PROTOCOL

Caffeine prophylaxis to improve intermittent hypoxaemia in infants born late preterm: a randomised controlled dosage trial (Latte Dosage Trial)

Short Title: The Latte Dosage Trial

Registration: The Latte Dosage Trial will be registered with ANZCTR (ACTRN12618001745235)

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This study protocol follows the SPIRIT checklist
## Version Record

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1. PROJECT SUMMARY

Late preterm infants (34^{0}-36^{6} weeks’ gestational age (GA)) are the most common of all preterm infants, constituting 6% of all births or 3,700 births annually in New Zealand. These infants have a 30% increased risk of severe long-term neurodevelopmental impairment compared to infants born at term. While there has been progress in improving neurodevelopmental outcomes for infants born more preterm, it is only recently that late preterm infants have been recognised as being at risk of significant problems. Remarkably, there has been very little research on how to improve the long term outcomes of infants born late preterm.

In very preterm infants, both apnoea (pauses in breathing) and intermittent hypoxaemia (recurrent drops in oxygen saturation) are common and are associated with worse neurodevelopmental outcomes. Treatment with caffeine not only reduces apnoea and intermittent hypoxaemia in very preterm infants but also improves long-term neurodevelopment. While apnoea of prematurity is less common in late preterm infants, we have recently identified that infants born late preterm are also at increased risk of intermittent hypoxaemia. Caffeine has been shown to improve intermittent hypoxaemia in very preterm infants at 35 weeks’ and 36 weeks’ post-menstrual age, but there are no data to show if caffeine improves long-term neurodevelopmental outcomes in infants born late preterm.

The most effective dose of caffeine at this gestational age is uncertain. Very preterm infants have limited ability to metabolise caffeine, but this increases with greater gestational age. Therefore, late preterm infants are likely to need a higher dose of caffeine than very preterm infants. However, if the dose is too high it may have increased side effects and will not be tolerated. This randomised, placebo-controlled, dosage trial of prophylactic caffeine treatment will determine the most effective and best tolerated dose of caffeine to reduce intermittent hypoxia in late preterm infants.
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2. INTRODUCTION

2.1 Background

Late preterm infants (34+0-36+6 weeks’ GA), constitute 83% of the preterm population, and approximately 6% of all infants born annually in New Zealand, or 3,700 infants per year\(^1\). Despite late preterm infants making up by far the largest proportion of infants born preterm, the majority of research efforts have concentrated on improving survival and long-term outcomes of infants born very (<32 weeks’ GA) and extremely (<28 weeks’ GA) preterm\(^2-4\). Many late preterm infants are managed in a similar manner to term infants on postnatal wards, rather than in neonatal units\(^5, 6\) 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. Recently, it has become apparent that late preterm infants have higher rates of mortality\(^7, 8\), cerebral palsy\(^9, 10\), developmental delay\(^11, 12\), cognitive impairment\(^13-15\), and behavioural disorders\(^16\) compared to term infants (>37 weeks’ GA). The identification of late preterm infants as a high-risk group has been described as an important public health breakthrough\(^5\), yet there have been few interventional studies designed to improve long-term outcomes in late preterm infants\(^17\). A recently published trial of antenatal betamethasone for women at risk of late preterm delivery, demonstrating a reduction in respiratory morbidity in infants born late preterm when the mother had been prophylactically treated with antenatal steroids, is an example of a treatment known to be effective in very preterm infants\(^18\) that is also effective in late preterm infants\(^19\).

Very preterm infants are at high risk of apnoea of prematurity and intermittent hypoxaemia\(^20, 21\). Apnoea of prematurity refers to prolonged pauses in breathing, of 20 seconds or more, which may cause a reduction in the oxygen saturation and bradycardia and is associated with an increased incidence of brain injury\(^22\) and neurodevelopmental impairment\(^23\). Late preterm infants also experience apnoea of prematurity, although less frequently than in very preterm infants\(^20\). Recently, we have demonstrated that late preterm infants have frequent episodes of intermittent hypoxaemia, i.e., brief 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 2 weeks’ postnatal age, before reducing to baseline levels at term corrected age (Fig 1)\(^24\).

Studies in adults have consistently shown that even brief exposure to hypoxia, whether from high altitude\(^25\) or carbon monoxide poisoning\(^26\), 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\(^27-29\). Intermittent hypoxaemia is associated with altered brain development in neonatal mice\(^30\) and reduced cognition and behavioural deficits in neonatal rats\(^31\). In humans, intermittent hypoxaemia is associated with poor neurodevelopmental outcomes in extremely preterm infants\(^32\) and in children with sleep disordered breathing\(^33\) and congenital heart disease\(^34\).

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\(^3, 35, 36\). Importantly, 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\textsuperscript{37, 38}. While some of the long term beneficial
effects of caffeine may be due to its effect in reducing the incidence of chronic lung disease\textsuperscript{39},
there is also benefit from reducing the amount of time that infants are hypoxic, independent of
the effect on chronic lung disease\textsuperscript{40}. 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
also very well tolerated.

