24 hour period.	18	*Loading:* 20 mg/kg IM caffeine citrate (0.8 mL) *Maintenance:* 5 mg/kg/day PO caffeine citrate	No treatment	Apnoea	

Schmidt 2006 (Doyle et al., 2014, 2017; Mürner-Lavanchy et al., 2018; Schmidt et al., 2006, 2007, 2012, 2017, 2019)	Australia, Canada, Germany Israel, The Netherlands, Sweeden, Switzerland, UK, USA	BW 500-1250 g; Day 1-10 of life; infant considered a candidate for methylxanthine therapy by clinical staff	2006	*Loading:* 20 mg/kg IV caffeine citrate: *Maintenance:* 5 mg/kg/day IV or PO (when tolerating full enteral feeds) caffeine citrate	*Loading & Maintenance:* Equivolume doses of saline 0.9%	Neurocognitive impairment (composite of death, cerebral palsy, cognitive delay, deafness or blindness) at a corrected age of 18 – 21 months	If apnoea persisted, the daily dose of caffeine could be increased to 10mg/kg/day.
Comparison 2: High dose vs low dose caffeine							
Kori 2021 (Kori et al., 2021)	Malaysia	GA 26 – 32; within 24 h periextubation period if ventilated	78	*Loading:* 20 mg/kg PO caffeine citrate *Maintenance:* 10 mg/kg PO caffeine citrate	*Loading:* 40 mg/kg PO caffeine citrate *Maintenance:* 20 mg/kg PO caffeine citrate	Apnoea	
Mohammed 2015 (Mohammed et al., 2015)	Egypt	GA < 32 weeks'; AOP in first 10 days of life	120	*Loading:* 20 mg/kg IV caffeine citrate *Maintenance:* 10 mg/kg/day IV or PO caffeine citrate, until 7 days post-extubation	*Loading:* 40 mg/kg IV caffeine citrate *Maintenance:* 20 mg/kg/day IV/ PO caffeine citrate, until 7 days post-extubation	Extubation failure in mechanically ventilated infants	
Oliphant 2022 (Oliphant et al., 2020, 2023)	New Zealand	GA 34-36 weeks'; <72 h old	132	*Loading:* Caffeine citrate 10, 20, 30 or 40 mg/kg/day PO *Maintenance:* Caffeine citrate 5, 10, 15 or 20 mg/kg/day PO	*Loading & Maintenance:* Equivolume doses of PO water	Intermittent hypoxaemia	Trial included multiple doses and placebo arm. Data included in both comparisons (all caffeine groups vs placebo in Comparison 1; 5 & 10 mg/kg/day groups vs 15 & 20 mg/kg/day in

							Comparison 2).

Scanlon 1992 (Scanlon et al., 1992)	England	GA < 31 weeks'; either ≥10 apnoea in 8 h or ≥4 apnoea in 1 h.	30	*Loading:* 25 mg/kg PO caffeine citrate	*Loading:* 50 mg/kg PO caffeine citrate (given as 2 x 25mg/kg doses 1 h apart)	Apnoea	A third group receiving theophylline was also included in the trial, but not considered in this systematic review
				Maintenance: 6 mg/kg/day PO caffeine citrate	*Maintenance:* 12 mg/kg/day PO caffeine citrate		
Steer 2003 (Steer et al., 2003)	Australia	GA ≤ 31 weeks'; received/were anticipated to receive ≥48 h mechanical ventilation	127	*Loading:* 30 or 60 mg/kg IV caffeine citrate	*Loading:* 6 mg/kg IV caffeine citrate	Extubation failure in mechanically ventilated infants	
				Maintenance: 15 or 30 mg/kg IV caffeine citrate (OG if enterally fed)	*Maintenance:* 3 mg/kg IV caffeine citrate (OG if enterally fed)		
Steer 2004 (Steer et al., 2004)	Australia	Infants requiring methylxanthines for treatment of apnoea of prematurity or as a part of peri-extubation management	287	*Loading:* 80 mg/kg caffeine citrate	*Loading:* 20 mg/kg caffeine citrate	Extubation failure in mechanically ventilated infants	Route of administration not specified
				Maintenance: 20 mg/kg caffeine citrate every 24 h, starting 24 h after loading dose	*Maintenance:* 5 mg/kg caffeine citrate every 24 h, starting 24 h after loading dose		

Zhao 2016 (Zhao et al., 2016)	China	GA ≤ 32 weeks'; primary apnoea	164	*Loading:* 20 mg/kg IV caffeine citrate	*Loading:* 20 mg/kg IV caffeine citrate	Apnoea	
				Maintenance: 15 mg/kg/day IV caffeine citrate	*Maintenance:* 5 mg/kg/day IV caffeine citrate		
				Maintenance: 15 mg/kg/day IV caffeine citrate	*Maintenance:* 5 mg/kg/day IV caffeine citrate		

[a]All trials were randomized on a 1:1 basis (1:1:1 and 1:1:1:1:1 for the multi-arm studies Scanlon 1992 and Oliphant 2022 respectively) [b]Outcomes are for the neonatal/infant epoch unless otherwise stated.

Table 6.2 Overall risk of bias of included studies

Study	Risk of bias							
	D1	D2	D3	D4	D5	D6	D7	Overall
Armanian 2016	+	−	×	+	+	+	−	−
Bucher 1988	+	+	+	+	+	−	+	−
Erenberg 2000	+	−	−	−	+	−	+	−
Fakoor 2019	−	−	×	×	×	×	−	×
Iranpour 2022	+	+	×	×	×	−	−	×
Kori 2021	+	+	+	+	+	−	−	+
Liu 2020	+	−	−	−	×	−	+	−
Mohammed 2015	+	+	+	+	+	−	+	+
Murat 1981	−	−	×	×	×	×	+	×
Oliphant 2022	+	+	+	+	×	+	+	+
Scanlon 1992	−	×	×	−	×	−	+	×
Schmidt 2006	+	+	+	+	+	+	+	+
Steer 2003	+	+	+	+	+	−	+	+
Steer 2004	+	−	+	+	+	+	+	+
Zhao 2016	+	−	−	−	+	×	−	−

Key: × high, − unclear + low risk of bias for D1: Random sequence generation, D2: Allocation concealment, D3: Blinding of participants and personnel, D4: Blinding of outcome assessment, D5: Incomplete outcome data, D6: Selective reporting, D7: Other sources of bias.

6.4.3 Caffeine vs. placebo/no treatment

6.4.3.1 Primary outcome

Neonatal/infancy: For the primary outcome of apnoea (dichotomous), evidence of very low certainty from five trials showed possible benefit from receiving caffeine compared to placebo or no treatment (risk ratio [RR] 0.59, 95% confidence interval [CI] 0.46, 0.75, 453 infants)(Table 6.3)(Armanian et al., 2016; Erenberg et al., 2000; Fakoor et al., 2019; Iranpour et al., 2022; Oliphant et al., 2023). There was statistical heterogeneity (I^2=78%) among trials, although the direction of effect consistently favoured caffeine (Figure 6.2). In sensitivity analysis, exclusion of two trials at high risk of bias (Fakoor et al., 2019; Iranpour et al., 2022), did not substantially alter the results (RR 0.62, 95% CI 0.50, 0.77, three trials, 263 infants).

Early childhood: For the primary outcome of neurocognitive impairment, evidence of low certainty from one trial could not exclude clinical benefit or harm from receiving caffeine compared to placebo (RR 0.98, 95% 0.63, 1.51, 1518 children)(Table 6.3)(Schmidt et al., 2012).

Middle childhood: For the primary outcome of neurocognitive impairment, evidence of moderate certainty showed possible benefit from receiving caffeine compared to placebo (RR 0.84, 95% 0.71, 1.01, 1 trial, 920 children)(Table 6.3)(Schmidt et al., 2017).

There were no data for the primary outcome of neurocognitive impairment in adolescence.

6.4.3.2 Secondary outcomes

Moderate certainty evidence indicated probable clinical benefit of receiving caffeine compared to placebo or no treatment for BPD (RR 0.77, 95% CI 0.69, 0.86, three trials,

2059 infants, I^2=31%) and patent ductus arteriosus (RR 0.67, 95% CI 0.60, 0.74, four trials, 2242 infants, I^2=0%)(Table 6.3), and motor impairment in middle childhood (RR 0.72 95% CI 0.57, 0.91, one trial, 930 infants)(Table 6.3). Caffeine therapy may reduce neurocognitive impairment and cerebral palsy (Table 6.3). It is possible that caffeine reduces weight gain velocity after birth, but it does not appear to affect body size in childhood (Table 6.3). The evidence was too uncertain to determine the effect of caffeine on intermittent hypoxaemia, respiratory support, feeding, other major neonatal morbidities, death, other developmental outcomes in childhood, and asthma/wheeze (Table 6.3; Figure 6.3).

6.4.3.3 Secondary analysis

There were insufficient data to undertake the planned subgroup analyses.

Table 6.3 GRADE summary of findings for caffeine vs placebo comparison

	Certainty Assessment					Number of patients[a]		Effect		Certainty	Importance
	Number of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Caffeine	Placebo / no treatment	Relative (95% CI)	Absolute (95% CI)		
Neonatal and infant epoch[b]											
Apnoea (dichotomous outcome)	5 (Armanian et al., 2016; Erenberg et al., 2000; Fakoor et al., 2019; Iranpour et al., 2022; Oliphant et al., 2023)	Very Serious[c]	Serious[d]	Not serious	Serious[e]	47/271 (17.3%)	71/182 (39.0%)	**RR 0.59** (0.46 to 0.75)	160 fewer per 1,000 (from 211 fewer to 98 fewer)	⊕◯◯◯ Very low	Critical
Apnoea (continuous outcome)	2 (S. Liu et al., 2020; Murat et al., 1981)	Very Serious[f]	Very Serious[g]	Not serious	Serious[e]	1.8 events / day (N=89)	1.9 events / day (N=86)	-	MD 0.7 lower (1.1 lower to 0.2 lower)	⊕◯◯◯ Very low	Critical
Intraventricular haemorrhage	3 (Armanian et al., 2016; Fakoor et al., 2019; Iranpour et al., 2022)	Very Serious[h]	Not serious	Not serious	Very Serious[i]	9/121 (7.4%)	5/121 (4.1%)	**RR 1.80** (0.64 to 5.03)	33 more per 1,000 (from 15 fewer to 167 more)	⊕◯◯◯ Very low	Critical
Death before primary hospital discharge	7 (Armanian et al., 2016; Erenberg et al., 2000; Fakoor et al., 2019; Iranpour et al., 2022; S. Liu et al., 2020; Oliphant et al., 2023; Schmidt et al., 2006)	Serious[j]	Not serious	Not serious	Serious[e]	70/1370 (5.1%)	69/1275 (5.4%)	**RR 1.00** (0.73 to 1.38)	0 fewer per 1,000 (from 15 fewer to 21 more)	⊕⊕◯◯ Low	Critical
Intermittent hypoxaemia	2 (Bucher & Duc, 1988; Oliphant et al., 2023)	Not serious	Not serious	Serious[k]	Serious[e]	1.0 events / day (N=110)	0.7 events / day (N=47)	-	MD 0.3 higher (0.2 higher to 0.4 higher)	⊕⊕◯◯ Low	Important
Bronchopulmonary dysplasia	3 (Armanian et al.,	Serious[l]	Not serious	Not serious	Not serious	363/1034 (35.1%)	466/1025 (45.5%)	**RR 0.77** (0.69 to	105 fewer per 1,000	⊕⊕⊕◯ Moderate	Important

	2016; Iranpour et al., 2022; Schmidt et al., 2006)							0.86)	(from 141 fewer to 64 fewer)		
Duration of mechanical ventilation	2 (Fakoor et al., 2019; S. Liu et al., 2020)	Very Serious[m]	Serious[n]	Not serious	Serious[e]	5.0 days (N=130)	4.3 days (N=130)	-	MD 1.2 lower (2.5 lower to 0.1higher)	⊕◯◯◯ Very low	Important
Duration of positive pressure support	2 (Fakoor et al., 2019; Iranpour et al., 2022)	Very Serious[o]	Serious[d]	Not serious	Serious[e]	2.6 days (N=95)	3.0 days (N=95)	-	MD 0.2 lower (1.0 lower to 0.6 higher)	⊕◯◯◯ Very low	Important
Tachycardia	5 (Armanian et al., 2016; Bucher & Duc, 1988; Iranpour et al., 2022; S. Liu et al., 2020; Oliphant et al., 2023)	Serious[p]	Not serious	Not serious	Very Serious[i]	21/262 (8.0%)	14/198 (7.1%)	**RR 1.50** (0.81 to 2.79)	35 more per 1,000 (from 13 fewer to 127 more)	⊕◯◯◯ Very low	Important
Patent ductus arteriosus	4 (Armanian et al., 2016; Fakoor et al., 2019; Iranpour et al., 2022; Schmidt et al., 2006)	Serious[q]	Not serious	Not serious	Not serious	352/1122 (31.4%)	525/1120 (46.9%)	**RR 0.67** (0.60 to 0.74)	155 fewer per 1,000 (from 188 fewer to 122 fewer)	⊕⊕⊕◯ Moderate	Important
Growth velocity – weight gain	1 (Oliphant et al., 2023)	Not serious	Serious[r]	Not serious	Very Serious[i]	6.2 g/kg/d (N=77)	8.8 g/kg/d (N=20)	-	MD 2.6 lower (4.2 lower to 1.0 lower)	⊕◯◯◯ Very low	Important
Growth velocity – linear growth	1 (Oliphant et al., 2023)	Not serious	Serious[r]	Not serious	Very Serious[i]	0.8 cm/week (N=77)	0.7 cm/week (N=20)	-	MD 0.1 higher (0.2 lower to 0.4 higher)	⊕◯◯◯ Very low	Important
Growth velocity – head circumference	1 (Oliphant et al., 2023)	Not serious	Serious[r]	Not serious	Very Serious[i]	0.5 cm/week (N=77)	0.6 cm/week (N=20)	-	MD 0.1 lower (0.2 lower to 0.0 higher)	⊕◯◯◯ Very low	Important

Time to establish full enteral feeds	1 (Iranpour et al., 2022)	Very serious[s]	Not serious	Not serious	Serious[e]	154 h (N=45)	171 h (N=45)	-	MD 17 fewer hours (43 fewer to 8 more)	⊕◯◯◯ Very low	Less important

Early childhood epoch[t]											
Neurocognitive impairment	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Serious[e]	38/768 (4.9%)	38/750 (5.1%)	**RR 0.98** (0.63 to 1.51)	1 fewer per 1,000 (from 19 fewer to 26 more)	⊕⊕◯◯ Low	Critical
Death	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Serious[e]	59/867 (6.8%)	58/837 (6.9%)	**RR 0.98** (0.69 to 1.39)	1 fewer per 1,000 (from 21 fewer to 27 more)	⊕⊕◯◯ Low	Critical
Survival without neurosensory impairment	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Not serious	657/833 (78.9%)	607/807 (75.2%)	**RR 1.05** (0.99 to 1.11)	38 more per 1,000 (from 8 fewer to 83 more)	⊕⊕⊕◯ Moderate	Critical
Motor Impairment	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Serious[e]	13/803 (1.6%)	21/773 (2.7%)	**RR 0.60** (0.30 to 1.18)	11 fewer per 1,000 (from 19 fewer to 5 more)	⊕⊕◯◯ Low	Critical
Cerebral palsy	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Serious[e]	40/909 (4.4%)	66/901 (7.3%)	**RR 0.60** (0.41 to 0.88)	29 fewer per 1,000 (from 43 fewer to 9 fewer)	⊕⊕◯◯ Low	Critical
Hearing impairment	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Serious[e]	22/798 (2.8%)	25/773 (3.2%)	**RR 0.85** (0.48 to 1.50)	5 fewer per 1,000 (from 17 fewer to 16 more)	⊕⊕◯◯ Low	Critical
Visual Impairment	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Serious[e]	7/792 (0.9%)	7/763 (0.9%)	**RR 0.96** (0.34 to 2.73)	0 fewer per 1,000 (from 6 fewer to 16 more)	⊕⊕◯◯ Low	Critical
Emotional-behavioural difficulties	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Serious[e]	42/773 (5.4%)	53/748 (7.1%)	**RR 0.77** (0.52 to 1.14)	16 fewer per 1,000 (from 34 fewer to 10 more)	⊕⊕◯◯ Low	Important
Growth - Weight	1	Not serious	Serious[r]	Not serious	Not serious	-0.19 Z-score	-0.16 Z-score	-	MD 0.03 lower	⊕⊕⊕◯	Important

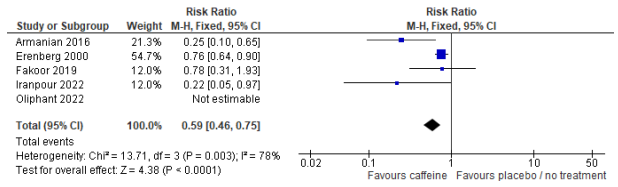
	(Schmidt et al., 2012)					(N=798)	(N=763)		(0.08 lower to 0.02 higher)	Moderate	
Growth - Height	1 (Schmidt et al., 2012)	Not serious	Serious[r]	Not serious	Not serious	-0.04 Z-score (N=793)	-0.04 Z-score (N=759)	-	MD 0.03 lower (0.08 lower to 0.02 higher)	⊕⊕⊕◯ Moderate	Important
Middle childhood epoch[u]											
Neurocognitive impairment	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Not serious	145/457 (31.7%)	174/463 (37.6%)	**RR 0.84** (0.71 to 1.01)	60 fewer per 1,000 (from 109 fewer to 4 more)	⊕⊕⊕◯ Moderate	Critical
Motor Impairment	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Not serious	90/457 (19.7%)	130/473 (27.5%)	**RR 0.72** (0.57 to 0.91)	77 fewer per 1,000 (from 118 fewer to 25 fewer)	⊕⊕⊕◯ Moderate	Critical
Cerebral palsy	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Serious[e]	21/484 (4.3%)	29/484 (6.0%)	**RR 0.72** (0.42 to 1.25)	17 fewer per 1,000 (from 35 fewer to 15 more)	⊕⊕◯◯ Low	Critical
Hearing impairment	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Serious[e]	16/484 (3.3%)	13/484 (2.7%)	**RR 1.23** (0.60 to 2.53)	6 more per 1,000 (from 11 fewer to 41 more)	⊕⊕◯◯ Low	Critical
Visual Impairment	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Very serious[i]	4/484 (0.8%)	1/484 (0.2%)	**RR 4.00** (0.45 to 35.66)	6 more per 1,000 (from 1 fewer to 72 more)	⊕◯◯◯ Very low	Critical
Emotional-behavioural difficulties	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Very serious[i]	52/476 (10.9%)	40/481 (8.3%)	**RR 1.31** (0.89 to 1.94)	26 more per 1,000 (from 9 fewer to 78 more)	⊕◯◯◯ Very low	Important
Asthma/Wheeze	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Very serious[i]	10/88 (11.4%)	17/80 (21.3%)	**RR 0.53** (0.26 to 1.10)	100 fewer per 1,000 (from 157	⊕◯◯◯ Very low	Important

									fewer to 21 more)		
Growth - Weight	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Not serious	-0.18 Z-score (N=474)	-0.10 Z-score (N=479)	-	MD 0.08 lower (0.24 lower to 0.08 higher)	⊕⊕⊕◯ Moderate	Important
Growth - Height	1 (Schmidt et al., 2017)	Not serious	Serious[r]	Not serious	Not serious	-0.20 Z-score (N=474)	-0.21 Z-score (N=478)	-	MD 0.01 higher (0.13 lower to 0.15 higher)	⊕⊕⊕◯ Moderate	Important

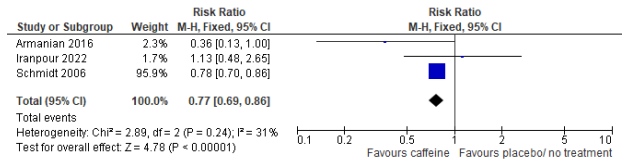
[a]For continuous outcomes values represent weighted mean. [b]In the neonatal and infant epoch, the critical outcomes of death before one year of age, neurocognitive impairment, survival without neurosensory impairment and cerebral palsy and the important outcome of retinopathy of prematurity were not reported by any included studies. [c]Two included studies (Fakoor 2019, Iranpour 2022) were judged to have high overall risk of bias; two (Armanian 2016, Erenberg 2000) were judged to have some concerns overall and one (Oliphant 2022) was judged to have a low overall risk of bias for this outcome. [d]I^2 = 78%. [e]OIS criteria not met (total population less than optimal information size [OIS] resulting in downgrading by one step). [f]One included study (Liu 2020) was judged to have high overall risk of bias for this outcome, and the other (Murat 191) was judged to have some concerns overall for this outcome. [g]I^2 = 97%. [h]Two included studies (Fakoor 2019 & Iranpour 2022) were judged to have high overall risk of bias for this outcome and one (Armanian 2016) was judged to have some concerns overall for this outcome. [i]OIS criteria not met (total population less than half of OIS, resulting in downgrading by two steps). [j]Two included studies (Fakoor 2019 & Iranpour 2022) were judged to have high overall risk of bias for this outcome; three (Armanian 2016, Erenberg 2000 & Liu 2020) was judged to have some concerns overall for this outcome; and two (Oliphant 2022 & Schmidt 2006) was judged to have low risk of bias overall for this outcome. [k]Patient populations of the two included studies were substantially different: Bucher 1988 included infants under 32 weeks' gestation (mean 30.3 weeks) while Oliphant 2022 included infants 34-36 weeks' gestation. Intermittent hypoxaemia is known to vary by gestational age. [l]One included study (Iranpour) was judged to have high overall risk of bias for this outcome; one (Armanian 2016) was judged to have some concerns overall for this outcome; and one (Schmidt 2006) was judged to have low risk of bias overall for this outcome. [m]One included study (Fakoor 2019) was judged to have high overall risk of bias for this outcome; and one (Liu 2020) was judged to have some concerns overall for this outcome. [n]I^2 = 43%. [o]Both included studies (Fakoor 2019 & Iranpour 2022) were judged to have high overall risk of bias for this outcome. [p]One included study (Iranpour 2022) was judged to have high overall risk of bias for this outcome; three (Armanian 2016, Bucher 1988 & Liu 20202) were judged to have some concerns overall for this outcome; and one (Oliphant 2022) was judged to have low risk of bias overall for this outcome. [q]Two included studies (Fakoor 2019 & Iranpour 2022) were judged to have high overall risk of bias for this outcome; one (Armanian 2016) was judged to have some concerns overall for this outcome; and one (Schmidt 2006) was judged to have low risk of bias overall for this outcome. [r]Results from a single study only. [s]The only included study (Iranpour 2022) was judged to have high overall risk of bias for this outcome. [t]In the early childhood epoch, the important outcome of asthma / wheeze was not reported by any included studies. [u]In the middle childhood epoch, the critical outcomes of death before one year of age and survival without neurosensory impairment were not reported by any included studies.

Caffeine vs placebo/no treatment

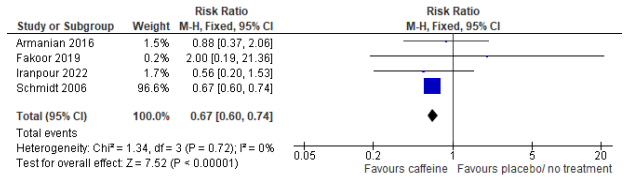
Apnoea – dichotomous[a]



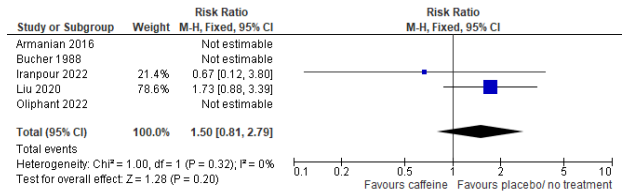
Bronchopulmonary Dysplasia



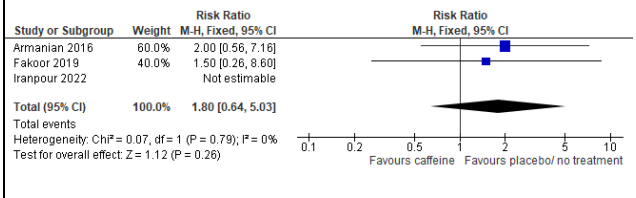
Patent ductus arteriosus



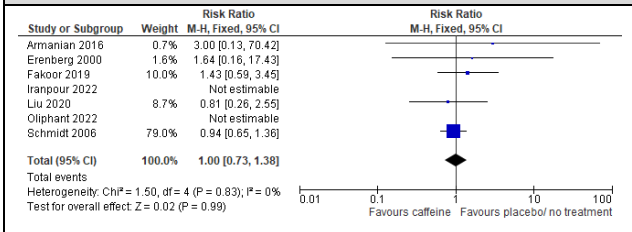
Tachycardia



Intraventricular hemorrhage

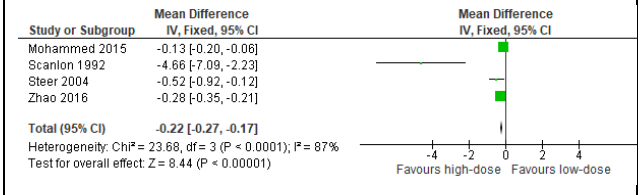


Death before primary discharge[b]

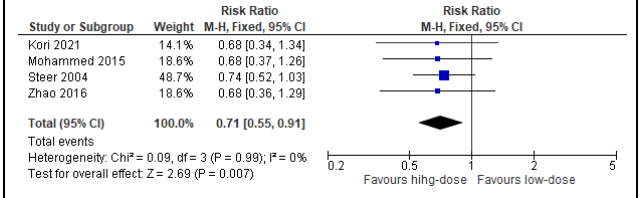


High vs low dose caffeine

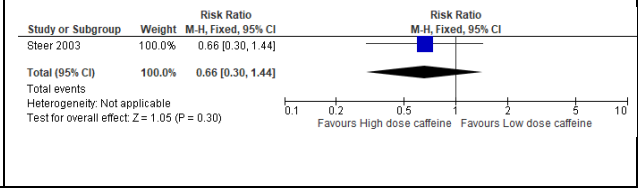
Apnoea – continuous[a]



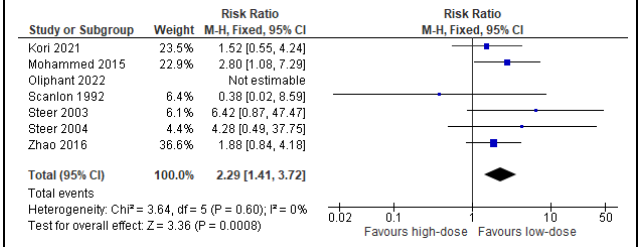
Bronchopulmonary Dysplasia



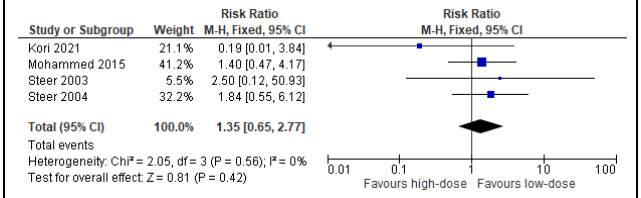
Patent ductus arteriosus



Tachycardia



Intraventricular hemorrhage



Death before primary discharge[b]

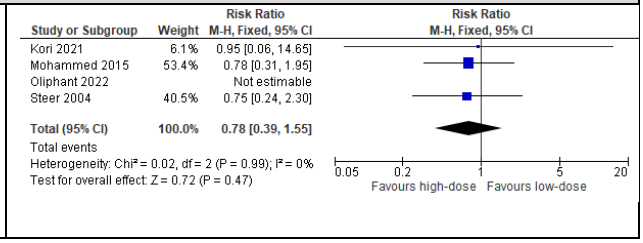


Figure 6.2 Forest plots of the neonatal/infant primary outcome, and critical and selected important secondary outcomes

[a]Apnoea results are presented as a dichotomous measure (for caffeine vs placebo comparison) or a continuous measure (for high vs low dose comparison), based on how apnoea was measured in the majority of studies in each comparison. The forest plot for the alternate measure for each comparison is presented in Figure 6.3. [b]Death before one year of age was also considered a critical outcome, but only 1 study reported this measure (in the low vs. high dose comparison). This data is included in Figure 6.3, with other secondary outcomes.

