Effect of whole body hypothermia on inflammation and surfactant function in asphyxiated neonates

T*o the Editor:*

Hypothermia has become an evidence-based treatment for neonates with hypoxic-ischaemic encephalopathy (HIE) [1]. The usefulness of hypothermia is due to several mechanisms and among these the reduction in inflammation seems to play a relevant role [2, 3]. When applied as whole body hypothermia (WBH), cooling may affect other organs as well. Recent data showed better respiratory outcomes and trends towards lower inflammation in WBH-treated preterm lambs [4], suggesting its possible usefulness to reduce lung injury through the modulation of the inflammatory pathway. By contrast, experiments with hibernating animals have shown that temperature induces significant adaptive changes to the surfactant composition and structure [5]. Nevertheless, no data are currently available in humans. Two case reports have recently described an infant [6] and an adult [7] with severe lung injury, whose ventilation had been facilitated by concurrent hypothermia. Since hypothermia is an accepted therapy only for HIE, we designed a preliminary translational study to investigate the effect of WBH on inflammation and surfactant status in neonates with HIE unaffected by any pulmonary injury.

Eligible babies were neonates with HIE who required WBH according to TOBY (total body hypothermia trial) criteria [8]. Control babies were normothermic neonates matched for gestational age and SNAPPE-II (Score for Neonatal Acute Physiology and Perinatal Extension-II) score, born within 2 months of the HIE cases and needing intubation for surgical procedures during the first day of life. Both cases and controls had to be free from any pulmonary disease and fulfil the following criteria: 1) normal chest radiograph and auscultation; 2) inspiratory oxygen fraction of 0.21 to achieve arterial oxygen saturation $\geqslant$95%; 3) normal amniotic fluid; 4) no signs of infection; and 5) no congenital lung disease or complex malformations.

WBH was started in HIE neonates within the first 6 h of life and targeted at 33.5°C [8] using a whole body servo-controlled mattress, with continuous oesophageal temperature monitoring (Criticool; MTRE Mennen Medical, Feasterville-Trevose, PA, USA). Following our institutional protocol, neonates were mechanically ventilated for 72 h (until the rewarming), in order to reduce their metabolic demand. Time-cycled, pressure-regulated, assisted-controlled ventilation was set with a tidal volume $\leqslant$5 mL·kg^{-1}. Blood gas analysis (α-stat) was performed from indwelling arterial lines before the onset of WBH (pre-WBH; within the first 6 h of life) and after 24, 48 and 72 h. Within 3 h from the blood sampling, nonbronchoscopic bronchoalveolar lavage (BAL) was performed as soon as neonates needed to be suctioned for clinical reasons. BAL was carried out using a standardised technique as previously described [9], in accordance with the advice of the European Respiratory Society Paediatric Task Force [10]. BAL and blood samples were centrifuged (700 × *g*, 10 min, 4°C), and serum and supernatant were separated and immediately frozen at -80°C. Control babies were sampled only once within 6 h from intubation. The institutional review board (Dept of Critical Care, University Hospital "A.Gemelli", Rome, Italy) approved the study and informed consent was obtained.

BAL and serum samples were assayed for tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8 and IL-10 using a multiple customised chemiluminescent assay [11], which had a mean coefficient of variation $\leqslant$10%. The serum/BAL urea ratio was used to correct for dilution and obtain epithelial lining fluid (ELF) concentrations [12]. Surfactant function in BAL was studied *via* fluorescence analysis of adsorption kinetics into the air/liquid interface, as described previously [13]. Results are provided as background-corrected relative fluorescent units. Experiments were performed both at the original neonate's temperature (33.5°C) and at 37°C. Fluorescence was continuously assayed and measured at 15, 45 and 90 min. All experiments were performed in triplicate, by investigators blinded to the infants' clinical data. Cytokines data were analysed with repeated measures ANOVA. Data from the surfactant study were analysed using multifactorial ANOVA, having surfactant adsorption as the response variable, and the duration of hypothermia at the sample collection (pre-WBH, 24 h, 48 h and 72 h) and the time of the experiment at the fluorescence readings (15 min, 45 min and 90 min) as the independent variables. Bonferroni *post hoc* tests were applied for paired comparisons.

Significant reductions in IL-6 and IL-8 levels in ELF during WBH were demonstrated (fig. 1a and b). Similarly, serum IL-8 was significantly decreased (mean±SE pre-WBH: 194±115 pg·mL^{-1}, 24 h: 100±73.5 pg·mL^{-1}, 48 h: 41±24.5 pg·mL^{-1}, 72 h: 42±242 pg·mL^{-1}; p=0.003). No other differences in cytokines levels were observed during WBH, or between cases and controls before the onset of WBH (data not shown). In addition, there was no change in oxygenation index during WBH (data not shown).

The surfactant function assay showed no change after 24 h of WBH, a significant increase after 48 h and a significant reduction after 72 h of hypothermia (fig. 1c). These results were similar and always significant at 15, 45 and 90 min fluorescence readings, and at both at 33.5°C and 37°C (data not shown).

WBH seems to reduce some proinflammatory mediators and have some impact on surfactant function in the ELF of neonates unaffected by any lung injury. These preliminary results are important for fostering new studies investigating more deeply the effect of hypothermia on the lung and its possible applications.

Observational data showed no change in ventilatory parameters and lung mechanics during WBH in neonates mainly without any pulmonary disease [14]. Thus, it is likely that changes in inflammatory and surfactant status might only be clinically evident in diseased lungs: in fact, we did not observe any change in oxygenation or clinical respiratory conditions during WBH. Interestingly, hypothermia modified the inflammatory status in neonatal animal models [4]. Thus, a reduction in inflammatory cytokines could partially explain the positive outcome described in recent case reports of WBH-treated severe lung injury [6, 7].