In the last decade, there have been multiple studies demonstrating that late preterm infants
have an increased risk of poor neurodevelopmental outcomes\textsuperscript{41-43}. However, there have been
few studies on interventions which aim to improve these outcomes. 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 elimination is from the P450 1A cytochrome subgroup in the
liver\textsuperscript{44}. However, in newborn preterm infants, hepatic metabolism of caffeine is almost absent,
and most caffeine is eliminated via the kidneys. Therefore, caffeine metabolism is slow in
extremely preterm infants, and the half-life of caffeine is long. With increasing gestational age,
the hepatic metabolism of caffeine increases\textsuperscript{31}, and larger doses may be needed to maintain a
therapeutic effect. Further, 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\textsuperscript{45}.

There is a wide range in the dose of caffeine citrate given to extremely preterm infants, from
daily doses of 5 mg/kg\textsuperscript{35} to 20 mg/kg\textsuperscript{46}. The CAP trial used a dose of 5 mg/kg, which could be
increased to 10 mg/kg if necessary to control apnoea of prematurity\textsuperscript{46}. The Rhein trial 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\textsuperscript{21}. 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\textsuperscript{46}. Caffeine also causes reduced neonatal weight gain
compared to placebo\textsuperscript{35}, 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)\textsuperscript{46}. 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 therefore propose the Latte Dosage Trial, a randomised, placebo-controlled dosage trial of
oral caffeine citrate from birth to term equivalent age to reduce intermittent hypoxaemia in late
preterm infants.

\textbf{2.2 Objective}

To determine the most effective dose of caffeine to reduce intermittent hypoxaemia in late
preterm infants.
2.3 Hypothesis
Caffeine citrate administered to late preterm infants will reduce the incidence of intermittent hypoxaemia compared to controls in a dose-dependent manner.

2.4 Study Design
A phase IIb, double-blind, five-arm, parallel, randomised controlled trial to compare the effect of 4 different doses of oral caffeine citrate versus placebo on the frequency of intermittent hypoxaemia in late preterm infants.

3. METHODS
3.1 Participants, Interventions and Outcomes
3.1.1 Study Setting
Participants will be recruited at Auckland City Hospital and Middlemore Hospital, but may reside within any of the three Auckland District Health Board (DHB) regions (Auckland, Counties Manukau or Waitemata). Infants will be randomised and receive the initial overnight pulse oximetry and loading dose of the study drug while in hospital. Once the infant is discharged the parents will continue to give a daily dose of the study drug until term equivalent age. A staff member (research nurse or PhD student) will perform the follow-up visits at 2 weeks’ post randomisation and term equivalent age.

3.1.2 Eligibility Criteria
Infants born between 34+0 – 36+6 weeks’ GA without contradiction to caffeine treatment.

3.1.3 Exclusion criteria
- 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 DHB regions

3.1.4 Study Intervention
Infants will be assigned randomly by online randomisation to either caffeine citrate 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg or placebo, with 1:1:1:1:1 allocation, and stratified by study site and then for gestational age at birth (34, 35 or 36 weeks) (Figure 2). Infants will be randomised within 72 hours of birth. Infants from multiple births will be randomised to the same treatment group.

The staff member randomizing the infant will receive a study allocation letter (A to E or F to J)

![Figure 2. Flow diagram of randomisation schedule](image-url)
that corresponds to pre-labelled study bottles containing either caffeine citrate or water (identical appearance). Four different concentrations of the caffeine citrate (5 mg/ml, 10 mg/ml, 15 mg/ml and 20 mg/ml) will be used so that all infants receive the same dose volume (1 ml/kg, once daily), to maintain blinding.

The infant will be given a 2 ml/kg 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) at 7-9 am on the first morning after the infant reaches 72 hours of age, followed by a daily dose of 1 ml/kg each morning (5 mg/kg, 10 mg/kg, 15 mg/kg or 20 mg/kg of caffeine citrate or placebo) until until term equivalent age (40 weeks’ post-menstrual age). The dose will be recalculated for the infants’ weight gain weekly after the infant has regained birth weight.

The study drug is 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.

3.1.5 Open Label Caffeine Citrate

If an infant in the trial has apnoea or intermittent hypoxaemia clinicians will be encouraged to use oxygen or positive pressure ventilation (high flow or CPAP) as a first line treatment where appropriate. If necessary, clinicians can give a loading dose of caffeine citrate as an open label medication. If a clinician decides to continue caffeine treatment, they can discuss the option of partially un-blinding the infant (caffeine or placebo) with the Site Principal Investigator (see 2.2.4). Open label caffeine treatment will be recorded, and these infants will be included in the intention-to-treat analysis.

3.1.6 Compliance

Parents of the infants in the trial will be asked to keep a drug diary, detailing the volume of the study drug that was given, the time of the dose, and any vomiting or reflux with the medication. This will be checked by the research nurse at two weeks visit and collected at the last visit. At the two weeks visit the research nurse will give the parents a new bottle of the study drug and collect the current bottle to measure compliance. On the last visit (term equivalent age) the research nurse will ask what treatment they thought their infant received, to assess the adequacy of study blinding, and collect the remaining study drug for measurement to determine the remaining volume. Good compliance will be defined as <20% of the expected study drug volume remaining.

