CLINICAL RESEARCH ARTICLE

Electrical activity of the diaphragm following a loading dose of caffeine citrate in ventilated preterm infants

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BACKGROUND: Administration of caffeine citrate can facilitate extubation. Our aim was to determine whether a loading dose of caffeine citrate given to ventilated, preterm infants affected the diaphragm electrical activity.
METHODS: Infants <34 weeks of gestational age were recruited if requiring mechanical ventilation and prescribed a loading dose of caffeine citrate. Surface electrodes recorded the electrical activity of the diaphragm (dEMG) before and after administration of intravenous caffeine citrate. The mean amplitude of the EMG (dEMG) trace and the mean area under the EMG curve (aEMGc) were calculated.
RESULTS: Thirty-two infants were assessed with a median gestational age of 29 (27–31) weeks. The dEMG amplitude increased, peaking at 25 min post administration ($p = 0.006$), and the increase in aEMGc ($p = 0.004$) peaked at 30 min; the differences were not significant after 60 min. At 20 min, there was an increase in minute volume ($p = 0.034$) and a reduction in the peak inspiratory pressure ($p = 0.049$).
CONCLUSIONS: We have demonstrated a transient increase in both electrical activity of the diaphragm and respiratory function following an intravenous loading dose of caffeine citrate.

INTRODUCTION

Mechanical ventilation is frequently used to support very prematurely born infants who have immature, underdeveloped lungs and inadequate respiratory drive. Yet, prolonged ventilation is associated with complications, such as bronchopulmonary dysplasia and pulmonary interstitial emphysema,[1,2] and hence, it is important to successfully extubate infants as soon as possible. Caffeine citrate is a methylxanthine and administration can facilitate successful extubation through a number of mechanisms.[3] Caffeine citrate is a respiratory stimulant that acts centrally and antagonises inhibitory adenosine A_1 receptors.[4] It increases the ventilatory response to hypercapnia in preterm infants.[5] Furthermore, administration of caffeine citrate can increase respiratory muscle strength and improve lung compliance.[6] Intravenous caffeine citrate has also been shown to increase the diaphragmatic activity of spontaneously breathing preterm infants with a corresponding increase in tidal volume.[7] Whether, however, the effects of caffeine citrate administration on diaphragm electrical activity are similar in ventilated, preterm infants is currently unknown.

Electromyography (EMG) is a global measure of the neural impulses that are translated into muscle fibre action potentials, with the processed signal giving the amplitude of electrical activity of a muscle.[8] The amplitude of the electrical activity of the diaphragm (EAdi) is helpful in monitoring asynchrony and respiratory muscle loading of ventilated patients.[8] The EAdi can also be used to assess neural respiratory drive;[9,10] inspiratory drive has been shown to increase in response to an increased work of breathing.[11] We hypothesised that administration of a loading dose of caffeine citrate to ventilated, prematurely born infants would result in an increase in the amplitude of the diaphragm electromyogram and improved respiratory function. Our aim was to test those hypotheses.