6.4.4 High-dose vs. low-dose caffeine

6.4.4.1 Primary outcome

Neonatal/infancy: For the primary outcome of apnoea (continuous), evidence of very low certainty from four trials showed possible benefit from receiving high-dose caffeine compared to low-dose caffeine, although the effect size was small (mean difference [MD] -0.2, 95% CI -0.3, -0.2, 560 infants)(Table 6.4)(Mohammed et al., 2015; Scanlon et al., 1992; Steer et al., 2004; Zhao et al., 2016). There was statistical heterogeneity (I^2=87%) among trials, although the direction of effect consistently favoured high-dose caffeine

Figure 6.2). In sensitivity analysis, exclusion of one trial at high risk of bias (Scanlon et al., 1992), did not alter the results (MD -0.2, 95% CI -0.3, -0.2, 530 infants, I^2=81%).

Other epochs: No trials of high-dose vs. low-dose caffeine reported on neurocognitive impairment.

6.4.4.2 Secondary outcomes

Moderate certainty evidence from four trials showed probable benefit for BPD with high-dose vs. low-dose caffeine (RR 0.71 95% CI 0.55, 0.91, 586 infants, I^2=0%)(Table 6.4). Evidence of very low certainty from seven trials suggested that high-dose vs. low-dose caffeine may increase the rate of tachycardia (RR 2.29 95%CI 1.41, 3.72, 839 infants, I^2=0%)(Table 6.4). The evidence was too uncertain to determine the effect of high-dose vs. low-dose caffeine on other neonatal outcomes (Table 6.4;

Figure 6.3). For the critical outcome of survival without neurosensory impairment in early childhood low certainty evidence from one trial means benefit of high-dose vs. low-dose caffeine could not be excluded (RR 0.92 95%CI 0.82, 1.03, 236 children)(Table 6.4).

6.4.4.3 Secondary analysis

There were insufficient data to undertake the planned subgroup analyses.

Table 6.4 GRADE summary of findings for low vs high dose caffeine comparison

Outcome	Certainty Assessment					Number of patients[a]		Effect		Certainty	Importance
	Number of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	High dose caffeine	Low dose caffeine	Relative (95% CI)	Absolute (95% CI)		
Neonatal and infant epoch[b]											
Apnoea (dichotomous outcome)	2 (Kori et al., 2021; Oliphant et al., 2023)	Not serious	Serious[c]	Not serious	Very serious[d]	16/94 (17.0%)	15/89 (16.9%)	**RR 1.01** (0.59 to 1.75)	2 more per 1,000 (from 69 fewer to 126 more)	⊕◯◯◯ Very low	Critical
Apnoea (continuous outcome)	4 (Mohammed et al., 2015; Scanlon et al., 1992; Steer et al., 2004; Zhao et al., 2016)	Serious[e]	Very serious[f]	Not serious	Not serious	0.1 events / day (N=276)	0.5 events / day (N=284)	-	MD 0.2 lower (0.3 lower to 0.2 lower)	⊕◯◯◯ Very low	Critical
Intraventricular haemorrhage	4 (Kori et al., 2021; Mohammed et al., 2015; Steer et al., 2003, 2004)	Not serious	Not serious	Not serious	Very serious[d]	16/305 (5.2%)	11/266 (4.1%)	**RR 1.35** (0.65 to 2.77)	14 more per 1,000 (from 14 fewer to 73 more)	⊕⊕◯◯ Low	Critical
Death before primary hospital discharge	5 (Kori et al., 2021; Mohammed et al., 2015; Oliphant et al., 2023; Steer et al., 2004)	Not serious	Not serious	Not serious	Very serious[d]	21/356 (5.9%)	28/357 (7.8%)	**RR 0.76** (0.44 to 1.30)	19 fewer per 1,000 (from 44 fewer to 24 more)	⊕⊕◯◯ Low	Critical
Death before one year of age	1 (Steer et al., 2004)	Serious[g]	Serious[h]	Not serious	Very serious[d]	7/116 (6.0%)	10/120 (8.3%)	**RR 0.72** (0.29 to 1.84)	23 fewer per 1,000 (from 59 fewer to 70 more)	⊕◯◯◯ Very low	Critical
Intermittent hypoxaemia	1 (Oliphant et al., 2023)	Not serious	Serious[h]	Not serious	Very serious[d]	4.8 events/hr (N=41)	4.6 events/hr (N=44)	-	MD 0.2 higher (2.0 lower to 2.4 higher)	⊕◯◯◯ Very low	Important
Bronchopulmonary dysplasia	4 (Kori et al., 2021;	Not serious	Not serious	Not serious	Serious[i]	71/289 (24.6%)	104/297 (35.0%)	**RR 0.71** (0.55 to 0.91)	102 fewer per 1,000 (from 158	⊕⊕⊕◯ Moderate	Important

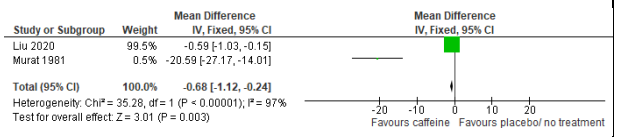
	Mohammed et al., 2015; Steer et al., 2004; Zhao et al., 2016)								fewer to 32 fewer)		
Duration of mechanical ventilation	4 (Mohammed et al., 2015; Steer et al., 2003, 2004; Zhao et al., 2016)	Serious[j]	Serious[k]	Not serious	Not serious	2.8 days (N=347)	1.7 days (N=310)	-	MD 0.54 lower (1.3 lower to 0.2 higher)	⊕⊕◯◯ Low	Important
Duration of positive pressure support	3 (Kori et al., 2021; Mohammed et al., 2015; Steer et al., 2004)	Not serious	Not serious	Not serious	Very serious[d]	3.8 days (N=220)	4.3 days (N=224)	-	MD 1.1 lower (2.9 lower to 0.7 higher)	⊕⊕◯◯ Low	Important
Tachycardia	7 (Kori et al., 2021; Mohammed et al., 2015; Oliphant et al., 2023; Scanlon et al., 1992; Steer et al., 2003, 2004; Zhao et al., 2016)	Serious[l]	Not serious	Not serious	Very serious[d]	54/435 (12.4%)	21/404 (5.2%)	**RR 2.29** (1.41 to 3.72)	67 more per 1,000 (from 21 more to 141 more)	⊕◯◯◯ Very low	Important
Patent ductus arteriosus	1 (Steer et al., 2003)	Not serious	Serious[h]	Not serious	Very serious[d]	12/85 (14.1%)	9/42 (21.4%)	**RR 0.66** (0.30 to 1.44)	73 fewer per 1,000 (from 150 fewer to 94 more)	⊕◯◯◯ Very low	Important
Retinopathy of Prematurity	2 (Mohammed et al., 2015; Steer et al., 2004)	Not serious	Not serious	Not serious	Very serious[d]	8/153 (5.2%)	14/163 (8.6%)	**RR 0.60** (0.26 to 1.40)	34 fewer per 1,000 (from 64 fewer to 34 more)	⊕⊕◯◯ Low	Important
Growth velocity – weight gain	4 (Mohammed et al., 2015; Oliphant et al., 2023; Steer et al., 2003, 2004)	Not serious	Very serious[m]	Not serious	Not serious	8.6 g/kg/d (N=302)	9.5 g/kg/d (N=268)	-	MD 0.3 lower (1.2 lower to 0.7 higher)	⊕⊕◯◯ Low	Important
Growth velocity – linear growth	1 (Oliphant et al., 2023)	Not serious	Serious[h]	Not serious	Very serious[d]	0.8 cm/week (N=37)	0.7 cm/week (N=40)	-	MD 0.1 higher (0.1 lower to 0.3 higher)	⊕◯◯◯ Very low	Important
Growth velocity –	1	Not	Serious[h]	Not serious	Very serious[d]	0.5 cm/week	0.5 cm/week	-	MD 0.0	⊕◯◯◯	Important

head circumference	(Oliphant et al., 2023)	serious				(N=37)	(N=40)		(0.1 lower to 0.1 higher)	Very low	
Time to full enteral feeds	2 (Kori et al., 2021; Mohammed et al., 2015)	Not serious	Not serious	Not serious	Serious[i]	12.3 days (N=100)	11.5 days (N=98)	-	MD 1.5 fewer days (3.4 fewer to 0.4 more)	⊕⊕⊕◯ Moderate	Less important
Early childhood epoch[n]											
Survival without neurosensory impairment	1 (Steer et al., 2004)	Not serious	Serious[h]	Not serious	Serious[i]	95/120 (79.2%)	100/116 (86.2%)	**RR 0.92** (0.82 to 1.03)	69 fewer per 1,000 (from 155 fewer to 26 more)	⊕⊕◯◯ Low	Critical

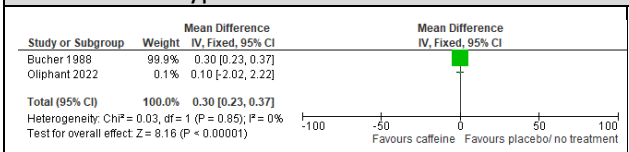
[a]For continuous outcomes values represent weighted mean. [b]In the neonatal and infant epoch, the critical outcomes of neurocognitive impairment, survival without neurosensory impairment and cerebral palsy were not reported by any included studies. [c]Although 2 studies reported apnoea as an outcome, the apnoea outcome did not occur in any participants in Oliphant 2022, and hence only a single study (Kori 2021) contributed data to this analysis. [d]OIS criteria not met (total population less than half of OIS, resulting in downgrading by two steps). [e]One included studies (Scanlon 1992) was judged to have high overall risk of bias for this outcome, two (Steer 2004 & Zhao 2016) were judged to have some concerns overall for this outcome and one (Mohammed 2015) was judged to have a low overall risk of bias for this outcome. [f]I^2 = 87%. [g]Data are from a single study (Steer 2004) with a high risk of incomplete outcome data. [h]Results from a single study only. [i]OIS criteria not met (total population less than OIS, resulting in downgrading by one step). [j]Two included studies (Steer 2004 & Zhao 2016) were judged to have some concerns overall for this outcome and two (Mohammed 2015, Steer 2003) were judged to have a low overall risk of bias for this outcome. [k]I^2 = 49%. [l]One included study (Scanlon 1992) was judged to have high overall risk of bias for this outcome; two (Steer 2004 & Zhao 2016) were judged to have some concerns overall for this outcome; and the remaining four studies (Kori 2021, Mohammed 2015, Oliphant 2022 & Steer 2003) were judged to have a low overall risk of bias for this outcome. [m]I^2 = 69%. [n]In the early childhood epoch, the critical outcomes of death, neurocognitive impairment, motor impairment, cerebral palsy, hearing impairment and visual impairment, and the important outcomes of emotional behavioural difficulties, asthma/wheeze, growth – height and growth - height were not reported by any included studies.

Caffeine vs placebo/no treatment

Apnoea - continuous



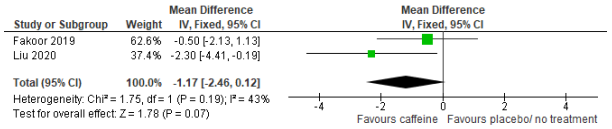
Intermittent hypoxaemia



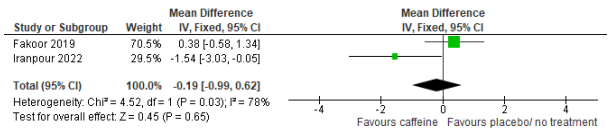
Retinopathy of Prematurity

No studies with data

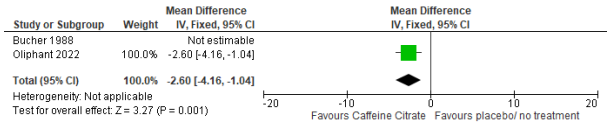
Duration of mechanical ventilation



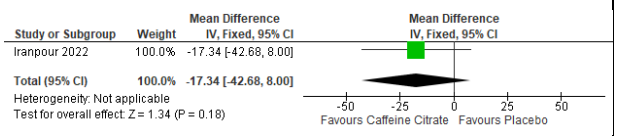
Duration of positive pressure support



Growth velocity - weight gain



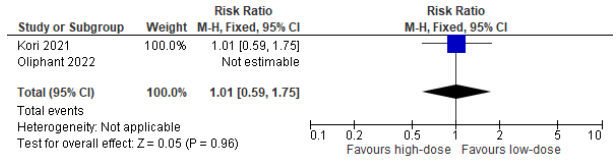
Time to establish full oral feeds



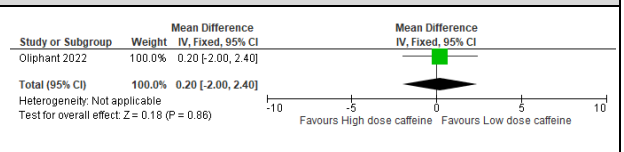
Death before one year of age

High vs low dose caffeine

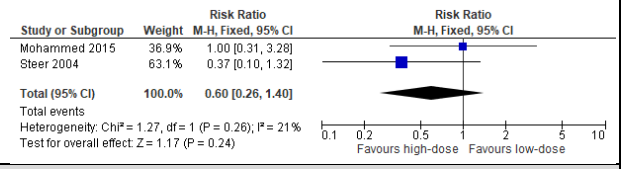
Apnoea - dichotomous



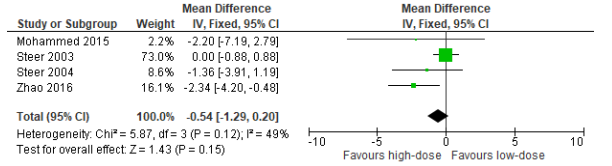
Intermittent hypoxaemia



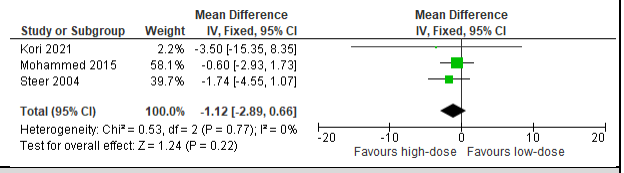
Retinopathy of Prematurity



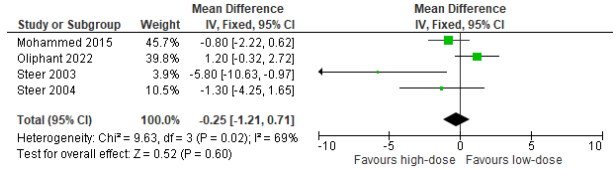
Duration of mechanical ventilation



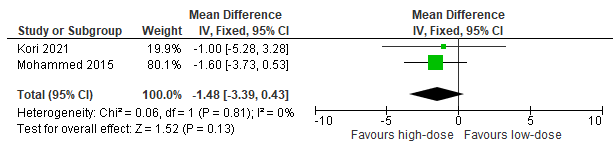
Duration of positive pressure support



Growth velocity - weight gain



Time to establish full oral feeds



Death before one year of age

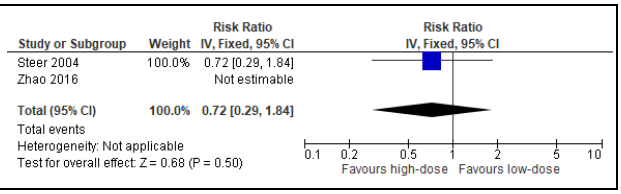
No studies with data	

Figure 6.3 Forest plots of additional neonatal/infant secondary outcomes

6.5 Discussion

Currently, there is no high certainty evidence for use of caffeine in preterm neonates for any critical or important outcomes from birth to adolescence. However, in very preterm neonates, caffeine therapy probably reduces the rate of BPD and PDA; possibly increases survival without neurosensory impairment in early childhood and reduces cerebral palsy; and probably reduces the rate of neurocognitive impairment and motor impairment in middle childhood. Although traditionally given for apnoea of prematurity, the evidence supporting this benefit of caffeine was of very low certainty, given the considerable heterogeneity in contributing studies, RoB inherent in these studies and the relatively small number of infants for whom data are available.

In general, evidence for the relative effectiveness of high vs. low dose caffeine is even less certain, but moderate certainty evidence indicates higher doses probably reduce the rate of BPD more than lower ones, and very low certainty evidence suggests higher doses may cause more tachycardia.

Quantifying the effect of caffeine on longer-term outcomes is limited by the available studies, with only two trials presenting any outcome data beyond the neonatal/infancy period (one in each comparison) and only one of those reporting significant follow-up assessments and results. As a result, meta-analysis was not possible in epochs beyond neonatal/infancy, and the certainty of the findings is limited. No information was available comparing the effects of high and low dose caffeine on neurodevelopmental outcomes.

This review provides a current and comprehensive summary of the available literature on the use of caffeine in preterm infants and included 15 RCTs covering 3,530 premature infants. In contrast to previous systematic reviews, we included all studies enrolling preterm infants (<37 weeks' PMA), rather than limiting the population to

infants born at earlier gestational ages (Brattström et al., 2018; Bruschettini et al., 2023; Vliegenthart et al., 2018). This was because moderate and late preterm infants may experience apnoea of prematurity (Henderson-Smart, 1981) and are known to have episodes of intermittent hypoxemia (Williams et al., 2018) , and so may also benefit from caffeine therapy, though the evidence in this are remains uncertain. Previous systematic reviews have addressed a single question (either caffeine vs. placebo, or high vs. low dose regimens), rather than considering both together as in this review, and have often included trials of other methylxanthines which are no longer routinely used in addition to caffeine. Furthermore, these older systematic reviews did not apply the explicit and comprehensive GRADE criteria to the assessment of the quality of the evidence, and so have perhaps overstated the certainty of the evidence underlying their recommendations (Henderson-Smart & De Paoli, 2010b, 2010a). Recently published Cochrane reviews present GRADE analysis for only a subset of outcomes (Bruschettini et al., 2023; Marques et al., 2023), whereas in this review GRADE analysis was performed for all outcomes with available data.

The Cochrane Neonatal Group have recently published reviews of caffeine dosing regimens in preterm infants (Bruschettini et al., 2023) and of methylxanthines vs placebo / no treatment (Marques et al., 2023) . However, this later Cochrane review includes a substantial number of trials that used other methylxanthines (aminophylline and theophylline) no longer routinely used in clinical practice, and does not include some of the more recent trials of caffeine (Fakoor et al., 2019; Iranpour et al., 2022; Oliphant et al., 2023) included in this review. Both the Cochrane and other reviews of caffeine low dose vs high dose caffeine therapy have concluded that higher doses of caffeine are (Chen et al., 2018) or may be (Brattström et al., 2018; Bruschettini et al., 2023) more effective in reducing the occurrence of extubation failure. Analysis of the evidence for the important outcome of BPD has resulted in different conclusions in different reviews; either that higher doses reduce the rate of BPD compared with lower doses (Bruschettini et al., 2023; Chen et al., 2018; Vliegenthart et al., 2018) or that higher

doses do not alter the rate of BPD (Brattström et al., 2018). In contrast to previously published reviews (Bruschettini et al., 2023; Chen et al., 2018; Vliegenthart et al., 2018), we pre-defined high (>10 $mg.kg^{-1}day^{-1}$ caffeine citrate equivalent) and low doses (≤10 $mg.kg^{-1}day^{-1}$) of caffeine on the basis of maintenance dose, avoiding cross-over of doses included in the comparison groups and hence producing a more meaningful comparison. This may explain the differences in findings, as some other reviews have included trials where the only difference in dose was in the loading dose (Brattström et al., 2018; Vliegenthart et al., 2018), or where both doses used would be considered low doses in current clinical practice (Vliegenthart et al., 2018). We also included all trials where infants received caffeine, regardless of indication, as we wished to include apnoea given for the prevention of neurodevelopmental impairment, as well as solely for the prevention or treatment of apnoea, or to assist in extubation.

As a systematic review, the robustness of the conclusions is limited by the quality and quantity of the included studies. The caffeine vs. placebo comparison identified and included a number of recent studies that have not previously been included in published meta-analysis (Fakoor et al., 2019; Iranpour et al., 2022; Zhao et al., 2016), but some of these studies have domains with high risk of bias and there was a high degree of heterogeneity between studies, limiting the quality of the evidence. Furthermore, this comparison is dominated by a single study, which contributed over 2000 infants of the 2,592 participants identified (Schmidt et al., 2007) We had planned to undertake subgroup analysis to assess the effectiveness of caffeine based on the indication for use (prophylaxis, treatment of apnoea or for extubation, late hypoxaemia or established lung disease) and by gestation (extremely, very, moderately or late preterm) but were unable to undertake these analyses due to the lack of data broken down by these variables in the identified studies. The lack of data on the effectiveness of caffeine in these different subgroups remains an important evidence gap, and further research is needed to inform evidence-based decision-making in clinical care.

While caffeine is widely used in neonatal units, the evidence remains uncertain and other reviews on the topic have called for further clinical trials in this area (Brattström et al., 2018; Chen et al., 2018; Moschino et al., 2020). We join previous authors in this call for further research, and this systematic review indicates the evidence gaps where more information is required to guide clinical practice. In particular, there is a lack of data on long term outcomes following different doses of caffeine in the neonatal period, and longer-term follow-up of infants in recent trials should be conducted to address this evidence gap. This is particularly important given the indications in this and other (Brattström et al., 2018; Chen et al., 2018; Vliegenthart et al., 2018) meta-analyses that higher doses may be more effective in improving short term outcomes, as use of higher doses in clinical practice should be preceded by evidence of the long-term safety of such doses. In addition to dose, more information is required on how the indication for treatment, infant gestation, duration of treatment/stopping and timing of initiation and discontinuation influence outcomes. Whether caffeine should be used during mechanical ventilation should also be ascertained, and if the dose should be decreased with tachycardia or increased with gestational age.

6.6 Conclusion

Caffeine administered to preterm infants probably reduces BPD, PDA, and motor impairment, with higher doses probably conferring additional benefit in reducing BPD but possibly increasing the occurrence of tachycardia. However, most of the current evidence is of low certainty and establishing the optimal dose requires more research, including long-term outcome assessment.

7. Discussion

7.1 What this thesis adds to current knowledge

This thesis describes a dose-finding and feasibility randomised controlled trial of caffeine citrate for the prevention of intermittent hypoxaemia in late preterm babies. The development and application of laboratory processes necessary to support the trial are also presented, including formulation and stability testing of trial medication and quantification of salivary caffeine concentrations in trial participants. It also includes a systematic review of caffeine use in preterm infants, synthesising and summarising the evidence for this common neonatal medicine and highlighting the areas where evidence is lacking, and further research is required.

The Latte Dosage trial demonstrated the effectiveness of caffeine at doses of 10 and 20 $mg.kg^{-1}.day^{-1}$ in reducing intermittent hypoxaemia, without significant adverse effects on growth velocity, sleep or gastrointestinal reflux, but with an increase in tachycardia. Laboratory work in support of this trial characterised an oral formulation of caffeine citrate suitable for neonatal consumption, with chemical, physical and microbiological stability data to support its use at concentrations ranging from 5 $mg.mL^{-1}$ to 20 $mg.mL^{-1}$. The Latte Dosage Trial was a five-armed trial, with medication administered by nursing and midwifery staff and/or parents daily for three to six weeks. Maintaining blinding required that the same volume of medication be administered to all infants. As the range of concentrations required to achieve this were not available commercially, the formulation was manufactured at hospital sites at four different concentrations, to allow a standard volume of 2 $mL.kg^{-1}$ as a loading dose followed by 1 $mL.kg^{-1}$ daily maintenance dose to be administered to all infants in the trial. This is a novel approach for a neonatal trial, where dose-finding trials of this scope and duration are unusual. This approach was effective in maintaining blinding but did require considerable support from the pharmacy departments at the trial sites. In addition, balancing stock availability at all strengths with the short expiry associated with medications

manufactured extemporaneously resulted in wastage and required a level of stock management that may not be sustainable in a larger trial or where there was less intensive academic clinician involvement.

Laboratory work also involved the development of a method for extracting caffeine from small-volume saliva samples. This was applied to determine the concentration of caffeine in saliva in trial participants, allowing estimation of the relationship between caffeine dose and resulting caffeine concentration in late preterm infants. Though there were some challenges in the collection of saliva from infants using swabs (see section 7.3.1 below), obtaining saliva samples was relatively straightforward and acceptable to parents. The method was effective in determining the caffeine concentration and allowed us to be confident that the different doses used in the trial resulted in different caffeine concentrations within the infants, thereby accounting for the differences in outcomes observed between the groups. Knowledge of the caffeine concentrations attained in the different groups became particularly important when trying to understand the reasons for the unexpected findings in the 15 $mg.kg^{-1}.day^{-1}$ group, namely, it allowed us to rule out non-compliance as a reason for the results in this group not following the dose-response relationship seen with the other doses used.

Application of research findings to clinical practice requires review of the available research and synthesis that brings together all the relevant evidence. This is commonly in the form of a systematic review and meta-analysis, often using methods developed by the Cochrane Collaboration. Existing Cochrane reviews relating to caffeine use for apnoea in preterm infants were outdated, addressed a single question and often included other methylxanthines, such as theophylline, no longer used in clinical practice (Henderson-Smart & Davis, 2010; Henderson-Smart & De Paoli, 2010b, 2010a; Henderson-Smart & Steer, 2010). Though the Cochrane review relating to high vs low doses of caffeine has been recently updated (Bruschettini et al., 2023), other questions were not in scope, and required synthesis of the current evidence to answer. Therefore,

a comprehensive systematic review of caffeine for apnoea and the prevention of neurodevelopmental impairment in preterm infants was performed.

The systematic review (Chapter 6) provides a current and comprehensive summary of the available literature and details the evidence for both caffeine vs. placebo, and high vs. low dose regimens. It included 15 randomised controlled trials (nine in the caffeine versus placebo comparison and seven in the high versus low dose comparison, with one contributing to both), which enrolled a total of 3,530 infants. Despite this, and widespread use of caffeine in clinical practice (Grainge et al., 2022; Gray & Chauhan, 2016; Puia-Dumitrescu et al., 2019; Siddhi & Foster, 2023a), the effect estimates obtained by meta-analysis were not of high certainty for any of the pre-specified outcomes.