3.1.7 Primary Outcome

Frequency of intermittent hypoxaemia (events/hour, defined as oxygen saturation concentration ≥10% below baseline for any duration) on overnight oximetry, two weeks after randomisation.

3.1.8 Secondary Outcomes

- Frequency of intermittent hypoxaemia on overnight oximetry at term equivalent age (40 weeks’ post-menstrual age)
- Mean overnight oxygen saturation 2 weeks after randomisation and at term equivalent age
- Weight gain from birth to term equivalent age, measured as growth velocity, g.kg⁻¹.day⁻¹
- Length and head circumference growth from birth to term equivalent age, measured as growth velocity, cm.kg⁻¹.day⁻¹
- Tachycardia, measured as percentage of time HR >180 beats/min on overnight pulse oximetry, 2 weeks after randomisation and at term equivalent age
- Feed intolerance 2 weeks after randomisation and at term equivalent age, reported by parents
- Sleeping and arousal 2 weeks after randomisation and at term equivalent age, as measured by subscale 9 on the Infant Behaviour Questionnaire-Revised (IBQ-R), modified for neonates (Appendix 8.7)
- Maternal and infant salivary caffeine concentration at two weeks after randomisation
- Maternal daily caffeine intake 2 weeks after randomisation and at term equivalent age
- Maternal mental health (Edinburgh postnatal depression score)
- Duration of tube feeding (days)
- Pre-specified adverse events (see section 5)
- Readmission to hospital, and reason(s) for admission (defined as admission to postnatal, medical or surgical ward, or emergency department stay >12 hours) until 44 weeks post-menstrual age
- Open label caffeine use
- Use of respiratory support, including oxygen, until term equivalent age.

### 3.1.9 Participant Timeline

The study schedule is as follows:

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<th>Baseline</th>
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3.1.10 Sample Size

Based on our previous study we estimate a mean (SD) frequency of 6.9 (3.4) episodes per hour of intermittent hypoxaemia ≥10% below baseline 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 arm x 5 arms = 120 infants, with two-sided alpha of 0.05.

3.1.11 Recruitment

Potential parents of late preterm infants on the antenatal wards, and parents of late preterm infants will be identified from the whiteboard of the neonatal units, postnatal wards and from the hospital database. Parents of eligible infants will be approached by a research nurse/midwife or investigator, or a member of the neonatal clinical team either antenatally or in the first 72 hours after birth. Parents will be informed about the trial and given the Parent Information Sheet (PIS). They will be given time to consider the trial, ask questions and discuss the study with family and friends. Parents who agree for their infant to participate in the trial will be asked to sign the consent form, one copy of which will be given to the parents and another placed in the clinical record. After birth and completion of written informed consent, the infant will be randomised.

3.1.12 Discontinuation of Study Drug

The allocated treatment can be stopped at any time at the request of the parents, or by the clinician caring for the infant if (s)he feels that stopping the treatment would be in the best interest of the infant. If treatment is stopped due to an acute illness or problem that resolves, consideration should be given to restarting the allocated treatment. Infants should continue in the study and complete all planned assessments, regardless of treatment status, unless they are formally withdrawn from the study. Data collected up to the point of withdrawal will be reported (with consent) and analysis will be according to the intention-to-treat principle.

3.1.13 Withdrawal

If a parent wishes to remove their infant from the study they will be given the option of 1) discontinuation of study drug and collection of minimum outcome data (as agreed with the parents); 2) withdrawal from the study and discontinuation of further data collection, with use of data collected prior to withdrawal; or 3) complete withdrawal from the study including previously collected outcome data.

3.1.14 Co-interventions

All clinical care, other than study interventions and assessments, will be determined by the local clinical team, according to local guidelines.

Clinicians will be advised to use oxygen as first line treatment for persistent hypoxaemia.

3.1.15 Safe sleep

The research team will be trained in safe sleep practices (e.g. back to sleep, flat sleeping surface, no bed-sharing). If the infant does not have a safe sleeping environment a research team member will offer the family a referral to a safe sleep provider for consideration of provision of a Pepi-pod.
3.1.16 Re-admission

Infants in the trial will have an “alert” in the hospital database placed on their file to inform clinicians that the infant is in the trial, and to give them the contact details of the Site Principal Investigator and Research Nurse. Clinicians will be asked to continue the study drug during the admission, unless there is a clinical indication not to do so, and to manage apnoea and hypoxaemia as per the protocol (see 2.1.4). They will also be informed that the infant may receive a loading dose of caffeine citrate, in addition to the study drug, if clinically appropriate and urgent without prior discussion with the Site Principal Investigator. Before giving ongoing caffeine citrate the clinician should discuss partial unblinding (caffeine or placebo) with the Site Principle Investigator.

3.2 Assignment of interventions

3.2.1 Allocation sequence generation

The allocation sequence will be generated by the study statistician, with variable block sizes and priority stratification for study site (ADHB or CMDHB) and gestational age at birth (34, 35 and 36 weeks’ GA). Multiples will be allocated to the same study group.