WBH may also modulate surfactant function, although a time-dependent effect seems to be important. Interestingly, the clinical cases reported to date received a short course of WBH (⩽48 h) [6, 7]. In fact, interfacial adsorption is enhanced after 48 h of hypothermia, suggesting that adaptive mechanisms could have been acting to modulate surfactant behaviour. Our findings subsequently showed impaired adsorption at 72 h, suggesting that any adaptive mechanism cannot be sustained for an unlimited time. Adaptive surfactant changes may include qualitative modifications in phospholipid composition and three-dimensional structure. Animals undergoing relatively rapid body temperature fluctuations, for example circadian rhythms in bats or very rapid temperature decays in starving dunnarts, have a surfactant that is less rich in saturated phospholipid species and can cope with a wider temperature range, since its composition is finely tuned through rapid changes in cholesterol [15]. By contrast, animals undergoing long-term seasonal changes of temperature, for example hibernating squirrels, modify their metabolism to produce a totally different surfactant [5, 15]. Our findings seem to suggest that the phospholipid surfactant composition may not change rapidly enough to adapt to lower temperature long-term. Thus, the improvement in adsorption at 48 h of WBH could possibly be due to structural reorganisation, which may include the mobilisation of certain species, such as cholesterol, between different surfactant stores. The subsequent decay may indicate that the compensatory mechanisms cannot be sustained long-term.

Moreover, the depth of hypothermia might also be important, as various changes both in surfactant status and cytokine release have been demonstrated at different temperatures in animals [5].

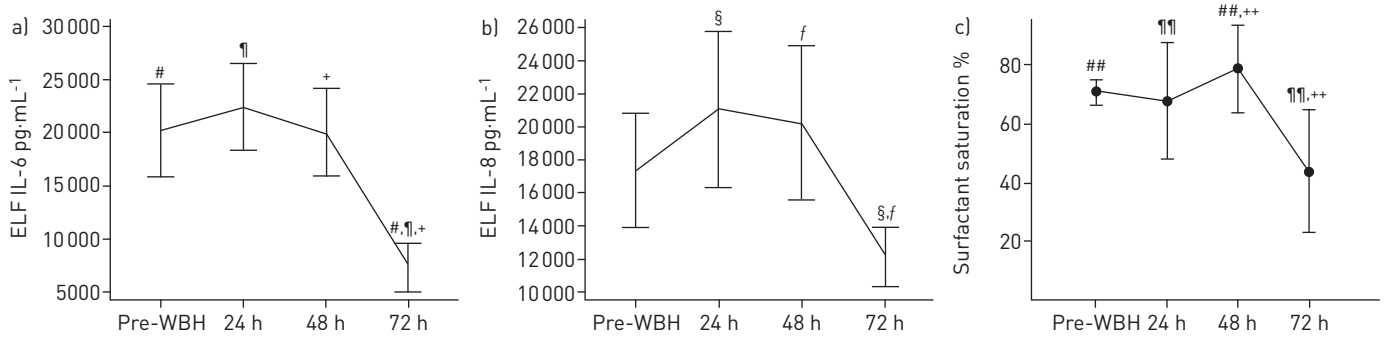

FIGURE 1 Inflammatory mediators and surfactant function in asphyxiated neonates under whole body hypothermia (WBH). Levels of a) interleukin (IL)-6 and b) IL-8 in epithelial lining fluid (ELF). c) Surfactant function presented as the percentage saturation of the interphase upon surfactant adsorption (% of material upon maximal adsorption) at the end of the experiment (90 min). Data are presented as mean±SE. Raw data were as follows. IL-6: pre-WBH 20.2±10.5 pg·mL^{-1}, 24 h 22.5±9.8 pg·mL^{-1}, 48 h 20±10 pg·mL^{-1}, 72 h 7.3±5.6 pg·mL^{-1}; overall p=0.005. IL-8: pre-WBH 17.3±8.6 pg·mL^{-1}, 24 h 21±11.5 pg·mL^{-1}, 48 h 20±11.3 pg·mL^{-1}, 72 h 12.1±4.4 pg·mL^{-1}; overall p=0.046. Surfactant adsorption: pre-WBH 0.70±0.04 RFU, 24 h 0.68±0.19 RFU, 48 h 0.79±0.15 RFU, 72 h 0.44±0.20 RFU; overall p<0.001. RFU: relative fluorescence unit. Symbols indicate significant *post-hoc* comparisons between different time points following WBH. $^{\#}$: IL-6 pre-WBH *versus* 72 h p=0.016; ¶: IL-6 at 24 h *versus* 72 h p=0.008; $^{+}$: IL-6 at 48 h *versus* 72 h p=0.01; §: IL-8 at 24 h *versus* 72 h p=0.03; ^{f}: 48 h *versus* 72 h p=0.04; $^{\#\#}$: surfactant adsorption pre-WBH *versus* 48 h p=0.005; ¶¶: surfactant adsorption at 24 h *versus* 72 h p=0.019; $^{++}$: surfactant adsorption at 48 h *versus* 72 h p<0.001.

Our study is based on a small population and control babies were sampled only once for ethical reasons. However, we must keep in mind that, if ventilation would have had an effect in an extended normothermic control group it would have been towards raising inflammation. These are the first human data on this subject and the next research steps would be clarifying modifications in surfactant composition/structure and investigating the effect of WBH in neonates who are also affected by severe lung injury. This could eventually lead to hypothesises for possible uses of WBH for critical respiratory conditions.

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