Intermittent hypoxia and caffeine in infants born preterm: the ICAF Randomized Clinical Trial

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► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/archdischild-2025-329230).

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Received 13 June 2025
Accepted 6 November 2025

ABSTRACT

Objective To determine whether extending caffeine therapy through 43 weeks' postmenstrual age (PMA) decreases intermittent hypoxia (IH) in convalescing preterm infants. Secondary objectives were to assess caffeine effects on changes in inflammation-related plasma biomarkers and brain MRI.

Design Multicentre masked randomised trial.

Setting 16 US hospitals.

Patients Infants at <30 weeks + 6 days gestational age on caffeine between 32 weeks and 36+5 days PMA in room air with routine caffeine discontinuation prior to 36 weeks +6 days.

Intervention Randomisation to caffeine or placebo and treated through 42 completed weeks. Pulse oximetry was recorded from enrolment through 1 week after stopping study drug. Blood for 12 inflammation-related biomarkers obtained at enrolment and 38 weeks' PMA and brain imaging after enrolment or <3 days of randomisation, and study end.

Main outcome measure Seconds/hour of oxygen saturation <90% from randomisation to study end.

Results Randomised 160 subjects, 78 placebo, 82 caffeine. IH was less at every PMA with caffeine treatment from 34 (172.7 (123.4, 241.7); 84.7 (64.4, 111.4, p<0.01) through 41 weeks (73.0 (51.3, 103.7); 26.6 (18.5, 38.2, p<0.001). Adjusted TNF-α levels were 23% lower at follow-up in the caffeine group compared with placebo (p<0.02), without other biomarker differences. Paired brain imaging found no significant differences.

Conclusions Extended caffeine reduced the burden of IH in very preterm infants and may reduce inflammation. Further study is needed to determine if this effect of caffeine is associated with reduced risk of adverse outcomes.

Trial registration number NCT03321734.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Intermittent hypoxia (IH) events are common in preterm infants after discontinuing routine caffeine treatment and usually do not reach levels similar to term infants until 42–43 weeks' postmenstrual age (PMA).
⇒ IH is known to be proinflammatory both in human adults and in animal models.
⇒ It is unknown whether treatment with extended caffeine in preterm infants until 42–43 weeks' PMA reduces the burden of IH and affects biomarkers of inflammation or MRI/diffusion tensor imaging (DTI)/magnetic resonance spectroscopy (MRS) evidence of acute brain injury.

WHAT THIS STUDY ADDS

⇒ In this randomised trial, extended caffeine significantly reduced IH at all PMAs from 34 weeks to 41 completed weeks.
⇒ There was a larger reduction over time of TNF-α in the caffeine-treated subjects compared with placebo, with no other changes in 11 other inflammation-related biomarkers. Paired brain MRI/DTI/MRS at study start and end in a subset of subjects found no significant differences in selected regional brain volumes, DTI or metabolites.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Further research is needed to assess whether these beneficial effects of extended caffeine on IH and potentially on inflammation improve longer-term preterm infant outcomes, including neurodevelopment.

To cite: Eichenwald E, Corwin M, McEntire B, *et al*. *Arch Dis Child Fetal Neonatal Ed* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/archdischild-2025-329230

INTRODUCTION

Episodes of intermittent hypoxia (IH) are brief repetitive cycles of hypoxia and reoxygenation. IH occurs frequently in convalescing preterm infants secondary to persistence of immature respiratory control and does not reach a level similar to term infants until approximately 42 weeks' postmenstrual age (PMA).[1] Animal and human studies show that IH causes oxidative stress, free radical production, release of proinflammatory cytokines and central nervous system injury.[2–7] In addition, secondary analysis of the Canadian Oxygen Trial of extremely preterm infants demonstrated a link between more severe IH over the first 10 postnatal weeks and motor and neurocognitive impairment at 18 months.[8] It is unknown, however, whether persisting and often clinically inapparent IH may be another risk factor for disabilities.

Caffeine reduces the incidence of apnoea of prematurity. It is typically prescribed soon after preterm birth and continued until approximately 34 weeks' PMA.[9] Caffeine improves both motor and cognitive outcomes in such infants, with unclear mechanisms.[10–12] We previously reported that clinically inapparent IH events are common in

preterm infants after discontinuing routine caffeine treatment and can be ameliorated by extended caffeine therapy through 38 weeks' PMA.[13 14] In this multicentre, masked randomised placebo-controlled study, our primary objective was to assess caffeine effects on IH through 43 weeks' PMA. Secondary objectives were to assess caffeine effects on changes in inflammation-related plasma biomarkers and brain MRI/diffusion tensor imaging/magnetic resonance spectroscopy (MRI/DTI/MRS).