3.2.2 Allocation concealment mechanism

Infants will be assigned randomly via an internet randomisation service to the placebo or one of four caffeine doses with equal allocation ratio (Figure 2).

3.2.3 Implementation

During randomisation the infant will be allocated to a study group (A, B, C, D or E) that will correspond to a pre-labelled study drug bottle which contains either water or identical appearing caffeine citrate at one of four different concentrations (5 mg/ml, 10 mg/ml, 15 mg/ml or 20 mg/ml). After 50% of the infants have been recruited the letters corresponding to each study group will change (F, G, H, I, J) to ensure continued blinding of the research team. The infant’s hospital sticker will be applied to the bottle and the drug will only be dispensed to that infant. Only the Data Manager, Study Statistician and Site Pharmacist will know which dose of caffeine citrate or placebo an infant has been allocated.

3.2.4 Blinding

The research team (investigators, nurses, coordinator, students, research assistants), clinical staff and the infant’s parents will be blinded to treatment allocation. This will be achieved by dispensing caffeine in identical bottles with identical study drug solutions (caffeine and water).

3.2.5 Emergency unblinding

The infant may be un-blinded if judged clinically necessary by the clinical team caring for the infant, in agreement with the Site Principal Investigator. To unblind the infant’s study drug, the Site Principal Investigator will contact the Data Manager and ask them to contact the responsible clinician and/or hospital pharmacist with the study allocation. In the first instance this will be by partial unblinding (designated as either placebo or caffeine), unless the actual dose of study drug is required for the infant’s care.
4. DATA COLLECTION, MANAGEMENT AND ANALYSIS

4.1 Data Collection Methods

4.1.1 Pulse Oximetry

Infants will have an overnight pulse oximetry (Rad 8, Masimo Corp., Irvine, CA) using a 2-second averaging time and a 2-second resolution recorded from either foot between 48 and 72 hours of age, two weeks after randomisation and at term equivalent age. Clinicians and parents will be blinded to the oximetry reading with the oximeters set to a sleep mode, with no alarms or visual displays available, unless the infant requires pulse oximetry for clinical reasons, in which case the oximetry display will be available. The pulse oximetry sensor is attached to the infant’s foot in the evening once the infant is settled and will remain in situ for 12 hours. The research nurse will instruct and demonstrate to parents how to attach the oximeter probe.

The parents will complete an overnight diary of events, e.g., feeding, crying, that may affect the oximetry trace. The overnight oximetry recording will be downloaded with PROFOX oximetry software (version Masimo 2011.27D, PROFOX Associates Inc., Escondido, CA), and edited to remove readings distorted by artefact. Recordings with less than 6 hours of edited data will be repeated the following night (estimated to be 15% of recordings). Only recordings with 6 hours or more of edited data will be included in the analysis. The downloaded recording from the PROFOX software will include the number of episodes of intermittent ≥10% and ≥3% from the baseline, the mean SpO₂, the mean pulse rate, and the proportion of time <95%, <90%, <80%. The mean duration of episodes of intermittent hypoxaemia will be calculated, and the mean high and low event saturation. Intermittent hypoxaemia will be defined as a drop in oxygen saturation concentration of ≥10% from the baseline.

If the baby is unwell with an infectious illness at the time the pulse oximetry is scheduled the oximetry can be delayed for up to a week, i.e., if unwell at 2 weeks post randomisation, the oximetry can be done at 3 weeks post randomisation. If the baby remains unwell the oximetry data for this time point will be considered missing.

4.1.2 Anthropometry

Weight, length and head circumference will be measured at study entry, two weeks after randomisation and term equivalent age by the research nurse. Birthweight and neonatal centiles will be calculated using Fenton-WHO growth charts for preterm infants. Growth velocity will be calculated from weight at birth and term equivalent age using an exponential model. Between 2 weeks post randomisation and term equivalent age the parents will be phoned weekly by a member of the research team, and the dose of the study drug re-calculated using the latest weight measured by usual clinical care providers.

4.1.3 Neonatal Salivary Caffeine

At 2 weeks’ post randomisation age saliva will be collected from all infants using a mouth swab and salivary caffeine concentrations will be measure by ELISA (Appendix 8.6). Saliva will be collected on the morning after the overnight oximetry prior to caffeine dosing. Breastfeeding mothers will be asked to refrain from consuming any caffeine from midnight on the morning of sampling.

Saliva and plasma caffeine concentrations are highly correlated in infants. One study in late preterm infants reported no significant mean bias between plasma and saliva caffeine concentrations and 95% limits of agreement of approximately 9 mg/L at plasma concentrations from 9 to 54 mg/L. This relationship was not affected by weight, postnatal age or caffeine concentration. Another study in very preterm infants found that there was a small negative
bias of approximately 2 mg/L between saliva and serum caffeine concentrations. Preliminary data suggest that plasma caffeine concentrations can be more accurately predicted from salivary concentrations using a three-compartment recirculation model.

Salivary caffeine concentrations will be related to caffeine dose and frequency of intermittent hypoxia to assist in determining optimal therapeutic target concentrations and caffeine dose in late preterm infants.