METHODS

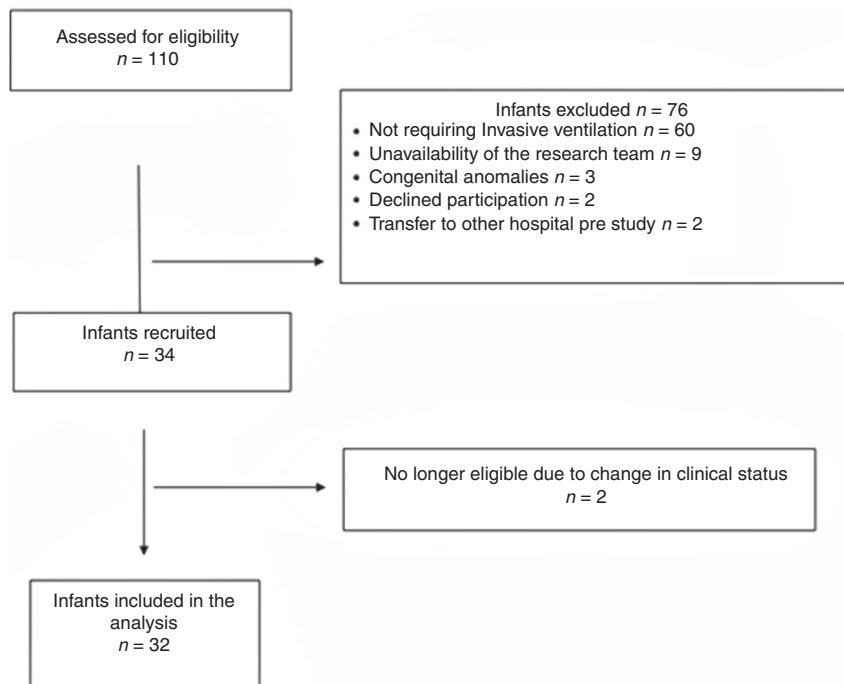### Study design and subjects

A prospective, observational cohort study took place between 01/09/2018 and 30/06/2019 at the two neonatal units within King's College Hospital NHS Foundation Trust (KCH), London UK. This NHS Foundation Trust comprises of King's College Hospital, London and the Princess Royal University Hospital, Orpington. The London Brent Research Ethics Committee approved the study and the parents gave written, informed consent for their infant to take part.

Infants born <34 weeks of completed gestational age (GA) who were prescribed a loading dose of caffeine citrate by the clinical team were eligible for recruitment into the study if they were receiving invasive mechanical ventilation. Infants with chromosomal or major congenital anomalies were excluded. Baseline demographic data were collected including gender, ethnicity, antenatal exposure to corticosteroids and magnesium sulphate, GA and mode of delivery, mode of ventilation prior to caffeine administration and use of postnatal surfactant.

At KCH, ventilatory support was delivered by an SLE 6000 ventilator (SLE limited, Croydon UK). Preterm infants were usually

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Fig. 1 Consort flow diagram of recruitment process

supported by patient-triggered ventilation with a targeted tidal volume. Targeted tidal volumes were set between 5 and 6 mL/kg. The clinical team routinely prescribed a 20-min loading dose caffeine citrate (20 mg/kg) to ventilated infants <34 weeks of GA as per Trust guidelines and in agreement with recent evidence showing the benefit of early therapy (before day 3 of postnatal life) in decreasing the composite outcome of bronchopulmonary dysplasia or all-cause mortality.[12]
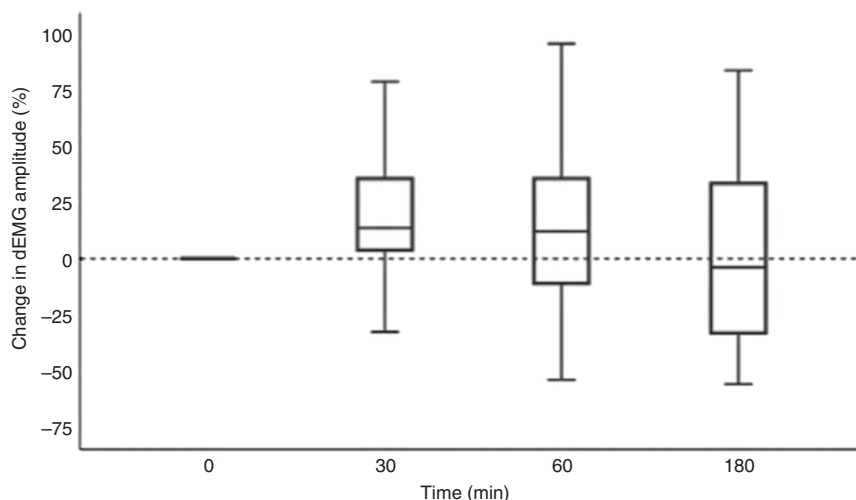
Diaphragmatic electrical activity was measured continuously 15 min prior to the administration of a loading dose of caffeine citrate and until 180 min after the loading dose. The peak inspiratory pressure (PIP), positive end expiratory pressure, expired tidal volume, fraction of inspired oxygen, inspiratory time and mean airway pressure were recorded during the same time periods as the EMG analysis.

The activity of the diaphragm was measured via transcutaneous EMG of the diaphragm using surface electrodes. All infants were assessed in the supine position. The transcutaneous diaphragm EMG was monitored using a portable 16-channel digital physiological amplifier (Dipha-16; Inbiolab, Groningen, the Netherlands) and three surface electrodes (Kendall H59P cloth electrodes; Covidien, MA). Two electrodes were placed vertically in line with the nipples on the right and left chest wall at the costo-abdominal margin and one electrode placed at the centre of the sternum. The electrodes were connected to a portable physiological amplifier (DEMCON; Macawi Medical Systems, the Netherlands) that generated raw signals correlating to the diaphragm EMG at a sampling rate of 500 Hz. The physiological amplifier wirelessly transmitted to a bedside computer running Polybench (Applied Biosignals, Weener, Germany). Raw signals were filtered and processed automatically within the Polybench software including the gating technique. The method used to filter the cardiac electrical activity was similar to the gating technique described by O'Brien.[13] EMG data were imported into MATLAB from Poly5 format using the TMSi MATLAB Interface (Twente Medical Systems International, Oldenzaal, the Netherlands). The complete recording of each infant was reviewed and points at which the infants was moving or being handled were identified by the presence of interference to the signal, these were excluded from analysis. EMG

