## Pulmonary surfactant membranes of hibernating ground squirrels possess increased fluidity but are capable of maintaining an ordered membrane structure at low temperatures

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Pulmonary surfactant, a mixture of lipids and proteins, regulates the surface tension at the air-liquid interface of the lung. Reduced body temperature during hibernation is accompanied in 13-lined ground squirrels (Ictidomys tridecemlineatus) by an increase in fluid monounsaturated phosphatidylcholine (PC) species (e.g. PC 16:0/16:1, PC 16:0/18:1) and phosphatidylglycerol (PG) species (e.g. PG 16:0/18:1, PG 18:0/18:2), but fewer disaturated PC and PG species (Possmayer et al., 2010). Previously we speculated that altered surfactant lipid composition during metabolic depression states such as torpor or hibernation will reduce the phase transition temperature (T<sub>m</sub>) of the mixture, enabling pulmonary surfactant to remain fluid over a broader range of temperatures and thereby maintaining respiratory function (Lang et al., 2005). Here we analyze thermodynamic properties and behavior of surfactant from hibernating and summer-active 13-lined ground squirrels in relation to natural porcine surfactant, using differential scanning calorimetry and LAURDAN fluorescence spectroscopy. In addition we conducted epifluorescence studies to visualize changes in phase coexistence of surfactant films of hibernating and summer-active animals. Surfactant membranes of hibernators showed gel-to-fluid transitions at lower T<sub>m</sub> with reduced enthalpy relative to membranes from summer-active squirrels. Both exhibited lower enthalpy than porcine surfactant. LAURDAN fluorescence and epifluoresence suggested possible structural rearrangements of surfactant membrane lipids and films, respectively, in hibernators. These exhibited a similarly dehydrated and condensed highly packed ordered phase as for summer active squirrels, despite differences in composition and T<sub>m</sub>. In conclusion, pulmonary surfactant composition changes in hibernating squirrels to increase overall fluidity, but to maintain an ordered membrane structure at low temperature.

- Possmayer F, McCaig L, Yao L, Zhao L, Staples J, Orgeig S, Veldhuizen RA. (2010). Coping with the cold: effect of hibernation on pulmonary surfactant in the thirteen-lined ground squirrel. *Biophysical Journal* **98**, 76a.
- Lang CJ, Postle AD, Orgeig S, Possmayer F, Bernhard W, Panda AK, Jürgens KD, Milsom WK, Nag K & Daniels CB. (2005). Dipalmitoylphosphatidylcholine is not the major surfactant phospholipid species in all mammals. *American Journal of Physiology. Regulatory, Integrative and Comparitive Physiology* **289**, R1426-R1439.