Moderate-certainty evidence supports the use of caffeine for the prevention of BPD (RR 0.77; 95% CI 0.69,0.86) and PDA (RR 0.67; 95% CI 0.60, 0.74), and indicates that higher doses of caffeine may be more effective for preventing BPD than lower ones (RR 0.71; 95% CI 0.55, 0.91). However, for many other critically important short- and long-term outcomes the evidence was of low or very-low certainty, meaning we cannot be sure that the summary effect estimate is close to the true effect. This includes use for apnoea, the prevention or treatment of which is generally the indication for prescribing caffeine, and ongoing instances of which are frequently used as an indication for dose increase. While caffeine appears to reduce the rate and occurrence of apnoea compared to placebo, the evidence for this is of very low certainty, and the evidence for higher doses over lower ones is conflicting.

Findings such as this highlight the areas in which data are lacking and further research is required, especially for a drug that is so widely used in clinical practice (Grainge et al., 2022; Gray & Chauhan, 2016). In particular, caffeine is given for at least three different indications: facilitation of extubation, prevention of apnoea in infants

considered at high risk (generally based on gestational age) and treatment of documented apnoea (Grainge et al., 2022; Gray & Chauhan, 2016). The review protocol planned for subgroup analysis by the primary indication for which caffeine was prescribed, but the majority of trials did not provide these data, making subgroup analysis impossible. Likewise, subgroup analysis by gestation length (extremely, very, moderate and late preterm) was not possible. Data to inform evidence-based prescribing in these areas are still required, and further trials are needed to establish the effectiveness of caffeine for each separate indication, and to determine the optimal dose for extremely, very and moderately preterm infants.

7.2 Follow-on research underway: The Latte Trial

The Latte Dosage Trial demonstrated the effectiveness of caffeine in reducing intermittent hypoxaemia, but ultimately it is long-term neurodevelopment that is of most clinical significance.

The Latte trial (Alsweiler, 2022), which follows on from the Latte Dosage Trial, is a two-arm, double-blind multi-centre phase III randomised controlled trial designed to determine if a daily dose of 20 $mg.kg^{-1}$ caffeine citrate (combined with a loading dose of 40 $mg.kg^{-1}$) in late preterm infants improves neurodevelopmental outcomes, as assessed using the cognitive, language (receptive and expressive) and motor (gross and fine motor) scales of the Bayley Scales of Infant and Toddler Development, Fourth Edition (BSID-IV) at 2 ½ years corrected age. It has received funding from the Health Research Council of NZ (HRC 22/305) and began recruitment at two sites in Auckland in May 2023, with a number of additional sites across New Zealand currently working through trial set-up procedures. Recruitment of the 478 babies required to adequately power this trial is projected to take two years, with a follow up period for each participant of 2½ years.

In demonstrating that caffeine reduced the occurrence of intermittent hypoxaemia in late preterm infants, hence preventing periods of hypoxaemia that may affect brain development, we have demonstrated a mechanism by which caffeine might be expected to improve neurodevelopmental outcomes. The dose-finding and feasibility study reported herein has determined an appropriate dose for use in this population, and generated learnings in many aspects of the study (such as the need for a more palatable formulation) that are being applied to the full study. Completion of the Latte trial is important to establish if caffeine, given at doses sufficient to reduce intermittent hypoxaemia, does in fact improve long term neurodevelopmental outcomes. This trial will add significantly to knowledge of the long-term effects of caffeine, as currently only a single trial (the CAP trial) has assessed children and reported longer-term outcomes following caffeine therapy.

Indigenous infants in affluent countries are more likely to be born prematurely than non-indigenous infants, and have higher rates of infant mortality (Edmonds et al., 2021; Smylie et al., 2010). In New Zealand, Māori account for 28.3% of all infants born late preterm and in a study of NZ births between 2010-2014, 4.7% of Māori infants were born late preterm, compared with 4.2% of non-Māori infants, indicating health inequity between these two groups (Edmonds et al., 2021). The Latte Dosage Trial recruited 14% Māori participants overall, resulting in under-representation of Māori in the study. In order to ensure equal explanatory power (mana whakamārama) for both groups, the Latte Trial has a strong focus on cultural safety and recruitment of Māori participants and aims to recruit equal numbers of Māori and non-Māori infants.

7.3 Research challenges

7.3.1 Laboratory analysis

As part of the Latte Dosage Trial, we aimed to determine the caffeine concentrations in participating infants, to confirm that the different doses of caffeine resulted in different

caffeine concentrations among groups and was not unduly affected by maternal intake in breastfeeding infants. This was achieved by analysing saliva samples for caffeine concentrations as described in Chapters 3 and 4.

While mothers participating in the trial were able to expectorate saliva directly into a marked test-tube which allowed immediate visualisation of the volume collected, infants (aged 36-38 weeks PMA at the time) required the use of swabs to collect saliva directly from within the mouth. Sample collection was conducted by the researcher (or the parent if they preferred) at the two-week visit, using SalivaBio Infant Swabs (Salimetrics, Carlsbad, CA, USA). The swabs were then placed in the top portion of the collection tube and returned to the laboratory, where they were centrifuged to extract the saliva from the swab and collect it in the lower portion of the tube. The top portion of the tube and the swab was then discarded, and the extracted saliva frozen until analysis was undertaken.

Experience with the SalivaBio swabs was variable and presented challenges for the research team. Saliva-laden swabs do not appear appreciably different from dry ones, and it was thus impossible to know if sufficient saliva had been obtained until the swab had been centrifuged in the laboratory, by which stage it was too late to obtain further samples if the volume of saliva extracted proved insufficient. In an attempt to overcome this, research staff adapted the collection method to use both ends of the swab, holding each end in the infant's mouth for 2 minutes, or as long as was tolerated. We also refined the laboratory analysis method to use the smallest possible volume of saliva for infant samples (10µL in cases where the sample was insufficient for the standard 50µL volume), but there still remained some infants for whom the saliva collection failed (no saliva could be extracted from the collection swab). There were no infant characteristics identified that predicted failed sample collection.

7.3.2 Practical challenges within the Latte Dosage Trial

7.3.2.1 Recruitment

Recruitment of babies to the Latte Dosage Trial occurred within the first 72 hours after birth and included both babies admitted to the neonatal intensive care units at Starship Child Health and Kidz First Children's Hospital and those rooming-in with their mothers on the corresponding postnatal wards at National Women's Health and Middlemore Hospital in Auckland, New Zealand. These two different settings in which preterm infants were cared for influenced recruitment rates in different ways, with recruitment on the postnatal wards presenting additional challenges over that occurring in NICU.

Babies admitted to NICU may be viewed by their parents as being particularly unwell (compared to an objective assessment based on a standardised scoring system)(Brooks et al., 2012) and parents who report their baby's medical condition as more serious have significantly higher rates of participation in clinical trials (Morley et al., 2005; Weiss et al., 2021). Even when trials do not provide direct benefit to the baby participating, parents of babies in neonatal units consider that their participation will be of benefit to other babies in the future (Morley et al., 2005). The neonatal units involved in this trial participate in several clinical trials at any one time, and staff within the unit are used to supporting these. Furthermore, members of the Latte Dosage Trial team worked clinically on each of the neonatal units involved in the study and have longstanding relationships with other neonatal staff. Given the acuity of the neonatal unit, nursing staff working there typically care for fewer patients at any one time and spend more time with each baby and parent than their colleagues on postnatal wards, allowing them to develop trusting relationships. This encourages participation in the clinical trial and assists in retention of participants as support is provided in administering medication and completing trial measurements (such as oximetry recordings), as well of reinforcing the potential benefits of clinical trial participation.

In contrast, on the postnatal wards mothers and babies are cared for primarily by midwives, whose professional philosophy prioritises the normality of birth and the postnatal period. Midwife-to-patient ratios are significantly lower on postnatal wards than the nurse-to- patient ratio in the NICU, and this was particularly pronounced during the course of the Latte Dosage trial, which coincided with a period of midwifery staffing crisis in Auckland (Auckland District Health Board, 2019). This resulted in less time being available for clinical staff to support new parents with tasks such as trial medication administration, and anecdotally lower overall levels of support for the trial. However, there are no available data investigating the success of clinical trial recruitment between NICUs and postnatal wards and this is a potential area for further research. Documenting any difference in recruitment rates and investigating the reasons for these would allow issues to be addressed in future clinical trials to optimise recruitment.

Recruitment was also affected by the circumstances leading to trial eligibility. In many cases, late preterm delivery had been unexpected. Parents were often shocked to have had their baby born earlier than they expected and were not necessarily aware of the implications of preterm birth on long-term outcomes. While for some parents, explanation of these implications meant they were keen to participate in a trial that may improve long term development, for others the idea was too overwhelming at the time of recruitment. In addition, the short period available for trial enrolment, while necessary for medication effect, limited the time available for parents to consider the decision to participate or not. Depending on the day and time of birth, some infants were not able to be identified and seen by research staff in sufficient time for their parents to make an informed decision within the 72-hour window.

7.3.2.2 Administration of the trial medication

A number of parents found administering the trial medication to their babies difficult due to the volume involved (1 $mL.kg^{-1}.day^{-1}$, which averaged 2.6 mL per dose at the start

of the trial), despite advice and support from medical, nursing, midwifery and/or pharmacy staff members. Consideration was given to manufacturing a more concentrated formulation of caffeine citrate to overcome this. However, the bitter taste of caffeine meant that at 20 $mg.mL^{-1}$ (the highest concentration used in the clinical trial, and equivalent to that available commercially in NZ) the oral liquid tasted unpleasant, and attempts at further concentrating the solution to 40 $mg.mL^{-1}$ (which would have enabled reducing the volume to 0.5 $mL.kg^{-1}.day^{-1}$) resulted in a solution that was considered by all investigators to be too unpalatable for use.

Several parents reported that their infants appeared not to like the taste of the trial medicine. This is understandable, given the bitter taste of the solution, though this is comparable to the formulation available commercially and used clinically in NZ. This is in accordance with recommendations to avoid excipients in neonatal medicines where there is not a clear pharmaceutical requirement for their use, to minimise the risk of harm (Turner & Shah, 2015). However, current clinical use of caffeine most often occurs in very and extremely premature infants, who generally have nasogastric feeding tubes *in situ* which can be utilised for the administration of enteral medicines, bypassing the taste buds and avoiding the issue of taste.

Further trials in this area would be aided by the use of a more concentrated and palatable formulation with the hope that this would result in easier administration to participating babies, hence reducing withdrawal rates. Routine clinical use in a larger group of breastfeeding (or bottle feeding) infants would also be aided by a more appropriate formulation than that currently available in NZ. This may be achieved by importation of formulations licensed in overseas jurisdictions (which may have different taste characteristics, but are not readily available in more concentrated formulations), or by working with the NZ manufacturer to formulate a more palatable, and preferably more concentrated, solution. For the follow-on Latte trial, we have worked with a local pharmaceutical manufacturing company to formulate a more

palatable 20 mg.mL^{-1} formulation, utilising cherry flavouring and syrup to disguise the bitter taste of caffeine.

7.3.2.3 Withdrawals and missing data

The withdrawal rate for the Latte Dosage Trial was higher than was expected, at 33/132 (25%) overall, resulting in primary outcome data being unavailable for 19% of infants, compared to the 10% allowed for during power calculations. As a result, enrolment was increased from the originally calculated 120 infants to 132 infants to ensure the trial remained adequately powered.

Most parents who chose to withdraw cited difficulties in administering the trial medication to their baby and/or their struggle to cope with a late preterm baby following discharge as their reason for doing so. In addition to this, as a result of the COVID-19 pandemic we were forced to pause recruitment to the Latte Dosage Trial during periods of government-mandated lockdown and to withdraw some participants from the trial where adequate arrangements could not be made for support, trial assessments and follow-up to be completed in a timely manner.

New Zealand entered a strict 'Level 4' lockdown on 25 March 2020. Of the two patients participating in the trial at that point, one was close to discharge and had to be withdrawn as home visits and even travel to collect oximeters (should one have been sent home with the patient) was prohibited. The second participant was a recently enrolled 34-week gestation baby who was remaining in NICU for some time, and so was able to remain in the trial with the 2 week visit requirements conducted as a mix of trial assessments completed by staff involved in clinical care within the unit, and surveys completed remotely by parents utilising electronic data collection forms and email and phone contact with research staff.

Subsequent to the easing of restrictions, and the restarting of recruitment, the Auckland region was subject to a second, lower-level regional lockdown. This 'Level 3' lockdown did not necessitate the withdrawal of any patients, though recruitment was paused, and some visits had to be conducted with no physical patient contact, meaning anthropometric measurements could not be conducted for assessments occurring during this period. While only being directly responsible for a very small number of withdrawals, the reduction in physical and face-to-face practical support, both from the clinical trial staff and from other services and whānau, during the pandemic may have compounded the struggle in coping with a late preterm infant that many parents reported, and so indirectly may have contributed to the withdrawal of more participants than had been expected. However, any pandemic-related effects will have affected all groups equally, and so while the pandemic caused delays to the completion of the trial, it is unlikely to have affected the results.

Overall, withdrawal rates varied between a low of 17% in the placebo group, and a high of 30% in the 15 $mg.kg^{-1}.day^{-1}$ group. Fifteen infants across the four caffeine groups, but none in the placebo group, were withdrawn due to difficulties administering the study drug, the infant not tolerating the drug (spitting medication out or spilling) or parental or investigator concerns about side effects. While the rate of withdrawal was higher in the caffeine group, this is unlikely to have significantly influenced the primary outcome, which relied on an objective measurement coupled with blinding of parents and trial personnel, meaning the estimate of effectiveness is unlikely to have been affected. It is possible that the withdrawal of infants whose parents considered they had side effects or were not tolerating the drug may have affected the results of sleep and reflux questionnaires which were parent-scored. If parents who thought their child had side effects withdrew, leaving those without any perceived side effects in the trial, it is possible that the scores reported for the caffeine group could be better than if those who withdrew had also been included. However, the number of withdrawals due to intolerance or presumed side effects was small (the majority of the withdrawals were

due either to the parents changing their mind about participating in the trial, or experiencing difficulties with administering the medication), so the effect of withdrawal on these metrics is likely to be minimal. Additionally, I-GERQ-R scores were lower in the caffeine groups (representing fewer symptoms with caffeine than placebo), so even if this was under-representing the true incidence of symptoms, we can be reasonably confident that caffeine is not causing significant gastroesophageal symptoms.

7.3.2.4 Side effects

Irritability, restlessness and gastro-oesophageal reflux are considered possible side effects of caffeine citrate in infants, as in adults (Howell et al., 1981). It was thus noteworthy that in the Latte Dosage Trial there was no significant difference in parent-reported sleep scores (calculated using subscale nine on the Infant Behaviour Questionnaire-Revised, modified for neonates (Gartstein & Rothbart, 2003)) between caffeine (at any dose or overall) and placebo (Oliphant et al., 2023). Furthermore, symptoms of gastro-oesophageal reflux (measured using parental report assessed using I-GERQ-R (Kleinman et al., 2006) were clinically and statistically significantly lower in the 10 and 15 $mg.kg^{-1}$ groups compared to placebo at the two week timepoint, with no significant differences between caffeine and placebo in other dose groups or at term.

One participant in the Latte Dosage trial was found to have significant tachycardia, with a mean heart rate of 198 beats per minute and heart rate of >199 beats per minute for 48.9% of the time on overnight oximetry at 2 weeks of age. The infant was a patient on the post-natal ward at the time and, as a result of the overnight oximetry findings, was referred to the neonatal team and admitted to the NICU for an electrocardiogram, blood tests and monitoring. The clinical team were partially unblinded to treatment allocation (caffeine vs placebo), trial medication was withheld, and the infant's heart rate normalised. The baby remained in the trial but did not receive further trial medication, and was discharged home where the final oximetry recording was

completed. As a result of this case, the Data Monitoring Committee advised the Steering Committee to modify the protocol to include assessing the heart rate at 1-week post randomisation. This extra safety check was in place for the remainder of the Latte Dosage trial and has been incorporated into the follow-on Latte trial.

Tachycardia is a possible side effect of caffeine and other stimulant medications, with our systematic review finding evidence of very low certainty from seven trials to suggest that high-dose caffeine may increase the rate of tachycardia over that of low-dose caffeine (RR 2.29 95%CI 1.41, 3.72, 839 infants, I^2=0%). Tachycardia in neonates is often considered concerning as it may be a symptom of infection, cardiac pathology, dehydration, electrolyte disturbance or thyroid dysfunction, and can result in poor feeding, tachypnoea, sweating, lethargy and irritability (Moak, 2000). Heart rate is an important contributor to cardiac output, in conjunction with stroke volume (Desai & Macrae, 2022). In neonates, cardiac tissue is less compliant than in older children and adults due to a higher proportion of extracellular matrix and a higher ratio of type I to type II collagen within this matrix (Marijianowski et al., 1994). As a result, the ability to regulate stroke volume is limited in the neonate, and hence cardiac output is primarily dependant on heart rate (Ord & Griksaitis, 2019). An increase in heart rate thus increases cardiac output, improving perfusion and oxygen delivery to the tissues (Cohen et al., 2012). It is possible that caffeine exerts its effect, at least in part, by causing tachycardia and hence increasing oxygen delivery to the tissues.

Further research is needed to understand the mechanism of action of caffeine and its cardio- and cerebrovascular effects. The planned Latte Hearts and Minds study (a mechanistic sub-study within the Latte Study) will investigate this by assessing cardiac function, cerebral blood flow and regional oxygen saturation following administration of the trial medication (caffeine 20mg.kg^{-1} or placebo), to assess cerebral haemodynamic changes that may explain the mechanism of caffeine in affecting neurodevelopmental outcomes.

7.3.2.5 Dose selection

Overall, results from the Latte Dosage Trial were much as had been expected, with the 5 $mg.kg^{-1}.day^{-1}$ dose routinely used in very and extremely preterm infants proving ineffective in late preterm infants, and a dose-response relationship evident for most outcomes across the 5, 10 and 20 $mg.kg^{-1}.day^{-1}$ dose groups. However, the results for the 15 $mg.kg^{-1}.day^{-1}$ group did not fit this dose-response relationship and were not statistically significantly different from placebo for many outcomes. We investigated lack of compliance with trial medication as a possible reason for this discrepancy, but compliance assessed based on returned drug was comparable to other groups, and infant salivary caffeine concentrations in the 15 $mg.kg^{-1}.day^{-1}$ group were intermediate to those in the 10 and 20 $mg.kg^{-1}.day^{-1}$ group, indicating these infants did receive the study medication. No other significant differences between infants in this group and those in the other groups were identified, and we concluded that the results observed in this group were due to chance as a result of the small numbers in this trial.

The unexpected results in the 15 $mg.kg^{-1}.day^{-1}$ dose group complicated the selection of a dose for the main Latte Trial. Though 10 $mg.kg^{-1}.day^{-1}$ was effective in reducing the rate of intermittent hypoxaemia in the dosage trial, the lack of significant effect at 15 $mg.kg^{-1}.day^{-1}$ meant that we were concerned that 10 $mg.kg^{-1}.day^{-1}$ may ultimately prove too low to have a clinically significant long-term effect, and conducting the full trial with this dose ran the risk of dismissing a potentially effective treatment due to using an inadequate dose. After discussion with a multi-disciplinary team, a dose of 20 $mg.kg^{-1}.day^{-1}$ was selected for the main trial. This highest of the doses used in the dosage trial was the most effective in reducing the rate of intermittent hypoxaemia, increasing mean SpO_2 and reducing percentage of time with SpO_2<90%, without being associated with an increase in reflux symptoms or sleep disturbance, increase in duration of tube feeding or hospital stay, or reduction in growth velocity. Though the time with heart rate > 180 beats per minute was highest in this group, the mean heart rate was not

significantly different from other caffeine groups. In light of the recommendations from the Data Monitoring Committee during the dosage trial, a clinical check of the heart rate of infants in the trial has been included at one-week post-randomisation, and provision has been included to reduce the dose of caffeine to 10 $mg.kg^{-1}.day^{-1}$ if an infant is tachycardic.

7.3.3 Systematic review

The primary outcome for the neonatal epoch was apnoea, which was predefined this using the widely-accepted American Academy of Pediatrics definition (a *"pause in breathing of ≥20 seconds, or <20 seconds with bradycardia (HR <100 bpm), cyanosis or pallor"* (American Academy of Paediatrics, 2003)), with other definitions used by authors also being acceptable. However, during data extraction it became apparent that the issue was not with the definition of apnoea, but rather the way in which it was reported. Different trials variously reported apnoea as a rate over a range of periods of time, as a reduction of at least a given percentage from baseline, or as the occurrence of any apnoea or a given number of apnoeic events after treatment, and reported both medians and means of outcome measures. Statistical methods for converting medians to means were used to transform data (Wan et al., 2014), with rates calculated over a standardised 24-hour period for the purposes of meta-analysis. However, even with these statistical transformations, it was impossible to combine all apnoea outcomes into a single format, and instead apnoea was reported using two different measures – one as a continuous measure (in cases where the outcome was reported as a rate, and we were able to calculate from the reported results the mean number of apnoea per 24 hours), and the other as a dichotomous measure (in cases where we were able to establish from the presented data that apnoea had improved from baseline). Both outcomes were presented separately in the final review.

7.4 Further research opportunities

The most direct area of further research is the full Latte Trial, which followed on from this dose-finding and feasibility trial. The Latte Trial will establish if caffeine at a dose now known to decrease intermittent hypoxaemia improves long-term neurodevelopmental outcomes in late preterm infants. This study is already underway, and is described more fully in section 7.2 above. Other areas of potential future research arising from this thesis are described below.

7.4.1 Long-term follow up of Latte Dosage infants

The systematic review of caffeine reported in this thesis highlights the evidence gap that remains around the long-term effects of caffeine. Despite widespread clinical use, only one trial provides data from caffeine-exposed infants beyond two years of age, and then only for caffeine vs placebo. Long-term follow up of preterm infants in clinical trials is essential to fully understand the implications of interventions delivered in the neonatal period (MacBean et al., 2019) as long-term outcomes may be at odds with short-term ones (J. Davis et al., 2003; Zivanovic et al., 2014).

The Latte Dosage trial provides a cohort of infants who were randomised to different doses of caffeine and placebo and, while the study was not powered for neurodevelopmental outcomes, long term neurodevelopmental follow up of these infants would add to the available body of evidence on the topic, and could be included in future meta-analyses. In particular, assessment of motor skills would be important, given the long-term differences observed in this area in children treated with caffeine in the CAP trial (Doyle et al., 2014; Schmidt et al., 2017). In addition, there is the potential to include those who were in the placebo and 20mg.kg^{-1} groups in longer-term follow up alongside infants enrolled in the Latte trial.

7.4.2 Pharmacokinetic analysis at caffeine discontinuation using salivary caffeine levels

The Latte Dosage Trial included analysis of salivary caffeine concentrations in both mothers and infants and collection of maternal dietary intake of caffeine-containing foods and beverages. In the context of the trial, this data provided reassurance that the different doses of caffeine resulted in different salivary concentrations of caffeine in infants and allowed us to be confident that the differences in outcomes observed between groups were likely as a result of the different caffeine doses. However, it also identified that the concentration of caffeine was significantly higher in infants in the 20mg.kg^{-1}.day^{-1} group, with the relationship between caffeine dose and salivary caffeine concentration following an exponential relationship.

The half-life of caffeine in preterm infants is prolonged, and metabolic pathways and excretion changes with post-natal maturation of the renal and hepatic systems (Aranda & Beharry, 2020; Charles et al., 2008). It is common practice for infants to remain in hospital with monitoring for recurrence of apnoea following discontinuation of caffeine, though the duration of this monitoring period varies widely between units and neonatologists. It is also somewhat arbitrary, with 5-7 days being most common (Gray & Chauhan, 2016; Ji et al., 2020), though the caffeine level remains therapeutic for 11-12 days post-discontinuation of low-dose caffeine (Doyle et al., 2015). Given the issues associated with repeated blood sampling for pharmacokinetic assessments, studies assessing caffeine concentrations in infants post-discontinuation of therapeutic caffeine have generally been retrospective analyses of infants who had had caffeine levels measured at the discretion of their clinical team following discontinuation of treatment (Chung et al., 2022; Tabacaru et al., 2017). These infants are unlikely to be representative of infants discontinuing the clinical use of caffeine, and studies have not included the higher doses of caffeine that are becoming more common in clinical practice.

The method for determining salivary caffeine concentration in the Latte Dosage Trial could be applied to the determination of caffeine concentrations in infants weaning off caffeine, as it avoids the need for blood tests and can be used for collections both in hospital and in the home setting (Oliphant et al., 2023). This would allow prospective determination of the half-life of caffeine, and the period for which caffeine remains in the therapeutic range following discontinuation, in a more representative sample of infants who are ceasing caffeine therapy. This is particularly important, given the higher doses now being used more commonly in clinical practice and the higher concentrations found to be associated with these doses, and could provide an evidence base on which decisions about pre-discharge observation periods could be made.

7.4.3 Caffeine formulation work

Difficulties in administering caffeine liquids orally in the Latte Dosage trial highlighted the need for appropriate formulations of medicines for children and neonates, who are often poorly served by conventional pharmaceutical manufacturing. The formulation of caffeine citrate currently used clinically in New Zealand minimises excipients and therefore exposure of preterm infants to potentially harmful and avoidable substances, but the taste characteristics hindered oral administration (compared to nasogastric administration which is common in more premature infants). In addition, use in larger infants requires larger doses and hence a greater volume to be administered, which also proved problematic. Furthermore, the pH of the compounded trial product had a very low pH, with baseline values ranging between 2.43 (5 $mg.mL^{-1}$) and 1.90 (20 $mg.mL^{-1}$). This is comparable to results obtained from testing the commercial product available in NZ, though the nature of this product (being manufactured by a local compounding company, rather than a full-scale commercial manufacturer) means it is not registered by Medsafe in New Zealand, and consequently product specifications in the form of a medicine data sheet are not available. Commercially available oral caffeine products registered overseas have a considerably higher pH of 4.2-5.2 (Chiesi Pharmaceuticals, 2014; Phebra Pty Ltd, 2021). However, there is a lack of national or international

guidance on appropriate pH for medications administered by different routes in the neonatal population. This is an area where further research and the development of appropriate standards is required. This knowledge will guide further paediatric formulation development, both in this area and in wider neonatal clinical practice.

For the Latte trial we have worked with a local pharmaceutical manufacturer to develop a caffeine citrate formulation in a cherry flavoured syrup base to improve the taste, and thus the ability to administer the medication. Experience in administering this medication during the Latte trial will guide further developments in caffeine formulation. Additional research to develop and validate an improved formulation would aid translation to clinical practice if the Latte trial has a positive outcome, and use in the late preterm population is proven beneficial. Such a formulation would ideally have minimal excipients (specifically avoiding those known to be harmful to neonates, such as propylene glycol and ethanol)(Cuzzolin, 2017), be as concentrated as possible (40 $mg.mL^{-1}$ would be preferable as this would halve the volume required to 0.5 $mL.kg^{-1}.day^{-1}$), and be palatable for infants. Furthermore, as late preterm birth is common and widespread (Blencowe et al., 2012) and oral caffeine is well tolerated and can be administered outside of high-resource settings (World Health Organization, 2022), an inexpensive formulation with a long shelf-life and demonstrated stability under unfavourable environmental conditions would enable widespread use, including throughout the developing world, thus contributing to equity (Access to Medicine Foundation, 2022; Nabwera et al., 2021; Ozawa et al., 2019).

