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Hyperglycemia-induced stimulation of glucose transport in skeletal muscle measured by PET-[¹⁸F]6FDG and [¹⁸F]2FDG

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Abstract

A physiologically based model proposed by our group has been developed to assess glucose transport and phosphorylation in skeletal muscle. In this study, we investigated whether our model has the ability to detect a glucose-induced increase in glucose transport in skeletal muscle. In particular, we used small-animal positron emission tomography (PET) data obtained from [¹⁸F]6-fluoro-6-deoxy-D-glucose ([¹⁸F]6FDG). A two-hour PET scan was acquired following a bolus injection of [¹⁸F]6FDG in rats currently under euglycemic or hyperglycemic conditions, while somatostatin was infused during both conditions in order to prevent a rise in the endogenous plasma insulin concentration. We were thus able to assess the effect of hyperglycemia per se. For a comparison of radiopharmaceuticals, additional rats were studied under the same conditions, using [¹⁸F]2-fluoro-2-deoxy-D-glucose ([¹⁸F]2FDG). When [¹⁸F]6FDG was used, the time-activity curves (TACs) for skeletal muscle had distinctly different shapes during euglycemic and hyperglycemic conditions. This was not the case with [¹⁸F]2FDG. For both [¹⁸F]6FDG and [¹⁸F]2FDG, the model detects increases in both interstitial and intracellular glucose concentrations, increases in the maximal velocity of glucose transport, and increases in the rate of glucose transport, all in response to hyperglycemia. In contrast, there was no increase in the maximum velocity of glucose phosphorylation or in the glucose phosphorylation rate. Our model-based analyses of the PET data, obtained with either [¹⁸F]6FDG or [¹⁸F]2FDG, detect physiologic changes consistent with established behaviors. Moreover, based on differences in the TAC shapes, [¹⁸F]6FDG appears to be superior to [¹⁸F]2FDG for evaluating the effect of hyperglycemia on glucose metabolism in skeletal muscle.

Keywords

glucose transport; kinetic model; PET; glucose clamp; hyperglycemia

1. Introduction

Over the past two decades, the use of dynamic *in vivo* positron emission tomography (PET) imaging to assess skeletal muscle glucose metabolism has been increasing (Kelley *et al* 2001, Ng *et al* 2009). Unlike the hyperinsulinemic euglycemic clamp that assesses whole-body glucose metabolism (DeFronzo *et al* 1979), the modeling of dynamic PET data allows the assessment of tissue-specific glucose metabolism (Kelley *et al* 1996, 1999). To further

characterize the individual transport and phosphorylation steps of glucose metabolism in skeletal muscle, dynamic PET imaging with sequential injections of radiolabeled non-phosphorylatable and phosphorylatable glucose analogs has been proposed by Bertoldo *et al* (2006). In fact, their study showed that the multi-tracer PET imaging method has the ability to separately quantify glucose transport and phosphorylation in skeletal muscle (Bertoldo *et al* 2006). However, the multi-tracer protocol requires two tracers which may not be available at every research institution. In addition, the kinetic modeling of this protocol usually requires many blood samples in order to determine the input functions. Finally, the standard PET models (Sokoloff *et al* 1977, Phelps *et al* 1979, Bertoldo *et al* 2001) which estimate the rate constants of the radiolabeled glucose analog provide general physiological information without the specificity for resolving transport and phosphorylation and for resolving intracellular and interstitial concentration. In contrast, the physiologically based kinetic model proposed by our group (Huang *et al* 2011) provides physiological quantities including the cellular influx and efflux of glucose, its phosphorylation rate, and its maximal transport and phosphorylation capacities. As such, our model may provide insight that is not otherwise available.

In this study, we investigated whether our model using single-tracer PET data can be used to individually evaluate the steps of glucose transport and phosphorylation in skeletal muscle. To achieve this objective, dynamic *in vivo* PET data were acquired following the injection of a radiolabeled non-phosphorylatable glucose analog, i.e. [^{18}F]6-fluoro-6-deoxy-D-glucose ([^{18}F]6FDG), or a radiolabeled phosphorylatable glucose analog, i.e. [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]2FDG), using rats currently under either euglycemic or hyperglycemic conditions (clamp technique). We tested the effect of hyperglycemic conditions to further validate the ability of our model to assess the effect of acute hyperglycemia on glucose uptake in skeletal muscle. To our knowledge, this is the first study to use the modeling of dynamic PET imaging to assess the glucose-induced increase in glucose transport in skeletal muscle.