Table 1. Baseline demographics

n	32
Gestational age (weeks)	29.71 (27.27–31.49)
Birth weight (kg)	1.15 (0.84–1.44)
Antenatal steroids	29 (90.6)
Male sex	13 (40.6)
Apgar Score at	
1 min	6 (3–7)
5 min	8 (7–9)
10 min	9 (8.3–10)
Reason for intubation	
Increased FiO$_2$	4 (12.5)
Increased work of breathing	8 (25)
Poor respiratory effort	11 (34.4)
Chest X-ray changes	1 (3.1)
Apnoea	8 (25)
Where intubated	
Delivery suite	30 (93.8)
NNU	2 (6.3)
Time of delivery suite intubation post birth (min)	6 (4.0–10.3)
Time post birth to caffeine (min)	288 (133–746)
Postnatal surfactant	32 (100)
Mode of ventilation	
PTV	30 (93.8)
SIMV	2 (6.3)
Added TTV	28 (87.5)
FiO$_2$ at the time of study	0.3 (0.23–0.40)
PEEP (cm H$_2$O)	5.2 (5.0–5.7)

Data are presented as median (interquartile range) or n (%)
GA gestational age, *BW* birth weight, *FiO$_2$* fraction of inspired oxygen, *PEEP* positive end expiratory pressure

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Fig. 2 Boxplot of percentage change in diaphragm amplitude over time. The ends of each box represent the upper and lower quartiles with the median being marked by a horizontal line inside the box. The whiskers are the two lines outside the box that extend to the highest and lowest observations
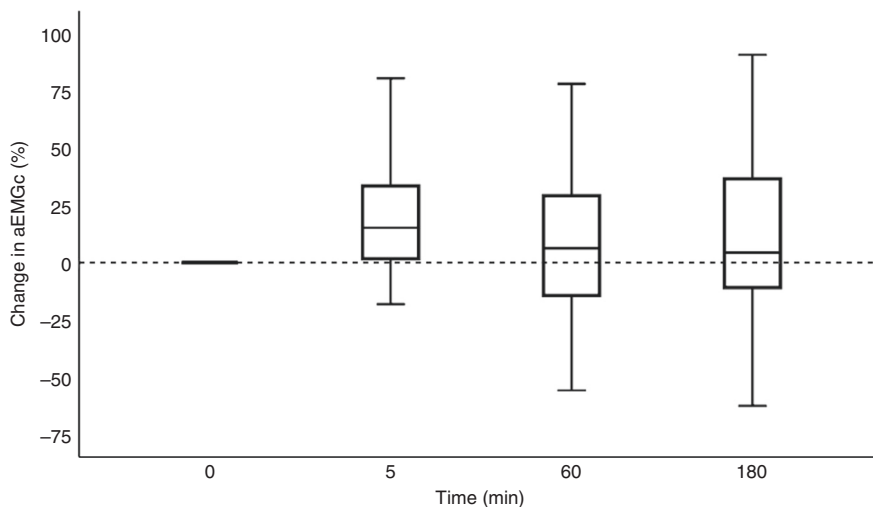
Fig. 3 Boxplot of percentage change in area under the EMG curve over time. The ends of each box represent the upper and lower quartiles with the median being marked by a horizontal line inside the box. The whiskers are the two lines outside the box that extend to the highest and lowest observations

analyses of periods of 30-s artefact-free recordings were performed at set time intervals. Three sets of stable 30-s recordings in a time period beginning 5 min prior to administration of the loading dose of caffeine citrate and at 5-, 20-, 25-, 30-, 60-, 120- and 180-min time intervals after the start of the 20-min caffeine citrate loading dose infusion were analysed. For each artefact-free 30-s recording, the mean amplitude of the EMG (dEMG) trace and the mean area under the EMG curve (aEMGc)[14,15] were calculated using MATLAB Statistics Toolbox Release 2015b (The MathWorks, Inc., Natick, MA, USA). At each time interval, the change from the baseline for each measurement was calculated with each infant acting as their own control.

Analysis
The data were tested for normality using Shapiro–Wilk test and found to be non-normally distributed. Therefore, data are presented as median (interquartile range) and differences assessed for statistical significance using Wilcoxon rank-sum test for paired changes post caffeine administration. Statistical analyses were undertaken using the SPSS software Version 25 (SPSS Inc., Chicago IL).

