The effects of caffeine following hypoxic-ischemic encephalopathy: A systematic review of animal studies

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ABSTRACT

Background: Caffeine is believed to be neuroprotective in preterm and term infants, despite the conflicting data on its effects on the developing brain in animal models. We aimed to conduct a systematic review with meta-analysis assessing the effects of caffeine on the prevention and treatment of neurological morbidity caused by hypoxic-ischemic encephalopathy (HIE) in preclinical studies.

Methods: Randomized and non-randomized control studies in animal models of HIE reporting caffeine administration within the first ten days of life were included. Primary outcomes were behavioral tests that served as surrogates for cognition, memory, motor coordination, and gait; secondary outcomes pertained to structural neurologic changes. Screening for inclusion, risk of bias and data extraction were performed independently by two authors.

Results: Seven studies met inclusion: 5 studies were conducted in rats and 2 in mice. All studies were performed in full-term animals, and the majority of studies used animals of both sexes (5/7). In six studies, caffeine was administered intraperitoneally to the pups, while in the remaining study, it was delivered via the drinking water of the lactating dams. The doses of caffeine ranged from 5 to 20 mg/kg; in one study, caffeine dosage was 0.3 mg/L in the drinking water of lactating dam. The mortality rate was reported only in three studies. Caffeine had a positive effect on overall functional outcome (SDM 0.92 (95%CI 0.25 to 1.59)). Animals treated with caffeine performed better on Morris water maze and rotarod tests (SDM 1.39 (95%CI 0.36 to 2.41)) and (SDM 1.03 (95%CI 0.03 to 2.04)), respectively. Caffeine treated animals performed worse on open field test compared to the controls (SDM -1.11 (95%CI -3.01 to 0.80)). The overall quality of the included studies was limited.

Conclusions: Early caffeine exposure in preclinical rodent models of HIE is associated with improved selective functional and neurological outcomes, although the certainty of the evidence is limited. To validate the therapeutic efficacy of caffeine as a neuroprotective adjuvant, there is a need to explore its effects in larger animal models, which will help guide the design of relevant clinical trials.

1. Background

Hypoxic-ischemic encephalopathy (HIE) is the major cause of mortality and disability in full-term and near-term infants (Jacobs et al., 2013). The incidence of any HIE in developed countries varies between 1.5 and 3.0 per 1000 live births (Kurinczuk et al., 2010; Shipley et al., 2021) and 2.3–30.6 per 1000 life birth in developing countries (Kurinczuk et al., 2010; Namusoke, 2018). The only available treatment strategy for newborns with moderate to severe HIE is therapeutic hypothermia, which has been shown to reduce mortality and neurological disability (Jacobs et al., 2013). However, therapeutic hypothermia requires initiation within the first six hours after birth, and it is available only in tertiary level neonatal intensive care units (Jacobs et al., 2013). There is an urgent need to develop alternative treatment strategies that may improve survival and neurological disability and are readily accessible.

Caffeine (methyl theobromine) is a standard treatment across all neonatal intensive care units (NICUs) (Dobson and Hunt, 2013).
Methylxanthines have been used successfully over the past 40 years to treat apnea of prematurity. Caffeine has now largely replaced other treatments for apnea, such as theophylline and aminophylline (Natarajan et al., 2007). This is associated with its longer half-life, more comprehensive therapeutic range, cost-effectiveness and decreased need for drug monitoring. In the landmark study “Caffeine for Apnea of Prematurity trial (CAP)”, infants who received caffeine demonstrated a reduced incidence of bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA) requiring medical and/or surgical treatment, and severe retinopathy of prematurity (ROP) (Schmidt et al., 2006; Schmidt et al., 2007). An 18-month follow-up study revealed a neuroprotective outcome associated with caffeine intake, marked by a lower incidence of cerebral palsy and cognitive delay (Schmidt et al., 2007). Furthermore, the CAP trial 11-year follow-up showed that caffeine treatment was associated with improved vision (Mürner-Lavanchy et al., 2018) and reduced motor disability (Schmidt et al., 2007) without affecting intelligence, attention, or behavior, emphasizing its long-term safety (Schmidt et al., 2007; Mürner-Lavanchy et al., 2018). Many NICUs have thus changed their practice toward the earlier initiation of caffeine therapy.