2. Materials and Methods

2.1 Animals

Fourteen, male, Sprague-Dawley rats, each weighting between 200 and 270 g, were purchased from Harlan Laboratories (Indianapolis, IN). They were randomly divided into two groups, one studied under euglycemic conditions and the other studied under hyperglycemic conditions. The animals were prepared for scanning by cannulating the left carotid artery and the right jugular vein for, respectively, blood sampling and administration of radiopharmaceuticals and glucose for the clamp (Spring-Robinson *et al* 2009). On the evening before the scanning, their food was removed. For each rat, a PET scan was performed following a bolus injection of [^{18}F]6FDG which was synthesized (Neal *et al* 2005) by members of our University's Imaging Center. For comparison, the study was repeated with fourteen additional rats, i.e. seven per physiologic condition, using [^{18}F]2FDG which was purchased commercially (PETNET Solutions, Cleveland, OH). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University.

2.2 Euglycemic and hyperglycemic clamp studies

Rats were PET-scanned during euglycemic and hyperglycemic clamp conditions. Thirty minutes after the induction of anesthesia (1.5% isoflurane in oxygen), infusion of somatostatin (0.5 $\mu\text{g}/\text{min}$, Sigma-Aldrich) was begun via the venous catheter and was kept constant (2 $\mu\text{l}/\text{min}$) throughout the entire study. After ten minutes of somatostatin infusion, the plasma glucose concentrations ($[P_g]$) were measured every 5 min in 1- μl blood samples

(Aviva; ACCU-CHEK) taken from the cut tail, and euglycemia (~6 mmol/l) was maintained by adjusting the infusion rate of 6% dextrose between 0 and 50 $\mu\text{l}/\text{min}$. Hyperglycemia (~20 mmol/l) was similarly maintained using 25% dextrose. Four blood samples (~50 μl each) were taken starting 30 min following the induction of anesthesia, and then 10, 60, and 120 min after starting the PET scan in order to determine the insulin concentration using an Ultra Sensitive Rat Insulin ELISA Kit (Crystal Chem, Inc. Downers Grove, IL).

2.3 PET scans and data analysis

PET scanning was performed on an Inveon dedicated small-animal PET scanner (Siemens Preclinical Solutions, Knoxville, TN) (Bao *et al* 2009). Each PET scan started with a 15-min transmission scan using a ^{57}Co point source followed by a two-hour emission scan acquired beginning with the intravenous injection of 37 MBq [^{18}F]6FDG (or [^{18}F]2FDG). Using Fourier rebinning, the list-mode data were binned into a total of 56 frames with 5 $\times$ 2-s, 10 $\times$ 5-s, 12 $\times$ 30-s, 8 $\times$ 60-s, and 21 $\times$ 300-s frames. A 2-dimensional, ordered subset expectation maximization algorithm (Hudson and Larkin 1994) with 16 subsets and 12 iterations, was then used to reconstruct dynamic image sequences of 128 $\times$ 128 $\times$ 159 voxels with a spacing of 0.78 $\times$ 0.78 $\times$ 0.80 mm. During the reconstruction, we performed corrections for attenuation, scatter, radioactive decay, and dead-time. Using the COMKAT Image Tool (Muzic *et al* 2001, Fang *et al* 2010), regions of interest (~40 mm 3) were drawn over the biceps and triceps, and tissue time-activity curves (TACs) were generated.

In order to determine the input function for the first two minutes post-injection, the blood from the arterial catheter was continuously drawn through a syringe pump at a rate of 0.2 ml/min and was counted by a flow-through radiation detector using consecutive 0.1-sec intervals (Nelson *et al* 1990). Corrections for sensitivity, delay and dispersion were performed to yield blood activity concentration (Nelson *et al* 1990, Nelson *et al* 1993, Muzic *et al* 1993). After the first two minutes, nine samples (15 μl each) were obtained manually at 3, 5, 10, 15, 20, 30, 60, 90, and 120 min and were counted with a well counter (Wallac LKB 1282). The radioactivity data from the flow-through detector as well as the manually acquired samples were linearly interpolated in order to produce a single input function. To determine the plasma activity fraction and the hematocrit, an extra blood sample was taken 120 min post-injection. The total amount of blood collected was approximately 0.8 mL which represents approximately 5% of the total blood volume given that the total volume is estimated as 15 mL (Lee and Blafox, J Nucl Med, 26:72–76, 1985).

