**Introduction:** The high cell densities required for adequate signal to noise ratios in reasonable time intervals pose unique and difficult design and optimization problems for NMR studies with perfused isolated cell systems. Oxygenation is a major issue especially when investigations of oxidative metabolism are desired. The vast majority of NMR studies of cells have focused on 31P-NMR measurements. Despite their value 13C-NMR studies of perfused cells are scarce and have traditionally been performed with highly glycolytic tumor cells, where [3-13C]-lac was the major product of [1-13C]-glut metabolism. Here, we describe an NMR compatible bioreactor and a support perfusion system, which was designed to allow the simultaneous acquisition of high resolution 31P and 13C-NMR spectra from intact, well-oxygenated, alginate-entrapped cells. A specific and rather stringent design requirement was that the achievable 13C-NMR spectral resolution should be sufficient to allow for the observation of the splitting of the [4-13C]-glut resonance. Based on methodology described by Malloy and co-workers for cellular extracts and tissues (1-2), and with the use of appropriate 13C-labeled nutrients, this would allow for the first time the non invasive monitoring of glucose and fatty acid oxidation from intact, perfused cells. The insulin secreting RINm5F cell line was chosen as a model for these studies due to the ascribed importance of the relative rates of glucose and fatty acid oxidation in β-cell dysfunction and Diabetes.

**Materials and Methods:** All NMR measurements were performed on a Bruker DMX-400 Wide-Bore spectrometer. RINm5F monolayers were obtained from ATCC. Cells were entrapped in alginate/Poly-L-lysine (AP) beads and perfused as detailed elsewhere (3). The initial cell density in beads was 1.5x10^8 cells/mL alginate. Beads were loaded into a modified 10-mm NMR glass tube and perfused with serum supplemented (10% V/V) DMEM at 12mL/min. DMEM contained 14mM [1-13C]-glut and was saturated with humidified gas (95%O2/5%CO2).

**Figure #1:** Schematic of the perfusion system used for the 31P- and 13C-NMR measurements. Key components included a combined heat and gas exchanger (1), a bubble trap (2), flow-through cells (3), (5) for temperature and oxygen concentration measurements at the inlet and outlet of the bioreactor (4), and a personal computer for continuous data acquisition.

**Results:** The 31P-NMR spectra from entrapped RINm5F cells in 3-mm beads were of good quality (Fig 2A) with strong signals originating from NTP (primarily attributed to ATP). The 13C NMR spectra from the same cells, however, demonstrated very little 13C-label incorporation into TCA cycle intermediates (Fig 2B). Decreasing the bead size to 1-mm and maintaining all other parameters constant resulted in well-oxygenated cells. This was demonstrated by the appreciable incorporation of label into TCA cycle intermediates (Fig 3A-D), the higher [4-13C]-glut/[3-13C]-lac ratio which was similar to that measured in normoxic monolayers, and the low (1.2) molar ratio of lactate production to glucose consumption (Yl/lG). The incorporation of Acetyl CoA from [1-13C]-glut and [1,2,3,4-13C]-octanoate into 13C-lactate was monitored from the intensities of signals arising from [4,13C]-glut and [3,4,5-13C]-glut (from glucose), and [4,5-13C]-glut (from octanoate). The ratio of the intensities of the [4-13C]-glut/[5-13C]-glut provided a measure of the relative rates of glucose and fatty acid oxidation (Fig. 3D). Significant increases of this ratio were reproducibly observed upon addition of 1mM Dichloroacetate, a known stimulator of pyruvate dehydrogenase (PDH), establishing the feasibility of the performance of such measurements with our system.

**Conclusions:** 1) Equilibration of the perfusion medium with a gas phase of 95%O2/5%CO2 does not guarantee good oxygenation of cells within beads at high cell densities. 2) 13C-NMR is an excellent indicator of RINm5F oxygenation status. 3) The presence and stability of high levels of ATP may not always be a good measure of oxygenation status. 4) The spectral resolution achievable in intact perfused cells is of sufficient quality to allow simultaneous non-invasive studies of glucose and fatty acid oxidation.