MRI and ^{31}P NMR studies of brain metabolism in European ground squirrels during hibernation and arousal

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Abstract
The cellular background of the drastic drop in metabolic rate and body temperature observed in mammalian hibernation is poorly understood *in vivo*. We applied flow-, T_2*-weighted MRI and ^{31}P-MRS to hibernating European ground squirrels in torpor and during arousal to euthermy. Blood flow was low in arteries supplying the torpid brain, however, T_2*-weighted images revealed high oxygenation. ATP levels increased during arousal to euthermy, and were accompanied by a decrease of PCr and intracellular acidosis. The results indicate that brain metabolism during torpor is reduced without limitations in oxygen supply, despite low blood flow.

Introduction
Hibernation is a fascinating behaviour, especially in endothermic animals. Hibernating endotherms compromise their normal euthermic cellular physiology by tolerating extremely low body temperatures in natural hypothermia (torpor). Not much is known about the cellular changes in energy supply in torpor and during arousal. In this study, we investigated effects of torpor on blood flow and tissue oxygenation, and cellular energy metabolism in torpor and during arousal in the brain of non-restrained European ground squirrels, using *in vivo* MR imaging and spectroscopy.

Material and methods
Hibernating European ground squirrels (*Spermophilus citellus*, n= 4) were used. Animals were housed at 0°C in their home cage with a nest box to allow normal hibernation. For NMR recording, torpid animals were placed in a perspex container with bedding material (dried hemp) in a perspex cooling chamber, and inserted into the 40 cm diameter magnet bore of a Bruker Biospec MR scanner (200 MHz, 4.7T). The chamber was aerated using outside air. Chamber temperature was monitored continuously using a fiber-optic sensor. Flow weighted MRI using a flow compensated gradient echo sequence (parameters: matrix size 128x128, Field of view (FOV) 4 cm, 5 slices of 2 mm thickness, distance 2.5 mm, repetition time TR= 101.5 ms, echo time TE= 9.8 ms, excitation pulse: 80° sinc3 of 2000 µs length, 2 averages, acquisition time of 51s) and T_2* weighted MR imaging (gradient echo sequence, parameters: matrix size 64x64, FOV= 4 cm, 1 slice of 2 mm thickness, TR= 100 ms, TE= 30 ms, 22.5 ° sinc3 of 2000 µs length, 2 averages, acquisition time of 25s) were conducted on two torpid animals , and during subsequent arousal initiated artificially by external warming. After NMR recording , animals were returned to their home cage and readily returned to torpor. For *in vivo* ^{31}P-NMR spectroscopy, animals were placed in a perspex container with bedding onto a 5 cm surface coil in their normal (head down) torpor position with the head located on top of the coil. Scout images were collected to check the position of the animal and sets of *in vivo* ^{31}P-NMR spectra were acquired (parameters: resonance frequency 81 MHz, acquisition size 4k, sweep width= 4000 Hz, 100 µs bp32 pulse, TR= 0.8 s, ns= 300, acquisition time of 4 min). After subsequent determination of the rectal body temperature with a thermometer , animals were aroused by warming and handling. The experiments were conducted three times during the early stage of arousal, at average body temperatures of 8, 11, and 16°C (n=3). Spectra were calibrated relative to phosphocreatine (PCr), all signal integrals were specified relative to α-ATP/α-ADP.

Results and Discussion
Scanner noise did not appear to affect the hibernating animals, since no movement artifacts were observed in MR images. Several sets of transversal multi-slice flow weighted MR images were acquired through the brain of the animals, combined with coronal T_2*-weighted MR images. In the flow weighted images blood-supplying arteries could be identified. Their intensity differed only slightly from the surrounding tissue, indicating very low blood flow through the arteries perfusing the brain. At higher body temperature, blood flow increased as indicated by higher signal intensities. T_2*-weighted image contrast in the coronal brain slices showed negligible differences in signal intensities between MR images collected during hibernation and after subsequent arousal. This indicates that good tissue oxygenation of the brain is maintained even during deep hibernation.
In vivo ^{31}P-NMR spectra during early arousal at 8°C showed a smaller β-ATP signal in comparison to the other two ATP signals indicating a relatively small amount of free ATP in torpor. Free ATP may be down regulated during hibernation, because of the reduced energy turnover. The β-ATP signal increased with body temperature, accompanied by an initial decrease in phosphocreatine (PCr), a decrease of the sugarphosphate/AMP signal as well as a slight increase of the inorganic phosphate signal (Pi)(Figure 1). Intracellular pH decreased during arousal (ΔpH= 0.25). The increase in energy requirements together with the decrease in pH_i would explain the drop in phosphagen levels. The increased level of free ATP may partly arise from the phosphorylation of AMP, indicated by the decrease of the SP/AMP signal.

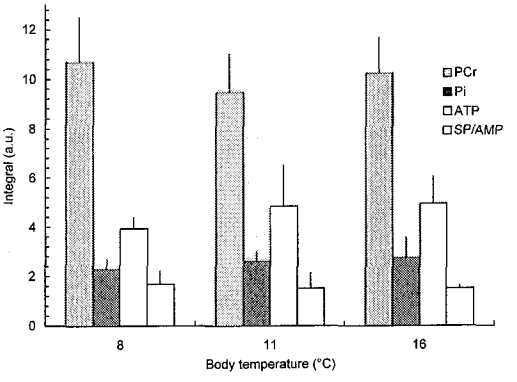

Figure 1: Changes in ^{31}P-NMR integrals of various phosphorus metabolite signals detected in the arousing European ground squirrel at different body temperatures. The increase in free ATP levels during arousal correlates with a drop in AMP and PCr concentrations.

Conclusions
These first analyses of hibernating animals inside an MRI scanner have shown that, despite MRI scanner noise, *in vivo* MRI experiments can be conducted during hibernation as well as during arousal under well-controlled, non-retrained, non-invasive physiological conditions. Low blood flow and reduced ATP levels are in line with a reduction of energy requirements in torpor. During arousal, increased energy demand is reflected by a degradation of PCr which goes hand in hand with the restoration of ATP.