Evidence suggests that caffeine may have a protective and positive neurodevelopmental effect on the development of the brain in preterm infants. However, animal studies have shown conflicting results regarding caffeine’s role in neurodevelopment (Jacobson et al., n.d.). Beneficial and harmful caffeine effects have been reported, both across and within similar animal models. Data from small and large animal studies play an integral role in the development of therapies that are currently commonplace in clinics. To better understand the impact of caffeine on the brain, we opted to assess the outcomes observed in preclinical literature. Therefore, our goal was to conduct a systematic review with meta-analysis to examine the effect of caffeine on neurodevelopment in animal models of HIE that recapitulate human neonates. The findings of this systematic analysis have the potential to impact clinical care and the design of future preclinical and clinical work.

2. Methods

The protocol was developed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-P checklist (Moher et al., 2015), which was prospectively registered and is available on the International Prospective Register of Systematic Reviews (PROSPERO) (Bruschettini, 2020). We included randomized and non-randomized controlled studies of animal models of HIE that delivered caffeine in the first 10 days of life. All mammals, irrespective of sex, were included; we excluded non-interventional studies, studies in which we were un

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able to stratify information between control and treatment groups, case studies, cross-over studies, or studies that only included in vitro work.

We conducted a literature search using PubMed, Embase, and Web of Science, with no time or language restrictions. The search strategies for each database are presented in Supplemental material 1. The titles and abstracts of the search results were individually screened and selected based on the inclusion and exclusion criteria. Full texts of all potentially eligible studies were retrieved and reviewed for eligibility independently by four members of the team working in pairs (OR, MB, AA, ABP) and data extracted from each study using standardized Microsoft excel spreadsheet forms (version 2019 (16.0). Disagreements between reviewers were resolved by consensus or by a third member.

As much of the data was available in figures and not in numerical form, we used a validated graphical digitizer (Web Plot – Digitizer, version 4.3: Ankit Rohatgi), a web-based semi-automated tool that operates with a diversity of plot types and images. First, the figures' images for the relevant outcome from all included studies were saved as screenshots. Then, the copies were uploaded to the tool. The first step of the analysis consisted of defining the type of graph analyzed, typically a two-dimensional bar plot and calibrating the axis by assigning four points of known values on the axes. The data points were then obtained by a manual method, data points were added by directly clicking on the graph, and WebPlotDigitizer calculated the specific coordinates of each point. This approach was utilized to calculate the mean and standard deviation/standard error of the mean for each graph.

The extracted data for the primary outcomes were behavioral tests related to cognition, memory, motor coordination impairment and gait disturbances. For the secondary outcomes, we extracted data related to inflammation markers for the brain, time to open eyes, seizures and hyperthermia-induced seizures, lesion size as measured by neuro-imaging and immunohistochemistry and degree of myelination.

Meta-analysis was conducted using a random-effects model to generate forest plots. The estimated effect size of caffeine on functional neurologic outcome and lesion size after HIE was determined using standardized mean difference (SMD) with a 95% confidence interval (CI). SMD, an ideal measure for continuous data, is calculated by dividing the mean difference in each study by that study’s standard deviation. Pooled data from all neurobehavioral studies were also examined to determine the overall effect size.

Statistical heterogeneity between studies was calculated using the I2 metric, with I2 > 50% suggesting evident heterogeneity. The presence of publication bias was evaluated using funnel plots and Egger’s tests. Funnel plots were visually assessed for asymmetry. For Egger’s tests, p < 0.05 was considered significant to confirm the presence of a small study size.

All statistical analyses were performed in R v.4.1.0 with a p-value < 0.05. Neurologic outcomes with less than two studies were described in a narrative fashion.

The risk of bias was assessed by two reviewers (MB, ABP) for each included study, using SYRCLE’s Risk of Bias tool (an adaptation of the Cochrane Risk of Bias Tool) for animal studies (Hooijmans, 2014). We extracted study characteristics related to the construct and external validity: age, sex, strain and animal species, timing, dose and mode of caffeine administration, the use of any co-interventions and other characterization of animal properties at baseline.

3. Results

Our literature search resulted in 40 articles based on the utilized key search terms. A total of 25 remained after duplicates were removed. After screening by title and abstract, 7 studies investigated caffeine use for HIE (Fig. 1). All of the studies were used in the meta-analysis.

3.1. Study characteristics

Studies included in this review were published between the years 2013–2020. Regarding countries conducting the studies, three of the studies were completed in the United States, two from Sweden, and one each from China and Turkey. Further details are described in Table 1. Rodents were used in all studies, with five of the studies (71.4%) completed in rats. Both sexes of animals were used in the experiments in 71.4% (n = 5 studies).