7.4.4 Other caffeine trials

Despite widespread use, the only trial of caffeine in preterm infants to report significant follow-up remains the CAP trial, which compared caffeine (at a dose of 5-10 $mg.kg^{-1}.day^{-1}$) and placebo started within the first 10 days of life, in infants weighing less than 1250g at birth (Schmidt et al., 2006). Clinical practice has undergone significant 'creep' since this study was first published, with higher doses commonly used in a wider population,

and increasing interest in early initiation – in some cases starting in the delivery room (Schmidt, 2023). However, one study of high (80 $mg.kg^{-1}$) vs standard (20 $mg.kg^{-1}$) loading doses of caffeine found a higher incidence of cerebellar haemorrhage in infants receiving a high dose of caffeine within 24 hours of birth, causing concern that early initiation of high doses may not be beneficial (McPherson et al., 2015). Many questions remain to be conclusively answered, including the optimal dose (and whether this differs at different gestational and postnatal ages), whether there are any differences based on indication for treatment, and the best time for starting and stopping therapy.

7.5 Conclusions

The Latte Dosage trial has demonstrated that caffeine citrate at a dose of 10 and 20 $mg.kg^{-1}.day^{-1}$ reduces intermittent hypoxaemia in late preterm infants and is well tolerated, though associated with an increase in tachycardia. Salivary caffeine concentrations are a reliable and acceptable way to assess caffeine concentrations in neonates. The ongoing Latte trial will determine if, as hypothesised, this reduction in intermittent hypoxaemia in turn leads to improvement in long term neurodevelopmental outcomes.

The systematic review of caffeine use for apnoea and neurodevelopment in preterm infants found that caffeine probably reduces BPD, PDA, and motor impairment, with higher doses probably conferring additional benefit in reducing BPD but possibly increasing the occurrence of tachycardia. However, it also highlighted the low certainty of much of the current evidence for neonatal and especially long-term outcomes. In particular, further research is required to identify the optimal dose of caffeine for the prevention and treatment of apnoea in very and extremely preterm infants.

8. References

Abitbol, C. L., Seeherunvong, W., Galarza, M. G., Katsoufis, C., Francoeur, D., Defreitas, M., Edwards-Richards, A., Master Sankar Raj, V., Chandar, J., Duara, S., Yasin, S., & Zilleruelo, G. (2014). Neonatal kidney size and function in preterm infants: What is a true estimate of glomerular filtration rate? *Journal of Pediatrics*, *164*(5), 1026-1031.e2. https://doi.org/10.1016/j.jpeds.2014.01.044

Access to Medicine Foundation. (2022). *Access to Medicine Foundation*. https://accesstomedicinefoundation.org/sectors-and-research/big-pharma

Adams-Chapman, I. (2006). Neurodevelopmental outcome of the late preterm infant. *Clinics in Perinatology*, *33*, 947–967. https://doi.org/10.1016/j.clp.2006.09.004

Adamson, S. L. (1991). Regulation of breathing at birth. *Journal of Developmental Physiology*, *15*(1), 45–52.

Adler, D., & Janssens, J. P. (2019). The pathophysiology of respiratory failure: Control of breathing, respiratory load, and muscle capacity. *Respiration*, *97*(2), 93–104. https://doi.org/10.1159/000494063

Agostoni, C., Buonocore, G., Carnielli, V. P., De Curtis, M., Darmaun, D., Decsi, T., Domellöf, M., Embleton, N. D., Fusch, C., Genzel-Boroviczeny, O., Goulet, O., Kalhan, S. C., Kolacek, S., Koletzko, B., Lapillonne, A., Mihatsch, W., Moreno, L., Neu, J., Poindexter, B., … Ziegler, E. E. (2010). Enteral nutrient supply for preterm infants: Commentary from the european society of paediatric gastroenterology, hepatology and nutrition committee on nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, *50*(1), 85–91. https://doi.org/10.1097/MPG.0B013E3181ADAEE0

Ainsworth, S. B. (Ed.). (2014). *Neonatal formulary 7 : drug use in pregnancy and the first year of life* (7th ed.). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118819494

Al-Turkait, A., Szatkowski, L., Choonara, I., & Ojha, S. (2022). Drug utilisation in neonatal units in England and Wales: a national cohort study. *European Journal of Clinical Pharmacology*, *78*(4), 669–677. https://doi.org/10.1007/S00228-021-03267-X/FIGURES/4

Alcorn, D., Adamson, T. M., Lambert, T. F., Maloney, J. E., Ritchie, B. C., & Robinson, P. M. (1977). Morphological effects of chronic tracheal ligation and drainage in the fetal lamb lung. *Journal of Anatomy*, *123*(3), 649–660.

Alhersh, E., Abushanab, D., Al-Shaibi, S., & Al-Badriyeh, D. (2020). Caffeine for the treatment of apnea in the neonatal intensive care unit: A systematic overview of meta-analyses. *Pediatric Drugs*, *22*(4), 399–408. https://doi.org/10.1007/S40272-020-00404-4

Ali, N., Pitson, D., & Stradling, J. R. (1996). Sleep disordered breathing: effects of adenotonsillectomy on

behaviour and psychological functioning. *European Journal of Pediatrics*, *155*(1), 56–62. https://doi.org/10.1007/bf02115629

Ali, S., Mohammed, N., Qureshi, N., & Gupta, S. (2021). Oxygen therapy in preterm infants: recommendations for practice. *Paediatrics and Child Health*, *31*(1), 1–6. https://doi.org/10.1016/j.paed.2020.10.001

Alkaysi, H. N., Salem, M. S., & El-Sayed, Y. M. (1988). High performance liquid chromatographic analysis of caffeine concentrations in plasma and saliva. *Journal of Clinical Pharmacy and Therapeutics*, *13*, 109–110.

Allegaert, K., Van De Velde, M., & Van Den Anker, J. (2014). Neonatal clinical pharmacology. *Pediatric Anesthesia*, *24*(1), 30–38. https://doi.org/10.1111/PAN.12176

Almendros, I., Wang, Y., & Gozal, D. (2014). The polymorphic and contradictory aspects of intermittent hypoxia. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *307*(2), L129-40. https://doi.org/10.1152/ajplung.00089.2014

Alsweiler, J. M. (2022). Investigating the effect of caffeine to improve neurodevelopmental outcomes in babies born late preterm: a randomised controlled trial. In *Australian New Zealand Clinical Trials Registry [Internet]: ACTRN12622001344785*. NHMRC Clinical Trials Centre (Australia) University of Sydney. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=384799&isReview=true

Álvarez-Fuente, M., Arruza, L., Muro, M., Zozaya, C., Avila, A., López-Ortego, P., González-Armengod, C., Torrent, A., Gavilán, J. L., & del Cerro, M. J. (2017). The economic impact of prematurity and bronchopulmonary dysplasia. *European Journal of Pediatrics*, *176*(12), 1587–1593. https://doi.org/10.1007/s00431-017-3009-6

American Academy of Paediatrics. (2003). Policy statement: Apnea, sudden infant death syndrome, and home monitoring. *Pediatrics*, *111*, 914–917. www.aappublications.org/news

Anderson, B. J., Gunn, T. R., Holford, N. H. G., & Johnson, R. (1999). Caffeine overdose in a premature infant: clinical course and pharmacokinetics. *Anaesthesia and Intensive Care*, *27*(3), 307–311.

Anderson, B. J., & Holford, N. H. G. (2018). Negligible impact of birth on renal function and drug metabolism. *Pediatric Anesthesia*, *28*(11), 1015–1021. https://doi.org/10.1111/PAN.13497

Andersson, S., Pitkänen, O., Janér, C., & Helve, O. (2010). Lung fluid during postnatal transition. *Chinese Medical Journal*, *123*(20), 2919–2923.

Aranda, J. V., Cook, C. E., Gorman, W., Collinge, J. M., Loughnan, P. M., Outerbridge, E. W., Aldridge, A., & Neims, A. H. (1979). Pharmacokinetic profile of caffeine in the premature newborn infant with

apnea. *The Journal of Pediatrics*, *94*(4), 663–668. https://doi.org/10.1016/S0022-3476(79)80047-5

Aranda, J. V., Gorman, W., Bergsteinsson, H., & Gunn, T. (1977). Efficacy of caffeine in treatment of apnea in the low-birth-weight infant. *The Journal of Pediatrics*, *90*(3), 467–472. https://doi.org/10.1016/S0022-3476(77)80718-X

Aranda, J. V., Sitar, D. S., Parsons, W. D., Loughnan, P. M., & Neims, A. H. (1976). Pharmacokinetic aspects of theophylline in premature newborns. *New England Journal of Medicine*, *295*(8), 413–416. https://doi.org/10.1056/NEJM197608192950803

Aranda, J. V, & Beharry, K. D. (2020). Pharmacokinetics, pharmacodynamics and metabolism of caffeine in newborns. *Seminars in Fetal and Neonatal Medicine*, *25*, 101183. https://doi.org/10.1016/j.siny.2020.101183

Aranda, J. V, Collinge, J. M., Zinman, R., & Watters, G. (1979). Maturation of caffeine elimination in infancy. *Archives of Disease in Childhood*, *54*, 946–949. https://doi.org/10.1136/adc.54.12.946

Armanian, A.-M., Iranpour, R., Faghihian, E., & Salehimehr, N. (2016). Caffeine administration to prevent apnea in very premature infants. *Pediatrics and Neonatology*, *57*, 408–412. https://doi.org/10.1016/j.pedneo.2015.10.007

Arnaud, M. J. (2011). Pharmacokinetics and metabolism of natural methylxanthines in animal and man. In B. B. Fredholm (Ed.), *Methylxanthines. Handbook of Experimental Pharmacology* (pp. 33–91). Springer. https://doi.org/10.1007/978-3-642-13443-2_3

Ashorn, P., Ashorn, U., Muthiani, Y., Aboubaker, S., Askari, S., Bahl, R., Black, R. E., Dalmiya, N., Duggan, C. P., Hofmeyr, G. J., Kennedy, S. H., Klein, N., Lawn, J. E., Shiffman, J., Simon, J., Temmerman, M., Okwaraji, Y., Krasevec, J., Bradley, E., … Hayashi, C. (2023). Small vulnerable newborns—big potential for impact. *The Lancet*, *401*(10389), 1692–1706. https://doi.org/10.1016/S0140-6736(23)00354-9

Askie, L. M., Darlow, B. A., Finer, N., Schmidt, B., Stenson, B., Tarnow-Mordi, W., Davis, P., Carlo, W. A., Brocklehurst, P., Davies, L. C., Das, A., Rich, W., Gantz, M. G., Roberts, R. S., Whyte, R. K., Costantini, L., Poets, C., Asztalos, E., Battin, M., … Simes, R. J. (2018). Association between oxygen saturation targeting and death or disability in extremely preterm infants in the neonatal oxygenation prospective meta-analysis collaboration. *Journal of the American Medical Association*, *319*(21), 2190–2201. https://doi.org/10.1001/JAMA.2018.5725

Aslaksen, A., Bakke, O., & Vigander, T. (1981). Comparative pharmacokinetics of theophylline and aminophylline in man. *British Journal of Clinical Pharmacology*, *11*(3), 269–273. https://doi.org/10.1111/j.1365-2125.1981.tb00533.x

Atik, A., Harding, R., De Matteo, R., Kondos-Devcic, D., Cheong, J., Doyle, L. W., & Tolcos, M. (2017). Caffeine for apnea of prematurity: Effects on the developing brain. *Neurotoxicology*, *58*, 94–102. https://doi.org/10.1016/j.neuro.2016.11.012

Auckland District Health Board. (2019). *Midwifery Recruitment and Retention Oversight Report*.

Axelrod, J., & Reichenthal, J. (1953). The fate of caffeine in man and a method for its estimation in biological material. *Journal of Pharmacology and Experimental Therapeutics*, *107*(4), 519–523.

Bahrke, M. S., & Shukitt-Hale, B. (1993). Effects of altitude on mood, behaviour and cognitive functioning. A review. *Sports Medicine*, *16*(2), 97–125. https://doi.org/10.2165/00007256-199316020-00003

Balain, M., & Oddie, S. (2014). Management of apnoea and bradycardia in the newborn. *Paediatrics and Child Health*, *24*(1), 17–22. https://doi.org/10.1016/j.paed.2013.06.004

Bancalari, E., Abdenour, G. E., Feller, R., & Gannon, J. (1979). Bronchopulmonary dysplasia: Clinical presentation. *The Journal of Pediatrics*, *95*(5), 819–823. https://doi.org/10.1016/S0022-3476(79)80442-4

Bancalari, E., & Jain, D. (2019). Bronchopulmonary dysplasia: 50 Years after the original description. *Neonatology*, *115*(4), 384–391. https://doi.org/10.1159/000497422

Barnes, A. R., Hebron, B. S., & Smith, J. (1994). Stability of caffeine oral formulations for neonatal use. *Journal of Clinical Pharmacy and Therapeutics*, *19*(6), 391–396. https://doi.org/10.1111/j.1365-2710.1994.tb00699.x

Barrington, K., & Finer, N. (1990). Periodic breathing and apnea in preterm infants. *Pediatric Research*, *27*(2), 118–121. https://doi.org/10.1203/00006450-199002000-00003

Barrington, K., & Finer, N. (1991). The natural history of the appearance of apnea of prematurity. *Pediatric Research*, *29*(4), 372–375. https://doi.org/10.1038/pr.1991.72500

Barrington, K., Finer, N. N., Peters, K. L., & Barton, J. (1986). Physiologic effects of doxapram in idiopathic apnea of prematurity. *The Journal of Pediatrics*, *108*(1), 124–129. https://doi.org/10.1016/S0022-3476(86)80786-7

Barrington, K., Finer, N. N., Torok-Both, G., Jamali, F., & Coutts, R. T. (1987). Dose-response relationship of doxapram in the therapy for refractory idiopathic apnea of prematurity. *Pediatrics*, *80*(1), 22–27. www.aappublications.org/news

Bass, J. L., Corwin, M., Gozal, D., Moore, C., Nishida, H., Parker, S., Schonwald, A., Wilker, R. E., Stehle, S., & Kinane, T. B. (2004). The effect of chronic or intermittent hypoxia on cognition in childhood:

A review of the evidence. *Pediatrics*, *114*(3), 805–816. https://doi.org/10.1542/peds.2004-0227

Basurto Ona, X., Osorio, D., & Bonfill Cosp, X. (2015). Drug therapy for treating post-dural puncture headache. *Cochrane Database of Systematic Reviews*, *7*. https://doi.org/10.1002/14651858.CD007887.pub3

Bell, E. F., Strauss, R. G., Widness, J. A., Mahoney, L. T., Mock, D. M., Seward, V. J., Cress, G. A., Johnson, K. J., Kromer, I. J., & Zimmerman, M. B. (2005). Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. *Pediatrics*, *115*(6), 1685–1691. https://doi.org/10.1542/peds.2004-1884

Berlin, C. M., Denson, M. H., Daniel, C. H., & Ward, R. M. (1984). Disposition of Dietary Caffeine in Milk, Saliva, and Plasma of Lactating Women. *Pediatrics*, *73*(1), 59–63.

Berry, M. J., Foster, T., Rowe, K., Robertson, O., Robson, B., & Pierse, N. (2018). Gestational age, health, and educational outcomes in adolescents. *Pediatrics*, *142*(5). https://doi.org/10.1542/PEDS.2018-1016

Bhongsatiern, J., Stockmann, C., Yu, T., Constance, J. E., Moorthy, G., Spigarelli, M. G., Desai, P. B., & Sherwin, C. M. T. (2016). Renal function descriptors in neonates: Which creatinine-based Formula best describes vancomycin clearance? *Journal of Clinical Pharmacology*, *56*(5), 528–540. https://doi.org/10.1002/jcph.650

Bhutani, V. K., & Johnson, L. (2006). Kernicterus in late preterm infants cared for as term healthy infants. *Seminars in Perinatology*, *30*(2), 89–97. https://doi.org/10.1053/j.semperi.2006.04.001

Blencowe, H., Cousens, S., Oestergaard, M. Z., Chou, D., Moller, A. B., Narwal, R., Adler, A., Vera Garcia, C., Rohde, S., Say, L., & Lawn, J. E. (2012). National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. *The Lancet*, *379*(9832), 2162–2172. https://doi.org/10.1016/S0140-6736(12)60820-4

Blunden, S. L., & Beebe, D. W. (2006). The contribution of intermittent hypoxia, sleep debt and sleep disruption to daytime performance deficits in children: Consideration of respiratory and non-respiratory sleep disorders. *Sleep Medicine Reviews*, *10*, 107–118. https://doi.org/10.1016/j.smrv.2005.11.003

Bock, K. W., Schrenk, D., Forster Adelheid, Griese, E.-U., Mörike, K., Brockmeier, D., & Eichelbaum, M. (1994). The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes. *Pharmacogenetics.*, *4*(4), 209–218.

Bogialli, S., Corcia, A. Di, & Nazzari, M. (2007). Extraction procedures. In *Food Toxicants Analysis* (pp. 269–297). Elsevier. https://doi.org/10.1016/B978-044452843-8/50010-9

Bosma, M. S., van Tuijl, S. G. L., Mooij, M. G., Kamerbeek, A., & Geerlings, C. J. C. (2012). Therapeutic drug monitoring of caffeine in preterm neonates. *European Journal of Hospital Pharmacy*, *19*(2), 191.2-191. https://doi.org/10.1136/ejhpharm-2012-000074.280

Bosquet Enlow, M., White, M. T., Hails, K., Cabrera, I., & Wright, R. J. (2016). The Infant Behavior Questionnaire-Revised: Factor structure in a culturally and sociodemographically diverse sample in the United States. *Infant Behavior and Development*, *43*, 24–35. https://doi.org/10.1016/j.infbeh.2016.04.001

Boylan, P., & Lewis, P. J. (1980). Fetal breathing in labor. *Obstetrics and Gynecology*, *56*(1), 35–38.

Boylan, S., Cade, J. E., Kirk, S. F. L., Greenwood, D. C., White, K. I. M., Shires, S., Simpson, N. A. B., Wild, C. P., & Hay, A. W. M. (2008). Assessing caffeine exposure in pregnant women. *British Journal of Nutrition*, *100*(4), 875–882. https://doi.org/10.1017/S0007114508939842

Boyle, E. M., Johnson, S., Manktelow, B., Seaton, S. E., Draper, E. S., Smith, L. K., Dorling, J., Marlow, N., Petrou, S., & Field, D. J. (2015). Neonatal outcomes and delivery of care for infants born late preterm or moderately preterm: a prospective population-based study. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, *100*(6), F479–F485. https://doi.org/10.1136/fetalneonatal-2014-308136

Bradford, S. (2022). *Birth characteristics: 2020 Dataset*. https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/datasets/birthcharacteristicsinenglandandwales

Brattström, P., Russo, C., Ley, D., & Bruschettini, M. (2018). High-versus low-dose caffeine in preterm infants: a systematic review and meta-analysis. *Acta Paediatrica*, *108*(3), 401–410. https://doi.org/10.1111/apa.14586

Brice, C., & Smith, A. (2001). Caffeine levels in saliva: associations with psychosocial factors and behavioural effects. *Human Psychopharmacology Hum Psychopharmacol Clin Exp*, *16*, 507–521. https://doi.org/10.1002/hup.326

Brooks, S., Rowley, S., Broadbent, E., & Petrie, K. J. (2012). Illness perception ratings of high-risk newborns by mothers and clinicians: Relationship to illness severity and maternal stress. *Health Psychology*, *31*(5), 632–639. https://doi.org/10.1037/a0027591

Brouillette, R. T., Morrow, A. S., Weese-Mayer, D. E., & Hunt, C. E. (1987). Comparison of respiratory inductive plethysmography and thoracic impedance for apnea monitoring. *The Journal of Pediatrics*, *111*(3), 377–383. https://doi.org/10.1016/S0022-3476(87)80457-2

Broussard, D. L. (1995). Gastrointestinal motility in the neonate. *Clinics in Perinatology*, *22*(1), 37–59. https://doi.org/10.1016/s0095-5108(18)30300-2

Brown, M. J., Olver, R. E., Ramsden, C. A., Strang, L. B., & Walters, D. V. (1983). Effects of adrenaline and of spontaneous labour on the secretion and absorption of lung liquid in the fetal lamb. *The Journal of Physiology*, *344*(1), 137–152. https://doi.org/10.1113/JPHYSIOL.1983.SP014929

Bruschettini, M., Brattström, P., Russo, C., Onland, W., Davis, P., & Soll, R. (2023). Caffeine dosing regimens in preterm infants with or at risk for apnea of prematurity. *Cochrane Database of Systematic Reviews*, *2023*(4). https://doi.org/10.1002/14651858.CD013873.PUB2

Bucher, H. U., & Duc, G. (1988). Does caffeine prevent hypoxaemic episodes in premature infants? - A randomized controlled trial. *European Journal of Pediatrics*, *147*(3), 288–291. https://doi.org/10.1007/BF00442697/METRICS

Buckingham, R. (Ed.). (2018). Caffeine. In *Martindale: The Complete Drug Reference* ([online]). Pharmaceutical Press.

Bühler, E., Lachenmeier, D. W., Schlegel, K., & Winkler, G. (2014). Development of a tool to assess the caffeine intake among teenagers and young adults. *Ernaehrungs Umschau International*, *61*(4), 58–63. https://doi.org/10.4455/eu.2014.011

Burns, C. M., Rutherford, M. A., Boardman, J. P., & Cowan, F. M. (2008). Patterns of Cerebral Injury and Neurodevelopmental Outcomes After Symptomatic Neonatal Hypoglycemia. *Pediatrics*, *122*(1), 65–74. https://doi.org/10.1542/PEDS.2007-2822

Butcher-Puech, M. C., Henderson-Smart, D. J., Holley, D., Lacey, J. L., & Edwards, D. A. (1985). Relation between apnoea duration and type and neurological status of preterm infants. *Archives of Disease in Childhood*, *60*(10), 953–958. https://doi.org/10.1136/adc.60.10.953

Butler, M. A., Lang, N. P., Young, J. F., Caporaso, N. E., Vineis, P., Hayes, R. B., Teitel, C. H., Massengill, J. P., Lawsen, M. F., & Kadlubar, F. F. (1992). Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics*, *2*(3), 116–127. https://doi.org/10.1097/00008571-199206000-00003

Calvaresi, V., Escuder, D., Minutillo, A., Bastons-Compta, A., García-Algar, O., Alonso, C. R. P., Pacifici, R., & Pichini, S. (2016). Transfer of nicotine, cotinine and caffeine into breast milk in a smoker mother consuming caffeinated drinks. *Journal of Analytical Toxicology*, *40*(6), 473–477. https://doi.org/10.1093/jat/bkw034

Carlo, W. A., Finer, N. N., Walsh, M. C., Rich, W., Gantz, M. G., Laptook, A. R., Yoder, B. A., Faix, R. G., Das, A., Poole, W. K., Schibler, K., Newman, N. S., Ambalavanan, N., Frantz, I. D., Piazza, A. J., Sánchez, P. J., Morris, B. H., Laroia, N., Phelps, D. L., … Higgins, R. (2010). Target ranges of oxygen saturation in extremely preterm infants. *New England Journal of Medicine*, *362*(21), 1959–1969.

https://doi.org/10.1056/NEJMoa0911781

Carrillo, J. A., Christensen, M., Ramos, S. I., Alm, C., Dahl, M. L., Benítez, J., & Bertilsson, L. (2000). Evaluation of caffeine as an in vivo probe for CYP1A2 using measurements in plasma, saliva, and urine. *Therapeutic Drug Monitoring*, *22*(4), 409–417. https://doi.org/10.1097/00007691-200008000-00008

Chaabanea, A., Chioukhb, F. Z., Chadlia, Z., Ben Fredja, N., Ben Ameurb, K., Ben Hmidab, H., Boughattasa, N. A., Monastirib, K., & Aouama, K. (2017). Therapeutic drug monitoring of caffeine in preterm infants: Could saliva be an alternative to serum? *Therapie*, *72*, 685–689.

Challis, J. R. G., Dilley, S. R., Robinson, J. S., & Thorburn, G. D. (1976). Prostaglandins in the circulation of the fetal lamb. *Prostaglandins*, *11*(6), 1041–1052. https://doi.org/10.1016/0090-6980(76)90011-3

Charles, B. G., Townsend, S. R., Steer, P. A., Flenady, V. J., Gray, P. H., & Shearman, A. (2008). Caffeine citrate treatment for extremely premature infants with apnea: Population pharmacokinetics, absolute bioavailability, and implications for therapeutic drug monitoring. *Therapeutic Drug Monitoring*, *30*(6), 709–716. https://doi.org/10.1097/FTD.0b013e3181898b6f

Chen, J., Jin, L., & Chen, X. (2018). Efficacy and safety of different maintenance doses of caffeine citrate for treatment of apnea in premature infants: a systematic review and meta-analysis. *BioMed Research International*, 9061234. https://doi.org/10.1155/2018/9061234

Cheong, J. L., Doyle, L. W., Burnett, A. C., Lee, K. J., Walsh, J. M., Potter, C. R., Treyvaud, K., Thompson, D. K., Olsen, J. E., Anderson, P. J., & Spittle, A. J. (2017). Association between moderate and late preterm birth and neurodevelopment and social-emotional development at age 2 years. *JAMA Pediatrics*, *171*(4), e164805–e164805. https://doi.org/10.1001/jamapediatrics.2016.4805

Chiesi Pharmaceuticals. (2014). *Peyona 20 mg/mL solution for infusion and oral solution data sheet*. https://www.ema.europa.eu/en/documents/product-information/peyona-epar-product-information_en.pdf

Chiruvolu, A., George, R., Stanzo, K. C., Kindla, C. M., & Desai, S. (2022). Effects of Placental Transfusion on Late Preterm Infants Admitted to a Mother-Baby Unit. *American Journal of Perinatology*, *39*(16), 1812–1819. https://doi.org/10.1055/s-0041-1726387

Chung, J., Tran Lopez, K., Amendolia, B., Bhat, V., Nakhla, T., Slater-Myer, L., Saslow, J., & Aghai, Z. H. (2022). Stopping caffeine in premature neonates: how long does it take for the level of caffeine to fall below the therapeutic range? *Journal of Maternal-Fetal and Neonatal Medicine*, *35*(3), 551–555. https://doi.org/10.1080/14767058.2020.1729117

Clark, R. H., Bloom, B. T., Spitzer, A. R., & Gerstmann, D. R. (2006). Reported medication use in the

neonatal intensive care unit: Data from a large national data set. *Pediatrics*, *117*(6), 1979–1987. https://doi.org/10.1542/peds.2005-1707

Clement, M. (1989). Caffeine and babies. *BMJ : British Medical Journal*, *298*(6685), 1461.