METHODS

Enrolment

Infants <30 weeks + 6 days gestational age receiving caffeine between 32 weeks +0 days to 36+6 days PMA were screened for enrolment between January 2019 and July 2023 at 16 US sites. Eligibility criteria included the ability to tolerate enteral medications and in room air for at least 12 hours without ventilatory support. Exclusion criteria included severe intraventricular haemorrhage, congenital disorders, treatment for seizures or cardiac arrhythmias, renal/hepatic dysfunction or issues that could affect protocol compliance. All sites had institutional review board approval. If eligible prior to 36 weeks+6 days PMA, parents were approached for written informed consent, including for blood sampling and non-sedated MRIs.

Randomisation

Randomisation occurred after discontinuation of routine caffeine treatment. A randomly permuted blocked randomisation design was used, with block sizes of 4 and 8, stratified by site and birth gestational age categories: (<28 weeks; 28 weeks +0 through 30 weeks +6) prepared by the study's statistician (TCH). Multiple births were randomised to the same treatment. Investigational pharmacists at each site were provided with a randomisation table for each gestational age stratum to assign subjects to the assigned study drug.

Study intervention

Caffeine base was used as a study drug; its dose equivalent is one-half of caffeine citrate (ie, 5 mg of caffeine base=10 mg caffeine citrate). We chose caffeine base due to cost and ease of placebo preparation. Following randomisation, infants received once daily caffeine base at 5 mg/kg/day in Syrspend or equivalent volume of placebo (Syrspend alone), with escalation at 36 weeks' PMA to 5 mg/kg of caffeine base two times per day or placebo, weight-adjusted weekly until discharge. This caffeine dose was based on our previous study.[14] Only pharmacists were aware of the study assignment. We continued the study drug until 42 weeks' + 6 PMA. Salivary caffeine levels were obtained at 1 week after starting two times per day dosing, then at 40–41 weeks' PMA and batched for later analysis.[15]

Oximetry

After enrolment, continuous pulse oximetry recording commenced using a study oximeter with expanded internal memory (Masimo Rad-97) using 2 s averaging and 1 s sampling time. The study oximeter was preset in sleep lab mode, with alerts only for 'probe off' and 'low battery'. Recordings continued until 43 completed weeks' PMA. Time-stamped oximetry data were downloaded periodically. Our primary outcome was number of seconds with oxygen saturation (SaO_2) below 90% per hour of recording time, measured during each PMA week. The number of seconds below 85% and 80% per hour was examined as secondary outcomes.

Plasma biomarkers

Baseline blood samples (~0.8 mL) were obtained after randomisation and again at the earlier of 38 weeks' PMA or discharge. Assays were performed at Children's National Hospital in duplicate on 96-well plates and scanned on a calibrated Meso QuickPlex SQ 120 reader. No samples were thawed more than three times before assay. Data were derived from standard curves that performed as expected for each assay.

Brain MRI/DTI/MRS

MRIs were obtained at 10 sites with equivalent 3T MRI scanners for both inpatient and outpatient scans. Non-sedated scans were conducted after enrolment or within 3 days after randomisation, and outpatient scans after completion of the study drug at 43 weeks' PMA, but no later than 46 weeks' PMA. All sites used the same acquisition protocol. Analyses were performed at the Developing Brain Institute at Children's National Hospital (see online supplemental materials for acquisition and analysis protocols).

Safety parameters

Prespecified postrandomisation clinical outcomes of interest included: need for resuming treatment with clinical caffeine, supplemental O_2 or other respiratory support; days from randomisation until discharge, PMA at discharge and weight gain/day from randomisation until discharge. Discharge timing was determined by the site clinicians.