As per our inclusion criteria, models of HIE were performed in animals within the first ten days of age. Investigators surgically ligated one of the common carotid arteries to create an asphyxia model. Five of the studies (71.4%) also exposed the animals to a period of hypoxia.

3.2. Caffeine details

Caffeine citrate was the formulation of caffeine used in three studies (42.9%), while four of the studies did not specify the caffeine type. Six studies (85.7%) introduced caffeine into the animals via the intraperitoneal route. Dosages of caffeine ranged between 5 mg/kg to 20 mg/kg per dose.
3.3. Risk of bias

Fig. 2 depicts the risk of bias summary. The SYRCLE’S Risk of Bias contains ten entries related to selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. The studies are scored as ‘low’ risk, ‘high’ risk, and ‘unclear’ risk. None of the studies met the criteria for low risk of bias across all domains.

In evaluating randomization to caffeine administration or placebo, the studies were considered an unclear risk of bias, as were the sequence generation and allocation concealment. None of the studies provided a sample size calculation, nor did they provide enough evidence to deem whether intervention and control groups were similar at the start of experiments.

Most of the studies 85% (6/7) reported an unclear risk of bias under blinded assessment of outcome by the administration of caffeine. Again, it was also unclear whether the caregivers and researchers assessing the outcomes were blinded to the treatment group (detection bias).

In respects to random outcome assessment, two studies were considered low risk of bias (Winerdal et al., 2017; Xu, 2015) while the rest were assessed under unclear risk. For incomplete outcome data, one study was low risk (Alexander et al., 2013), one was high risk (Alexander et al., 2013), and the rest remained unclear.

Overall, in the vast majority of studies, 85% (6/7) were categorized as unclear risk of bias due to insufficient information, including allocation sequence, housing of animals, blinding, and prior registration of a protocol and one was at high risk (Sun et al., 2020).

3.4. Meta-analysis:

3.4.1. Functional neurological outcome (refer to Fig. 3)

Rotarod: Three studies assessed sensorimotor outcomes using the rotarod test (Winerdal et al., 2017; Di Martino et al., 2020; Potter et al., 2018). Although caffeine demonstrated benefit [SMD 1.03, (95% CI 0.03, 2.04)], heterogeneity was high at 71%. Only one study did not show improvement in the rotarod test after caffeine was given to HIE animals (Di Martino et al., 2020).

Water maze: Two studies (Alexander et al., 2013; Potter et al., 2018) assessed the cognitive effects of caffeine in animals via the water maze test. In a total of 59 animals, caffeine provided improvement in time to the water maze platform with an SMD of −1.11 (95% CI, −3.01; 0.80).

Open field: Two studies assessed cognitive performance in animals with HIE after caffeine administration (Winerdal et al., 2017; Di Martino et al., 2020). The SMD did not show caffeine benefit over placebo [SMD −1.11 (95% CI, −3.01; 0.80)].

Overall: When combining the three tests (nine comparisons), caffeine improved functional neurologic outcomes with an SMD of 0.92 (95% CI,
Table 1
Study characteristics.