4.1.4 Maternal Caffeine Consumption

Maternal plasma caffeine concentrations peak 50 to 60 minute after oral ingestion. Caffeine is rapidly absorbed into breast milk with delay in peak concentrations between plasma and breastmilk of 30 to 60 minutes. The area-under-the-curve ratio between plasma and breastmilk caffeine concentrations is approximately 80%, but daily infant uptake from breastmilk is affected by many factors, including maternal caffeine clearance milk volume, frequency of feeds, and timing of feeds in relation to maternal caffeine ingestion.

With usual maternal coffee consumption, infant caffeine uptake via breastmilk is relatively low and has been estimated to range from 0.03 to 0.2 mg/kg/d. Even with high maternal consumption of 500 mg per day (approximately 5 to 8 cups of coffee), infant caffeine intake from breast milk ranges from 0.3 to 1 mg/kg/d.

Maternal caffeine intake will be estimated from a questionnaire on caffeine intake in the previous 24 hours at baseline, on the day before the two-week post randomisation overnight oximetry and at term equivalent age. In addition, mothers will be asked to collect three saliva samples on the same day at 10 am, 2 pm and 6 pm. The mean of the three values will be used to determine average daytime maternal salivary caffeine concentration, which is closely related to plasma concentrations. Maternal salivary concentrations will serve as a check to the accuracy of estimated maternal caffeine intake.

4.1.5 Questionnaires

Mothers will complete questionnaires to provide the following information:

- Demographics at enrolment: address and contact information; maternal age, parity, ethnicity, height and weight, and smoking history; and infant ethnicity.
- Infant sleeping and arousal behaviour (IBQ-R, subscale 9), feed tolerance (I-GERD-R), two weeks after randomisation and (Appendix 8.7 and 8.8)
- Feeding status two weeks after randomisation and at term equivalent age.
- Maternal mental health (EPDS) at enrolment and term equivalent age.
- Maternal caffeine intake at two weeks after randomisation.
- Diary of study drug administration.

4.1.6 Neonatal Morbidity

Data will be collected on neonatal morbidity, including supplemental oxygen, respiratory support, and apnoea requiring stimulation, from the neonatal records. Exposure to antenatal corticosteroids will be recorded.

4.2 Data Management

The Maternal and Perinatal Central Coordinating Research Hub (CCRH), University of Auckland (wiki.auckland.ac.nz/researchhub), will provide data management, including online randomisation, development of the database, data entry, data reports, drug management and data extraction.
4.3 Statistical Methods

4.3.1 Descriptive Statistics

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.

4.3.2 Treatment Effect

The primary analysis will compare primary and secondary outcomes between the placebo and each caffeine group using mixed generalised models with adjustment for gestational age at birth, multiple comparisons (Dunnett), and non-independence of multiples (random effect). Linear trends will be tested using orthogonal contrasts. Edinburgh Postnatal Depression Scale scores will be adjusted for baseline values. 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.

4.3.3 Secondary Analysis

The following secondary analyses will be performed:

Compliance:

A sensitivity analysis will be performed for the primary outcome that includes only those infants who were compliant with the study drug (see section 2.1.5).

Open-label caffeine treatment

A sensitivity analysis will be performed for the primary outcome that includes only those infants who did not receive open-label caffeine treatment (see section 2.1.4)

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, maternal caffeine exposure will be assumed to be zero.

5. DATA MONITORING

A Data Safety Monitoring Committee (DSMC) consisting of a neonatologist and paediatrician experienced in large randomised trials and a statistician has been convened. The DSMC will monitor recruitment, sample size assumptions, completeness of data acquisition, and evidence for group differences in the safety outcome measures. The DSMC will convene after the first 60 patients have been randomised and advise the Steering Committee on trial continuation or protocol modification.

All serious adverse events (seizures, deaths) will be reported to the DSMC within 24 hours. The DSMC will review all adverse events every 6 months and make recommendations to the Steering Committee about any required alterations to the study protocol.

Members of the DSMC:
Professor Jane Harding (Chair, Neonatologist)
Professor Stuart Dalziel (Paediatric Emergency Medicine Specialist)
Mr Greg Gamble (Biostatistician)
5.1.1 Interim Analysis

It is envisaged that the trial will be completed as planned. There will be no interim efficacy analysis.

5.1.2 Harms

The following Serious Adverse Events and Adverse Events will be reported to the Data Safety Monitoring Committee (DSMC):

**Serious Adverse Events (notified to the DSMC within 24 hours)**

- Neonatal or infant death
- Seizures requiring anti-convulsant treatment until term equivalent age

**Adverse Events (reported to the DSMC at 6 monthly intervals)**

- Failure to regain birth weight by two weeks’ postnatal age
- Tachycardia (mean heart rate >160 beats/minute) on overnight oximetry at 2 weeks post randomisation age or term equivalent age
- Study drug stopped due to presumed side effects

6. ETHICS AND DISSEMINATION

6.1 Research Ethics Approval

Ethical approval will be sought from the Health and Disability Ethics Committees (HDEC).

