## The effects of temperature on the biophysical properties of warm- and cold-adapted pulmonary surfactant

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Pulmonary surfactant (PS), comprising lipids and proteins, lines the entire alveolar surface and regulates interfacial surface tension of the lung. Small changes in temperature have dramatic effects on surfactant composition, structure and function (Langman *et al.*, 1996; Codd *et al.*, 2002; Lang *et al.*, 2005). A dipalmitoyl phosphatidylcholine (DPPC) or PC 16:0/16:0 enriched monolayer is thought to be present at the alveolar air-water interface where it is responsible for lowering surface tension at low lung volumes during the breathing cycle. However, heterothermic mammals, that experience low and variable body temperatures, have an unusual surfactant composition with large amounts of fluid lipids including unsaturated phospholipids and neutral lipids like cholesterol (Lang *et al.*, 2005). It is unclear how such a surfactant is able to attain low surface tension at low temperatures. We characterized the thermodynamic, structural and functional properties of surfactant of heterothermic mammals, 13-lined ground squirrels (*Ictidomys tridecemlineatis*) and fat-tailed dunnarts (*Sminthopsis crassicaudata*), and compared these with porcine surfactant. Fluorescence spectroscopy by LAURDAN (6-dodecanoyl-2-dimethyl-aminonaphtalene), and differential scanning calorimetry were conducted to understand the phase behaviour and lipid packing. Microscopic studies were conducted with epifluorescence microscopy to visualise phase coexistence in surfactant films. Functional studies were conducted for squirrel PS with captive bubble surfactometry (CBS) at different temperatures. We also mixed simple artificial lipid mixtures, broadly based on the natural PS composition of heterothermic or homeothermic mammals and conducted the above mentioned biophysical studies at different temperatures along with compression driven pressure-area isotherms. Surfactant membranes of torpid animals possessed lower enthalpy compared to warm-active animals indicating a more fluid surfactant. Fluorescence spectroscopy studies showed that surfactant from torpid animals possessed a dehydrated, solid-like ordered phase similar to that of warm-active animals at low temperatures. Epifluorescence microscopic studies revealed structural differences in morphology and distribution of compression-driven segregated lipid domains between surfactant films of torpid and warm-active animals. CBS studies showed that PS functions at low and high temperatures (3, 25 and 37°C) in heterothermic mammals, whereas porcine surfactant showed poorer function at 3°C. Studies with artificial lipid mixtures confirmed the above results and also indicated that PPPC forms tighter phases with cholesterol at low temperatures. Our results suggest that in torpid animals, surfactant composition is altered as an adaptation to reduced body temperatures but function is retained by structural re-arrangements of the surfactant film. The higher proportion of the unsaturated phospholipid palmitoylpalmitoleoyl PC (PPPC) or PC16:0/16:1 may confer a greater thermal flexibility on heterothermic relative to homeothermic mammals, enabling PS to pack tightly at low body temperatures. The combination of PPPC plus cholesterol possibly complements DPPC phases in forming stable surfactant films during the dynamic breathing process. This may be the most critical feature to sustain stability of the respiratory interface at low lung volumes. Understanding surfactant structure-function relations under physiological extremes may aid in developing surfactant therapy for respiratory distress associated with hypothermic surgical procedures or fever.

Codd JR, Schurch S, Daniels CB & Orgeig S. (2002). *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* **1580**, 57-66.

Lang CJ, Postle AD, Orgeig S, Possmayer F, Bernhard W, Panda AK, Jurgens KD, Milsom WK, Nag K & Daniels CB. (2005). *American Journal of Physiology* **289**, R1426-R1439.

Langman C, Orgeig S & Daniels CB. (1996). *American Journal of Physiology* **271**, R437-R445.

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Animal Experiments: Lung lavage samples were collected after killing the animals with a lethal dose of pentobarbitone (Lethabarb, i.p. injection). All experiments were performed under animal ethics permits (133/07 and 147/08), approved by The Institute of Medical and Veterinary Science South Australia Animal Ethics Committee and approved by the University of Western Ontario animal use subcommittee and were in accordance with the Canadian Council of Animal Care's guidelines.