# NITROGEN CYCLING IN HIBERNATING BEARS

R.R.Wolfe, R.A.Nelson, M.H.Wolfe, L.Rogers
Shriners Burns Institute, Mass. General Hospital, Boston, MA 02114

Hibernating bears take in virtually no food or water for up to seven months, but maintain a body temperature or 97°, and excrete virtually no nitrogenous wastes. During hibernation there is no loss of lean body mass and urination is not required for several months. It is therefore hypothesized that these bears have a unique ability to re-utilize urea nitrogen. To probe this hypothesis, a tracer amount of di-$^{15}N$ urea was injected into two black bears hibernating in the wilderness area of northern Minnesota, and blood samples were drawn in the first hours and in the following two weeks as snow conditions permitted. The hibernating state of the bears was not disturbed.

Plasma urea enrichment was measured by GC-MS with SIM at M+2 to measure the rate of new urea production. The plasma urea was also measured at M+1 to estimate the re-cycling of nitrogen back into urea. Nitrogen enrichment in plasma albumin and fibrinogen was measured by isotope ratio mass spectrometry after reduction of these proteins to $N_2$ via ammonia. Free ammonia enrichment was measured by atomic emission. And finally, nitrogen enrichment was measured in 23 plasma amino acids by SIM-GC-MS.

Significant $^{15}N$ enrichment was detected in several amino acids including ornithine, arginine, glycine, tyrosine, phenylalanine, and threonine. Re-incorporation of amino acids into plasma protein was confirmed by significant levels of 15N in albumin and fibrinogen. Urea production was found to occur continuously since the urea M+2 level declined with time. Free ammonia 15N enrichment was also found to increase. Total body water was measured with a $^3H_2O$ tracer and lean body mass estimated. Total urea concentration was measured by enzymatic assay. The urea production rate was found to be 1/10th that of a normal bear in summer, awake state.

The combination of GC-MS and IR-MS has led to the elucidation of urea nitrogen re-utilization in a unique mammal, the hibernating black bear. What are normally considered irreversible pathways in the urea cycle appear to be reversible in this hibernating state, and certain keto-acids are transaminated to form what are normally essential amino acids. Artificial stimulation of these pathways would reduce the need for dialysis in anuric patients.

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Annotation: Hibernating black bears survive for 7 months or more without eating, drinking, or urinating and experience no loss of lean body mass. Tests of 2 wild hibernating bears showed that they produced urea at 1/10th the awake rate, broke the urea down, and re-utilized the nitrogen from it to synthesize amino acids, including what normally are essential amino acids.