Description of the TBR System

The computer utilizes feedback from the pH probe to adjust the gas exchanger CO_2 partial pressure (pCO_2) while maintaining a constant O_2 partial pressure (pO_2). Temperature control is maintained with a circulating water bath (Fisher-Scientific, St. Louis, MO). The cell chamber, degassing vessel, feed and waste bottles, inline septa, and flow line water jacket fittings are custom blown glassware that were designed by the authors and produced by MidRivers Glassblowing, St. Charles, MO.

Input Methods

The TBR accepts bolus, step-pulse, and constant composition as three general modes of input to the system. A bolus of tracer, drug, metabolic substrate, etc. is injected through the inline septum located upstream of the gas exchanger and mixing bottle. A pulse-step input function is achieved using a syringe connected to a 3-way stopcock upstream of the input pump. A constant composition input function is achieved by adding (or removing) the compound or metabolic substrate to the media feed at the desired concentration.

Collection of Media through Fraction Collector

The effluent is collected continuously as "mixed cup" samples in the fraction collector, which sends a signal to the computer for time stamping with each tube change. Under normal operation the recycle perfusion flow rate (50 ml/min) is about 100 times greater than the feed input flow rate (0.5 ml/min) so that the effluent concentration profile is identical to that of a perfectly mixed tank, and the average residence time is equal to the reactor volume/input flow rate. Therefore, metabolic or production rates, or tracer concentration curves based on effluent sample analyses can be readily calculated using the constant stirred tank reactor model (CSTR) (*1*).

Preparation of Media

The media powder and 3.9g of sodium bicarbonate were dissolved in 900 mL deionized water and filter sterilized. To this media were added the following supplements to reach the final concentration (prepared media): 10% fetal bovine serum (FBS) (Atlanta Biological, Lawrenceville, GA), 2 mM L-glutamine and 1mM sodium pyruvate (Cellgro, Manassas, VA), 100 IU/mL penicillin and 10 μg/mL streptomycin (Invitrogen, Carlsbad, CA), and 2 ng/mL insulin (Sigma-Aldrich, St. Louis, MO). As needed, the prepared media was further supplemented with combinations of glucose,1.39 or 5.55 mM, 100 μmol/liter of chemically defined lipid mixture (Gibco-Life Sciences, Carlsbad, CA), and 1μg/mL pioglitazone from a 10 mg/mL stock in Dimethyl sulfoxide (Sigma-Aldrich).

Glucose Stock Solution

The concentrated glucose stock (382 mg/mL) used to prepare the feed media and bolus injections was prepared by dissolving a measured amount of glucose (20.1 gm) in a measured amount of water (40 mL)), and measuring the final volume after mixing (52.6 mL). The concentration was confirmed by measuring dilutions of the stock by the method below.

Estimation of Metabolic Rates

The global consumption and production rates (glucose, lactate, free fatty acid) were calculated using the discrete form of the continuity equation for a perfectly mixed stirred tank reactor (*1*) (Equation 1) and the fitted input and effluent concentration profiles derived from assays.

$$\text{rate} = (C_{\text{feed}} - C_{\text{eff}})\frac{q_{\text{feed}}}{V_{\text{BR}}} - \frac{dC_{\text{eff}}}{dt} \quad (1)$$

where C_{feed} =[substrate] in feed, C_{eff} =[substrate] in effluent, q_{feed} =feed flow rate, and V_{BR} =total fluid volume of bioreactor

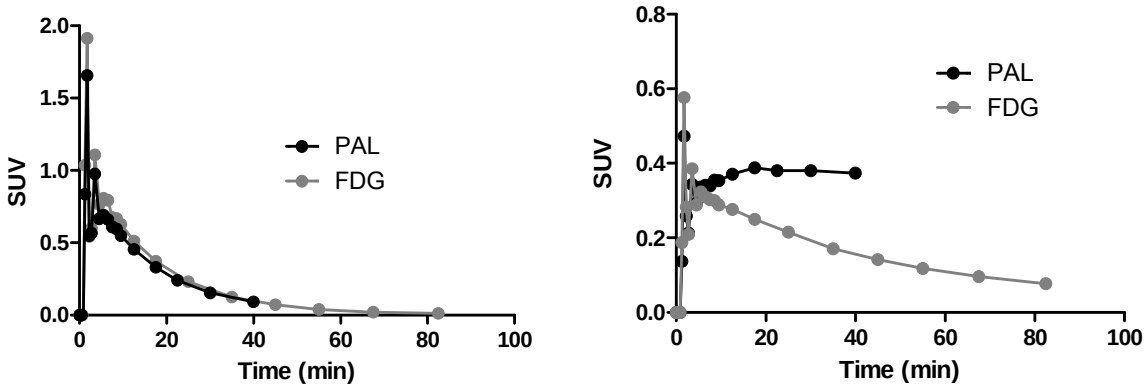
The oxygen consumption rates were calculated using the discrete form of the continuity equation for a plug flow reactor (Equation 2) (*1*) and the DO curves for the cell chamber inlet and outlet.

$$\text{rate}_{O_2} = (DO_{in} - DO_{out})\frac{q_{rcy}}{V_{bed}}C_{sat} \quad (2)$$

where DO_{in} = DO at chamber inlet, DO_{out} = DO at chamber outlet. q_{rcy} =media recycle flow rate, V_{bed} ,=(bead volume)(voidage), and C_{sat} = 1 atm/Henry's law constant at 37°C.

Comparison of FDG and [^{11}C]Palmitate kinetics in TBR

To study differences in the kinetics of radiopharmaceuticals, FDG (1 mCi) and [^{11}C]Palmitate (1 mCi) were administered in two separate setups of the system. The kinetics of FDG and [11C]Palmitate are depicted in Supplemental Figure 1 in both media and the TBR chamber. The data reflects rapid uptake of FDG followed by clearance of FDG in comparison to [^{11}C]Palmitate which is retained in tissue. Clearance of FDG may be attributed to the presence of glucose-6-phosphotase in hepatocytes but absent in most peripheral tissues (*2*).



Supplemental Figure 1: The kinetics of FDG and [^{11}C]Palmitate in the (A) media and the (B) chamber (whole chamber ROI).

References

1. Levenspiel O. *Chemical reaction engineering*. 3 ed. Hoboken, NJ: John Wiley & Sons; 1999:94-107.

2. Southworth R, Parry CR, Parkes HG, Medina RA, Garlick PB. Tissue-specific differences in 2-fluoro-2-deoxyglucose metabolism beyond fdg-6-p: A 19f nmr spectroscopy study in the rat. *NMR in Biomedicine.* 2003;16:494-502.