Coffey, C., Figiel, G., & Weiner, R. (1990). Caffeine augmentation of ECT. *The American Journal of Psychiatry*, *147*(5), 579–585.

Cohen, G., Katz-Salamon, M., & Malcolm, G. (2012). A key circulatory defence against asphyxia in infancy – the heart of the matter! *The Journal of Physiology*, *590*(23), 6157. https://doi.org/10.1113/JPHYSIOL.2012.239145

Costeloe, K. L., Hennessy, E. M., Haider, S., Stacey, F., Marlow, N., & Draper, E. S. (2012). Short term outcomes after extreme preterm birth in England: Comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). *BMJ (Online)*, *345*(7886). https://doi.org/10.1136/bmj.e7976

Council of Europe. (2019). *European Pharmacopoeia (Ph. Eur.)* (10th ed.). Council of Europe.

Cox, J. L., Holden, J. M., & Sagovsky, R. (1987). Detection of Postnatal Depression: Development of the 10-item Edinburgh Postnatal Depression scale. *British Journal of Psychiatry*, *150*, 782–786. https://doi.org/10.1192/bjp.150.6.782

Cross, K. W., & Oppé, T. E. (1952). The effect of inhalation of high and low concentrations of oxygen on the respiration of the premature infant. *The Journal of Physiology*, *117*(1), 38–55. https://doi.org/10.1113/jphysiol.1952.sp004731

Crowther, C. A., Ashwood, P., Middleton, P. F., McPhee, A., Tran, T., Harding, J. E., Group, M. S., Alsweiler, J., Baker, E., Eaglen, D., Groom, K., Hauch, H., Mackay, L., Pacella, M. J., Preest, A., Taylor, J., Williamson, K., Tottman, A., Austin, N., … Zhang, Y. (2023). Prenatal Intravenous Magnesium at 30-34 Weeks' Gestation and Neurodevelopmental Outcomes in Offspring: The MAGENTA Randomized Clinical Trial. *Journal of the American Medical Association*, *330*(7), 603–614. https://doi.org/10.1001/JAMA.2023.12357

Cuzzolin, L. (2017). Neonates exposed to excipients: concern about safety. *Journal of Pediatric and Neonatal Individualized Medicine*, *7*(1), e70112. https://doi.org/10.7363/070112

Czaba-Hnizdo, C., Olischar, M., Rona, Z., Weninger, M., Berger, A., & Klebermass-Schrehof, K. (2014). Amplitude-integrated electroencephalography shows that doxapram influences the brain activity of preterm infants. *Acta Paediatrica*, *103*(9), 922–927. https://doi.org/10.1111/apa.12681

Darlow, B. A., Graham, P. J., & Rojas-Reyes, M. X. (2016). Vitamin A supplementation to prevent mortality and short- and long-term morbidity in very low birth weight infants. *Cochrane Database*

of Systematic Reviews, *2016*(8). https://doi.org/10.1002/14651858.CD000501.PUB4

Darlow, B. A., Horwood, L. J., Wynn-Williams, M. B., Mogridge, N., & Austin, N. C. (2009). Admissions of all gestations to a regional neonatal unit versus controls: 2-year outcome. *Journal of Paediatrics and Child Health*, *45*(4), 187–193. https://doi.org/10.1111/j.1440-1754.2008.01457.x

Davidson, L. M., & Berkelhamer, S. K. (2017). Bronchopulmonary dysplasia: Chronic lung disease of infancy and long-term pulmonary outcomes. *Journal of Clinical Medicine*, *6*(4). https://doi.org/10.3390/jcm6010004

Davis, J., Parad, R. B., Michele, T., Allred, E., Price, A., & Rosenfeld, W. (2003). Pulmonary outcome at 1 year corrected age in premature infants treated at birth with recombinant human CuZn superoxide dismutase. *Pediatrics*, *111*(3), 469–476. https://doi.org/10.1542/peds.111.3.469

Davis, P., Schmidt, B., Roberts, R. S., Doyle, L. W., Asztalos, E., Haslam, R., Sinha, S., & Tin, W. (2010). Caffeine for apnea of prematurity trial: benefits may vary in subgroups. *Journal of Pediatrics*, *156*(3), 382–387. https://doi.org/10.1016/j.jpeds.2009.09.069

de Vries, J. I. P., Visser, G. H. A., Mulder, E. J. H., & Prechtl, H. F. R. (1987). Diurnal and other variations in fetal movement and heart rate patterns at 20–22 weeks. *Early Human Development*, *15*(6), 333–348. https://doi.org/10.1016/0378-3782(87)90029-6

de Vries, J. I. P., Visser, G. H. A., & Prechtl, H. F. R. (1982). The emergence of fetal behaviour. I. Qualitative aspects. *Early Human Development*, *7*, 301–322.

de Vries, J. I. P., Visser, G. H. A., & Prechtl, H. F. R. (1985). The emergence of fetal behaviour. II. Quantitative aspects. *Early Human Development*, *12*(2), 99–120.

Dekker, J., van Kaam, A. H., Roehr, C. C., Flemmer, A. W., Foglia, E. E., Hooper, S. B., & te Pas, A. B. (2019). Stimulating and maintaining spontaneous breathing during transition of preterm infants. *Pediatric Research 2019 90:4*, *90*(4), 722–730. https://doi.org/10.1038/S41390-019-0468-7

Derry, C. J., Derry, S., & Moore, R. A. (2014). Caffeine as an analgesic adjuvant for acute pain in adults. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD009281.pub3

Desai, A., & Macrae, D. (2022). Cardiovascular Physiology in Infants, Children, and Adolescents. In *Pediatric and Congenital Cardiology, Cardiac Surgery and Intensive Care* (pp. 1–16). Springer, London. https://doi.org/10.1007/978-1-4471-4999-6_255-1

Di Fiore, J., Bloom, J. N., Orge, F., Schutt, A., Schluchter, M., Cheruvu, V. K., Walsh, M., Finer, N., & Martin, R. (2010). A higher incidence of intermittent hypoxemic episodes is associated with severe retinopathy of prematurity. *Journal of Pediatrics*, *157*(1), 69–73.

https://doi.org/10.1016/j.jpeds.2010.01.046

Di Fiore, J., MacFarlane, P. M., & Martin, R. (2019). Intermittent Hypoxemia in Preterm Infants. *Clinics in Perinatology*, *46*(3), 553–565. https://doi.org/10.1016/j.clp.2019.05.006

Di Fiore, T. (2005). Use of sleep studies in the neonatal intensive care unit. *Neonatal Network*, *24*(1), 23–30. https://doi.org/10.1891/0730-0832.24.1.23

Dickson, K. A., Maloney, J. E., & Berger, P. J. (1986). Decline in lung liquid volume before labor in fetal lambs. *Journal of Applied Physiology*, *61*(6), 2266–2272. https://doi.org/10.1152/jappl.1986.61.6.2266

Dobson, N., & Hunt, C. E. (2018). Caffeine: an evidence-based success story in VLBW pharmacotherapy. *Pediatric Research 2018 84:3*, *84*(3), 333–340. https://doi.org/10.1038/s41390-018-0089-6

Dobson, N., Liu, X., Rhein, L., Darnall, R., Corwin, M., McEntire, B., Ward, R., James, L., Sherwin, C., Heeren, T., & Hunt, C. (2016). Salivary caffeine concentrations are comparable to plasma concentrations in preterm infants receiving extended caffeine therapy. *British Journal of Clinical Pharmacology*, *82*(3), 754–761. https://doi.org/10.1111/bcp.13001

Dobson, N., Rhein, L., Darnall, R., Corwin, M., Heeren, T., Eichenwald, E., James, L., McEntire, B., Hunt, C., Consenstein, L., White, R. D., & White, R. D. (2017). Caffeine decreases intermittent hypoxia in preterm infants nearing term-equivalent age. *Journal of Perinatology*, *37*(10), 1135–1140. https://doi.org/10.1038/jp.2017.82

Domellöf, M., Braegger, C., Campoy, C., Colomb, V., Decsi, T., Fewtrell, M., Hojsak, I., Mihatsch, W., Molgaard, C., Shamir, R., Turck, D., & Van Goudoever, J. (2014). Iron requirements of infants and toddlers. *Journal of Pediatric Gastroenterology and Nutrition*, *58*(1), 119–129. https://doi.org/10.1097/MPG.0000000000000206

Dong, Y., & Speer, C. P. (2015). Late-onset neonatal sepsis: recent developments. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *100*(3), F257–F263. https://doi.org/10.1136/archdischild-2014-306213

Doyle, L. W., Cheong, J., Hunt, R. W., Lee, K. J., Thompson, D. K., Davis, P., Rees, S., Anderson, P. J., & Inder, T. E. (2010). Caffeine and brain development in very preterm infants. *Annals of Neurology*, *68*(5), 734–742. https://doi.org/10.1002/ana.22098

Doyle, L. W., Cheong, J. L., Hay, S., Manley, B. J., & Halliday, H. L. (2021). Late (≥ 7 days) systemic postnatal corticosteroids for prevention of bronchopulmonary dysplasia in preterm infants. *The Cochrane Database of Systematic Reviews*, *11*. https://doi.org/10.1002/14651858.CD001145.PUB5

Doyle, L. W., Cheong, J. L., Hay, S., Manley, B. J., Halliday, H. L., & Soll, R. (2021). Early (started within 6

days) systemic postnatal corticosteroids for preventing bronchopulmonary dysplasia in preterm infants. *The Cochrane Database of Systematic Reviews*, *11*(10). https://doi.org/10.1002/14651858.CD001145.pub5

Doyle, L. W., Crowther, C. A., Middleton, P., Marret, S., & Rouse, D. (2009). Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus. *Cochrane Database of Systematic Reviews*, *1*. https://doi.org/10.1002/14651858.CD004661.PUB3

Doyle, L. W., Davidson, D., Katz, S., Varela, M., Demeglio, D., & DeCristofaro, J. (2015). Apnea of prematurity and caffeine pharmacokinetics: potential impact on hospital discharge. *Journal of Perinatology 2016 36:2*, *36*(2), 141–144. https://doi.org/10.1038/jp.2015.167

Doyle, L. W., Ranganathan, S., & Cheong, J. L. (2017). Neonatal caffeine treatment and respiratory function at 11 years in children under 1,251 g at birth. *American Journal of Respiratory and Critical Care Medicine*, *196*(10), 1318–1324. https://doi.org/10.1164/rccm.201704-0767OC

Doyle, L. W., Schmidt, B., Anderson, P. J., Davis, P., Moddemann, D., Grunau, R. E., O'Brien, K., Sankaran, K., Herlenius, E., & Roberts, R. (2014). Reduction in developmental coordination disorder with neonatal caffeine therapy. *Journal of Pediatrics*, *165*(2), 356–359. https://doi.org/10.1016/j.jpeds.2014.04.016

Dukhovny, D., Lorch, S. A., Schmidt, B., Doyle, L. W., Kok, J. H., Roberts, R. S., Kamholz, K. L., Wang, N., Mao, W., Zupancic, J. A., & for the Caffeine for Apnea of Prematurity Trial Group. (2011). Economic evaluation of caffeine for apnea of prematurity. *Pediatrics*, *127*(1), e146-155. https://doi.org/10.1542/peds.2010-1014

Edelman, N. H., Epstein, P. E., Lahiri, S., & Cherniack, N. S. (1973). Ventilatory responses to transient hypoxia and hypercapnia in man. *Respiration Physiology*, *17*(3), 302–314. https://doi.org/10.1016/0034-5687(73)90005-4

Edmonds, L. K., Sibanda, N., Geller, S., Cram, F., Robson, B., Filoche, S., Storey, F., Gibson-Helm, M., & Lawton, B. (2021). He Tamariki Kokoti Tau: Tackling preterm incidence and outcomes of preterm births by ethnicity in Aotearoa New Zealand 2010–2014. *International Journal of Gynecology & Obstetrics*, *155*(2), 239–246. https://doi.org/10.1002/IJGO.13855

Edwards, T., Alsweiler, J. M., Crowther, C. A., Edlin, R., Gamble, G. D., Hegarty, J. E., Lin, L., McKinlay, C. J. D., Rogers, J. A., Thompson, B., Wouldes, T. A., & Harding, J. E. (2022). Prophylactic Oral Dextrose Gel and Neurosensory Impairment at 2-Year Follow-up of Participants in the hPOD Randomized Trial. *Journal of the American Medical Association*, *327*(12), 1149–1157. https://doi.org/10.1001/JAMA.2022.2363

Edwards, T., Liu, G., Battin, M., Harris, D. L., Hegarty, J. E., Weston, P. J., & Harding, J. E. (2022). Oral dextrose gel for the treatment of hypoglycaemia in newborn infants. *Cochrane Database of Systematic Reviews*, *2022*(3). https://doi.org/10.1002/14651858.CD011027.PUB3

Edwards, T., Liu, G., Hegarty, J. E., Crowther, C. A., Alsweiler, J., & Harding, J. E. (2021). Oral dextrose gel to prevent hypoglycaemia in at-risk neonates. *The Cochrane Database of Systematic Reviews*, *5*(5). https://doi.org/10.1002/14651858.CD012152.PUB3

Eichenwald, E. C., & Committee on Fetus and Newborn. (2016). Apnea of prematurity. *Pediatrics*, *137*(1), 1–7. https://doi.org/10.1542/peds.2015-3757

El-Yazigi, A., Shabib, S., Al-Rawithi, S., Yusuf, A., Legayada, E. S., & Al-Humidan, A. (1999). Salivary clearance and urinary metabolic pattern of caffeine in healthy children and in pediatric patients with hepatocellular diseases. *Journal of Clinical Pharmacology*, *39*(4), 366–372. https://doi.org/10.1177/00912709922007930

Elsayed, N. A., Boyer, T. M., & Burd, I. (2021). Fetal Neuroprotective Strategies: Therapeutic Agents and Their Underlying Synaptic Pathways. In *Frontiers in Synaptic Neuroscience* (Vol. 13, p. 680899). Frontiers Media S.A. https://doi.org/10.3389/fnsyn.2021.680899

Engle, W. A., Tomashek, K. M., & Wallman, C. (2007). "Late-preterm" infants: a population at risk. *Pediatrics*, *120*(6), 1390–1401. https://doi.org/10.1542/peds.2007-2952

Erenberg, A., Leff, R. D., Haack, D. G., Mosdell, K. W., Hicks, G. M., & Wynne, B. A. (2000). Caffeine citrate for the treatment of apnea of prematurity: A double-blind, placebo-controlled study. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *20*(6), 644–652. https://doi.org/10.1592/PHCO.20.7.644.35167

Erenberg, A., Leff, R., & Wynne, B. (1998). Results of the first double blind placebo (pl) controlled study of caffeine citrate (cc) for the treatment of apnea of prematurity (AOP). *Pediatric Research 1998 43:4*, *43*(4), 172–172. https://doi.org/10.1203/00006450-199804001-01022

Eriksen, P. S., Gennser, G., Lofgren, O., & Nilsson, K. (1983). Acute effects of maternal smoking on fetal breathing and movements. *Obstetrics and Gynecology.*, *61*(3), 367–372.

Evans, S. M., & Griffiths, R. R. (1992). Caffeine tolerance and choice in humans. *Psychopharmacology*, *108*(1–2), 51–59. https://doi.org/10.1007/BF02245285/METRICS

Eyal, F., Alpan, G., Sagi, E., Glick, B., Peleg, O., Dgani, Y., & Arad, I. (1985). Aminophylline versus doxapram in idiopathic apnea of prematurity: A double-blind controlled study. *Pediatrics*, *75*(4), 709–713. https://doi.org/10.1542/peds.75.4.709

Fakoor, Z., Makooie, A. A., Joudi, Z., & Asl, R. G. (2019). The effect of venous caffeine on the prevention of apnea of prematurity in the very preterm infants in the neonatal intensive care unit of Shahid Motahhari Hospital, Urmia, during a year. *Journal of Advanced Pharmaceutical Technology & Research*, *10*(1), 16. https://doi.org/10.4103/JAPTR.JAPTR_334_18

Falcão, A. C., de Gatta, M. M. F., Iribarnegaray, M. F. D., Buelga, D. S., García, M. J., Dominguez-Gil, A., & Lanao, J. M. (1997). Population pharmacokinetics of caffeine in premature neonates. *European Journal of Clinical Pharmacology*, *52*(3), 211–217. https://doi.org/10.1007/s002280050276

Falconer, O., Ivy, S., Carpentier, D. Le, Gavlak, J., Liddle, N., Senior, E., Lowe, P., Crowley, P., Young, A., Johnson, M. J., Beattie, R. M., & Evans, H. J. (2023). Longitudinal pilot study of oxygen saturation indices in healthy preterm infants. *Pediatric Research*. https://doi.org/https://doi.org/10.1038/s41390-023-02741-9

Faridy, E. E. (1983). Instinctive resuscitation of the newborn rat. *Respiration Physiology*, *51*(1), 1–19. https://doi.org/10.1016/0034-5687(83)90098-1

Faudone, G., Arifi, S., & Merk, D. (2021). The medicinal chemistry of caffeine. *Journal of Medicinal Chemistry*, *64*(11), 7156–7178. https://doi.org/10.1021/ACS.JMEDCHEM.1C00261

Fenton, T. R., & Kim, J. H. (2013). A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatrics*, *13*(1), 59. https://doi.org/10.1186/1471-2431-13-59

Fischer, C., Ferdynus, C., Gouyon, J.-B., & Semama, D. S. (2013). Doxapram and hypokalaemia in very preterm infants. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *98*(5), F416–F418. https://doi.org/10.1136/ARCHDISCHILD-2012-303089

Flint, A., & Davies, M. W. (2018). The use of overnight oximetry in neonates: A literature review. *Journal of Paediatrics and Child Health*, *54*(7), 720–727. https://doi.org/10.1111/JPC.13935

Fogarty, M., Osborn, D. A., Askie, L., Seidler, A. L., Hunter, K., Lui, K., Simes, J., & Tarnow-Mordi, W. (2018). Delayed vs early umbilical cord clamping for preterm infants: a systematic review and meta-analysis. *American Journal of Obstetrics and Gynecology*, *218*(1), 1–18. https://doi.org/10.1016/J.AJOG.2017.10.231

Fox, H. E., Inglis, J., & Steinbrecher, M. (1979). Fetal breathing movements in uncomplicated pregnancies. *American Journal of Obstetrics and Gynecology*, *134*(5), 544–546. https://doi.org/10.1016/0002-9378(79)90837-8

Fuhr, U., Klittich, K., & Staib, A. (1993). Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. *British Journal of Clinical Pharmacology*, *35*(4), 431–436. https://doi.org/10.1111/j.1365-2125.1993.tb04162.x

Garner, A., Ben-Sira, I., Deutman, A., Fledelius, H., Flynn, J., Gole, G., Hindle, N. W., Ideta, H., Kingham, J., Koerner, F., Konen, W., Majima, A., Mccormick, A., Mushin, A., Nissenkorn, I., Palmer, E., Quinn, G., Rosenbaum, A., Schaffer, D., … Tanaka, Y. (1984). An international classification of retinopathy of prematurity. *Pediatrics*, *74*(1), 127–133.

Gartstein, M. A., & Rothbart, M. K. (2003). Studying infant temperament via the Revised Infant Behavior Questionnaire. *Infant Behavior and Development*, *26*(1), 64–86. https://doi.org/10.1016/S0163-6383(02)00169-8

George, J., Murphy, T., Roberts, R., Cooksley, W. G. E., Halliday, J. W., & Powell, L. W. (1986). Influence of alcohol and caffeine consumption on caffeine elimination. *Clinical and Experimental Pharmacology and Physiology*, *13*(10), 731–736. https://doi.org/10.1111/j.1440-1681.1986.tb02414.x

Georgieff, M. K. (2011). Long-term brain and behavioral consequences of early iron deficiency. *Nutrition Reviews*, *69*(SUPPL. 1), S43. https://doi.org/10.1111/j.1753-4887.2011.00432.x

Ginsberg, G., Hattis, D., Russ, A., & Sonawane, B. (2004). Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: Implications for assessing children's risks from environmental agents. *Journal of Toxicology and Environmental Health - Part A*, *67*(4), 297–329. https://doi.org/10.1080/15287390490273550

Glasgow, M. J., Edlin, R., & Harding, J. E. (2020). Cost-utility analysis of prophylactic dextrose gel vs standard care for neonatal hypoglycemia in at-risk infants. *Journal of Pediatrics*, *226*, 80-86.e1. https://doi.org/10.1016/j.jpeds.2020.06.073

Gluckman, P. D., Gunn, T. R., & Johnston, B. M. (1983). The effect of cooling on breathing and shivering in unanaesthetized fetal lambs in utero. *The Journal of Physiology*, *343*(1), 495. https://doi.org/10.1113/JPHYSIOL.1983.SP014905

Goldenberg, R. L., Culhane, J. F., Iams, J. D., & Romero, R. (2008). Epidemiology and causes of preterm birth. *Lancet (London, England)*, *371*(9606), 75. https://doi.org/10.1016/S0140-6736(08)60074-4

Gorodischer, R., Burtin, P., Hwang, P., Levine, M., & Koren, G. (1994). Saliva versus blood sampling for therapeutic drug monitoring in children: patient and parental preferences and an economic analysis. *Therapeutic Drug Monitoring*, *16*(5), 437–536.

Gorodischer, R., & Karplus, M. (1982). Pharmacokinetic aspects of caffeine in premature infants with apnoea. *European Journal of Clinical Pharmacology*, *22*(1), 47–52. https://doi.org/10.1007/BF00606424

Grainge, S., Nair, V., & Kannan Loganathan, P. (2022). National survey on caffeine use in neonatal units across the United Kingdom. *Acta Paediatrica*. https://doi.org/10.1111/APA.16635

Gray, P. H., & Chauhan, M. (2016). Use of caffeine for preterm infants in Australia and New Zealand: A survey. *Journal of Paediatrics and Child Health*, *52*(12), 1121–1122. https://doi.org/10.1111/JPC.13366

Gray, P. H., Flenady, V. J., Charles, B. G., & Steer, P. A. (2011). Caffeine citrate for very preterm infants: Effects on development, temperament and behaviour. *Journal of Paediatrics and Child Health*, *47*(4), 167–172. https://doi.org/10.1111/j.1440-1754.2010.01943.x

Griffith, R., Hegarty, J. E., Alsweiler, J. M., Gamble, G. D., May, R., McKinlay, C. J. D., Thompson, B., Wouldes, T. A., & Harding, J. E. (2021). Two-year outcomes after dextrose gel prophylaxis for neonatal hypoglycaemia. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *106*(3), 278–285. https://doi.org/10.1136/ARCHDISCHILD-2020-320305

Guyatt, G. H., Oxman, A. D., Kunz, R., Brozek, J., Alonso-Coello, P., Rind, D., Devereaux, P. J., Montori, V. M., Freyschuss, B., Vist, G., Jaeschke, R., Williams, J. W., Murad, M. H., Sinclair, D., Falck-Ytter, Y., Meerpohl, J., Whittington, C., Thorlund, K., Andrews, J., & Schünemann, H. J. (2011). GRADE guidelines 6. Rating the quality of evidence—imprecision. *Journal of Clinical Epidemiology*, *64*(12), 1283–1293. https://doi.org/10.1016/J.JCLINEPI.2011.01.012

Gyamfi-Bannerman, C., Thom, E. A., Blackwell, S. C., Tita, A. T. N., Reddy, U. M., Saade, G. R., Rouse, D. J., McKenna, D. S., Clark, E. A. S., Thorp, J. M., Chien, E. K., Peaceman, A. M., Gibbs, R. S., Swamy, G. K., Norton, M. E., Casey, B. M., Caritis, S. N., Tolosa, J. E., Sorokin, Y., … Jain, L. (2016). Antenatal betamethasone for women at risk for late preterm delivery. *New England Journal of Medicine*, *374*(14), 1311–1320. https://doi.org/10.1056/nejmoa1516783

Hallak, M., & Cotton, D. B. (1993). Transfer of maternally administered magnesium sulfate into the fetal compartment of the rat: Assessment of amniotic fluid, blood, and brain concentrations. *American Journal of Obstetrics and Gynecology*, *169*(2), 427–431. https://doi.org/10.1016/0002-9378(93)90101-N

Hashiguchi, M., Fujimura, A., Ohashi, K. -i, & Ebihara, A. (1992). Diurnal Effect on Caffeine Clearance. *The Journal of Clinical Pharmacology*, *32*(2), 184–187. https://doi.org/10.1002/j.1552-4604.1992.tb03824.x

Hay, S., Ovelman, C., Zupancic, J. A., Doyle, L. W., Onland, W., Konstantinidis, M., Shah, P. S., & Soll, R. (2023). Systemic corticosteroids for the prevention of bronchopulmonary dysplasia, a network meta-analysis. *Cochrane Database of Systematic Reviews*, *2023*(8). https://doi.org/10.1002/14651858.CD013730.PUB2

He, Q., Yang, Q. C., Zhou, Q., Zhu, H., Niu, W. Y., Feng, J., Wang, Y., Cao, J., & Chen, B. Y. (2014). Effects of varying degrees of intermittent hypoxia on proinflammatory cytokines and adipokines in rats and 3T3-L1 adipocytes. *PLoS ONE*, *9*(1), e86326. https://doi.org/10.1371/journal.pone.0086326

Health Quality and Safety Commission New Zealand. (2011). *Medication alert: Caffeine citrate oral solution*. www.safeuseofmedicines.co.nz

Heinonen, K., Eriksson, J. G., Lahti, J., Kajantie, E., Pesonen, A. K., Tuovinen, S., Osmond, C., & Raikkonen, K. (2015). Late preterm birth and neurocognitive performance in late adulthood: A birth cohort study. *Pediatrics*, *135*(4), e818–e825. https://doi.org/10.1542/peds.2014-3556

Henderson-Smart, D. J. (1981). The effect of gestational age on the incidence and duration of recurrent apnoea in newborn babies. *Australian Paediatric Journal*, *17*(4), 273–276. https://doi.org/10.1111/j.1440-1754.1981.tb01957.x

Henderson-Smart, D. J., & Davis, P. (2010). Prophylactic methylxanthines for endotracheal extubation in preterm infants. *Cochrane Database of Systematic Reviews*, *12*. https://doi.org/10.1002/14651858.CD000139.pub2

Henderson-Smart, D. J., & De Paoli, A. G. (2010a). Methylxanthine treatment for apnoea in preterm infants. *Cochrane Database of Systematic Reviews*, *12*. https://doi.org/10.1002/14651858.CD000140.pub2

Henderson-Smart, D. J., & De Paoli, A. G. (2010b). Prophylactic methylxanthine for prevention of apnoea in preterm infants. *Cochrane Database of Systematic Reviews*, *12*. https://doi.org/10.1002/14651858.CD000432.pub2

Henderson-Smart, D. J., & Steer, P. A. (2000). Doxapram versus methylxanthine for apnea in preterm infants. *Cochrane Database of Systematic Reviews*, *4*. https://doi.org/10.1002/14651858.CD000075

Henderson-Smart, D. J., & Steer, P. A. (2010). Caffeine versus theophylline for apnea in preterm infants. *Cochrane Database of Systematic Reviews*, *1*. https://doi.org/10.1002/14651858.CD000273.pub2.