6.2 Locality Approval

Locality Approval will be sought from ADHB and CMDHB, the recruitment sites, and from WDHB as infants may be transferred to this region.

6.3 Protocol Amendments

All amendments to the final version of this protocol will require review and approval of the Steering Committee, and will be submitted to HDEC and DHB Research Offices, as appropriate. All amendments, including approval date, will be recorded with this protocol (Appendix 8.4).

6.4 Consent

Informed consent will be obtained by the research nurses, PhD Student, Clinical Staff or Investigators.

6.5 Confidentiality

Electronic databases are stored on secure servers and access is controlled by unique user ID and password, with full electronic tracking log. CRF/eCRFs are identifiable only by study ID, first and last initial, DOB and EDD. Forms do not contain identifiable information such as names, address, or NHI. Extracted data files will contain DOB and EDD, as these are necessary for analysis, but participant initials are removed. Contact and personal information is stored separately from CRF/eCRF data. Hard copy CRFs are stored in a locked cabinet. Study reports contain only summary data and individual participant data is not reported. Identifiable data is not released to any third party. Research staff will be certified in best practice for clinical trials (ICH-GCP E6 and PHRP).

At the completion of the study, all electronic data will be permanently digitally archived and accessible only to the study investigators. All hard copy records that have been digitally
scanned will be added to the archive, and then destroyed. Remaining hard copy records will be stored in a locked cabinet in a secure office, and will be accessible only to the study investigators. Records will be retained for 10 years after the age of majority.

6.6 Declaration of Interests

Investigators will declare any financial, intellectual or other potential conflicts of interest, as outlined by the ICMJE, to the Steering Committee. The Steering Committee will decide on how any conflicts of interest are to be managed.

6.7 Access to Data

The Steering Committee have access to the full dataset and oversee analysis, interpretation and reporting of results. Approval will be sought from the Latte Dosage Trial Steering Committee prior to publication of study data. Care is taken to avoid duplication in reporting of results.

7. TRIAL ORGANISATION

7.1 Steering Committee

The Steering Committee takes overall responsibility for all aspects of the study, meeting initially on a fortnightly basis, and then monthly. Matters arising between meetings may be dealt with by email. The Principal Investigator is responsible for maintaining a record of correspondence and minutes of meetings.

Members of the Steering Committee:
Dr Jane Alsweiler (Chair, Neonatologist)
Dr Chris McKinlay (Neonatologist)
Dr David McNamara (Paediatric Respiratory Specialist)
Ms Elizabeth Oliphant (Paediatric Pharmacist)

7.2 Management Committee

Ms Sarah Philipsen, Trial Coordinator, will oversee day-to-day running of the study. She will be supported by a Management Committee that will meet regularly.

Members of the Management Committee:
Dr Jane Alsweiler (Chair)
Dr Chris McKinlay
Ms Sarah Philipsen (Trial Co-ordinator)
Ms Elizabeth Oliphant
Ms Sabine Huth (Research Nurse)

Other members of the research team may be requested to attend the Management Committee meetings as required.

7.3 Site Principal Investigators

The Site Principal Investigator will have overall responsibility for the running of the study at their local site, including completion of local governance requirements, and local promotion and staff education. They will be supported by the Management Committee and other research staff.

Site Principal Investigators:
ADHB: Dr Jane Alsweiler
CMDHB: Dr Chris McKinlay
8. APPENDICES

8.1 Participant Documents

The following participant documents are to accompany this protocol:

<table>
<thead>
<tr>
<th>Title</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant Information Sheet and Consent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Enrollment Questionnaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant Follow-up Questionnaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Follow-up Questionnaire</td>
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</tr>
</tbody>
</table>

8.2 Case Report Forms

The following case report forms (CRF) are to accompany this protocol:

<table>
<thead>
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<tbody>
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</tbody>
</table>

8.3 Ethical and Locality Approval

The following letters of approval are to accompany this protocol:

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<thead>
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<tbody>
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<td></td>
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</tbody>
</table>

8.4 Protocol Amendments

<table>
<thead>
<tr>
<th>Protocol version, Date</th>
<th>Amendment(s)</th>
<th>Date accepted by Steering Group</th>
<th>Date ethics notified (or NA)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

8.5 Study Committee Terms of Reference

The following Terms of Reference are to accompany this protocol:

<table>
<thead>
<tr>
<th>Title</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steering Group Term of Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data Safety Monitoring Committee Term of Reference</td>
<td></td>
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</tr>
</tbody>
</table>

8.6 Salivary Caffeine

Parents will be asked in the Consent document if they would like the salivary samples to be disposed of with a Karakia prior to disposal. Samples of participants who select this option will be stored at the Liggins Institute and disposed of during a regularly scheduled Karakia ceremony with remaining tissue spread around a dedicate Liggins Institute tree.
8.7 Smoking Questionnaire

Did you smoke regularly – that is every day – before you were aware you were pregnant? (yes, no)

   If yes, How many cigarettes did you smoke per day, on average, before this pregnancy?
       1-5 per day
       6-10 per day
       11-15 per day
       16-20 per day
       21-25 per day
       26-30 per day
       31 or more a day

Did you smoke during this pregnancy? (yes, no)