Adverse events and serious adverse events were assessed throughout the study and reported by each site. Prespecified adverse events included Brief Resolved Unexplained Events (BRUEs), apnoea, gastro-oesophageal reflux, bradycardia, tachycardia and irritability. The Brief Infant Sleep Questionnaire (BISQ), a validated sleep quality instrument, was collected at 41 weeks' PMA.[16]

Statistical analyses

Caffeine effect on IH

As in our prior studies,[13 14] to reduce the possible impact of outliers with very short recording times, we required at least 10 hours of acceptable quality oximetry recording during each PMA week for analysis. We expected that the distribution of seconds/hour with SaO_2<90% would be highly skewed; thus, per protocol, analyses were performed on logged values. Descriptive statistics are reported as geometric means (calculated by exponentiating the mean of the logged values) and 95% CIs. Exponentiating differences in means of logged values was used to calculate per cent differences in geometric means. At each PMA week (33 through 42), a mixed effect regression model compared caffeine versus placebo infants, controlling for stratification variables, gestational age category and enrolment hospital (as a random effect) and accounting for correlation among sibships as a random effect. Exponentiating the treatment group parameter and CI from this model yields an adjusted per cent difference and CI for the geometric mean for caffeine versus placebo infants. For primary oximetry outcomes, multiple testing across the 10 PMA weeks was controlled through the experimentwise error rate with p values adjusted using Holm's procedure.[17]

Secondary efficacy analyses

Oximetry

To more fully understand the impact of caffeine on oxygen saturation levels, secondary analyses were performed similar to those

described for the primary outcome, to examine the seconds/hour below 85% and 80% SaO_2 levels.

Caffeine effect on plasma biomarkers and brain MRI biomarkers

Separate analyses were performed for the follow-up assessment of each biomarker using linear mixed effect regression models with treatment group, baseline biomarker level, gestational age strata and time from baseline to follow-up as independent variables, and hospital and sibship as random effects. Infants needed both baseline and follow-up data to be included in analyses. Data are summarised using means and 95% CIs. By protocol, based on a preliminary review of the data, due to skewness, plasma biomarker data were log transformed and summarised using geometric means. Exponentiating the parameter for the treatment group was used to give an estimate of the per cent difference in biomarker level at follow-up for the caffeine versus placebo group, controlling for baseline level and the other model covariates.

Multiple testing was controlled for through the false discovery rate, and p values were adjusted using the Benjamini and Hochberg method for the 12 plasma biomarkers, and for imaging outcomes within each study domain (6 volume, 6 MRS, 6 DTI measures).[18]

Sample size

Sample size considerations focused on the primary outcome of seconds/hour with SaO_2<90%. In our pilot studies, geometric mean seconds/hour with SaO_2<90% was 15.1 in extended caffeine infants versus 37.5 in controls (60% reduction), corresponding to a standardised effect size for log seconds/hour of Cohen's d=0.74. We anticipated that seconds/hour with SaO_2<90% in placebo infants would approach levels in the caffeine group with advancing PMA, so we powered the study to detect a moderate standardised effect of d=0.50, which would correspond to a 46% reduction in geometric mean seconds/hour with SaO_2<90% for the caffeine versus placebo group. A sample size of n=100 infants/group would give 82% power of detecting this difference (via a t-test on log seconds/hour with SaO_2<90%, with a Bonferroni adjustment for separate comparisons at 5 PMA weeks and a familywise error rate of 0.05). To allow for missing data, we targeted enrolment at n=110 per group.

Enrolment was lower than expected; we randomised 160 subjects, with approximately n=60 at each PMA week. At this sample size, we had 55% power of detecting the targeted effect of d=0.50 and had 80% power of detecting an underlying effect of d=0.63 (corresponding to geometric means of 15.1 versus 32.8 (54% reduction).

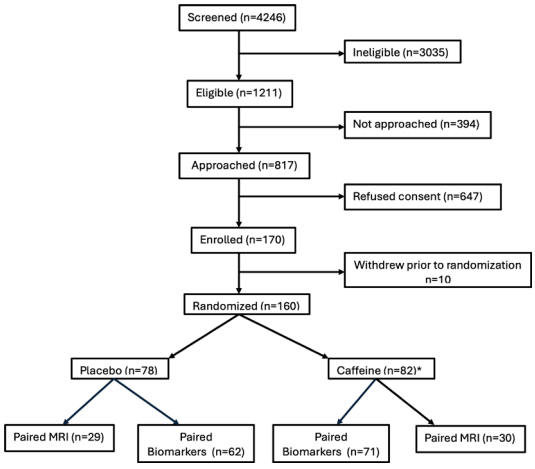

Figure 1 Consolidated Standards of Reporting Trials diagram. *One subject did not have oximetry data recorded