| Study | Animal species and strain | Animal sex (F = female, M = male, not reported) | Presence and degree of prematurity | Total nr. of animals at the beginning | Number of animals outcome data is reported for | Insult model if any | Age at caffeine administration | Caffeine type, dose, route and frequency of administration | Type of control | Outcomes | Mortality rate | Additional information | Funding |
|-------|--------------------------|-----------------------------------------------|-----------------------------------|--------------------------------------|---------------------------------------------|---------------------|--------------------------------|------------------------------------------------|----------------|----------------|----------------|----------------------|---------|
| 1     | Alexander 2013           | M                                             | Full-term                         | Not reported                         | 78                                          | Hypoxia-ischemia (right carotid artery cauterized) + 8% oxygen for two hours | P7                 | Caffeine formulation not reported; i. p. 10 mg/kg 120 min following insult | Saline                | From P90 to P95: WE, MWM | Not reported | None                   | Not reported |
| 2     | Xu 2015                  | Rats, Sprague-Dawley                          | F + M                             | Full-term                            | 48                                          | HIE (left common carotid artery ligation) + 8% oxygen for two hours           | P7-10               | Caffeine citrate, i. p., 20 mg/kg from P7 to P10 | Saline                | MBP expression, adenosine A1 receptor mRNA expression | Not reported | None                   | Not reported |
| 3     | Kilicdag 2014            | Rats, Wistar                                  | F + M                             | Full-term                            | 28                                          | Hypoxia-ischemia (right carotid artery ligation + a 2-h period of hypoxia (92% N2, 8% O2) in a hypoxia chamber) | P7-10               | Caffeine citrate, i. p., 20 mg/kg | Saline + Sham | P11: Neuronal apoptosis in hippocampus and parietal cortex | 14%                   | Sham group: A median neck incision was performed, but the rats were not subjected to ligation or hypoxia | None     |
| 4     | Potter 2018              | Rats, Wistar                                  | M                                 | Full-term                            | 64                                          | Hypoxia-ischemia (right carotid artery ligation + a 2-h period of hypoxia (92% N2, 8% O2) in a hypoxia chamber) | P6-7                | Caffeine citrate, i. p., 20 mg/kg | Saline                | P35: rota rod test P60: silent gap detection P90: non spatial water maze | Not reported | The University of Connecticut-Storrs Research Excellence Program (internal UConn funding), and the Connecticut Institute for Brain and Cognitive Sciences (IBACS) Medicinska Forskningsrådet (SE) regional agreement on medical training and clinical research, Marianne and Marcus Wallenberg foundation, Swedish Brain Foundation and Swedish Order of Freemasons in Stockholm | None     |
| 5     | Winerdal 2017            | Mice                                          | F + M                             | Full-term                            | 42                                          | HIE (unilateral electrocoagulation at 8 Watt of the right carotid artery, followed by hypoxia chamber (1 h of 10% O2 in 90 %N2) | P10                 | Caffeine formulation is not reported, i. p., 5 mg/kg | PBS                   | P24: Open field test activity; Rotarod: time spent: | Not reported | None                   | None     |

(continued on next page)
| Study | Animal species and strain | Animal sex (F = female, M = male, not reported) | Presence and degree of prematurity | Total nr. of animals at the beginning | Number of animals outcome data is reported for | Insult model if any | Age at caffeine administration | Caffeine type, dose, route and frequency of administration | Type of control | Outcomes | Mortality rate | Additional information | Funding |
|-------|--------------------------|-----------------------------------------------|----------------------------------|----------------------------------|-----------------------------------------------|-------------------|-----------------------------|-------------------------------------------------|-----------------|----------------|-----------------|--------------------------|---------|
| 6     | Sun 2020 Rats, Sprague-Dawley | F + M | Full-term | 60 | 44 | HIE (unilateral carotid ligation), followed by temperature-controlled hypoxia (2.5–3.0h 5.6%O2), either antenatally (not included in this review) or P3-16 | Caffeine formulation is not reported, Drinking water without caffeine | 20% for any individual litter following HIE | (Undamaged hemisphere area) | Brain activated CD69 + CD8 + T-lymphocyte population reduced | 0% mortality | Caffeine single dose at P10 either at 0, 6, 12 and 24 h post HI | NIH NINDS NS060896 and by NS107039 |
| 7     | Di Martino 2020 Mice Wild type C57/b6 specific pathogen-free | F + M | Full-term | Unclear | Caffeine formulation is not reported, i.p., 5 mg/kg | P24 OF: improved only for 0 h Caf group; no differences for 6 h, 12 h and 24 h Caf groups | PBS | 0% mortality | Caffeine single dose at P10 either at 0, 6, 12 and 24 h post HI | (continued on next page) | Brain electrical background activity: preserved upper aEEG margin on P8; restored burst duration and amplitude on P8; restores EEG power in delta frequency spectrum, unchanged in alpha, beta and gamma frequency spectrum | | |

P11: gen expression: IL6 downregulated, no differences in IL12, IL1b, Ifng
P15: global neuropathological score and striatum and hippocampus tissue loss: improved, the area

(continued on next page)
Table 1 (continued)

| Study | Animal species and strain | Animal sex (F = female, M = male, not reported) | Presence and degree of prematurity | Total nr. of animals at the beginning | Number of animals outcome data is reported for | Insult model if any | Caffeine type, dose, route and frequency of administration | Type of control | Outcomes | Mortality rate | Additional information | Funding |
|-------|--------------------------|-----------------------------------------------|-----------------------------------|--------------------------------------|-----------------------------------------------|---------------------|-------------------------------------------------|---------------|---------|-----------------|----------------------------|---------|
|       |                          |                                               |                                    |                                      |                                                |                     | covered by the glial scar in striatum, hippocampus and cortecies: reduced; TUNEL positive cells: reduced in cortex; microglial activation (Iba1): reduced in cortex and striatum; reduction in microglial cells size; MBP loss in striatum: reduced, no changes for MBP loss in corpus callosum, thalamus or cortex. P27: global neuropathological score and striatum tissue loss: improved only for 0 h Caf group; no differences for 6 h, 12 h and 24 h Caf groups |                     |                     |                   | Swedish Medical Society, the Swedish Brain Foundation (grant number FO2019- 0045), and The Philipson foundation. |