   If yes, How many cigarettes did you smoke per day, on average, during this pregnancy?
       1-5 per day
       6-10 per day
       11-15 per day
       16-20 per day
       21-25 per day
       26-30 per day
       31 or more a day

Did anyone smoke in the same room as you during pregnancy? (yes, no)

   If yes, How often? (Rarely (less than once a week), occasionally (a few times a week),
       Often (almost or every day of week))

Do you smoke now? (yes, no)

   If yes, How many cigarettes do you smoke per day, on average?
       1-5 per day
       6-10 per day
       11-15 per day
       16-20 per day
       21-25 per day
       26-30 per day
       31 or more a day
Does anyone else who lives in the house with your baby smoke (including outside)? (yes, no)

If yes, How often? (Rarely (less than once a week), occasionally (a few times a week), Often (almost or every day of week))

**8.8 Sleeping Questionnaire**

IBQ-R subscale 9 (Falling Reactivity/Rate of Recovery from Distress) will be used to assess sleep and reactivity. It quantifies rate of recovery from peak distress, excitement, or general arousal, and ease of falling asleep in the last 7 days. Higher scores indicate improved recovery from arousal and greater ease of falling asleep. Subscale 9 includes 13 Items, but the last item will be omitted as it is not relevant to neonates.

Please indicate how often you observed the following behavior in your baby in the LAST WEEK:

**Sleep:**

- When going to bed at night, how often did your baby: 
  - fall asleep within 10 minutes? 
  - have a hard time settling down to sleep? 
  - settle down to sleep easily? 

- When your baby woke at night, how often did s/he:
  - have a hard time going back to sleep? 
  - go back to sleep immediately? 

- When put down for a sleep during the day, how often did your baby:
  - stay awake for a long time? 
  - go to sleep immediately? 
  - settle down quickly? 
  - have a hard time settling down? 

**Daily Activities:**

- When frustrated with something, how often did your baby:
  - calm down within 5 minutes? 

- When your baby was upset about something, how often did s/he:
  - stay upset for up to 10 minutes or longer? 
  - stay upset for up to 20 minutes or longer? 

Responses are scored as follows:

- Never =1 (R=7)
- Very rarely =2 (R=6)
- Less than half the time =3 (R=5)
- About the half the time =4 (R=4)
- More than half the time =5 (R=3)
- Almost always=6 (R=2)
- Always =7 (R=1)

The total score is divided by the number of items receiving a numerical response. This subscale has a normative mean (SD) of 2.55 (1.09) for infants aged between 3 and 12 months\(^59\).

**8.9 Feeding Questionnaire**

Infant Gastroesophageal Reflux Questionnaire (I-GERQ-R)
1. During the past week, how often did the baby usually spit up (anything coming out of the mouth) during a 24-hour period?
   - Less than once
   - 1 to 3 times
   - 4 to 6 times
   - More than 6 times

2. During the past week, how much did the baby usually spit up (anything coming out of the mouth) during a typical episode?
   - Did not spit up
   - Less than 1 tablespoonful
   - 1 tablespoonful to 2 ounces
   - More than 2 ounces to half the feeding
   - More than half the feeding

3. During the past week, how often did spitting up (anything coming out of the mouth) seem to be uncomfortable for the baby, for example, crying, fussing, irritability, etc.?
   - Never
   - Rarely
   - Sometimes
   - Often
   - Always

4. During the past week, how often did the baby refuse a feeding even when hungry?
   - Never
   - Rarely
   - Sometimes
   - Often
   - Always

5. During the past week, how often did the baby stop eating soon after starting even when hungry?
   - Never
   - Rarely
   - Sometimes
   - Often
   - Always

6. During the past week, did the baby cry a lot during or within 1 hour after feedings?
   - Never
7. During the past week, did the baby cry or fuss more than usual?
   □ Never
   □ Rarely
   □ Sometimes
   □ Often
   □ Always

8. During the past week, on average how long did the baby cry or fuss during a 24 hour period?
   □ Less than 10 minutes
   □ 10 minutes to 1 hour
   □ More than 1 hour but less than 3 hours
   □ 3 or more hours

9. During the past week, how often did the baby have hiccups?
   □ Never
   □ Rarely
   □ Sometimes
   □ Often
   □ Always

10. During the past week, how often did the baby have episodes of arching back?
    □ Never
    □ Rarely
    □ Sometimes
    □ Often
    □ Always

11. During the past week, has the baby stopped breathing while awake or struggled to breathe?
    □ No
    □ Yes

12. During the past week, has the baby turned blue or purple?
    □ No
    □ Yes
8.10 Caffeine Intake Questionnaire

We would like to know how much caffeine you consume

Indicate in the table, how much you drank yesterday.
Put a line in the box for every cup/glass.
If you didn’t drink the item, leave the box empty.