Table 1 Baseline characteristics of randomised infants

Characteristic	Placebo N=78	Caffeine N=82
Gestational age at birth, mean (SD), weeks	28.4 (1.6)	28.5 (1.8)
Birth weight, mean (SD), g	1171 (306.5)	1166 (337.3)
Male infants, n (%)	36 (46.2)	38 (46.3)
Multiple birth, n (%)*	23 (29.5)	22 (26.8)
Apgar score (mean (SD), 1 min)	5.5 (2.2)	5.4 (2.3)
Apgar score (mean (SD), 5 min)	7.8 (1.3)	7.6 (1.5)
Maternal race/ethnicity category		
Hispanic	6 (7.7)	13 (15.9)
Non-Hispanic black	19 (24.4)	28 (34.1)
Non-Hispanic white	48 (61.5)	33 (40.2)
Other	5 (6.4)	8 (9.8)
Postmenstrual age at enrolment, mean (SD), weeks	34.0 (1.0)	34.0 (0.9)
Postmenstrual age at randomisation, mean (SD), weeks	34.7 (0.9)	34.6 (0.8)
Received mechanical ventilation, n (%)	38 (48.7)	44 (53.7)
Received CPAP, n (%)	77 (98.7)	81 (98.8)
Days of prerandomisation supplemental oxygen, mean (SD)	13.0 (15.9)	13.1 (18.0)

*Caffeine: 22 infants from 13 twins (randomised both 9, one 4).
*Placebo: 18 infants from 11 twins (randomised both 7; one 4); 5 infants from 3 triplets (randomised all 1, one 2).
CPAP, continuous positive airway pressure.

RESULTS

We enrolled 170 subjects (figure 1). 10 withdrew prior to randomisation, leaving 160 subjects for analysis. Enrolment stopped prior to reaching the planned sample size, with DSMB assent, due to loss of funding. Unmasking of subject assignment and all analyses were performed after the close of the study. There were no significant differences in demographic and clinical characteristics between the caffeine and placebo groups at enrolment (table 1). Median salivary caffeine levels in the caffeine group were in the therapeutic range at both sampling periods (18 µg/mL sample 1; 22 µg/mL sample 2).

Primary outcome

Oximetry analyses

Infants randomised to extended caffeine had fewer seconds/hour below a threshold of 90% SaO_2 at each PMA from 34 through 41 weeks (table 2), with a median reduction of 60% in the caffeine group compared with placebo (minimum reduction 42%, maximum reduction 65%). Oximetry data not included (<10 hours/week) were similar between groups (online supplemental eTables 1 and 2).

Secondary outcomes

Oximetry

Statistically significant decreases in seconds/hour below threshold were observed with SaO_2 thresholds of <85% and <80% (online supplemental eTables 3 and 4), which were similar in magnitude to those observed using a threshold of <90%.

Plasma biomarkers

62 and 71 subjects (placebo and caffeine, respectively) had paired plasma samples. TNF-α at 38 weeks was 23% lower in

Table 2 Mean seconds per hour below 90% SaO_2 and adjusted difference by postmenstrual age week, by treatment

	Placebo		Caffeine			
PMA, week	N*	Geometric mean† seconds per hour below 90% SaO_2 (95% CI)	N*	Geometric mean† seconds per hour below 90% SaO_2 (95% CI)	Adjusted % difference‡ (95% CI)	Adjusted p value§
33	12	200.4 (87.1 to 461.0)	10	54.5 (28.3 to 105.2)	−60 (−87 to 20)	0.1
34	41	172.7 (123.4 to 241.7)	49	84.7 (64.4 to 111.4)	−49 (−67 to −20)	0.01
35	60	161.6 (128.2 to 203.7)	65	63.1 (50.2 to 79.5)	−59 (−71 to −43)	<0.001
36	65	143.5 (116.8 to 176.4)	69	54.9 (43.3 to 69.7)	−60 (−71 to −45)	<0.001
37	61	114.3 (92.9 to 140.5)	57	39.7 (29.3 to 53.8)	−65 (−76 to −50)	<0.001
38	59	88.1 (72.4 to 107.1)	49	32.9 (24.7 to 43.8)	−61 (−72 to −44)	<0.001
39	49	84.8 (63.3 to 113.5)	47	31.0 (21.1 to 45.4)	−65 (−79 to −42)	<0.001
40	47	65.6 (49.5 to 86.9)	41	30.8 (21.7 to 43.8)	−51 (−67 to −28)	0.002
41	39	73.0 (51.3 to 103.7)	45	26.6 (18.5 to 38.2)	−64 (−78 to −41)	<0.001
42	37	53.2 (36.9 to 76.8)	38	30.2 (18.8 to 48.4)	−42 (−68 to 5)	0.1

*Infants with 10+ hours of analysable SaO_2 data.
†Geometric mean, reflecting values analysed on log scale.
‡Per cent difference in geometric mean seconds per hour below 90% SaO_2 for caffeine versus placebo infants, adjusted for GA strata, study hospital and multiple births through mixed effects regression analysis.
§Adjusted for multiple comparisons across 10 PMA weeks using the Holm's test to control experiment-wise error.
GA, gestational age; PMA, postmenstrual age; SaO2, oxygen saturation.

the caffeine group compared with placebo, adjusting for baseline and covariates (p<0.02, table 3). There were no significant differences between groups in any other measured plasma biomarkers.