Outcomes: (list of outcomes reported in the original papers amongst those listed in our review; if differences between groups, we used symbols or ↓ if caffeine improved or worsened the outcome, respectively. If arrows are in brackets, it means that the effect was transient.)
Heterogeneity for this analysis was high at 88%.

3.4.2. Lesion size (refer to Fig. 4)

Examination of HIE injury was evaluated under the following accounts:

- **Cortical volume:** was higher in the cohort of animals (n = 2 studies) that received caffeine after 95% HIE induction [SMD 1.07 (95% CI, 0.41; 1.73)] (Alexander et al., 2013; Potter et al., 2018).

- **Brain atrophy/tissue loss:** Two studies demonstrated that brain atrophy or tissue loss was lessened in the group of animals receiving caffeine [SMD –1.70 (95% CI, –2.33; –1.07)] (Winerdal et al., 2017; Di Martino et al., 2020).

- **Apoptosis:** A subset of 36 animals had apoptosis measured via TUNEL or caspase measurements. Caffeine reduced apoptosis by an SMD of –1.83 (95% CI, –2.71; –0.96) (Di Martino et al., 2020; Kilicdag et al., 2014).

3.4.3. Narrative findings

- **Infarct volume:** Caffeine given before injury, not after HIE induction, reduced infarct volume (Sun et al., 2020).
Microglia activation: Animals receiving caffeine after HIE significantly reduced microglial activation (Di Martino et al., 2020).

Myelination: Caffeine decreased the mean loss of grey intensity of myelin binding protein (MBP)-stained tissue (Di Martino et al., 2020). In another study, subcortical white matter expression of MBP was higher in the caffeine-exposed cohort (Xu, 2015).

Inflammation: One study examined the expression of inflammatory genes in animals after an HIE insult (Di Martino et al., 2020). Of the four genes, only one (interleukin-6) was significantly lower in the caffeine treated group when compared to the control cohort (Di Martino et al., 2020). Winderdal et al. also examined the immunomodulatory effects of caffeine in the brain (e.g., local effects) and spleen (e.g., systemic effects). They found that caffeine did not alter resident immune cells in the brain except for CD8+ CD69 + T-lymphocytes. Authors concluded that caffeine had no apparent long-term immune consequences as only one subpopulation of cells (from > 30 tested) were activated. No differences in immune cell activation were observed in the spleen.

Electrical activity: Caffeine restored normal background electrical activity (delta frequency) after injury (Sun et al., 2020).

Brain weights: no difference was observed between saline and caffeine groups in one study (Kilicdag et al., 2014).

Silent gap: is a learned task for processing speech detection; animals given caffeine had improved silent gap after HIE (Potter et al., 2018).

3.4.4. Publication bias

Supplemental file 2 shows funnel plots for the primary and secondary outcomes. Asymmetry was not detected, indicating the absence of publication bias; however, the limited number of included studies limits the reliability of the funnel plot. Egger’s test was performed for analyses that included more than two unique studies with a p-value > 5% (i.e., unlikely publication bias).

4. Discussion

This is the first systematic review on the effects of caffeine in the neonatal period following HIE, reporting the findings of seven preclinical studies on brain function/development. Overall, caffeine resulted in improved cognitive outcomes in animals following an HIE insult. However, animals treated with caffeine following HIE were more anxious and exhibited decreased exploration in the open field test. There are no clinical studies on caffeine effect in newborns with HIE, and therefore it is difficult to translate the data from our meta-analysis. Although the overall functional outcome was better in animals treated with caffeine, the heterogeneity was very high. It highlights the need for more homogeneous randomized animal studies to evaluate the effect of caffeine in HIE animal models, which may help design better clinical trials.

