SUPPLEMENTAL APPENDIX A

ART/CS Measurement of Myocardial 1-^{11}C-Glucose Metabolism

Abbreviations:

EF = extraction fraction; PF = production fraction; OF = oxidation fraction; GCF = glycolysis fraction; GNF = glycogen fraction; [GLU] and [^{11}C-GLU] = unlabeled and 1-^{11}C-labeled glucose concentrations in μmol/mL and counts/min/mL, respectively; [LA] and [^{11}C-LA] = unlabeled and ^{11}C-labeled lactate concentrations in μmol/mL and counts/min/mL, respectively; ART = arterial; CS = coronary sinus.

Key components of 1-^{11}C-glucose metabolism including glucose extraction, glycolysis (lactate production + oxidation), oxidation, lactate extraction/production, lactate oxidation, and glycogen synthesis were measured from 1-^{11}C-glucose, ^{11}C-lactate, and $^{11}CO_2$ arterial and coronary sinus activity (counts/min/mL). Using the Fick method, all metabolic quantities were calculated as fractional measurements.

Measurements of glucose metabolism after taking into account myocardial ^{11}C-lactate extraction and oxidation were performed based on the following assumptions: (a) $^{11}CO_2$ was produced from 1-^{11}C-glucose and/or ^{11}C-lactate only; (b) If net myocardial production of ^{11}C-lactate was observed during a given study, it was assumed that there was no net myocardial extraction of ^{11}C-lactate and, therefore, no net $^{11}CO_2$ production from ^{11}C-lactate; (c) To calculate $^{11}CO_2$ produced from extracted ^{11}C-lactate, it was assumed that 75% of extracted lactate was oxidized (*20,21,27*). The necessary equations therefore become:

1) Extracted 1-^{11}C-glucose:

$$\mathbf{EF(^{11}C\text{-}GLU)} = \{[^{11}\text{C-GLU}]_{ART} - [^{11}\text{C-GLU}]_{CS}\}/[^{11}\text{C-GLU}]_{ART} + \text{EF}[^{11}\text{C-LA}]_{GLU.} \quad (1A)$$

2) Extracted ^{11}C-lactate that is produced from 1-^{11}C-glucose:

$$\mathbf{EF(^{11}C\text{-}LA)_{GLU}} = \{[^{11}\text{C-LA}]_{ART} - [^{11}\text{C-LA}]_{CS}\}/[^{11}\text{C-GLU}]_{ART.} \quad (2A)$$

3) Total oxidation measured as myocardial $^{11}CO_2$ produced from 1-^{11}C-glucose and ^{11}C-lactate:

$$\mathbf{OF(^{11}C\text{-}GLU + {}^{11}C\text{-}LA)} = \{[^{11}CO_2]_{CS} - [^{11}CO_2]_{ART}\}/[^{11}\text{C-GLU} + {}^{11}\text{C- LA}]_{ART.} \quad (3A)$$

4) Lactate oxidation calculated as myocardial production of $^{11}CO_2$ from ^{11}C-lactate:

$$\mathbf{OF(^{11}C\text{-}LA)} = \{\text{EF(LA)} \bullet [^{11}\text{C-LA}]_{ART} \bullet 0.75\}/[^{11}\text{C-LA} + {}^{11}\text{C-GLU}]_{ART,} \quad (4A)$$

where EF(LA) is the fractional myocardial extraction of unlabeled lactate.

5) Glucose oxidation calculated as the difference between total and lactate oxidation:

$$\mathbf{OF(^{11}C\text{-}GLU)} = \{\text{OF}(^{11}\text{C-GLU} + {}^{11}\text{C-LA}) - \text{OF}(^{11}\text{C-LA})\} \bullet [^{11}\text{C-GLU} + {}^{11}\text{C-LA}]_{ART}/[^{11}\text{C-GLU}]_{ART.} \quad (5A)$$

If $\mathbf{OF(^{11}C\text{-}GLU)} < 0$, then $\mathbf{OF(^{11}C\text{-}GLU)} = 0$.

6) Fraction of 1-^{11}C-glucose that is converted to ^{11}C-LA production:

$$\mathbf{PF(^{11}C\text{-}LA)^*_{GLU}} = \{[^{11}\text{C-LA}]_{CS} - [^{11}\text{C-LA}]_{ART}\}/[^{11}\text{C-GLU}]_{ART.}$$

*Net production of ^{11}C-LA was observed in only 2 of 22 animals. (6A)

7) Fraction of extracted 1-^{11}C-glucose that goes through glycolysis:

$$\mathbf{GCF(^{11}C\text{-}GLU)^*} = \text{OF}(^{11}\text{C-GLU}) + \text{PF}(^{11}\text{C-LA}). \quad (7A)$$

*In 20 of 22 studies GCF(^{11}C-GLU) = OF(^{11}C-GLU).

8) Fraction of extracted 1-^{11}C-glucose that does not go through glycolysis and is assumed to be glycogen:

$$\mathbf{GNF(^{11}C\text{-}GLU)} = \text{EF}(^{11}\text{C-GLU}) - \text{GCF}(^{11}\text{C-GLU}). \quad (8A)$$

SUPPLEMENTAL APPENDIX B

PET Measurements of Myocardial 1-^{11}C-Glucose Metabolism

Key components of myocardial glucose metabolism—including rates of glucose uptake, glycolysis, oxidation, lactate production, and glycogen formation—were estimated from the myocardial kinetics of 1-^{11}C-glucose obtained with PET and compartmental modeling based on the assumptions used to derive our model to measure overall glucose uptake (*11*) and the assumptions described in the Materials and Methods. The differential equations describing the 2-step modeling approach (Fig. 1) and the calculations of key rates of glucose metabolism are described below.

