Dynamic response of intracellular reaction cascades to changing environments is a hallmark of living systems. As metabolism is complex, mechanistic models have gained popularity for describing the dynamic response of cellular metabolism and for identifying target genes for engineering. At the same time, the detailed tracking of transient metabolism in living cells on the sub-minute timescale has become amenable using dynamic nuclear polarization enhanced 13C NMR. Here, we suggest an approach combining in-cell NMR spectroscopy with perturbation experiments and modeling to obtain evidence that the bottlenecks of yeast glycolysis depend on intracellular redox state. In pre-steady state glycolysis, pathway bottlenecks shift from downstream to upstream reactions within a few seconds, consistent with a rapid decline in the NAD+/NADH ratio. Simulations using mechanistic models reproduce the experimentally observed response and help identify unforeseen biochemical events. Remaining inaccuracies in the computational models can be identified experimentally. The combined use of rapid injection NMR spectroscopy and in silico simulations provides a promising method for characterizing cellular reactions with increasing mechanistic detail.