However, a long-term follow-up study of preterm infants treated with caffeine demonstrated improved motor coordination, visuomotor integration, and visuospatial integration (Mürner-Lavanchy et al., 2018). Furthermore, no behavioral problems were reported in the infants treated with caffeine (Mürner-Lavanchy et al., 2018). It should be noted that the Morris water maze was performed at the advanced age of the animals at P90, which roughly corresponds to adolescence in humans, while the open field test was done at P24, which correlates with the infancy period. It is not known whether the anxiety behavior and exploration in the open field would be different if those animals would be tested at a later time point.

Furthermore, the data from included animal studies suggest that animals treated with caffeine following HIE had reduced apoptosis (Di Martino et al., 2020; Kilicdag et al., 2014), reduced brain atrophy (Winderdal et al., 2017; Di Martino et al., 2020), and increased cortical volume (Alexander et al., 2013; Potter et al., 2018). The apoptosis and brain atrophy were evaluated in animals at age P11–P24, while cortical volume at P 90–120. In human preterm infants, it has been shown that treatment with caffeine improved white matter structure on cerebral MRI at postmenstrual age 36–44 weeks (Doyle et al., 2010; Liu, 2020), but at 11 years of age, no differences were observed in brain volume and white matter structure (Kelly et al., 2018). The smaller corpus callosum was noted in the caffeine-treated infants at 11 years of age. Caffeine seems to improve the cerebral morphological structure; however, too few data are available so far, and the results’ interpretation should be cautious. Furthermore, if preterm infants treated with caffeine had a smaller corpus callosum at 11 years of age, the effects of caffeine treatment must be studied in animal models in the long term.

Each of the included studies reports some single data on caffeine effects, such as infarction volume (Sun et al., 2020); immunomodulatory effect (Winderdal et al., 2017; Di Martino et al., 2020), microglia activation (Di Martino et al., 2020), brain weights (Kilicdag et al., 2014), and brain electrical activity (Sun et al., 2020). Caffeine seems to influence these parameters, but more studies are needed to be able to interpret the results with confidence.

All included studies were affected by relevant study limitations, such as unclear information on randomization, blinding, and outcomes reporting. Moreover, none of the studies has a pre-registered protocol available online. Together with the imprecision of the estimates due to
the low sample size and wide confidence of intervals, the overall certainty of the evidence was very low, thus prohibiting drawing firm conclusions on the findings of this systematic review. The main limitation of this review is the post-hoc decision to restrict the inclusion criteria to studies on HIE, whereas the protocol registered in Prospero aimed to include all studies on caffeine effects on brain development. Moreover, characteristics of caffeine administration were affected by either heterogeneity or incomplete information regarding the dose (ranging from 5 to 20 mg/kg), formulation (either citrate or base) and timing (from P2 to P16), thus limiting the external validity of these findings.

This meta-analysis of preclinical studies indicates that caffeine treatment of rodents with hypoxic-ischemic injuries improves neuro-pathological and cognitive functional behaviors. Hypoxic-ischemic encephalopathy due to perinatal asphyxia is common and often fatal. Thus, there is an urgent, unmet public health need to develop adjuvant therapies to improve survival and neurodevelopmental outcomes in this population. Therapeutic caffeine may offer neuroprotection and reduces mortality and morbidity in infants with HIE. Further mechanistic studies in larger physiologically relevant animal models (e.g., non-human primates) are of immense value in delineating the short- and long-term effects of caffeine on cerebral white matter integrity following injury.

5. Conclusion
In sum, caffeine therapy for preterm neonates might be a promising neuroprotective candidate for mitigating the devastating effects of neonatal hypoxic-ischemic brain injury and may help decrease the burden of morbidities in this population. However, the certainty of the evidence is limited, and the translational value of the studies where caffeine is given prior to the hypoxic insult is uncertain. Clearly, further studies (preclinical in larger animal models and controlled prospective randomized perinatal trials) are warranted to delineate the most appropriate dosage and timings to capitalize on its therapeutic potential regarding hypoxic-ischemic brain injury translation to the clinical setting.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Contributions of interest
Matteo Bruschettini conceived the study, screened studies for inclusion, conducted risk of bias assessment, drafted the manuscript. Alvaro Moreira: conceived the study, screened studies for inclusion, extracted data, performed meta-analysis, reviewed the manuscript. Ana Beatriz Pizarro: screened studies for inclusion, conducted risk of bias assessment, reviewed the manuscript. Shamimunisa Mustafa: performed meta-analysis, reviewed the manuscript.

Olga Romantisk: conceived the study, screened studies for inclusion, extracted data, drafted the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2022.147990.

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