**Supplementary Material**

**Material and methods:**

*Animal protocols*

Time mated pregnant does (hybrid of New-Zealand White and Dendermonde) were obtained from the animalium of the group Biomedical Sciences at the KU-Leuven. Animals were treated according to current guidelines of animal well-being. Experiments were approved by the Ethics committee for Animal Experimentation of the Faculty of Medicine (P185/2011). Does were housed in separate cages prior to delivery, with free access to water and chow and a light-dark cycle of twelve hours. At 28 days of gestation (early saccular stage of lung development; term=31d), pups were delivered by cesarean section and housed as previously described [[1](#_ENREF_1)]. Briefly, after birth pups were dried, stimulated and placed in an incubator(Incubator 7310, Dräger®, Germany) at 32°C in 21% oxygen(normoxia) or >95% oxygen(hyperoxia). After one hour, the surviving pups were weighed and randomly assigned to placebo or caffeine treatment. They remained in the incubator for 5 days either in normoxia or hyperoxia according to the assigned group, being removed for less than 5 min/day for feeding and cleaning. Feeding was done by gavage with an orogastric tube twice per day with rabbit milk formula (40-50-75-100ml/kg bodyweight (BW) on day 0-1-2-3 to 5 respectively). The pups received intramuscular vitamin K1 on day 2(0.002mg/kg BW, Konakion pediatrique®; Roche, Switzerland) and from that point pups were given a daily intramuscular injection of benzylpenicillin(20,000I.U./kg BW day, Penicilline®; Kela, Belgium) and amikacin (20 mg/kgBW day, Amukin®; Bristol-Myers-Squibb) [[1](#_ENREF_1)].

*Dose finding groups and treatment groups*

In the first part of the experiment we tested the relationship between dose and serum levels of caffeine. We aimed for a target dose of 10-20μg/mL after the administration as described previously in humans [[2](#_ENREF_2)]. Serum caffeine concentration was measured in the dose finding groups after 5 days of intraperitoneal administration. Dose-1 group (3 pups) received within the first hour after birth a loading dose of intraperitoneal caffeine (Caffeine 25mg/2 mL; Sterop, Belgium) 10 mg/kg BW in 0.9% NaCl. From day 1 to 5 pups received a maintenance dose of 5mg/kg BW. Dose-2 group (3 pups) received a loading dose of 5 mg/kg BW followed by 2.5 mg/kg BW daily. Fetal blood samples were collected at 12 hours after the last caffeine administration by thoracotomy and cardiac puncture after anesthesia with intramuscular ketamine 35mg/kg BW (Ketamine 1000; CEVA Santé Animal, Belgium) and xylazine 6 mg/kg BW (Vexylan; CEVA Santé Animal, Belgium). The blood was centrifuged at 3000rpm for 5 min. Caffeine serum levels were measured in all survivors of the hyperoxia caffeine group to confirm the expected levels.

*Caffeine serum levels*

Caffeine levels were measured by an in-house developed and validated liquid chromatographic (LC)-tandem mass spectrometric (MS/MS) method. To 50 µL of serum, 25 µl of the internal standard working solution (25 µg/mL, IS, [2H9]-caffeine) and 925 µl of a 0.1 % formic acid solution in methanol were added and the sample was vortex mixed for 15 sec. Subsequently, the sample was centrifuged for 10 min at 7800 × g. An aliquot of the supernatant was further diluted by a factor 1/250 using a solution of 0.1 % formic acid in LC-MS grade water, followed by a filtration step using a Millex-GV PVDF syringe filter (0.22 µm, Merck, Overijse, Belgium). The filtrate was transferred to an autosampler vial and a 5-µL aliquot was injected onto the LC-MS/MS system.

The LC system consisted of an Acquity UPLC H-Class Quaternary Solvent Manager and Flow-Through-Needle Sample Manager with temperature controlled tray and column oven from Waters (Zellik, Belgium). Chromatographic separation was achieved on an Acquity UPLC HSS T3 (100 × 2.1 mm i.d., dp: 1.8 µm) column using a gradient elution with 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile as mobile phases A and B, respectively (flow-rate: 0.3 mL/min). The temperatures of the column oven and autosampler tray were set at 60°C and 8°C, respectively.

The LC column effluent was interfaced to a Xevo TQ-S® MS/MS system, equipped with an electrospray ionization (ESI) probe operating in the positive mode (all from Waters) Instrument parameters were optimised for both analytes (caffeine and IS) and the following multiple reaction monitoring (MRM) transitions were selected: caffeine: mass to charge ratio (m/z) = 194.9 > 137.8 (quantifier ion) and 109.9 (qualifier ion); IS: m/z = 204.0 > 143.9.