Higgins, J., Altman, D., Gøtzsche, P., Jüni, P., Moher, D., Oxman, A., Savović, J., Schulz, K., Weeks, L., Sterne, J., Cochrane Bias Methods Group, & Cochrane Statistical Methods Group. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*, *343*, d5928 doi:10.1136/bmj.d5928. https://doi.org/10.1136/bmj.d5928

Higgins, R., Jobe, A. H., Koso-Thomas, M., Bancalari, E., Viscardi, R. M., Hartert, T. V, Ryan, R. M., Kallapur, S. G., Steinhorn, R. H., Konduri, G. G., Davis, S. D., Thebaud, B., Clyman, R. I., Collaco, J. M., Martin, C. R., Woods, J. C., Finer, N. N., & Raju, T. N. K. (2018). Bronchopulmonary dysplasia: Executive summary of a workshop. *Journal of Pediatrics*, *197*, 300–308. https://doi.org/10.1016/j.jpeds.2018.01.043

Hildebrandt, R., & Gundert-Remy, U. (1983). Lack of pharmacological active saliva levels of caffeine in breast-fed infants. *Pediatric Pharmacology (New York, N.Y.)*, *3*(3–4), 237–244.

http://www.ncbi.nlm.nih.gov/pubmed/6677875

Hines, R. N. (2013). Developmental expression of drug metabolizing enzymes: Impact on disposition in neonates and young children. *International Journal of Pharmaceutics*, *452*, 3–7. https://doi.org/10.1016/j.ijpharm.2012.05.079

Hirvonen, M., Ojala, R., Korhonen, P., Haataja, P., Eriksson, K., Gissler, M., Luukkaala, T., & Tammela, O. (2014). Cerebral palsy among children born moderately and late preterm. *Pediatrics*, *134*(6), e1584–e1593. https://doi.org/10.1542/PEDS.2014-0945

Hitchings, L., Rodriguez, M., Persaud, R., & Gomez, L. (2022). The effect of delayed cord clamping on blood sugar levels on 34–36 week neonates exposed to late preterm antenatal steroids. *The Journal of Maternal-Fetal & Neonatal Medicine*, *35*(18), 3587–3594. https://doi.org/10.1080/14767058.2020.1832074

Hogg, M. I. J., Golding, R. H., & Rosen, M. (1977). The effect of doxapram on fetal breathing in the sheep. *BJOG: An International Journal of Obstetrics & Gynaecology*, *84*(1), 48–50. https://doi.org/10.1111/j.1471-0528.1977.tb12466.x

Hohimer, A. R., Bissonnette, J. M., Richardson, B. S., & Machida, C. M. (1983). Central chemical regulation of breathing movements in fetal lambs. *Respiration Physiology*, *52*(1), 99–111. https://doi.org/10.1016/0034-5687(83)90139-1

Hooper, S. B., & Harding, R. (1990). Changes in lung liquid dynamics induced by prolonged fetal hypoxemia. *Journal of Applied Physiology*, *69*(1), 127–135. https://doi.org/10.1152/jappl.1990.69.1.127

Hooper, S. B., Te Pas, A. B., Lang, J., Van Vonderen, J. J., Roehr, C. C., Kluckow, M., Gill, A. W., Wallace, E. M., & Polglase, G. R. (2015). Cardiovascular transition at birth: A physiological sequence. In *Pediatric Research* (Vol. 77, Issue 5, pp. 608–614). Nature Publishing Group. https://doi.org/10.1038/pr.2015.21

Horning, M. G., Brown, L., Nowlin, J., Lertratanangkoon, K., Kellaway, P., & Zion, T. E. (1977). Use of saliva in therapeutic drug monitoring. *Clinical Chemistry*, *23*(2), 157.

Howell, J., Clozel, M., & Aranda, J. V. (1981). Adverse effects of caffeine and theophylline in the newborn infant. *Seminars in Perinatology*, *5*(4), 359–369.

Hsieh, E. M., Hornik, C. P., Clark, R. H., Laughon, M. M., Benjamin, D. K., & Smith, P. B. (2014). Medication use in the neonatal intensive care unit. *American Journal of Perinatology*, *31*(9), 811. https://doi.org/10.1055/S-0033-1361933

Huddy, C. L. J., Johnson, A., & Hope, P. L. (2001). Educational and behavioural problems in babies of 32-

35 weeks gestation. *Arch Dis Child Fetal Neonatal Ed*, *85*(1), F23–F28. https://doi.org/10.1136/fn.85.1.F23

Hunter, P. J., Awoyemi, T., Ayede, A. I., Chico, R. M., David, A. L., Dewey, K. G., Duggan, C. P., Gravett, M., Prendergast, A. J., Ramakrishnan, U., Ashorn, P., Klein, N., Black, R. E., Lawn, J. E., Ashorn, U., Hofmeyr, G. J., Temmerman, M., & Askari, S. (2023). Biological and pathological mechanisms leading to the birth of a small vulnerable newborn. *The Lancet*, *401*(10389), 1720–1732. https://doi.org/10.1016/S0140-6736(23)00573-1

Hüppi, P. S., Warfield, S., Kikinis, R., Barnes, P. D., Zientara, G. P., Jolesz, F. A., Tsuji, M. K., & Volpe, J. J. (1998). Quantitative magnetic resonance imaging of brain development in premature and mature newborns. *Annals of Neurology*, *43*(2), 224–235. https://doi.org/10.1002/ana.410430213

Hyman, P. E., Clarke, D. D., Everett, S. L., Sonne, B., Stewart, D., Harada, T., Walsh, J. H., & Taylor, I. L. (1985). Gastric acid secretory function in preterm infants. *The Journal of Pediatrics*, *106*(3), 467–471. https://doi.org/10.1016/S0022-3476(85)80682-X

Iacobelli, S., & Guignard, J. P. (2021). Maturation of glomerular filtration rate in neonates and infants: an overview. *Pediatric Nephrology*, *36*(6), 1439–1446. https://doi.org/10.1007/S00467-020-04632-1

Iranpour, R., Armanian, A. M., Miladi, N., & Feizi, A. (2022). Effect of prophylactic caffeine on noninvasive respiratory support in preterm neonates weighing 1250–2000 g: A randomized controlled trial. *Archives of Iranian Medicine*, *25*(2), 98–104. https://doi.org/10.34172/AIM.2022.16

Irestedt, L., Dahlin, I., Hertzberg, T., Sollevi, A., & Lagercrantz, H. (1989). Adenosine concentration in umbilical cord blood of newborn infants after vaginal delivery and cesarean section. *Pediatric Research*, *26*(2), 106–108. https://doi.org/10.1203/00006450-198908000-00007

Jain, L., Chen, X. J., Ramosevac, S., Brown, L. A., & Eaton, D. C. (2001). Expression of highly selective sodium channels in alveolar type II cells is determined by culture conditions. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *280*(4 24-4). https://doi.org/10.1152/AJPLUNG.2001.280.4.L646

Jain, L., & Eaton, D. C. (2006). Physiology of fetal lung fluid clearance and the effect of labor. *Seminars in Perinatology*, *30*(1), 34–43. https://doi.org/10.1053/j.semperi.2006.01.006

Janvier, A., Khairy, M., Kokkotis, A., Cormier, C., Messmer, D., & Barrington, K. (2004). Apnea is associated with neurodevelopmental impairment in very low birth weight infants. *Journal of Perinatology*, *24*(12), 763–768. https://doi.org/10.1038/sj.jp.7211182

Jaryal, A. K., & Singh, A. (2020). Physiological modulation of respiration and respiratory reflexes. In H. Prabhakar & C. Mahajan (Eds.), *Brain and Lung Crosstalk* (pp. 41–83). Springer, Singapore.

https://doi.org/10.1007/978-981-15-2345-8_2

Jensen, E. A. (2020). What is bronchopulmonary dysplasia and does caffeine prevent it? *Seminars in Fetal & Neonatal Medicine*, *25*(6). https://doi.org/10.1016/J.SINY.2020.101176

Jensen, E. A., Foglia, E. E., & Schmidt, B. (2015). Evidence-based pharmacologic therapies for prevention of bronchopulmonary dysplasia: Application of the grading of recommendations assessment, development, and evaluation methodology. *Clinics in Perinatology*, *42*(4), 755–779. https://doi.org/10.1016/j.clp.2015.08.005

Jensen, E. A., & Schmidt, B. (2014). Epidemiology of bronchopulmonary dysplasia. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *100*(3), 145–157. https://doi.org/10.1002/bdra.23235

Ji, D., Smith, P. B., Clark, R. H., Zimmerman, K. O., Laughon, M., Ku, L., & Greenberg, R. G. (2020). Wide variation in caffeine discontinuation timing in premature infants. *Journal of Perinatology*, *40*(2), 288–293. https://doi.org/10.1038/s41372-019-0561-0

Jobe, A. (1999). The new BPD: An arrest of lung development. *Pediatric Research*, *46*(6), 641–643. https://doi.org/10.1203/00006450-199912000-00007

Jobe, A., & Bancalari, E. (2001). Bronchopulmonary dysplasia. *American Journal of Respiratory and Critical Care Medicine*, *163*(7), 1723–1729. https://doi.org/10.1164/ajrccm.163.7.2011060

Jones, R. A. K. (1982). Apnoea of immaturity 1. A controlled trial of theophylline and face mask continuous positive airways pressure. *Archives of Disease in Childhood*, *57*, 761–765. https://doi.org/10.1136/adc.57.10.761

Joosten, K., de Goederen, R., Pijpers, A., & Allegaert, K. (2017). Sleep related breathing disorders and indications for polysomnography in preterm infants. *Early Human Development*, *113*, 114–119. https://doi.org/10.1016/J.EARLHUMDEV.2017.07.005

Jordan, G. D., Themelis, N. J., Messerly, S. O., Jarrett, R. V, Garcia, J., & Frank, C. G. (1986). Doxapram and potential benzyl alcohol toxicity: A moratorium on clinical investigation? In *Pediatrics* (Vol. 78, Issue 3, pp. 540–541). https://doi.org/10.1542/peds.78.3.541

Jordan, N. Y., Mimpen, J. Y., van den Bogaard, W. J. M., Flesch, F. M., van de Meent, M. H. M., & Torano, J. S. (2015). Analysis of caffeine and paraxanthine in human saliva with ultra-high-performance liquid chromatography for CYP1A2 phenotyping. *Journal of Chromatography B*, *995–996*, 70–73. https://doi.org/10.1016/j.jchromb.2015.05.020

Joshi, S., & Kotecha, S. (2007). Lung growth and development. *Early Human Development*, *83*(12), 789–794. https://doi.org/10.1016/j.earlhumdev.2007.09.007

Kaiser, J. R., Bai, S., Gibson, N., Holland, G., Lin, T. M., Swearingen, C. J., Mehl, J. K., & ElHassan, N. O. (2015). Association between transient newborn hypoglycemia and fourth-grade achievement test proficiency: A population-based study. *JAMA Pediatrics*, *169*(10), 913–921. https://doi.org/10.1001/jamapediatrics.2015.1631

Kanaan, A., Farahani, R., Douglas, R. M., LaManna, J. C., & Haddad, G. G. (2006). Effect of chronic continuous or intermittent hypoxia and reoxygenation on cerebral capillary density and myelination. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *290*(4), R1105-14. https://doi.org/10.1152/ajpregu.00535.2005

Kanack, M. D., Nakra, N., Ahmad, I., & Vyas, R. M. (2022). Normal neonatal sleep defined: Refining patient selection and interpreting sleep outcomes for mandibular distraction. *Plastic and Reconstructive Surgery - Global Open*, *10*(1), E4031. https://doi.org/10.1097/GOX.0000000000004031

Katheria, A. C., Sauberan, J. B., Akotia, D., Rich, W., Durham, J., & Finer, N. N. (2015). A pilot randomized controlled trial of early versus routine caffeine in extremely premature infants. *American Journal of Perinatology*, *32*(9), 879–886. https://doi.org/10.1055/s-0034-1543981

Kawade, N., & Onishi, S. (1981). The prenatal and postnatal development of UDP-glucuronyltransferase activity towards bilirubin and the effect of premature birth on this activity in the human liver. *Biochemical Journal*, *196*(1), 257–260. https://doi.org/10.1042/bj1960257

Kenward, M., & Roger, J. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, *53*, 983–997.

Kenyon, S., Boulvain, M., & Neilson, J. P. (2013). Antibiotics for preterm rupture of membranes. *Cochrane Database of Systematic Reviews*, *2013*(12). https://doi.org/10.1002/14651858.CD001058

Kerstjens, J. M., Bocca-Tjeertes, I. F., De Winter, A. F., Reijneveld, S. A., & Bos, A. F. (2012). Neonatal morbidities and developmental delay in moderately preterm-born children. *Pediatrics*, *130*(2), e265-72. https://doi.org/10.1542/peds.2012-0079

Kesavan, K., Frank, P., Cordero, D. M., Benharash, P., & Harper, R. M. (2016). Neuromodulation of limb proprioceptive afferents decreases apnea of prematurity and accompanying intermittent hypoxia and bradycardia. *PLoS ONE*, *11*(6), e0157349. https://doi.org/10.1371/journal.pone.0157349

Kinney, H. C. (2006). The near-term (late preterm) human brain and risk for periventricular leukomalacia: A review. In *Seminars in Perinatology* (Vol. 30, Issue 2, pp. 81–88). https://doi.org/10.1053/j.semperi.2006.02.006

Kirpalani, H., Whyte, R. K., & Andersen, C. (2006). The premature infants in need of transfusion (PINT) study: A randomised, controlled trial of a restrictive (low) versus liberal (high) transfusion

threshold for extremely low birth weight infants. *The Journal of Pediatrics*, *149*(3), 301–307. https://doi.org/10.1016/j.jpeds.2006.05.011

Kitterman, J. A. (1988). Physiological factors in fetal lung growth. *Canadian Journal of Physiology and Pharmacology*, *66*(8), 1122–1128. https://doi.org/10.1139/y88-184

Kitterman, J. A. (1996). The effects of mechanical forces on fetal lung growth. *Clinics in Perinatology*, *23*(4), 727–740.

Kitterman, J. A., Ballard, P. L., Clements, J. A., Mescher, E. J., & Tooley, W. H. (1979). Tracheal fluid in fetal lambs: Spontaneous decrease prior to birth. *Journal of Applied Physiology Respiratory Environmental and Exercise Physiology*, *47*(5), 985–989. https://doi.org/10.1152/jappl.1979.47.5.985

Kitterman, J. A., Liggins, G. C., Clements, J. A., & Tooley, W. H. (1979). Stimulation of breathing movements in fetal sheep by inhibitors of prostaglandin synthesis. *Journal of Developmental Physiology*, *1*(6), 453–466.

Kitterman, J. A., Liggins, G. C., Fewell, J. E., & Tooley, W. H. (1983). Inhibition of breathing movements in fetal sheep by prostaglandins. *Journal of Applied Physiology Respiratory Environmental and Exercise Physiology*, *54*(3), 687–692. https://doi.org/10.1152/jappl.1983.54.3.687

Kleinman, L., Rothman, M., Strauss, R., Orenstein, S. R., Nelson, S., Vandenplas, Y., Cucchiara, S., & Revicki, D. A. (2006). The infant gastroesophageal reflux questionnaire revised: development and validation as an evaluative instrument. *Clinical Gastroenterology and Hepatology*, *4*(5), 588–596. https://doi.org/10.1016/j.cgh.2006.02.016

Klinger, G., Sirota, L., Lusky, A., & Reichman, B. (2006). Bronchopulmonary dysplasia in very low birth weight infants is associated with prolonged hospital stay. *Journal of Perinatology 2006 26:10*, *26*(10), 640–644. https://doi.org/10.1038/sj.jp.7211580

Koos, B. J., & Rajaee, A. (2014). Fetal breathing movements and changes at birth. *Advances in Experimental Medicine and Biology*, *814*, 89–101. https://doi.org/10.1007/978-1-4939-1031-1_8

Kori, A. M. M., Van Rostenberghe, H., Ibrahim, N. R., Yaacob, N. M., & Nasir, A. (2021). A randomized controlled trial comparing two doses of caffeine for apnoea in prematurity. *International Journal of Environmental Research and Public Health*, *18*(9). https://doi.org/10.3390/IJERPH18094509

Köroğlu, Ö. A., MacFarlane, P. M., Balan, K. V., Zenebe, W. J., Jafri, A., Martin, R., & Kc, P. (2014). Anti-inflammatory effect of caffeine is associated with improved lung function after lipopolysaccharide-induced amnionitis. *Neonatology*, *106*(3), 235–240. https://doi.org/10.1159/000363217

Krauss, A. N., Klain, D. B., Waldman, S., & Auld, P. A. (1975). Ventilatory response to carbon dioxide in

newborn infants. *Pediatric Research*, *9*, 46–50. https://doi.org/10.1097/00006254-197510000-00011

Kreutzer, K., & Bassler, D. (2014). Caffeine for apnea of prematurity: a neonatal success story. *Neonatology*, *105*(4), 332–336. https://doi.org/10.1159/000360647

Kuban, K. C. k., Leviton, A., Pagano, M., Fenton, T., Strassfeld, R., & Wolff, M. (1992). Maternal toxemia is associated with reduced incidence of germinal matrix hemorrhage in premature babies. *Journal of Child Neurology*, *7*(1), 70–76. https://doi.org/10.1177/088307389200700113

Kugelman, A., & Colin, A. A. (2013). Late preterm infants: Near term but still in a critical developmental time period. *Pediatrics*, *132*(4), 741–751. https://doi.org/10.1542/peds.2013-1131

Kuzemko, J. A., & Paala, J. (1973). Apnoeic attacks in the newborn treated with aminophylline. *Archives of Disease in Childhood*, *48*(5), 404–406. https://doi.org/10.1136/adc.48.5.404

Lackmann, G. M., Schnieder, C., & Bohner, J. (1998). Gestational age-dependent reference values for iron and selected proteins of iron metabolism in serum of premature human neonates. *Biology of the Neonate*, *74*(3), 208–213. https://doi.org/10.1159/000014026

Lagercrantz, H., Rane, A., & Tunell, R. (1980). Plasma concentration-effect relationship of theophylline in treatment of apnea in preterm infants. *European Journal of Clinical Pharmacology*, *18*(1), 65–68. https://doi.org/10.1007/BF00561480

Lando, A., Klamer, A., Jonsbo, F., Weiss, J., & Greisen, G. (2005). Doxapram and developmental delay at 12 months in children born extremely preterm. *Acta Paediatrica*, *94*(11), 1680–1681. https://doi.org/10.1080/08035250500254449

Laughon, M., Allred, E. N., Bose, C., O'Shea, T. M., Van Marter, L. J., Ehrenkranz, R. A., & Leviton, A. (2009). Patterns of respiratory disease during the first 2 postnatal weeks in extremely premature infants. *Pediatrics*, *123*(4), 1124–1131. https://doi.org/10.1542/peds.2008-0862

Lawn, J. E., Ohuma, E. O., Bradley, E., Okwaraji, Y. B., Yargawa, J., Blencowe, H., Idueta, L. S., Hazel, E., Erchick, D. J., Katz, J., Diaz, M., Black, R. E., Lee, A. C. C., Salasibew, M., Requejo, J., Hayashi, C., Moller, A. B., Borghi, E., Ashorn, P., … Prendergast, A. (2023). Small babies, big risks: global estimates of prevalence and mortality for vulnerable newborns to accelerate change and improve counting. *The Lancet*, *401*(10389), 1707–1719. https://doi.org/10.1016/S0140-6736(23)00522-6

Le Guennec, J. C., Billon, B., & Pare, C. (1985). Maturational changes of caffeine concentrations and disposition in infancy during maintenance therapy for apnea of prematurity: Influence of gestational age, hepatic disease, and breast-feeding. *Pediatrics*, *76*(5), 834–840.

Lee, S. M., Sie, L., Liu, J., Profit, J., & Lee, H. C. (2022). Evaluation of trends in bronchopulmonary

dysplasia and respiratory support practice for very low birth weight infants: A population-based cohort study. *Journal of Pediatrics*, *243*, 47-52.e2. https://doi.org/10.1016/j.jpeds.2021.11.049

Leon, A. E. C., Michienzi, K., Ma, C. X., & Hutchison, A. A. (2007). Serum caffeine concentrations in preterm neonates. *American Journal of Perinatology*, *24*(1), 39–47. https://doi.org/10.1055/S-2006-958163/ID/20/BIB

Li, W., Jian, W., & Fu, Y. (2019). Basic sample preparation techniques in LC-MS bioanalysis. In *Sample Preparation in LC-MS Bioanalysis* (pp. 1–30). Wiley. https://doi.org/10.1002/9781119274315.ch1

Liggins, G. C., & Howie, R. N. (1972). A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics*, *50*(4), 515–525. https://doi.org/10.1542/PEDS.50.4.515

Lista, G., Fabbri, L., Polackova, R., Kiechl-Kohlendorfer, U., Papagaroufalis, K., Saenz, P., Ferrari, F., Lasagna, G., Carnielli, V. P., & for the Peyona® PASS Group. (2016). The real-world routine use of caffeine citrate in preterm infants: a European postauthorization safety study. *Neonatology*, *109*(3), 221–227. https://doi.org/10.1159/000442813

Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J. E., Cousens, S., Mathers, C., & Black, R. E. (2015). Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: An updated systematic analysis. *The Lancet*, *385*(9966), 430–440. https://doi.org/10.1016/S0140-6736(14)61698-6

Liu, S., Zhang, X., Liu, Y., Yuan, X., Yang, L., Zhang, R., Zhang, X., Wang, X., Xu, F., & Zhu, C. (2020). Early application of caffeine improves white matter development in very preterm infants. *Respiratory Physiology & Neurobiology*, *281*, 103495. https://doi.org/10.1016/J.RESP.2020.103495

Maastrup, R., Hansen, B. M., Kronborg, H., Bojesen, S. N., Hallum, K., Frandsen, A., Kyhnaeb, A., Svarer, I., & Hallström, I. (2014). Breastfeeding progression in preterm infants is influenced by factors in infants, mothers and clinical practice: The results of a national cohort study with high breastfeeding initiation rates. *PLOS ONE*, *9*(9), e108208. https://doi.org/10.1371/JOURNAL.PONE.0108208

MacBean, V., Drysdale, S. B., Zivanovic, S., Peacock, J. L., & Greenough, A. (2019). Participant retention in follow-up studies of prematurely born children. *BMC Public Health*, *19*(1), 1–6. https://doi.org/10.1186/S12889-019-7575-6/TABLES/2

Mackay, D. F., Smith, G. C. S., Dobbie, R., & Pell, J. P. (2010). Gestational age at delivery and special educational need: Retrospective cohort study of 407,503 schoolchildren. *PLOS Medicine*, *7*(6), e1000289. https://doi.org/10.1371/JOURNAL.PMED.1000289

Magee, L. A., De Silva, D. A., Sawchuck, D., Synnes, A., & von Dadelszen, P. (2019). Magnesium sulphate for fetal neuroprotection. *Journal of Obstetrics and Gynaecology Canada*, *41*(4), 505–522. https://doi.org/10.1016/J.JOGC.2018.09.018

Maillard, C., Boutroy, M., Fresson, J., Barbe, F., & Hascoet, J. (2001). QT interval lengthening in premature infants treated with doxapram. *Clinical Pharmacology & Therapeutics*, *70*(6), 585–585. https://doi.org/10.1016/S0009-9236(01)95877-1

Makker, K., Alissa, R., Dudek, C., Travers, L., Smotherman, C., & Hudak, M. L. (2018). Glucose gel in infants at risk for transitional neonatal hypoglycemia. *American Journal of Perinatology*, *35*(11), 1050–1056. https://doi.org/10.1055/S-0038-1639338/ID/JR170409-17/BIB

Manapurath, R. M., Gadapani Pathak, B., Sinha, B., Upadhyay, R. P., Choudhary, T. S., Chandola, T. R., Mazumdar, S., Taneja, S., Bhandari, N., & Chowdhury, R. (2022). Enteral iron supplementation in preterm or low birth weight infants: A systematic review and meta-analysis. *Pediatrics*, *150*(Supplement 1), e2022057092I. https://doi.org/10.1542/peds.2022-057092I

March of Dimes, The Partnership for Maternal Newborn and Child Health, Save the Children, & World Health Organization. (2012). *Born too soon: the global action report on preterm birth*.