Sample size
A previous study found an increase in median peak electrical activity from 1.42 to 2.16 µV in non-ventilated infants following caffeine administration,[7] therefore, using an estimated sample mean of 1.4 ± 0.6 µV, to detect a 30% difference in peak diaphragm EMG with 80% power at the 5% significance level, a sample of 32 infants was recruited.

RESULTS
During the study period, 110 infants born <34 weeks of completed GA were admitted to the neonatal unit, and 32 were recruited (Fig. 1; Table 1). There were no significant differences in GA and birth weight (BW) between the included infants and those 15 infants who were eligible but not included (GA: 31.14 (25.0–32.71) weeks, $p = 0.817$; BW: 1.15 (0.73–1.91) kg, $p = 0.71$). In the included infants, the median time after birth to caffeine citrate administration was 288 (133–745) min (Table 1).
There was a significant increase in dEMG amplitude from baseline following the loading dose of caffeine citrate that peaked at 25 min post administration ($p = 0.006$) and a significant increase

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Table 2. Diaphragmatic activity and respiratory function before and after a loading dose of caffeine

	Baseline	5 min	20 min	25 min	30 min	1 h	2 h	3 h
dEMG (µV)	1.60 (0.77–2.25)	1.72 (0.84–2.67), **p = 0.015**	1.63 (0.87–2.45), p = 0.217	2.01 (0.92–2.52), **p = 0.006**	1.96 (0.98–2.40), **p = 0.011**	1.53 (0.82–2.45), p = 0.295	1.47 (0.78–2.26), p = 0.517	1.43 (0.82–2.19), p = 0.792
aEMGc (µV.S)	2.39 (1.58–2.66)	2.69 (2.02–3.15), **p < 0.001**	2.40 (1.68–3.16), p = 0.115	2.58 (1.95–3.18), **p = 0.005**	2.59 (1.96–3.27), **p = 0.004**	2.33 (1.74–2.96), p = 0.231	2.29 (1.70–2.86), p = 0.735	2.53 (1.79–2.91), p = 0.360
RR (bpm)	56.0 (42.7–70.7)	56.4 (48.0–67.9), p = 0.455	60.4 (53.8–79.5), p = 0.152	50.5 (39.9–61.8), p = 0.164	61.5 (45.0–73.1), **p = 0.021**	57.5 (40.1–73.1), p = 0.902	65.7 (53.4–77.9), p = 0.069	60.5 (51.9–73.0), p = 0.645
MV (mL/min)	377.5 (224.6–502.5)	320 (220.0–480.0), p = 0.479	395.6 (282.0–528.0), **p = 0.034**	405.26 (247.8–517.5), p = 0.124	380.0 (227.7–500.0), p = 0.163	369.9 (231.8–528.9), p = 0.759	370 (247.2–530.0), p = 0.469	410.0 (270–510.0), p = 0.879
TV (mL)	6.4 (4.9–8.1)	6.1 (4.28–7.35), p = 0.974	6.7 (4.98–7.63), p = 0.524	6.9 (4.7–7.7), p = 0.401	6.1 (4.8–7.8), p = 1.0	6.25 (4.8–8.3), p = 0.642	5.7 (4.53–7.33), p = -0.249	5.5 (4.1–7.8), p = 0.318
PIP (cm H$_2$O)	16.7 (12.1–20.0)	16.8 (12.8–20.0), p = 0.638	12.9 (10.3–19.8), **p = 0.049**	15.3 (13.8–20.0), p = 0.912	15.9 (12.1–20.0), p = 0.737	16.6 (11.6–19.8), p = 0.935	16.3 (11.2–20.0), p = 0.470	16.1 (11.4–20.0), p = 0.623

Data are presented as median (interquartile range)
RR respiratory rate, MV minute volume, TV tidal volume, PIP peak inspiratory pressure
Statistically significant p-values are in bold

in aEMGc from baseline peaking at 30 min post administration of caffeine citrate ($p = 0.004$). By 60 min, the loading dose of caffeine citrate had no significant effect on the EAdi (Figs. 2 and 3). At 20 min, there was a significant increase in minute volume ($p = 0.034$) from baseline and significant reduction from baseline in the PIP ($p = 0.049$), and at 30 min, a significant increase in the respiratory rate ($p = 0.021$) from baseline. There were no significant changes in tidal volume post caffeine administration (Table 2).