MRI/DTI/MRS analysis

88 MRIs were completed (45 placebo/43 caffeine), with 59 paired MRIs successfully analysed (29 placebo, 30 caffeine; 1 deleted from motion artefact). Unsuccessfully paired MRIs were due primarily to parental refusal of the second MRI scan. Not all paired scans were adequate for analyses due to quality: volumes (57; 29 placebo, 28 caffeine), DTI (47; 24 placebo, 23 caffeine) and MRS (41; 19 placebo, 22 caffeine). Results for the measured MRI volumes, DTI and metabolites assessed by MRS (online supplemental eTable 5) showed no significant differences in any measurements when corrected for multiple comparisons.

Safety analyses

Unexpected differences in the postrandomisation clinical outcomes prompted additional exploratory analysis. Fewer infants on caffeine required restarting supplemental oxygen compared with placebo 4 versus 14 (risk difference −13.1 (–2.9 to –23.9)). In addition, infants randomised to caffeine had fewer median days from randomisation to discharge 17 v. 27 (–8 (−2 to –14) and slower mean daily weight gain (g/day) from randomisation to discharge 27.0 versus 32.1 (−5.1 (−2.4 to –7.8)).

Adverse events

All adverse events reported in the study are provided in online supplemental eTable 6. No clinically important differences were observed in any adverse events including adverse events of special interest (table 4), adverse events reported as related to study drug (online supplemental eTable 7) or serious adverse events (online supplemental eTable 8).

Sleep quality

There were no differences between the caffeine and placebo groups in any parameters in the BISQ (online supplemental eTable 9).

DISCUSSION

This randomised placebo-controlled trial in preterm infants showed that extended caffeine therapy substantially reduced the burden of intermittent hypoxemia from randomisation at approximately 34 through 41 weeks' PMA. Although our smaller-than-planned sample size reduced the power of our study to detect a caffeine effect, the reduction of IH was large enough that we were able to detect a significant difference at most PMAs studied. Extended caffeine use was also associated with a greater reduction in TNF-α, without measurable differences in other inflammation-related biomarkers or MRI evidence of brain injury.

Our oximetry data expands our previous studies of extended caffeine compared with usual care in similar populations of preterm infants. In the first study, infants treated with 6 mg/kg/day of caffeine citrate had significantly reduced IH at 35-week and 36-week PMA, but not beyond.[13] In our second dose finding study, treatment was started with caffeine citrate at 10 mg/kg/day and increased to either 14 or 20 mg/kg/day versus usual care starting at 36 weeks' PMA. Oxygen saturation was analysed at 36-week, 37-week and 38-week PMA and compared with historical controls without treatment.[14] Salivary caffeine levels were also measured. At the doses used, therapeutic levels >20 µg/mL were maintained through study end, but the greatest reduction in IH through 38 weeks' PMA was observed in infants who received 20 mg/kg/day of caffeine citrate after 36 weeks PMA, which was the basis of the dosing used in the current study.

Studies in adults and children have shown that IH resulting from obstructive sleep apnoea is proinflammatory and associated with MRI evidence of brain injury and clinically with cognitive impairments.[2–4] However, no studies in preterm infants have explored whether reductions in IH with extended caffeine are associated with decreased biomarkers of inflammation or brain injury. Several neonatal animal studies, however, suggest that IH may increase inflammation and cause white matter injury.[5 6] In a study of rat pups subjected to cycles of hypoxia and normoxia mimicking IH in preterm infants, for example, both proinflammatory and brain injury biomarkers were increased in pups exposed to IH compared with room air controls; MRI scans showed white matter injury and thinning of the corpus

Table 3 Plasma biomarker concentrations at baseline* and follow-up†; adjusted difference by treatment group