<table>
<thead>
<tr>
<th>Portion sizes</th>
<th>Small cup 150 mL</th>
<th>Large cup 250 mL</th>
<th>Small glass 150 mL</th>
<th>Large glass 250 mL</th>
<th>Energy drink 250 mL can</th>
<th>Energy drink 500 mL can</th>
<th>Energy Shot 60 mL can</th>
<th>Espresso cup 60 mL</th>
<th>Chocolate bar 20g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cola, fizzy soft drink</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Chocolate</td>
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For example, like this:

<table>
<thead>
<tr>
<th></th>
<th>Coffee</th>
<th>Cola, fizzy soft drink</th>
<th>Chocolate</th>
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</thead>
<tbody>
<tr>
<td>Breakfast</td>
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</tbody>
</table>

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Name: __________________ Age: ______________ Gender □ Female □ male
Height: __________________ Weight: ______________ Date: ______________
8.11 Edinburgh Postnatal Depression Scale

Appendix

Edinburgh Postnatal Depression Scale (EPDS)
The Edinburgh Postnatal Depression Scale (EPDS) has been developed to assist primary care health professionals to detect mothers suffering from postnatal depression, a distressing disorder more prolonged than the 'blues' (which occur in the first week after delivery) but less severe than postpartum psychosis.

Previous studies have shown that postnatal depression affects at least 10% of women and that many depressed mothers remain untreated. These mothers may cope with their baby and with household tasks, but their enjoyment of life is seriously affected and it is possible that there are long-term effects on the family.

The EPDS was developed at health centres in Livingston and Edinburgh. It consists of ten short statements. The mother underlines which of the four possible responses is closest to how she has been feeling during the past week. Most mothers complete the scale without difficulty in less than 5 minutes.

The validity of the study showed that mothers who scored above a threshold 12/13 were likely to be suffering from a depressive illness of varying severity. Nevertheless the EPDS score should not override clinical judgement. A careful clinical assessment should be carried out for the diagnosis. The scale indicates how the mother has felt during the previous week, and in doubtful cases it may be usefully repeated after 2 weeks. The scale will not detect mothers with anxiety neuroses, phobias or personality disorders.

Instructions for users

1. The mother is asked to underline the response which comes closest to how she has been feeling in the previous 7 days.
2. All ten items must be completed.
3. Care should be taken to avoid the possibility of the mother discussing her answers with others.
4. The mother should complete the scale herself, unless she has limited English or has difficulty with reading.
5. The EPDS may be used at 4-8 weeks to screen postnatal women. The child health clinic, postnatal check-up or a home visit may provide suitable opportunities for its completion.

EDINBURGH POSTNATAL DEPRESSION SCALE (EPDS)
J. L. Cox, J. M. Holden, R. Sagovsky
Department of Psychiatry, University of Edinburgh

Name:
Address:
Baby's age:

As you have recently had a baby, we would like to know how you are feeling. Please UNDERLINE the answer which comes closest to how you have felt IN THE PAST 7 DAYS, not just how you feel today.

Here is an example, already completed.
I have felt happy:

Yes, all the time
Yes, most of the time
No, not very often
No, not at all

This would mean: "I have felt happy most of the time" during the past week. Please complete the other questions in the same way.

In the past 7 days:
1. I have been able to laugh and see the funny side of things
   As much as I always could
   Not quite so much now
   Definitely not so much now

2. I have looked forward with enjoyment to things
   As much as I ever did
   Rather less than I used to
   Definitely less than I used to
   Hardly at all

3. I have blamed myself unnecessarily when things went wrong
   Yes, most of the time
   Yes, some of the time
   Not very often
   No, never

4. I have been anxious or worried for no good reason
   No, not at all
   Hardly ever
   Yes, sometimes
   Yes, very often

5. I have felt scared or panicky for no very good reason
   Yes, quite a lot
   Yes, sometimes
   No, not much
   No, not at all

6. Things have been getting on top of me
   Yes, most of the time I haven't been able to cope at all
   Yes, sometimes I haven't been coping as well as usual
   No, most of the time I have coped quite well
   No, I have been coping as well as ever

7. I have been so unhappy that I have had difficulty sleeping
   Yes, most of the time
   Yes, sometimes
   Not very often
   No, not at all

8. I have felt sad or miserable
   Yes, most of the time
   Yes, quite often
   Not very often
   No, not at all

9. I have been so unhappy that I have been crying
   Yes, most of the time
   Yes, quite often
   Only occasionally
   No, never

10. The thought of harming myself has occurred to me
    Yes, quite often
    Sometimes
    Hardly ever
    Never

Response categories are scored 0, 1, 2, and 3 according to increased severity of the symptom.
Items marked with an asterisk are reverse scored (i.e. 3, 2, 1 and 0). The total score is calculated by adding together the scores for each of the ten items. Users may reproduce the scale without further permission providing they respect copyright (which remains with the British Journal of Psychiatry) by quoting the names of the authors, the title and the source of the paper in all reproduced copies.

*J. L. Cox, MA, DM, FRCPsych, Professor of Psychiatry, Department of Postgraduate Medicine, University of Keele. Consultant Psychiatrist, City General Hospital, Stoke-on-Trent, formerly Senior Lecturer, Department of Psychiatry, University of Keele. Research Psychologist: R. Sagovsky, MA, BSc (Hons), Research Psychologist, University of Edinburgh.
9. REFERENCES