DISCUSSION

We have demonstrated that in preterm, ventilated infants there was a significant increase in both the dEMG amplitude and aEMGc, which peaked within the first hour following a loading dose of caffeine citrate. This transient increase in diaphragmatic electrical activity post administration of intravenous caffeine citrate is in agreement with a recent study in non-ventilated newborns.[7] It may be explained by the initial high peak serum levels seen shortly after administration of the loading dose of caffeine. The timing of the increase in diaphragmatic activity was associated with improvements in lung function as shown by the increase in minute volume and decrease in the PIP.

The current study measured electrical activity and not contractions of the diaphragm. One in vivo study found, however, that administration of theophylline resulted in an increase in minute ventilation, which correlated with an increase in diaphragmatic contractility as well as inspiratory diaphragmatic neuromuscular output.[16] What is unknown, however, is the underlying mechanism by which caffeine acts to enhance this contractile nature. One adult study showed an increase in trans-diaphragmatic pressure post caffeine therapy implying that caffeine does indeed enhance muscle contractility of the diaphragm.[17] It has been suggested that caffeine acts centrally by rapidly crossing the blood–brain barrier[18] and, through transmission of neural impulses, has subsequent effects on respiratory function resulting in increased diaphragmatic muscle activity.[19] A further possibility is that caffeine increases sarcoplasmic reticulum calcium concentrations[17,20] and hence enhances the activation of contractile proteins by increasing the affinity of calcium activation sites for calcium ions.[21]

A previous study described a significant increase in tidal volume post caffeine citrate administration in non-invasively ventilated infants, which was shown to correlate with the increase in dEMG amplitude.[7] The majority of infants in the present study were supported with a predetermined targeted tidal volume, hence we would not have expected to see a change in the expiratory tidal volume. As lung mechanics change over time, the PIP required to achieve a target tidal volume will change. In this study, we observed a statistically significant decrease in PIP post caffeine administration with a corresponding increase in minute volume with an increase in respiratory rate demonstrating that the increase in diaphragmatic electrical activity favourably influenced respiratory function.

The elimination half-life of caffeine citrate is 101 h in infants,[22] compared to the 3–6 h half-life observed in adults.[4] It has been shown, however, that there is a high variation in the elimination half-life between neonates of relatively similar conceptional ages.[23] Moreover, one study showed that peak concentrations of both oral and intravenous caffeine citrate are observed between 30 min and 2 h following administration.[24] We speculate that the physiological effects of caffeine citrate in our study peaking within the first 1 h post administration are related to the time to peak effect (T_{max}) at the peak concentration (C_{max}) and not related to the maximum possible effect of the drug (E_{max}).[25]

Our study has strengths and some limitations. This study used non-invasive techniques for monitoring, with the use of surface electrodes, which were generally acceptable to parents with only two parents declining participation. There is ease and simplicity in using surface electrodes and these can be used for infants of all

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GAs.[26] Diaphragm electromyogram recording by surface electrodes could potentially be susceptible to interference from surrounding muscle groups, such as intercostal and abdominal. On analysis of the traces, however, little interference was seen. This is in agreement with one previous study of diaphragm electrical recordings that showed little crosstalk of intercostal and abdominal muscles with diaphragmatic activity.[27] Interventions such as neonatal procedures, nursing cares or position changes were not controlled for due to the acute setting and timing of the study, and these may have had some interference on the dEMG signal as movement artefacts; however; this limitation was accounted for by selecting the closest 30-s artefact-free period at each time interval studied. Overall, we saw a positive response in electrical activity following caffeine administration, but the response was variable. Our sample size, unfortunately, precludes sub-group analysis. A further limitation of this study was the non-randomisation of infants, but as caffeine has been shown to be beneficial to all premature infants,[28,29] it would be unethical to randomise infants.

In conclusion, we have demonstrated that a loading dose of caffeine citrate in ventilated preterm infants had a transient increase in the EAdi and improvement in respiratory function. The transient nature of the effects we describe have implications for neonatal clinicians, that is if extubation is not soon after the loading dose of caffeine citrate has been administered, it may be less effective in facilitating extubation, but there is no evidence that this would impinge on its proven effects on improving neurodevelopmental outcome.[30]

AUTHOR CONTRIBUTIONS
A.G. and K.A.H. designed the study; E.E.W., K.A.H., J.J. and R.S.-R. collected the data; E.E.W., T.D. and A.G. analysed the data and produced the first draft. All authors were involved in revising it critically for important intellectual content and gave final approval of the version to be published.

ADDITIONAL INFORMATION
Competing interests: A.G. has a non-conditional educational grant from SLE to support her research on optimising neonatal ventilation. The other authors declare no competing interests.

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