Measure	Placebo N‡	Placebo Baseline* Geometric mean§ (95% CI)	Placebo Follow-up† Geometric mean§ (95% CI)	Caffeine N‡	Caffeine Baseline* Geometric mean§ (95% CI)	Caffeine Follow-up† Geometric mean§ (95% CI)	Adjusted % difference at follow-up¶ (95% CI)	Adjusted p value**
Planned analyses								
TNF-α	55	982.3 (825.8 to 1168.5)	994.6 (882.3 to 1121.2)	58	1032.3 (906.5 to 1175.6)	794.6 (695.5 to 907.8)	−23 (−34 to −9)	0.02
IFN-γ	56	1003.5 (753.6 to 1336.2)	923.3 (707.4 to 1205.2)	65	868.4 (719.7 to 1047.8)	682.1 (553.4 to 840.7)	−21 (−41 to 7)	0.5
IL-12p70	44	802.5 (654.6 to 983.9)	785.3 (656.0 to 940.0)	48	858.8 (711.3 to 1036.8)	696.4 (576.2 to 841.8)	−15 (−29 to 2)	0.5
IL-10	55	3392.9 (2620.3 to 4393.4)	3210.4 (2629.6 to 3919.4)	57	2823.5 (2143.2 to 3719.9)	2678.4 (2177.8 to 3294.2)	−14 (−34 to 12)	0.5
IL-17	57	3469.0 (2833.6 to 4246.7)	2260.4 (1883.3 to 2713.2)	66	2855.8 (2385.3 to 3419.0)	1915.4 (1644.2 to 2231.3)	−9 (−25 to 10)	0.5
IL-4	54	99.7 (81.1 to 122.6)	88.8 (71.2 to 110.8)	60	71.4 (60.0 to 84.9)	69.5 (58.7 to 82.4)	−2 (−21 to 23)	>0.9
IL-6	54	2207.0 (1691.3 to 2880.0)	1603.7 (1225.7 to 2098.2)	60	2335.3 (1802.9 to 3024.9)	1539.4 (1160.5 to 2041.9)	1 (−32 to 50)	>0.9
IL-2	54	466.4 (372.6 to 583.8)	331.1 (290.3 to 377.7)	56	404.9 (363.0 to 451.6)	337.5 (302.2 to 377.0)	5 (−12 to 24)	0.8
IL-1β	57	602.3 (468.9 to 773.6)	732.5 (526.6 to 1018.8)	56	663.2 (498.7 to 881.9)	734.1 (550.1 to 979.6)	7 (−27 to 57)	0.9
Additional analyses								
Tau	29	491 143.9 (359890.3 to 670266.2)	409 734.1 (311006.0 to 539803.3)	35	483 943.7 (367489.4 to 637301.5)	370 973.9 (284091.2 to 484427.6)	−13 (−32 to 10)	0.5
NFL††	31	30.3 (24.3 to 37.9)	25.1 (20.2 to 31.2)	32	28.3 (20.4 to 39.3)	32.3 (25.2 to 41.3)	16 (−12 to 54)	0.5
CRP††	40	86 504.6 (33447.0 to 223728.7)	85 315.2 (35940.3 to 202521.6)	51	111 423.6 (51687.8 to 240196.2)	141 433.0 (62439.3 to 320364.0)	49 (−31 to 221)	0.5

*Baseline sample drawn as close as possible to randomisation, between consent and second calendar day after first dose of study drug.
†Follow-up sample obtained at PMA 38+0 (±3 days) or within two calendar days prior to hospital discharge, whichever came first.
‡Infants with analysable paired samples for each biomarker.
§Geometric mean, reflecting values analysed on log scale.
¶Per cent difference in geometric mean biomarker level at follow-up for caffeine versus placebo infants, adjusted for baseline value, time between samples GA strata, hospital and multiple births through mixed effects regression.
**Adjusted for multiple comparisons across n=12 biomarkers tested using the Benjamini-Hochberg method to control the false discovery rate.
††Not adjusted for multiple birth, due to small sample size.
GA, gestational age; PMA, postmenstrual age.

callosum in hypoxia-exposed pups.[19] These collective human and animal data suggest that IH could also be a cause of injury in preterm infants and further suggest that extended caffeine might ameliorate this injury through reductions in the incidence and severity of IH.