The transport of tracer from one compartment to another can be described by the following differential equations:

$$dq_{1B}/dt = K_{1A} \cdot C_{glu}(t) - (k_{2A} + k_{3A}) \cdot q_{1B}(t), \quad (1B)$$

$$dq_{2B}/dt = k_{3A} \cdot q_{1B}(t) - (k_{1B} + k_{3B} + k_{4B}) \cdot q_{2B} + (k_{2B}) \cdot q_{3B}(t), \quad (2B)$$

$$dq_{3B}/dt = k_{1B} \cdot q_{2B} - k_{2B} \cdot q_{3B}, \quad (3B)$$

$$dq_{4B}/dt = K_{5B} \cdot C_{la}(t) - (k_{6B} + k_{7B}) \cdot q_{4B}, \quad (4B)$$

$$dq_{5B}/dt = k_{7B} \cdot q_{4B} - (k_{4B} + k_{7B}) \cdot q_{5B}, \quad (5B)$$

where $C_{glu}(t)$ and $C_{la}(t)$ are 1-^{11}C-glucose and ^{11}C-lactate blood concentration (cpm) over time (input functions), K_{1A} (mL/g/min), k_{2A} (min^{-1}), and k_{3A} (min^{-1}) are the rate constants defining 1-^{11}C-glucose uptake and obtained from step 1 (Fig. 1A). k_{1B}–k_{4B} represent the rate constants associated with 1-^{11}C-glucose metabolism, K_{5B}–k_{7B} represent rate constants associated with ^{11}C-lactate metabolism, and $q_{nB}(t)$ is the concentration of tracer (counts/g) in compartment n.

Total tracer concentration in myocardium as a function of time was defined as the sum of tracer concentration in each compartment:

$$q_{TB}(t) = \sum q_{iB}(t),\ 1 \le i \le 5, \quad (6B)$$

and correction for partial-volume and spillover effects were accounted for within the model equation:

$$q_{TB(PET)}(t) = Fmm \bullet q_{TB}(t) + Fbm \bullet C_{blood(PET)}(t), \quad (7B)$$

where $q_{TB(PET)}(t)$ and $C_{blood(PET)}(t)$ are myocardial and blood PET ^{11}C activity (cpm).

After fixing Fmm to values obtained from the ^{15}O-water perfusion model (*11*,*12*), Equation 7B was then used to estimate model parameters k_{1B}–k_{4B}, k_{6B}, k_{7B} (min^{-1}), K_{5B} (mL/g/min), and Fbm using well-established numerical methods (*31*,*32*). Once model parameters were estimated, $q_{nB}(t)$ quantities were calculated as functions of the rate constants k_{1B}–k_{7B} by assuming quasi-steady-state conditions (differential equations are set to zero after assuming that glycogenolysis flux ($k_{5B} \bullet q_{3B}$, mL/g/min) is negligible during the study period).

$$q_{1B} = K_{1A}/(k_{2A} + k_{3A}). \quad (8B)$$

$$q_{2B} = k_{3A} \bullet q_{1B}/(k_{1B} + k_{3B} + k_{4B}). \quad (9B)$$

$$q_{3B} = k_{1B} \bullet q_{2B}. \quad (10B)$$

The following metabolic fluxes were then calculated:

$$\text{Glucose uptake (mL/g/min)} = K_{1A} \bullet k_{3A}/(k_{2A} + k_{3A}). \quad (11B)$$

$$\text{Glycolysis (lactate production + oxidation) (mL/g/min)} = (k_{3B} + k_{4B}) \bullet q_{2B}. \quad (12B)$$

$$\text{Glucose oxidation (mL/g/min)} = k_{3B} \bullet q_{2B}. \quad (13B)$$

$$\text{Lactate production (mL/g/min)} = k_{4B} \bullet q_{2B}. \quad (14B)$$

$$\text{Glycogen synthesis (mL/g/min)} = k_{1B} \bullet q_{2B}. \quad (15B)$$

Because it is assumed that myocardial 1-^{11}C-glucose either goes through glycolysis or is stored as glycogen, the fraction of myocardial glucose that ends up in the glycogen pool can be defined as follows:

$$\text{Glycogen content/Glucose uptake} = k_{1B} \cdot q_{2B}/K_{1A} \cdot q_{1B.} \quad (16B)$$

Fluxes of ^{11}C-lactate uptake and oxidation were not calculated because compartments 4 and 5 (Fig. 1B) were designed to remove the contribution of ^{11}C-lactate to myocardial ^{11}C activity and not to quantify ^{11}C-lactate kinetics.

Myocardial fractional quantities were calculated from metabolic fluxes by dividing a given flux by MBF (mL/g/min). Measurements of the myocardial fate of glucose (utilization, oxidation, lactate production, and glycogen synthesis, in mmol/g/min) can then be calculated as the product plasma glucose (μmol/mL) and the corresponding flux (mL/g/min).