Solving the metabolic adenylate kinase paradox with *in vivo* NMR spectroscopy

[1,2]**Rohwer J.M.**, [3]Atauri P., [2]Mulquiney P.J., [2]Bubb W.A., [2]Coen M. and [2]Kuchel P.W.

[1]Triple-J Group for Molecular Cell Physiology, Department of Biochemistry, Stellenbosch University, South Africa
[2]School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia
[3]Department of Biochemistry, University of Barcelona, Barcelona, Spain

The aim of this work was to resolve an apparent paradox in the equilibration of the adenine nucleotides ATP, ADP and AMP via adenylate kinase in actively metabolising but glucose-starved human erythrocytes (RBCs). Nuclear magnetic resonance (NMR) spectroscopy was used to follow online the *in vivo* concentrations of the most important intracellular metabolites involved in RBC energetics. Starvation of RBCs led to rapid depletion of intracellular ATP levels, whose recovery after prolonged starvation required co-addition of glucose and either adenosine or inosine to the cells (the latter compounds leading to ATP production without prior ATP investment in the "sparking" reactions of glycolysis). Notwithstanding this, the ^{31}P NMR spectra revealed an apparent failure of the redistribution of the adenine nucleotides via adenylate kinase. We provide definitive evidence using modern 2-dimensional heteronuclear NMR spectroscopy that the previously assumed AMP is largely IMP that has the same chemical shift, implying that the ^{31}P NMR resonance previously assigned to AMP was in fact a different compound. Hence there is no failure of the previously described metabolic-reaction pathways, including adenylate kinase, in the glucose-starved RBC. The significance of the work lies in mapping out the metabolic processes and their rates in purine nucleotide turnover as they occur in the intact RBC.