Table 4 Adverse events of special interest, by treatment group

Adverse event term	Overall N=160 N (%)	Placebo N=78 N (%)	Caffeine N=82 N (%)	Risk difference (95% CI)
Total AEs of special interest	28	14	14	
Number of infants with 1+AE of special interest	27 (16.9)	14 (17.9)	13 (15.9)	−2.1 (−13.7 to 9.5)
Gastro-oesophageal reflux disease	12 (7.5)	6 (7.7)	6 (7.3)	−0.4 (−8.5 to 7.8)
Tachycardia	6 (3.8)	4 (5.1)	2 (2.4)	−2.7 (−8.6 to 3.2)
BRUE	4 (2.5)	2 (2.6)	2 (2.4)	−0.1 (−5.0 to 4.7)
Apnoea	3 (1.9)	2 (2.6)	1 (1.2)	−1.3 (−5.6 to 2.9)
Irritability	2 (1.3)	0 (0)	2 (2.4)	2.4 (−0.9 to 5.8)
Bradycardia	1 (0.6)	0 (0)	1 (1.2)	1.2 (−1.2 to 3.6)

AE, adverse event; BRUE, Brief Resolved Unexplained Event.

We also expanded our previous observations by measuring plasma biomarkers of inflammation associated with brain injury and the possible ameliorating effects of caffeine. One major proinflammatory cytokine (TNF-α) had a 23% greater reduction over time in the caffeine-treated infants compared with placebo. Elevations in TNF-α have been associated with poor cognitive outcomes in very low weight preterm infants,[20–23] but no previous studies have evaluated blood biomarkers of inflammation in general or TNF-α specifically in infants born preterm with persisting IH, or any potential effects of caffeine. Caffeine is also widely recognised as anti-inflammatory in both animals and humans.[24 25] In a study of caffeine effect on inflammatory gene expression in THP-1 premonocytes exposed to lipopolysaccharide, caffeine in a dose-dependent manner decreased TNF-α gene expression at a clinically relevant concentration in preterm infants.[26]

Exploratory analysis showed several differences between the caffeine and placebo groups in their postrandomisation clinical course. Caffeine-treated subjects were less likely to restart supplemental oxygen, had slower predischarge weight

gain and were discharged sooner. In contrast, in a study of moderately preterm infants randomised to extended caffeine versus placebo, apnoea stopped 2 days sooner in infants on caffeine than in the infants randomised to placebo, but hospital length of stay was not shorter since it was more dependent in both groups on delayed attainment of mature feeding behaviour than on apnoea events.[27] In our study, we did not document the incidence of apnoea, but the increased apparent need for reinstitution of supplemental oxygen in the placebo group and the reduced extent of IH with caffeine were likely manifestations of improved respiratory control, which may have allowed earlier discharge. Other differences in our study were the inclusion of lower gestational ages and confirmed therapeutic caffeine levels, which also may have influenced discharge timing. A larger study is necessary to assess the validity of our preliminary observations, particularly on the length of hospital stay.

There were no important differences between treatment groups in serious adverse events or sleep disturbances. Our study suggested slower weight gain in the caffeine-treated infants, which is consistent with other studies of both clinical caffeine[28] and extended caffeine,[27] but in both studies post-caffeine catch-up growth occurred.

The strengths of our study include enrolment of the largest cohort to date of very preterm infants treated with extended caffeine and recorded high-resolution oximetry both in hospital and at home with documented therapeutic caffeine levels. Compliance with home study drug administration was high, given the persistent reduction of IH and measurement of therapeutic levels of caffeine after discharge. In addition, we measured plasma biomarker values on a substantial proportion of enrolled subjects, providing insight into potential effects of caffeine and reduced IH on the inflammatory milieux in convalescing preterm infants.

Limitations include the less-than-projected sample size. Infants with <10 hours of usable oximetry data per PMA week were excluded from analysis. However, rates of excluded data were minimal and similar for both groups. We did not find any significant differences in measurements on MRI/DTI/MRS, though power was limited by paucity of scans. There were unexpected challenges in our MRI aim, including that several sites could not participate, some parents declined the second scan and a few scans were complicated by motion artefact. Finally, we were limited to two plasma samples in this preterm population; more frequent sampling may have revealed a more comprehensive assessment of inflammatory changes with caffeine therapy than we observed.

We conclude that extended caffeine therapy at the doses we used in convalescing preterm infants in room air appears to be safe and results in a significant reduction in the amount of IH through 41 weeks' PMA. The observed reduction in TNF-α suggests caffeine may reduce inflammation, which contributes to the risk for adverse outcomes in preterm infants. A larger randomised trial examining extended caffeine effect on long-term adverse outcomes in preterm infants is warranted.