The method was in-house validated by evaluating the following parameters: linearity (1 - 50 µg/mL), within-run and between-run accuracy and precision (20 µg/ml), limit of quantification (LOQ, 1 µg/mL), limit of detection (LOD, 0.28 µg/mL), specificity and carry-over. The validation protocol and the acceptance criteria used were previously described [[3](#_ENREF_3)].

*Assessment of pulmonary mechanics*

Invasive lung function testing was performed using the forced oscillation technique with FlexiVent system (SCIREQ, Canada) as described in detail before [[1](#_ENREF_1)]. Briefly, pups were anesthetized with ketamine 35mg/kg BW and xylazine 6 mg/kg BW injected intramuscularly, a tracheostomy was performed for insertion of an 18-gauge metal needle connected to the FlexiVent device. The following automated perturbations were assessed at a PEEP of 3cmH2O: 1)Total lung capacity perturbation(mean displaced volume (Vend/wt)); 2)Snapshot perturbation(dynamic resistance, compliance and elastance); 3)Prime-8 forced oscillation(central airway resistance(Rn), tissue damping(G) and elastance(H)); and 4)Pressure-volume(PV) loops (total lung capacity(TLC), static compliance(Cst) and static elastance(Est)). The mean of three measurements was calculated and used for analysis.

*Lung morphometry*

After performing the lung function test, anesthetized pups were euthanized using an intramuscular injection of 0.1 mL of a mixture of embutramide 200mg, mebezonium 26mg and tetracaine 4mg/mL (T61®, Intervet, Mechelen, Belgium).The lungs were removed after thoracotomy, the left lung was removed and snap frozen for later analysis while the right lung was pressure fixed with 4% paraformaldehyde at a hydrostatic pressure of 25cmH20 for 24 hours [[4](#_ENREF_4)]. Paraffin sections were stained with hematoxylin and eosin (H&E) and Miller´s elastic stain. Lung morphometric measurements were done in 20 random fields in non-consecutive sections as described before [[4](#_ENREF_4)], including the linear intercept (index of alveolar size; Lm), the mean wall transection length (interalveolar septal thickness; Lmw) and the radial alveolar count (RAC). Histologic measurements were done at least 20 times per animal lung as described before and the mean was used for analyses[[5](#_ENREF_5)]. For vascular morphometry at least 15 peripheral arteries under 100 µm were measured per lung to obtain the internal (ID) and external (ED) diameter of the medial (muscular) layer [[6](#_ENREF_6)], using its mean for analysis. A lung injury scoring system (LIS) proposed by the American Thorax Society [[7](#_ENREF_7)] was used to compare lung tissue inflammation in both groups using 20 randomly chosen high power fields (400X); the operators being blinded to treatment allocation using a score based on the presence of five histological parameters (i.e. neutrophils in the alveolar space, neutrophils in the interstitial space, hyaline membranes, proteinaceous debris in the airspace and alveolar septal thickening).

*Immunohistochemistry*

Immunohistochemical staining was performed for proliferating cells (expressing the nuclear antigen Ki67) and were visualized by a MIB-1 staining (monoclonal anti-mouse MIB 1, Dako) as previously described [[1](#_ENREF_1)]. Quantification of positively stained cells was performed semi-automatically using ImageJ software (1.47v, NIH, Bethesda, Maryland, USA). Ten random images (400X) from each slide were processed using the Axioskop platform (Carl Zeiss, Oberkochen, Germany).

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**3 De Baere S, Goossens J, Osselaere A, Devreese M, Vandenbroucke V, De Backer P, Croubels S: Quantitative determination of t-2 toxin, ht-2 toxin, deoxynivalenol and deepoxy-deoxynivalenol in animal body fluids using lc-ms/ms detection. Journal of chromatography B, Analytical technologies in the biomedical and life sciences 2011;879:2403-2415.**

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**5 Emery JL, Mithal A: The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. Archives of disease in childhood 1960;35:544-547.**

**6 Roubliova XI, Van der Biest AM, Vaast P, Lu H, Jani JC, Lewi PJ, Verbeken EK, Tibboel D, Deprest JA: Effect of maternal administration of betamethasone on peripheral arterial development in fetal rabbit lungs. Neonatology 2008;93:64-72.**

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