Incomplete knowledge of the longitudinal relaxation time constant (T1) leads to incorrect assumptions in quantitative kinetic models of cellular systems, studied by hyper-polarized real-time NMR. Using an assay that measures the intracellular signal of small carboxylic acids in living cells, the intracellular T1 of the carboxylic acid moiety of acetate, keto-isocaproate, pyruvate, and butyrate was determined. The intracellular T1 is shown to be up to four-fold shorter than the extracellular T1. Such a large difference in T1 values between the inside and the outside of the cell has significant influence on the quantification of intracellular metabolic activity. It is expected that the significantly shorter T1 value of the carboxylic moieties inside cells is a result of macro-molecular crowding. An artificial cytosol has been prepared and applied to predict the T1 of other carboxylic acids. We demonstrate the value of this prediction tool.