Marcus, C. L., Meltzer, L. J., Roberts, R. S., Traylor, J., Dix, J., D'Ilario, J., Asztalos, E., Opie, G., Doyle, L. W., Biggs, S. N., Nixon, G. M., Narang, I., Bhattacharjee, R., Davey, M., Horne, R. S. C., Cheshire, M., Gibbons, J., Costantini, L., Bradford, R., & Schmidt, B. (2014). Long-term effects of caffeine therapy for apnea of prematurity on sleep at school age. *American Journal of Respiratory and Critical Care Medicine*, *190*(7). https://doi.org/10.1164/rccm.201406-1092OC

Marijianowski, M. M. H., van der Loos, C. M., Mohrschladt, M. F., & Becker, A. E. (1994). The neonatal heart has a relatively high content of total collagen and type I collagen, a condition that may explain the less compliant state. *Journal of the American College of Cardiology*, *23*(5), 1204–1208. https://doi.org/10.1016/0735-1097(94)90612-2

Marrocchella, S., Sestilli, V., Indraccolo, U., de Rosario, F., Castellana, L., Mastricci, A. L., Calo, A., Magaldi, R., Del Bianco, A., Greco, P., & Matteo, M. (2014). Late preterm births: A retrospective analysis of the morbidity risk stratified for gestational age. *SpringerPlus*, *3*, 114. https://doi.org/10.1186/2193-1801-3-114

Martín, I., López-Vílchez, M. Á., Mur, A., García-Algar, Ó., Rossi, S., Marchei, E., & Pichini, S. (2007). Neonatal withdrawal syndrome after chronic maternal drinking of mate. *Therapeutic Drug Monitoring*, *29*(1), 127–129. https://doi.org/10.1097/FTD.0B013E31803257ED

Martin, J., Hamilton, B. E., Osterman, M. J. K., Driscoll, A. K., & Drake, P. (2017). Births: final data for

2017. In *National Vital Statistics Reports: Vol. 67 (8)*. https://www.cdc.gov/nchs/data_access/Vitalstatsonline.htm

Martin, R. (2017). Pathophysiology of apnea of prematurity. In Richard A Polin, Steven H. Abman, David H. Rowitch, William E. Benitz, & William W. Fox (Eds.), *Fetal and Neonatal Physiology* (5th ed., pp. 1595–1604). Elsevier. https://doi.org/10.1016/B978-0-323-35214-7.00157-8

Martin, R. J., Wang, K., Köroğlu, Ö., Di Fiore, J., & Kc, P. (2011). Intermittent hypoxic episodes in preterm infants: Do they matter? In *Neonatology* (Vol. 100, Issue 3, pp. 303–310). https://doi.org/10.1159/000329922

Mathew, O. P. (2011). Apnea of prematurity: pathogenesis and management strategies. *Journal of Perinatology*, *31*(5), 302–310. https://doi.org/10.1038/jp.2010.126

Mcdonald, S. J., Middleton, P., Dowswell, T., & Morris, P. S. (2013). Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. *Cochrane Database of Systematic Reviews*. https://doi.org/https://doi.org/10.1002/14651858.CD004074.pub3

McDowall, R., Doyle, E., Murkitt, G., & Picot, V. (1989). Sample preparation for the HPLC analysis of drugs in biological fluids. *Journal of Pharmaceutical and Biomedical Analysis*, *7*(9), 1087–1096. https://doi.org/10.1016/0731-7085(89)80047-0

McGoldrick, E., Stewart, F., Parker, R., & Dalziel, S. R. (2020). Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database of Systematic Reviews*, *2020*(12). https://doi.org/https://doi.org/10.1002/14651858.CD004454.pub4

McIntire, D. D., & Leveno, K. J. (2008). Neonatal mortality and morbidity rates in late preterm births compared with births at term. *Obstetrics & Gynecology*, *111*(1), 35–41. https://doi.org/10.1097/01.AOG.0000297311.33046.73

McKinlay, C. J. D., Alsweiler, J. M., Anstice, N. S., Burakevych, N., Chakraborty, A., Chase, J. G., Gamble, G. D., Harris, D. L., Jacobs, R. J., Jiang, Y., Paudel, N., San Diego, R. J., Thompson, B., Wouldes, T. A., & Harding, J. E. (2017). Association of neonatal glycemia with neurodevelopmental outcomes at 4.5 years. *JAMA Pediatrics*, *171*(10), 972–983. https://doi.org/10.1001/jamapediatrics.2017.1579

McLeod, W., Brien, J., Loomis, C., Carmichael, L., Probert, C., & Patrick, J. (1983). Effect of maternal ethanol ingestion on fetal breathing movements, gross body movements, and heart rate at 37 to 40 weeks' gestational age. *American Journal of Obstetrics and Gynecology*, *145*(2), 251–257. https://doi.org/10.1016/0002-9378(83)90501-X

McNamara, D. G., Nixon, G. M., & Anderson, B. J. (2004). Methylxanthines for the treatment of apnea associated with bronchiolitis and anesthesia. *Paediatric Anaesthesia*, *14*(7), 541–550.

https://doi.org/10.1111/j.1460-9592.2004.01351.x

McPherson, C., Neil, J. J., Tjoeng, T. H., Pineda, R., & Inder, T. E. (2015). A pilot randomized trial of high-dose caffeine therapy in preterm infants. *Pediatric Research*, *78*(2), 198–204. https://doi.org/10.1038/pr.2015.72

Medicines Act, (1981). https://www.legislation.govt.nz/act/public/1981/0118/latest/DLM53790.html

Mehta, A. C. (1993). Practice research: Strategies for stability studies on hospital pharmaceutical preparations. *International Journal of Pharmacy Practice*, *2*(1), 49–52. https://doi.org/10.1111/j.2042-7174.1993.tb00720.x

Mercer, J. S. (2001). Current best evidence: A review of the literature on umbilical cord clamping. *Journal of Midwifery and Women's Health*, *46*(6), 402–412. https://doi.org/10.1016/S1526-9523(01)00196-9

Miao, Y., Zhou, Y., Zhao, S., Liu, W., Wang, A., Zhang, Y., Li, Y., & Jiang, H. (2022). Comparative efficacy and safety of caffeine citrate and aminophylline in treating apnea of prematurity: A systematic review and meta-analysis. *PLoS ONE*, *17*(9). https://doi.org/10.1371/JOURNAL.PONE.0274882

Micallef, S., Amzal, B., Bach, V., Chardon, K., Tourneux, P., & Bois, F. Y. (2007). Sequential updating of a new dynamic pharmacokinetic model for caffeine in premature neonates. *Clinical Pharmacokinetics*, *46*(1), 59–74. https://doi.org/10.2165/00003088-200746010-00003

Miller, E., Aiello, M. O., Fujita, M., Hinde, K., Milligan, L., & Quinn, E. A. (2013). Field and laboratory methods in human milk research. In *American Journal of Human Biology* (Vol. 25, Issue 1, pp. 1–11). John Wiley & Sons, Ltd. https://doi.org/10.1002/ajhb.22334

Miller, M., Carlo, W. A., & Martin, R. (1985). Continuous positive airway pressure selectively reduces obstructive apnea in preterm infants. *The Journal of Pediatrics*, *106*(1), 91–94. https://doi.org/10.1016/S0022-3476(85)80475-3

Mills, R. J., & Davies, M. W. (2012). Enteral iron supplementation in preterm and low birth weight infants. *Cochrane Database of Systematic Reviews*. https://doi.org/https://doi.org/10.1002/14651858.CD005095.pub2

Ministry of Health. (2019). *Report on Maternity 2017*. https://www.health.govt.nz/publication/report-maternity-2017

Ministry of Health. (2021). *Report on maternity web tool (NZ births 2009-2019)*. https://minhealthnz.shinyapps.io/report-on-maternity-web-tool/

Moak, J. P. (2000). Supraventricular tachycardia in the neonate and infant. *Progress in Pediatric Cardiology*, *11*(1), 25–38. https://doi.org/10.1016/S1058-9813(00)00033-3

Mohammed, S., Nour, I., Shabaan, A. E., Shouman, B., Abdel-Hady, H., & Nasef, N. (2015). High versus low-dose caffeine for apnea of prematurity: a randomized controlled trial. *European Journal of Pediatrics*, *174*(7), 949–956. https://doi.org/10.1007/s00431-015-2494-8

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., Elbourne, D., Egger, M., & Altman, D. G. (2010). CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Journal of Clinical Epidemiology*, *63*(8), e1–e37. https://doi.org/10.1016/j.jclinepi.2010.03.004

Möller, J., Austing, A., Püst, B., Kohl, M., Reiss, I., Iven, H., & Gortner, L. (1999). A comparative study about the therapeutic effect of theophylline and doxapram in apnoeic disorders [Article in German]. *Klinische Pädiatrie*, *211*(02), 86–91. https://doi.org/10.1055/s-2008-1043772

Morley, C. J., Lau, R., & Davis, P. (2005). What do parents think about enrolling their premature babies in several research studies? *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *90*(3), F225. https://doi.org/10.1136/adc.2004.061986

Morton, S. U., & Smith, V. C. (2016). Treatment options for apnoea of prematurity. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *101*(4), F352–F356. https://doi.org/10.1136/archdischild-2015-310228

Moschino, L., Zivanovic, S., Hartley, C., Trevisanuto, D., Baraldi, E., & Roehr, C. C. (2020). Caffeine in preterm infants: where are we in 2020? *ERJ Open Research*, *6*(1), 00330–02019. https://doi.org/10.1183/23120541.00330-2019

Mose, T., Kjaerstad, M. B., Mathiesen, L., Nielsen, J. B., Edelfors, S., & Knudsen, L. E. (2008). Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system. *Journal of Toxicology and Environmental Health - Part A: Current Issues*, *71*(15), 984–991. https://doi.org/10.1080/01932690801934513

Moss, I. R., & Scarpelli, E. M. (1981). Stimulatory effect of theophylline on regulation of fetal breathing movements. *Pediatric Research*, *15*(5), 870–873. https://doi.org/10.1203/00006450-198105000-00009

Moster, D., Lie, R. T., & Markestad, T. (2008). Long-term medical and social consequences of preterm birth. *The New England Journal of Medicine*, *359*(3), 262–273.

Muehlbacher, T., Gaertner, V. D., & Bassler, D. (2020). History of caffeine use in neonatal medicine and the role of the CAP trial. *Seminars in Fetal and Neonatal Medicine*, *25*, 101159. https://doi.org/10.1016/j.siny.2020.101159

Murat, I., Moriette, G., Blin, M. C., Couehard, M., Flouvat, B., De Gamarra, E., & Relier, J. P. (1981). The efficacy of caffeine in the treatment of recurrent idiopathic apnea in premature infants. *Journal of*

Pediatrics, *99*(6), 984–989.

Mürner-Lavanchy, I. M., Doyle, L. W., Schmidt, B., Roberts, R. S., Asztalos, E. V, Costantini, L., Davis, P. G., Dewey, D., D'Ilario, J., Grunau, R. E., Moddemann, D., Nelson, H., Ohlsson, A., Solimano, A., Tin, W., Anderson, P. J., Dix, J., Adams, B. A., Warriner, E., … Carnell, J. (2018). Neurobehavioral outcomes 11 years after neonatal caffeine therapy for apnea of prematurity. *Pediatrics*, *141*(5), e20174047. https://doi.org/10.1542/peds.2017-4047

Nabwera, H. M., Ekhaguere, O. A., Kirpalani, H., Burgoine, K., Ezeaka, C. V., Otieno, W., Allen, S. J., & Embleton, N. D. (2021). Caffeine for the care of preterm infants in sub-Saharan Africa: A missed opportunity? *BMJ Global Health*, *6*(12), 10–12. https://doi.org/10.1136/bmjgh-2021-007682

Nahata, M. C. (2009). Safety of "inert" additives or excipients in paediatric medicines. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *94*(6), F392–F393. https://doi.org/10.1136/ADC.2009.160192

Nair, V., Loganathan, P., & Soraisham, A. S. (2014). Azithromycin and other macrolides for prevention of bronchopulmonary dysplasia: A systematic review and meta-analysis. In *Neonatology* (Vol. 106, Issue 4, pp. 337–347). S. Karger AG. https://doi.org/10.1159/000363493

Narayanan, M., Owers-Bradley, J., Beardsmore, C. S., Mada, M., Ball, I., Garipov, R., Panesar, K. S., Kuehni, C. E., Spycher, B. D., Williams, S. E., & Silverman, M. (2012). Alveolarization continues during childhood and adolescence: New evidence from helium-3 magnetic resonance. *American Journal of Respiratory and Critical Care Medicine*, *185*(2), 186–191. https://doi.org/10.1164/rccm.201107-1348OC

Nelson, K. B., & Grether, J. K. (1995). Can magnesium sulfate reduce the risk of cerebral palsy in very low birthweight infants? *Pediatrics*, *95*(2), 263–269.

New Zealand Medicines Formulary Limited Partnership. (2018). *The New Zealand formulary for children (NZFC).* (78th ed.). https://www.nzfchildren.org.nz

New Zealand Ministry of Health. (2012). *Communicable Disease Control Manual*. New Zealand Ministry of Health. https://www.health.govt.nz/publication/communicable-disease-control-manual

Newman, T. B., Xiong, B., Gonzales, V. M., & Escobar, G. J. (2000). Prediction and prevention of extreme neonatal hyperbilirubinemia in a mature health maintenance organization. *Archives of Pediatrics and Adolescent Medicine*. https://doi.org/10.1001/archpedi.154.11.1140

Newton, R., Broughton, L. J., Lind, M. J., Morrison, P. J., Rogers, H. J., & Bradbrook, I. D. (1981). Plasma and salivary pharmacokinetics of caffeine in man. *European Journal of Clinical Pharmacology*, *21*(1), 45–52. https://doi.org/10.1007/BF00609587

Northway, W. H., Rosan, R. C., & Porter, D. Y. (1967). Pulmonary disease following respirator therapy of hyaline-membrane disease. *New England Journal of Medicine*, *276*(7), 357–368. https://doi.org/10.1056/nejm196702162760701

Odd, D. E., Lingam, R., Emond, A., & Whitelaw, A. (2013). Movement outcomes of infants born moderate and late preterm. *Acta Paediatrica*, *102*(9), 876–882. https://doi.org/10.1111/apa.12320

Oliphant, E. A., Mckinlay, C. J. D., Mcnamara, D., Cavadino, A., & Alsweiler, J. M. (2023). Caffeine to prevent intermittent hypoxaemia in late preterm infants: randomised controlled dosage trial. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *108*(2), 106–113. https://doi.org/10.1136/archdischild-2022-324010

Oliphant, E. A., McKinlay, C. J. D., McNamara, D. G., & Alsweiler, J. M. (2020). Caffeine prophylaxis to improve intermittent hypoxaemia in infants born late preterm: a randomised controlled dosage trial (Latte Dosage Trial). *BMJ Open*, *10*(10), 38271. https://doi.org/10.1136/bmjopen-2020-038271

Oliphant, E. A., Purohit, T. J., Alsweiler, J. M., Mckinlay, C. J. D., & Hanning, S. M. (2022). Validation and application of a simple and rapid stability-indicating liquid chromatographic assay for the quantification of caffeine from human saliva. *Journal of Liquid Chromatography and Related Technologies*, *45*(1–4), 10–17. https://doi.org/10.1080/10826076.2022.2095402

Oliveira, V. X. N., & Teng, A. Y. (2016). The clinical usefulness of sleep studies in children. *Paediatric Respiratory Reviews*, *17*, 53–56. https://doi.org/10.1016/j.prrv.2015.08.003

Olsen, I. E., Louise Lawson, M., Nicole Ferguson, A., Cantrell, R., Grabich, S. C., Zemel, B. S., & Clark, R. H. (2015). BMI curves for preterm infants. *Pediatrics*, *135*(3), e572–e581. https://doi.org/10.1542/peds.2014-2777

Olusanya, B. O., Kaplan, M., & Hansen, T. W. R. (2018). Neonatal hyperbilirubinaemia: a global perspective. *The Lancet Child and Adolescent Health*, *2*(8), 610–620. https://doi.org/10.1016/S2352-4642(18)30139-1

Onrot, J., Goldberg, M. R., Biaggioni, I., Hollister, A. S., Kincaid, D., & Robertson, D. (1985). Hemodynamic and humoral effects of caffeine in autonomic failure. *New England Journal of Medicine*, *313*(9), 549–554. https://doi.org/10.1056/nejm198508293130905

Oo, C. Y., Burgio, D. E., Kuhn, R. C., Desai, N., & McNamara, P. J. (1995). Pharmacokinetics of caffein and its demethylated metabolites in lactation: Predictions of milk to serum concentration ratios. *Pharmaceutical Research*, *12*(2), 313–316. https://doi.org/10.1023/A:1016207832591

Oranges, T., Dini, V., & Romanelli, M. (2015). Skin physiology of the neonate and infant: clinical implications. *Advances in Wound Care*, *4*(10), 587–595. https://doi.org/10.1089/wound.2015.0642

Ord, H., & Griksaitis, M. J. (2019). Cardiac output diversity. Are children just small adults? *Physiology News Magazine*, *115*, 39–41. https://doi.org/https://doi.org/10.36866/pn.115.39

Osterman, M. J. K., Hamilton, B. E., Martin, J., Driscoll, A. K., & Valenzuela, C. P. (2022). Births: Final data for 2020. In *National Vital Statistics Reports* (Vol. 70, Issue 17). https://www.cdc.gov/nchs/products/index.htm.

Ozawa, S., Shankar, R., Leopold, C., & Orubu, S. (2019). Access to medicines through health systems in low-and middle-income countries. *Health Policy and Planning*, *34*, iii1–iii3. https://doi.org/10.1093/heapol/czz119

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *British Medical Journal*, *372*, n71. https://doi.org/10.1136/bmj.n71

Pakvasa, M. A., Saroha, V., & Patel, R. M. (2018). Optimizing caffeine use and risk of bronchopulmonary dysplasia in preterm infants: A systematic review, meta-analysis, and application of grading of recommendations assessment, development, and evaluation methodology. *Clinics in Perinatology*, *45*(2), 273–291. https://doi.org/10.1016/j.clp.2018.01.012

Papile, L. A., Burstein, J., Burstein, R., & Koffler, H. (1978). Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *The Journal of Pediatrics*, *92*(4), 529–534.

Parker, R. A., Lindstrom, D. P., & Cotton, R. B. (1992). Improved survival accounts for most, but not all, of the increase in bronchopulmonary dysplasia. *Pediatrics*, *90*(5 I), 663–668. https://doi.org/10.1542/peds.90.5.663

Parsons, W. D., & Neims, A. H. (1978). Effect of smoking on caffeine clearance. *Clinical Pharmacology and Therapeutics*, *24*(1), 40–45. https://doi.org/10.1002/cpt197824140

Patel, A., Engstrom, J., Meier, P., Jegier, B., & Kimura, R. (2009). Calculating postnatal growth velocity in very low birth weight (VLBW) premature infants. *Journal of Perinatology*, *29*(9), 618–622.

Peliowski, A., & Finer, N. N. (1990). A blinded, randomized, placebo-controlled trial to compare theophylline and doxapram for the treatment of apnea of prematurity. *The Journal of Pediatrics*, *116*(4), 648–653. https://doi.org/10.1016/S0022-3476(05)81620-8

Perera, V., Gross, A. S., & McLachlan, A. J. (2010). Caffeine and paraxanthine HPLC assay for CYP1A2 phenotype assessment using saliva and plasma. *Biomedical Chromatography*, *24*(10), 1136–1144.

https://doi.org/10.1002/bmc.1419

Petrini, J. R., Dias, T., McCormick, M. C., Massolo, M. L., Green, N. S., & Escobar, G. J. (2009). Increased risk of adverse neurological development for late preterm infants. *Journal of Pediatrics*, *154*(2), 169–176. https://doi.org/10.1016/j.jpeds.2008.08.020

Phebra Pty Ltd. (2021). *Cafnea injection and oral solution product information*. https://www.ebs.tga.gov.au/ebs/picmi/picmirepository.nsf/pdf?OpenAgent=&id=CP-2010-PI-03813-3&d=20230814172310101&d=20230814172310101

Philip, A. G. S. (1975). Oxygen plus pressure plus time: the etiology of bronchopulmonary dysplasia. *Pediatrics*, *55*(1), 44–50. https://doi.org/10.1542/peds.55.1.44

Pillai, M., & James, D. (1990). Hiccups and breathing in human fetuses. *Archives of Disease in Childhood*, *65*, 1072–1075. https://doi.org/10.1136/adc.65.10_Spec_No.1072

Poets, C. F. (2010). Apnea of prematurity: What can observational studies tell us about pathophysiology? *Sleep Medicine*, *11*(7), 701–707. https://doi.org/10.1016/J.SLEEP.2009.11.016

Poets, C. F., Roberts, R. S., Schmidt, B., Whyte, R. K., Asztalos, E. V, Bader, D., Bairam, A., Moddemann, D., Peliowski, A., Rabi, Y., Solimano, A., Nelson, H., Shah, P., Brown, L., Wenger, L., Frye, S., Imbesi, F., Kelly, E., D'Ilario, J., … Gent, B. (2015). Association between intermittent hypoxemia or bradycardia and late death or disability in extremely preterm infants. *Journal of the American Medical Association*, *314*(6), 595–603. https://doi.org/10.1001/jama.2015.8841

Poets, C. F., Samuels, M. P., & Southall, D. P. (1992). Potential role of intrapulmonary shunting in the genesis of hypoxemic episodes in infants and young children. *Pediatrics*, *90*(3 I), 385–391. https://doi.org/10.1542/peds.90.3.385

Polson, C., Sarkar, P., Incledon, B., Raguvaran, V., & Grant, R. (2003). Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, *785*(2), 263–275. https://doi.org/10.1016/S1570-0232(02)00914-5

Pons, G., Carrier, O., Richard, M. O., Rey, E., D'Athis, P., Moran, C., Badoual, J., & Olive, G. (1988). Developmental changes in caffeine elimination in infancy. *Developmental Pharmacology and Therapeutics*, *11*(5), 258–264. https://doi.org/10.1159/000457700

Premji, S. S. (Ed.). (2019). *Late Preterm Infants*. Springer. https://doi.org/10.1007/978-3-319-94352-7

Preston, C. (Ed.). (2022). *Stockley's Drug Interactions [Online]*. Pharmaceutical Press. http://www.medicinescomplete.com/

Puia-Dumitrescu, M., Smith, P. B., Zhao, J., Soriano, A., Payne, E. H., Harper, B., Bendel-Stenzel, E., Moya, F., Chhabra, R., Ku, L., Laughon, M., Wade, K. C., Furda, G., Benjamin, D., Capparelli, E., Kearns, G. L., Paul, I. M., & Hornik, C. (2019). Dosing and Safety of Off-label Use of Caffeine Citrate in Premature Infants. *Journal of Pediatrics*, *211*, 27-32.e1. https://doi.org/10.1016/j.jpeds.2019.04.028

Quigley, M. A., Poulsen, G., Boyle, E., Wolke, D., Field, D., Alfirevic, Z., & Kurinczuk, J. J. (2012). Early term and late preterm birth are associated with poorer school performance at age 5 years: a cohort study. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *97*(3), F167-73. https://doi.org/10.1136/archdischild-2011-300888

Rabe, H., Gyte, G. M. L., Díaz-Rossello, J. L., & Duley, L. (2019). Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. *Cochrane Database of Systematic Reviews*, *2019*(9). https://doi.org/10.1002/14651858.CD003248.PUB4

Rakshasbhuvankar, A. A., Simmer, K., Patole, S. K., Stoecklin, B., Nathan, E. A., Clarke, M. W., & Pillow, J. J. (2021). Enteral vitamin A for reducing severity of bronchopulmonary dysplasia: A randomized trial. *Pediatrics*, *147*(1). https://doi.org/10.1542/PEDS.2020-009985/33450

Ramanathan, R., Corwin, M. J., Hunt, C. E., Lister, G., Tinsley, L. R., Baird, T., Silvestri, J. M., Crowell, D. H., Hufford, D., Martin, R., Neuman, M. R., Weese-Mayer, D. E., Cupples, L. A., Peucker, M., Willinger, M., & Keens, T. G. (2001). Cardiorespiratory events recorded on home monitors: Comparison of healthy infants with those at increased risk for SIDS. *Journal of the American Medical Association*, *285*(17), 2199–2207. https://doi.org/10.1001/jama.285.17.2199

Reynolds, P. (2013). Fetal to neonatal transition - how does it take place? *Surgery*, *31*, 106–109. https://doi.org/10.1016/j.mpsur.2013.01.004

Reynolds, W. J., & Milhorn, H. T. (1973). Transient ventilatory response to hypoxia with and without controlled alveolar Pco2. *Journal of Applied Physiology*, *35*(2), 187–196. https://doi.org/10.1152/jappl.1973.35.2.187

Rhein, L., Dobson, N., Darnall, R. A., Corwin, M. J., Heeren, T. C., Poets, C. F., McEntire, B. L., Hunt, C. E., Bada, H., Carlo, W., Consenstein, L., Gregory, M. L., Hendricks-Munoz, K., Hoy, D., Karamchandani, N., Klarr, J. M., Kumar, P., Leach, C. L., Richardson, J., … White, R. D. (2014). Effects of caffeine on intermittent hypoxia in infants born prematurely. *JAMA Pediatrics*, *168*(3), 250–257. https://doi.org/10.1001/jamapediatrics.2013.4371

Rhein, L., Simoneau, T., Davis, J., Correia, C., Ferrari, D., Monuteaux, M., & Gregory, M. L. (2012). Reference values of nocturnal oxygenation for use in outpatient oxygen weaning protocols in premature infants. *Pediatric Pulmonology*, *47*(5), 453–459. https://doi.org/10.1002/PPUL.21562

Ritchie, J., & Lakhani, K. (1980). Fetal breathing movements in response to maternal inhalation of 5% carbon dioxide. *American Journal of Obstetrics and Gynecology*, *136*(3), 386–388. https://doi.org/10.1016/0002-9378(80)90867-4

Ritchie, K. (1980). The fetal response to changes in the composition of maternal inspired air in human pregnancy. *Seminars in Perinatology*, *4*(4), 295–299.

Ritchie, K., Carrière, I., de Mendonca, A., Portet, F., Dartigues, J. F., Rouaud, O., Barberger-Gateau, P., & Ancelin, M. L. (2007). The neuroprotective effects of caffeine: a prospective population study (the Three City Study). *Neurology*, *69*(6), 536–545. https://doi.org/10.1212/01.wnl.0000266670.35219.0c

Rite, S., Martín de Vicente, C., García-Iñiguez, J. P., Couce, M. L., Samper, M. P., Montaner, A., & Ruiz de la Cuesta, C. (2022). The consensus definition of bronchopulmonary dysplasia is an adequate predictor of lung function at preschool age. *Frontiers in Pediatrics*, *10*(February), 830035. https://doi.org/10.3389/fped.2022.830035

Roberts, T., Campbell, A., Larsen, P., & Elder, D. (2017). Preterm infants at discharge: nap polysomnography versus 24-hour oximetry. *Acta Paediatrica*, *106*(11), 1754–1759. https://doi.org/10.1111/APA.13900

Romeo, M. G., Proto, N., Tina, L. G., Parisi, M. G., & Distefano, G. (1991). A comparison of the efficacy of aminophylline and doxapram in preventing idiopathic apnea in preterm newborn infants [Article in Italian]. *La Pediatria Medica e Chirurgica : Medical and Surgical Pediatrics*, *13*(1), 77–81.

Ronca, A. E., & Alberts, J. R. (1995). Cutaneous induction of breathing in perinatal rats. *Psychobiology*, *23*(4), 261–269.

Row, B. W., Kheirandish, L., Neville, J. J., & Gozal, D. (2002). Impaired spatial learning and hyperactivity in developing rats exposed to intermittent hypoxia. *Pediatric Research*, *52*(3), 449–453. https://doi.org/10.1203/00006450-200209000-00024

Rustin, J. (1989). Caffeine and babies. *BMJ : British Medical Journal*, *299*(6691), 121. https://doi.org/10.1136/bmj.299.6691.121-b

Ryu, J. (1985). Caffeine in human milk and in serum of breast-fed infants. *Developmental Pharmacology and Therapeutics*, *8*(6), 329–337.

Salem, F., Johnson, T. N., Hodgkinson, A. B. J., Ogungbenro, K., & Rostami-Hodjegan, A. (2021). Does "birth" as an event impact maturation trajectory of renal clearance via glomerular filtration? Reexamining data in preterm and full-term neonates by avoiding the creatinine bias. *Journal of Clinical Pharmacology*, *61*(2), 159–171. https://doi.org/10.1002/jcph.1725

Sarici, S. Ü., Serdar, M. A., Korkmaz, A., Erdem, G., Oran, O., Tekinalp, G., Yurdakök, M., & Yigit, S. (2004). Incidence, course, and prediction of hyperbilirubinemia in near-term and term newborns. *Pediatrics*, *113*(4 I), 775–780. https://doi.org/10.1542/peds.113.4.775

Saugstad, O. D., & Aune, D. (2013). Optimal oxygenation of extremely low birth weight infants: A meta-analysis and systematic review of the oxygen saturation target studies. *Neonatology*, *105*(1), 55–63. https://doi.org/10.1159/000356561

Scanlon, J. E. M., Chin, K. C., Morgan, M. E. I., Durbin, G. M., Hale, K. A., & Brown, S. S. (1992). Caffeine or theophylline for neonatal apnoea? *Archives of Disease in Childhood*, *67*, 425–428. https://doi.org/10.1136/adc.67.4_Spec_No.425

Schittny, J. C. (2017). Development of the lung. *Cell and Tissue Research*, *367*(3), 427. https://doi.org/10.1007/S00441-016-2545-0

Schmidt, B. (1999). Methylxanthine therapy in premature infants: Sound practice, disaster, or fruitless byway? *The Journal of Paediatrics*, *135*(4), 526–528.