Acknowledgements We wish to acknowledge the important contributions of the ICAF study coordinators at each of the participating sites: Heather White, MPH, PA-C, University of MA Medical Center, Worcester, Massachusetts, USA; Neha Thakkar, MS, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA; Megan Dhawan, CRNP, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA; Toni Mancini, RN, Pennsylvania Hospital, Philadelphia, Pennsylvania, USA; Judith Fitzpatrick, MSN, Walter Reed National Military Medical Center, Bethesda, Maryland, USA; Nikia Gray-Hutto, CCRP, Loma Linda University Children's Hospital, Loma Linda, California, USA; Micah Tong, Kapiolani Medical Center for Women and Children and John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA; Matt Butoryak, BSN, CRC, Magee Women's Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; Mary M. McNally, BSRT, RRT, CCRP, Karlyn Martini, RN, Department of Pediatrics, Geisel School of Medicine at Dartmouth and Dartmouth Hitchcock Medical Center, Lebanon, New Hampshire, USA; Amanda Sowden BSN, RN, Department of Neonatology, The Woman's Hospital of Texas, Houston, Texas, USA; The Pediatrix Center for Research, Education, Quality, and Safety, Sunrise, Florida, USA; Tamara Babushkin, RN, Johns Hopkins All Children's Hospital, St. Petersburg, Florida, USA; Jennifer Shepard, CRNP, MBA, Johns Hopkins Children's Center and Johns Hopkins Bayview Medical Center, Baltimore, Maryland, USA; Kimberly Quire, BSN, University of Kentucky Children's Hospital, Lexington, Kentucky, USA; Nilesh Dankhara, Maryland, USA; Chelsea Giachelli, University of Mississippi Medical Center, Jackson, Mississippi, USA; Melissa Tyree, Maryland, USA; Ann Pokelsek, RN, AdventHealth Hospital, Orlando, Florida, USA; Hany Aly, Maryland, USA; Mohsen Farghaly MD, Jennifer M. Perez, MSN, Jalal Abu-Shaweesh, MD, MBA, Cleveland Clinic Children's Hospital, Cleveland, Ohio, USA; Mary Revenis, MD, Children's National Hospital, Division of Neonatology, George Washington University, School of Medicine, Washington, DC, USA; Robert Darnall, MD, Geisel Department Pediatrics, Geisel School of Medicine at Dartmouth and Dartmouth Hitchcock Medical Center, Lebanon, New Hampshire, USA; Lisa Crowell, MPH, and Ariana Saroufim, MPH, Slone Epidemiology Center at Boston University, Boston, Massachusetts, USA. We also acknowledge these additional contributors to the ICAF study: American SIDS Institute for partial support of pilot studies and travel expenses for national meeting ICAF presentations; Masimo Corp, Irvine, California, USA, for providing R-97 pulse oximeters with extended internal memory; Mark Peucker, BS, for biomedical engineering support; Griner Bio-One, Monroe, North Carolina, USA, for providing blood collection tubes; Thu-Lan Luong, Clinical Laboratory Specialist, Biomedical Research Lab, Walter Reed National Military Medical Center, Bethesda, Maryland, USA for analysis of salivary caffeine concentrations. Data Safety Monitoring Board: Ronnie Guillet, MD PhD (Chair), Eduardo Bancalari, MD, Sonya Heltshe, PhD, Michele Shaffer, PhD

Collaborators The ICAF Study Group: Drs Lawrence Rhein, Ann Stark, Ivan Franz, Karen Puopolo, Nicole Dobson, Elizabeth Schulz, Mitchell Goldstein, Venkataraman Balaraman, Tyler Hartman, Kaashif Ahmad, Prem Fort, Maureen Gilmore, Elie G Abu Jawdeh, Nilesh Dankhara, Melissa Tyree, Hany Aly, Mohsen Farghaly, Mary Revenis, Robert Darnall, Christian Poets, Lisa Crowell.

Contributors EE and CEH were co-principal investigators of the study, developed the protocol and supervised all aspects of the randomised trial. SKn conducted the plasma biomarker analysis. CL and KK developed the MRI analysis software and conducted all data analysis of the MRI/diffusion tensor imaging/magnetic resonance spectroscopy (DTI/MRS). BM was the project director and CI was the project coordinator. TCH and SKe were statisticians who developed the statistical analysis plan of the protocol and performed statistical analysis of the data. MC was the director of the Data Coordinating Center. ICAF Study Group: all participated in IRB preparation and approval, subject recruitment, monitoring of study protocol, data downloads, sample collection, reporting of adverse events and investigator meetings. All authors contributed and approved the final manuscript. EE is the guarantor.

Funding This study was funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (1RO1HD089289) and the American SIDS Institute.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the University of Pennsylvania Institutional Review Board, IRB protocol # 828783. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data are housed at the Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA.

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