Schmidt, B. (2023). Caffeine for apnea of prematurity: Too much or too little of a good thing. *Journal of Pediatrics*, *259*, 113488. https://doi.org/10.1016/j.jpeds.2023.113488

Schmidt, B., Anderson, P. J., Asztalos, E. V, Doyle, L. W., Grunau, R. E., Moddemann, D., & Roberts, R. S. (2019). Self-reported quality of life at middle school age in survivors of very preterm birth: results from the caffeine for apnea of prematurity trial. In *JAMA Pediatrics* (Vol. 173, Issue 5, pp. 487–488). American Medical Association. https://doi.org/10.1001/jamapediatrics.2018.4853

Schmidt, B., Anderson, P. J., Doyle, L. W., Dewey, D., Grunau, R. E., Asztalos, E. V, Davis, P. G., Tin, W., Moddemann, D., Solimano, A., Ohlsson, A., Barrington, K. J., & Roberts, R. S. (2012). Survival without disability to age 5 years after neonatal caffeine therapy for apnea of prematurity. *Journal of the American Medical Association*, *307*(3), 275–282. https://doi.org/10.1001/jama.2011.2024

Schmidt, B., Roberts, R. S., Anderson, P. J., Asztalos, E. V, Costantini, L., Davis, P., Dewey, D., D'Ilario, J., Doyle, L. W., Grunau, R. E., Moddemann, D., Nelson, H., Ohlsson, A., Solimano, A., & Tin, W. (2017). Academic performance, motor function, and behavior 11 years after neonatal caffeine citrate therapy for apnea of prematurity. *JAMA Pediatrics*, *171*(6), 564. https://doi.org/10.1001/jamapediatrics.2017.0238

Schmidt, B., Roberts, R. S., Davis, P., Doyle, L. W., Barrington, K., Ohlsson, A., Solimano, A., & Tin, W. (2006). Caffeine therapy for apnea of prematurity. *New England Journal of Medicine*, *354*(20), 2112–2121.

Schmidt, B., Roberts, R. S., Davis, P., Doyle, L. W., Barrington, K., Ohlsson, A., Solimano, A., & Tin, W.

(2007). Long-term effects of caffeine therapy for apnea of prematurity. *New England Journal of Medicine*, *357*(19), 1893–1902.

Schoen, K., Yu, T., Stockmann, C., Spigarelli, M. G., & Sherwin, C. M. T. (2014). Use of methylxanthine therapies for the treatment and prevention of apnea of prematurity. *Pediatric Drugs*, *16*(2), 169–177. https://doi.org/10.1007/s40272-013-0063-z

Schünemann, H., Brożek, J., Guyatt, G., & Oxman, A. (Eds.). (2013). *GRADE handbook*. The GRADE Working Group. www.guidelinedevelopment.org/handbook

Scott, R. M., Whitwam, J. G., & Chakrabarti, M. K. (1977). Evidence of a role for the peripheral chemoreceptors in the ventilatory response to doxapram in man. *British Journal of Anaesthesia*, *49*(3), 227–231. https://doi.org/10.1093/bja/49.3.227

Scurry, J. P., Adamson, T. M., & Cussen, L. J. (1989). Fetal lung growth in laryngeal atresia and tracheal agenesis. *Australian Paediatric Journal*, *25*(1), 47–51. https://doi.org/10.1111/J.1440-1754.1989.TB01413.X

Seger, N., & Soll, R. (2009). Animal derived surfactant extract for treatment of respiratory distress syndrome. *Cochrane Database of Systematic Reviews*, *2*. https://doi.org/10.1002/14651858.CD007836

Seppä-Moilanen, M., Andersson, S., & Kirjavainen, T. (2022). Supplemental oxygen treats periodic breathing without effects on sleep in late-preterm infants. *Neonatology*, *119*(5), 567–574. https://doi.org/10.1159/000525196

Shah, R., Dai, D. W. T., Alsweiler, J. M., Brown, G. T. L., Chase, J. G., Gamble, G. D., Harris, D. L., Keegan, P., Nivins, S., Wouldes, T. A., Thompson, B., Turuwhenua, J., Harding, J. E., & McKinlay, C. J. D. (2022). Association of neonatal hypoglycemia with academic performance in mid-childhood. *Journal of the American Medical Association*, *327*(12), 1158–1170. https://doi.org/10.1001/jama.2022.0992

Shanks, A. L., Grasch, J. L., Quinney, S. K., & Haas, D. M. (2019). Controversies in antenatal corticosteroids. *Seminars in Fetal and Neonatal Medicine*, *24*(3), 182–188. https://doi.org/10.1016/j.siny.2019.05.002

Shennan, A. T., Dunn, M. S., Ohlsson, A., Lennox, K., & Hoskins, E. M. (1988). Abnormal pulmonary outcomes in premature infants: Prediction from oxygen requirement in the neonatal period. *Pediatrics*, *82*(4), 527–532. https://doi.org/10.1542/peds.82.4.527

Shi, Y., Stornetta, D. S., Reklow, R. J., Sahu, A., Wabara, Y., Nguyen, A., Li, K., Zhang, Y., Perez-Reyes, E., Ross, R. A., Lowell, B. B., Stornetta, R. L., Funk, G. D., Guyenet, P. G., & Bayliss, D. A. (2021). A brainstem peptide system activated at birth protects postnatal breathing. *Nature*, *589*(7842), 426–

430. https://doi.org/10.1038/s41586-020-2991-4

Siddhi, P., & Foster, E. (2023a). Letter regarding "national survey on caffeine use in neonatal units across the United Kingdom." *Acta Paediatrica*, *00*, 1. https://doi.org/10.1111/APA.16789

Siddhi, P., & Foster, E. (2023b). Neonatal caffeine use: a clinician experience survey within the United Kingdom and abroad. *Archives of Disease in Childhood*, *108*(Suppl 2), A151–A151. https://doi.org/10.1136/ARCHDISCHILD-2023-RCPCH.247

Siffel, C., Kistler, K. D., Lewis, J. F. M., & Sarda, S. P. (2021). Global incidence of bronchopulmonary dysplasia among extremely preterm infants: a systematic literature review. *Journal of Maternal-Fetal and Neonatal Medicine*, *34*(11), 1721–1731. https://doi.org/10.1080/14767058.2019.1646240

Sigma-Aldrich. (2014). *Caffeine solution (C6035) - Datasheet*. https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/195/539/c6035-slbj7833vdat.pdf

Sippell, W. G., Becker, H., Versmold, H. T., Bidlingmaier, F., & Knorr, D. (1978). Longitudinal studies of plasma aldosterone, corticosterone, deoxycorticosterone, progesterone, 17-hydroxyprogesterone, cortisol, and cortisone determined simultaneously in mother and child at birth and during the early neonatal period. I. spontaneous deli. *Journal of Clinical Endocrinology and Metabolism*, *46*(6), 971–985. https://doi.org/10.1210/jcem-46-6-971

Smylie, J., Crengle, S., Freemantle, J., & Taualii, M. (2010). Indigenous birth outcomes in Australia, Canada, New Zealand and the United States – an overview. *Open Womens Health Journal*, *4*, 7–17. https://doi.org/10.2174/1874291201004010007

Sreenan, C., Etches, P. C., Demianczuk, N., & Robertson, C. M. T. (2001). Isolated mental developmental delay in very low birth weight infants: Association with prolonged doxapram therapy for apnea. *The Journal of Pediatrics*, *139*(6), 832–837. https://doi.org/10.1067/MPD.2001.119592

Stark, A., Smith, P. B., Hornik, C. P., Zimmerman, K. O., Hornik, C. D., Pradeep, S., Clark, R. H., Benjamin, D. K., Laughon, M., & Greenberg, R. G. (2022). Medication use in the neonatal intensive care unit and changes from 2010 to 2018. *Journal of Pediatrics*, *240*, 66-71.e4. https://doi.org/10.1016/j.jpeds.2021.08.075

Stavchansky, S., Delgado, M., Joshi, A., Combs, A., & Sagraves, R. (1988). Pharmacokinetics of caffeine in breast milk and plasma after single oral administration of caffeine to lactating mothers. *Biopharmaceutics & Drug Disposition*, *9*(3), 285–299. https://doi.org/10.1002/BOD.2510090307

Steer, P., Flenady, V. J., Shearman, A., Lee, T. C., Tudehope, D. I., & Charles, B. G. (2003). Periextubation caffeine in preterm neonates: a randomized dose response trial. *Journal of Paediatrics and Child*

Health, *39*(7), 511–515. https://doi.org/10.1046/J.1440-1754.2003.00207.X

Steer, P., Flenady, V., Shearman, A., Charles, B., Gray, P. H., Henderson-Smart, D., Bury, G., Fraser, S., Hegarty, J., Rogers, Y., Reid, S., Horton, L., Charlton, M., Jacklin, R., & Walsh, A. (2004). High dose caffeine citrate for extubation of preterm infants: A randomised controlled trial. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *89*(6), F499-503. https://doi.org/10.1136/adc.2002.023432

Stenson, B., Brocklehurst, P., & Tarnow-Mordi, W. (2011). Increased 36-week survival with high oxygen saturation target in extremely preterm infants. *New England Journal of Medicine*, *364*(17), 1680–1682. https://doi.org/10.1056/NEJMc1101319

Stevenson, D. K. (2007). On the caffeination of prematurity. *New England Journal of Medicine*, *357*(19), 1967–1968. https://doi.org/10.1056/NEJMe078200

Stojanovska, V., Atta, J., Kelly, S. B., Zahra, V. A., Matthews-Staindl, E., Nitsos, I., Moxham, A., Pham, Y., Hooper, S. B., Herlenius, E., Galinsky, R., & Polglase, G. R. (2022). Increased prostaglandin E2 in brainstem respiratory centers is associated with inhibition of breathing movements in fetal sheep exposed to progressive systemic inflammation. *Frontiers in Physiology*, *13*, 841229. https://doi.org/10.3389/fphys.2022.841229

Strandvik, B., Wahlén, E., & Wikström, S. A. (1994). The urinary bile acid excretion in healthy premature and full-term infants during the neonatal period. *Scandinavian Journal of Clinical and Laboratory Investigation*, *54*(1), 1–10. https://doi.org/10.3109/00365519409086503

Swanson, J. R., & Sinkin, R. A. (2015). Transition from fetus to newborn. *Pediatric Clinics of North America*, *62*, 329–343. https://doi.org/10.1016/j.pcl.2014.11.002

Szatkowski, L., Fateh, S., Abramson, J., Kwok, T. C., Sharkey, D., Budge, H., & Ojha, S. (2023). Observational cohort study of use of caffeine in preterm infants and association between early caffeine use and neonatal outcomes. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *108*(5), 505–510. https://doi.org/10.1136/archdischild-2022-324919

Tabacaru, C. R., Jang, S. Y., Patel, M., Davalian, F., Zanelli, S., & Fairchild, K. D. (2017). Impact of caffeine boluses and caffeine discontinuation on apnea and hypoxemia in preterm infants. *Journal of Caffeine Research*, *7*(3), 103–110. https://doi.org/10.1089/jcr.2017.0002

Talge, N. M., Holzman, C., Wang, J., Lucia, V., Gardiner, J., & Breslau, N. (2010). Late-preterm birth and its association with cognitive and socioemotional outcomes at 6 years of age. *Pediatrics*, *126*(6), 1124–1131. https://doi.org/10.1542/peds.2010-1536

Tayman, C., Rayyan, M., & Allegaert, K. (2011). Neonatal pharmacology: Extensive interindividual variability despite limited size. *The Journal of Pediatric Pharmacology and Therapeutics*, *16*(3), 170–

184. https://doi.org/10.5863/1551-6776-16.3.170

Teune, M. J., Bakhuizen, S., Bannerman, C. G., Opmeer, B. C., Van Kaam, A. H., Van Wassenaer, A. G., Morris, J. M., & Mol, B. W. J. (2011). A systematic review of severe morbidity in infants born late preterm. *American Journal of Obstetrics and Gynecology*, *205*(4), 374.e1. https://doi.org/10.1016/j.ajog.2011.07.015

The Antenatal Magnesium Sulphate For Neuroprotection Guideline Development Panel. (2010). *Antenatal magnesium sulphate prior to preterm birth for neuroprotection of the fetus, infant and child: National clinical practice guidelines*. The University of Adelaide.

Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M., & Welch, V. (Eds.). (2022). *Cochrane handbook for systematic reviews of interventions* (6.3). Cochrane. www.training.cochrane.org/handbook

Thomson, A. H., Kerr, S., & Wright, S. (1996). Population pharmacokinetics of caffeine in neonates and young infants. *Therapeutic Drug Monitoring*, *18*(3), 245–253.

Tian, C., Li, D., & Fu, J. (2022). Molecular mechanism of caffeine in preventing bronchopulmonary dysplasia in premature infants. *Frontiers in Pediatrics*, *10*, 902437. https://doi.org/10.3389/fped.2022.902437

Tilley, S. L. (2011). Methylxanthines in asthma. *Handbook of Experimental Pharmacology*, *200*(200), 439–456. https://doi.org/10.1007/978-3-642-13443-2_17

Tin, W., Brunskill, G., Kelly, T., & Fritz, S. (2012). 15-Year follow-up of recurrent "hypoglycemia" in preterm infants. *Pediatrics*, *130*(6), e1497–e1503. https://doi.org/10.1542/peds.2012-0776

Tracy, T. S., Venkataramanan, R., Glover, D. D., Caritis, S. N., & Tracy, S. (2005). Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. *American Journal of Obstetrics and Gynecology*, *192*, 633–642. https://doi.org/10.1016/j.ajog.2004.08.030

Turner, M., & Shah, U. (2015). Why are excipients important to neonates? *Current Pharmaceutical Design*, *21*(39), 5680–5687. https://doi.org/10.2174/1381612821666150901110341

Tyrala, E. E., & Dodson, W. E. (1979). Caffeine secretion into breast milk. *Archives of Disease in Childhood*, *54*(10), 787–800. https://doi.org/10.1136/adc.54.10.787

United States Department of Health and Human Services, Centers for Disease Control and Prevention, & National Center for Health Statistics. (2021). *Natality public-use data 2016-2020, on CDC WONDER Online Database*. http://wonder.cdc.gov/natality-expanded-current.html

US Food and Drug Administration, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), & Center for Veterinary

Medicine (CVM). (2018). Bioanalytical Method Validation: Guidance for Industry. In *Biopharmaceutics*. https://www.fda.gov/media/70858/download

Vali, A., Asilian, A., Khalesi, E., Khoddami, L., Shahtalebi, M., & Mohammady, M. (2005). Evaluation of the efficacy of topical caffeine in the treatment of psoriasis vulgaris. *Journal of Dermatological Treatment*, *16*(4), 234–237. https://doi.org/10.1080/09546630510011801

van den Anker, J., & Allegaert, K. (2021). Considerations for drug dosing in premature infants. *Journal of Clinical Pharmacology*, *61*(S1), S141–S151. https://doi.org/10.1002/jcph.1884

Verma, R. P., McCulloch, K. M., Worrell, L., & Vidyasagar, D. (1996). Vitamin A deficiency and severe bronchopulmonary dysplasia in very low birthweight infants. *American Journal of Perinatology*, *13*(7), 389–393. https://doi.org/10.1055/S-2007-994376/BIB

Vliegenthart, R., Miedema, M., Hutten, G. J., Van Kaam, A. H., & Onland, W. (2018). High versus standard dose caffeine for apnoea: A systematic review. In *Archives of Disease in Childhood: Fetal and Neonatal Edition* (Vol. 103, Issue 6, pp. F523–F529). https://doi.org/10.1136/archdischild-2017-313556

Vliegenthart, R., Ten Hove, C. H., Onland, W., & Van Kaam, A. H. L. C. (2017). Doxapram treatment for apnea of prematurity: A systematic review. *Neonatology*, *111*, 162–171. https://doi.org/10.1159/000448941

Vohra, V., & Marraffa, J. (2019). Maternal use of acetaminophen-butalbital-caffeine product resulting in neonate butalbital exposure through breast milk. *Clinical Toxicology*, *57*(6), 549.

Völler, S., Flint, R. B., Simons, S. H. P., & Knibbe, C. A. J. (2021). Comment on: "preterm physiologically based pharmacokinetic model, part I and part II". In *Clinical Pharmacokinetics* (Vol. 60, Issue 5, pp. 677–679). Adis. https://doi.org/10.1007/s40262-021-00993-4

Wahlländer, A., Mohr, S., & Paumgartner, G. (1990). Assessment of hepatic function: Comparison of caffeine clearance in serum and saliva during the day and at night. *Journal of Hepatology*, *10*(2), 129–137. https://doi.org/https://doi.org/10.1016/0168-8278(90)90041-O

Walker, D. W., Lee, B., & Nitsos, I. (2000). Effect of hypoxia on respiratory activity in the foetus. *Clinical and Experimental Pharmacology and Physiology*, *27*(1–2), 110–113. https://doi.org/10.1046/j.1440-1681.2000.03201.x

Walsh, J. M., Doyle, L. W., Anderson, P. J., Lee, K. J., & Cheong, J. L. Y. (2014). Moderate and late preterm birth: Effect on brain size and maturation at term-equivalent age. *Radiology*, *273*(1), 232–240. https://doi.org/10.1148/radiol.14132410

Walters, A. G. B., Crowther, C., Dalziel, S. R., Eagleton, C., Gamble, G., McKinlay, C. J., Milne, B., Xu, D., & Harding, J. E. (2023). Fifty-year follow-up of the Auckland steroid trial: assessing the long-term impacts of antenatal corticosteroids. *Paediatric Academic Societies Conference (Washington, DC)*.

Walters, A. G. B., Lin, L., Crowther, C. A., Gamble, G. D., Dalziel, S. R., & Harding, J. E. (2023). Betamethasone for preterm birth: Auckland steroid trial full results and new insights 50 years on. *Journal of Pediatrics*, *255*, 80-88.e5. https://doi.org/10.1016/j.jpeds.2022.10.028

Wan, X., Wang, W., Liu, J., & Tong, T. (2014). Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Medical Research Methodology*, *14*(1), 1–13. https://doi.org/10.1186/1471-2288-14-135

Wang, M., Dorer, D. J., Fleming, M. P., & Catlin, E. A. (2004). Clinical outcomes of near-term infants. *Pediatrics*, *114*(2), 372–376. https://doi.org/10.1542/peds.114.2.372

Wang, Y. C., Chan, O. W., Chiang, M. C., Yang, P. H., Chu, S. M., Hsu, J. F., Fu, R. H., & Lien, R. (2017). Red blood cell transfusion and clinical outcomes in extremely low birth weight preterm infants. *Pediatrics and Neonatology*, *58*(3), 216–222. https://doi.org/10.1016/j.pedneo.2016.03.009

Ward, R. M. (2006). Drug disposition in the late preterm ("near-term") newborn. *Seminars in Perinatology*, *30*(1), 48–51. https://doi.org/10.1053/j.semperi.2006.01.013

Weaver, L. K., Hopkins, R. O., Chan, K. J., Churchill, S., Gregory Elliott, C., Clemmer, T. P., Orme, J. F., Thomas, F. O., & Morris, A. H. (2002). Hyperbaric oxygen for acute carbon monoxide poisoning. *New England Journal of Medicine*, *347*(14), 1057–1067. https://doi.org/10.1056/NEJMoa013121

Weiss, E. M., Olszewski, A. E., Guttmann, K. F., Magnus, B. E., Li, S., Shah, A. R., Juul, S. E., Wu, Y. W., Ahmad, K. A., Bendel-Stenzel, E., Isaza, N. A., Lampland, A. L., Mathur, A. M., Rao, R., Riley, D., Russell, D. G., Salih, Z. N., Torr, C. B., Weitkamp, J.-H., … Shah, S. K. (2021). Parental factors associated with the decision to participate in a neonatal clinical trial. *JAMA Network Open*, *4*(1), e2032106. https://doi.org/10.1001/jamanetworkopen.2020.32106

Wellington, G., Campbell, A., & Elder, D. (2019). Intermittent hypoxia in preterm infants: Measurement using the desaturation index. *Pediatric Pulmonology*, *54*(6), 865–872. https://doi.org/10.1002/ppul.24276

Wellington, G., Elder, D., & Campbell, A. (2018). 24-hour oxygen saturation recordings in preterm infants: editing artefact. *Acta Paediatrica*, *107*(8), 1362–1369. https://doi.org/10.1111/APA.14293

Welsh, E. J., Bara, A., Barley, E., & Cates, C. J. (2010). Caffeine for asthma. *Cochrane Database of Systematic Reviews*, *2012*(8). https://doi.org/10.1002/14651858.CD001112.PUB2

WHO ACTION Trials Collaborators. (2022). Antenatal dexamethasone for late preterm birth: A multi-centre, two-arm, parallel, double-blind, placebo-controlled, randomized trial. *EClinicalMedicine*, *44*, 101285. https://doi.org/10.1016/j.eclinm.2022.101285

Whyte, R., & Kirpalani, H. (2011). Low versus high haemoglobin concentration threshold for blood transfusion for preventing morbidity and mortality in very low birth weight infants. *Cochrane Database of Systematic Reviews*, *11*. https://doi.org/10.1002/14651858.CD000512.pub2

Wigglesworth, J. S., & Desai, R. (1979). Effect on lung growth of cervical cord section in the rabbit fetus. *Early Human Development*, *3*(1), 51–65. https://doi.org/10.1016/0378-3782(79)90020-3

Williams, L. Z. J., Mcnamara, D., & Alsweiler, J. M. (2018). Intermittent hypoxemia in infants born late preterm: a prospective cohort observational study. *The Journal of Pediatrics*, *204*, 89-95.e1. https://doi.org/10.1016/j.jpeds

Woodward, L. J., Anderson, P. J., Austin, N. C., Howard, K., & Inder, T. E. (2006). Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *New England Journal of Medicine*, *355*(7), 685–694. https://doi.org/10.1056/nejm0a053792

World Health Organization. (2014). *Guideline: Delayed umbilical cord clamping for improved maternal and infant health and nutrition outcomes*. World Health Organization.

World Health Organization. (2015). *WHO recommendations on interventions to improve preterm birth outcomes*. World Health Organization.

World Health Organization. (2022). *WHO recommendations for care of the preterm or low-birth-weight infant*. World Health Organization.

Woythaler, M. (2018). Neurodevelopmental outcomes of the late preterm infant. *Seminars in Fetal and Neonatal Medicine*, *24*, 54–59. https://doi.org/10.1016/j.siny.2018.10.002

Woythaler, M., McCormick, M. C., Mao, W. Y., & Smith, V. C. (2015). Late preterm infants and neurodevelopmental outcomes at kindergarten. *Pediatrics*, *136*(3), 424–431. https://doi.org/10.1542/peds.2014-4043

Woythaler, M., McCormick, M. C., & Smith, V. C. (2011). Late preterm infants have worse 24-month neurodevelopmental outcomes than term infants. *Pediatrics*, *127*(3), e622-9. https://doi.org/10.1542/peds.2009-3598

Wu, C. S., Chen, C. M., & Chou, H. C. (2017). Pulmonary hypoplasia induced by oligohydramnios: Findings from animal models and a population-based study. *Pediatrics and Neonatology*, *58*(1), 3–7. https://doi.org/10.1016/j.pedneo.2016.04.001

Wu, Y., Völler, S., Flint, R. B., Simons, S. H., Allegaert, K., Fellman, V., & Knibbe, C. A. (2022). Pre- and postnatal maturation are important for fentanyl exposure in preterm and term newborns: A pooled population pharmacokinetic study. *Clinical Pharmacokinetics*, *61*(3), 401–412. https://doi.org/10.1007/s40262-021-01076-0

Wyllie, J., Bruinenberg, J., Roehr, C. C., Rüdiger, M., Trevisanuto, D., & Urlesberger, B. (2015). European Resuscitation Council Guidelines for Resuscitation 2015: Section 7. Resuscitation and support of transition of babies at birth. *Resuscitation*, *95*, 249–263. https://doi.org/10.1016/J.RESUSCITATION.2015.07.029

Xu, H., Rajesan, R., Harper, P., Kim, R. B., Lonnerdal, B., Yang, M., Uematsu, S., Hutson, J., Watson-MacDonell, J., & Ito, S. (2005). Induction of cytochrome P450 1A by cow milk-based formula: a comparative study between human milk and formula. *British Journal of Pharmacology*, *146*(2), 296–305. https://doi.org/10.1038/SJ.BJP.0706319

Yao, A. C., Moinian, M., & Lind, J. (1969). Distribution of blood between infant and placenta after birth. *Lancet*, *2*(7626), 871–873. https://doi.org/10.1016/s0140-6736(69)92328-9

Ye, Y., Yang, X., Zhao, J., He, J., Xu, X., Li, J., Shi, J., & Mu, D. (2022). Early vitamin a supplementation for prevention of short-term morbidity and mortality in very-low-birth-weight infants: A systematic review and meta-analysis. *Frontiers in Pediatrics*, *10*, 788409. https://doi.org/10.3389/fped.2022.788409

Yeoshoua, E., Goldstein, I., Zlozover, M., & Wiener, Z. (2012). Sonographic study of the relationship between gestational diabetes mellitus and fetal activity. *The Journal of Maternal-Fetal and Neonatal Medicine*, *25*(6), 623–626. https://doi.org/10.3109/14767058.2011.597897

Yin, W., Döring, N., Persson, M. S. M., Persson, M., Tedroff, K., Ådén, U., & Sandin, S. (2022). Gestational age and risk of intellectual disability: A population-based cohort study. *Archives of Disease in Childhood*, *0*, 1–7. https://doi.org/10.1136/archdischild-2021-323308

Yost, C. S. (2006). A new look at the respiratory stimulant doxapram. *CNS Drug Reviews*, *12*(3–4), 236–249.

Yu, T., Balch, A. H., Ward, R. M., Korgenski, E. K., & Sherwin, C. M. T. (2016). Incorporating pharmacodynamic considerations into caffeine therapeutic drug monitoring in preterm neonates. *BMC Pharmacology and Toxicology*, *17*(1), 1–8. https://doi.org/10.1186/s40360-016-0065-x

Yuan, Y., Yang, Y., Lei, X., & Dong, W. (2022). Caffeine and bronchopulmonary dysplasia: Clinical benefits and the mechanisms involved. *Pediatric Pulmonology*, *57*(6), 1392–1400. https://doi.org/10.1002/PPUL.25898

Zhao, Y., Tian, X., & Liu, G. (2016). Clinical effectiveness of different doses of caffeine for primary apnea in preterm infants [Article in Chinese]. *Zhonghua Er Ke Za Zhi [Chinese Journal of Pediatrics]*, *54*(1), 33–36. https://doi.org/10.3760/cma.j.issn.0578-1310.2016.01.008

Zivanovic, S., Peacock, J., Alcazar-Paris, M., Lo, J. W., Lunt, A., Marlow, N., Calvert, S., & Greenough, A. (2014). Late outcomes of a randomized trial of high-frequency oscillation in neonates. *New England Journal of Medicine*, *370*(12), 1121–1130. https://doi.org/10.1056/nejmoa1309220