2.4 Compartmental model and parameter estimation

To assess glucose transport and phosphorylation in skeletal muscle, a physiologically based model was used, as we have previously described in detail (Huang *et al* 2011). Briefly, the kinetic model has three tissue compartments with five rate constants, as shown in Figure 1. The molar balance equations of the model are:

$$d[\text{IS}'_a]/dt = k_1[\text{P}_a] - k_2[\text{IS}'_a] - k_{3a}[\text{IS}'_a] + k_{4a}[\text{IC}'_a] \quad (1)$$

$$d[\text{IC}'_a]/dt = k_{3a}[\text{IS}'_a] - k_{4a}[\text{IC}'_a] - k_{5a}[\text{IC}'_a] \quad (2)$$

$$d[\text{IP}'_a]/dt = k_{5a}[\text{IC}'_a] \quad (3)$$

where $[\text{IS}'_a] = f_{\text{IS}}[\text{IS}_a]$, $[\text{IC}'_a] = f_{\text{IC}}[\text{IC}_a]$, and $[\text{IP}'_a] = f_{\text{IC}}[\text{IP}_a]$. f_{IS} and f_{IC} are the fractions of the total space occupied by interstitial and intracellular spaces, respectively. $[\text{P}_a]$, $[\text{IS}_a]$, and $[\text{IC}_a]$ represent molar concentrations of the glucose analog in the plasma and interstitial and

intracellular spaces, respectively, and $[IP_a]$ is the molar concentration of the intracellular phosphorylated analog. k_1 and k_2 are the rate constants for the reversible exchange of the glucose analog between plasma and interstitial space and are indicative of glucose delivery by blood. k_{3a} and k_{4a} are the effective rate constants for the influx and efflux transport of the glucose analog via facilitative glucose transporters (GLUTs), respectively. In fact, the rate constants are not actual constants as they are concentration-dependent, according to the Michaelis-Menten kinetics. Likewise, k_{5a} is the effective rate constant for the phosphorylation of the intracellular glucose analog catalyzed by hexokinase (HK). For non-phosphorylatable analogs, the value of k_{5a} is zero and (3) is omitted. The model output, the radioactivity concentration averaged over the time interval of scan frame i beginning at time t_b^i and ending at time t_e^i , is calculated as:

$$M_{PET,i} = \frac{1}{t_e^i - t_b^i} \int_{t_b^i}^{t_e^i} \{A([IS'_a] + [IC'_a] + [IP'_a]) + f_b \times b(t)\} dt \quad (4)$$

where A is the exponentially decaying specific activity (in units of MBq/pmol), f_b is the fraction of space that is blood, and $b(t)$ is the whole-blood activity.

As we have defined $k_2 = k_1/f_{IS}$ and $f_{IC} = 1 - f_{IS} - f_b$ (Huang *et al* 2011), there are six, unknown parameters, i.e. k_1 , f_{IS} , f_b , k_{3a} , k_{4a} , and k_{5a} . Based on competitive enzyme-substrate kinetics (Dixon and Webb 1979, Mori and Maeda 1997, Pardridge and Oldendorf 1975), rate constants, k_{3a} , k_{4a} , and k_{5a} , can be written as shown in figure 1 (Huang *et al* 2011). V_g^G and V^H are the maximal velocities of glucose transport and phosphorylation, respectively. K_g^G and K_a^G are the Michaelis constants of GLUT for glucose and its analog, respectively. Similarly, K_g^H and K_a^H are the Michaelis constants of HK for glucose and its analog, respectively. As PET scans were performed during the steady-state plasma glucose concentrations, the interstitial ($[IS_g]$) and intracellular ($[IC_g]$) glucose concentrations were assumed to be constant. Consequently, k_{3a} , k_{4a} , and k_{5a} may be treated as constants over the course of the scanning. In addition, under the presumed conditions of a glucose steady state, the molar balance equations (1–3) for glucose simplify to the following set of algebraic equations (Huang *et al* 2011):

$$V_g^G = k_1([P_g] - [IS_g]) / \{[IS_g]/(K_g^G + [IS_g]) - [IC_g]/(K_g^G + [IC_g])\} \quad (5)$$

$$V^H = k_1([P_g] - [IS_g]) / \{[IC_g]/(K_g^H + [IC_g])\} \quad (6)$$

assuming that glucose and its analogs have similar maximal velocities (Huang *et al* 2011), and that (5) and (6) can be substituted into the expressions for k_{3a} , k_{4a} and k_{5a} . As a result, k_1 , f_{IS} , f_b , $[IS_g]$, $[IC_g]$, $[P_g]$, K_g^G , K_a^G , K_g^H , and K_a^H are our model parameters. Given the measured $[P_g]$ and assumed Michaelis constants, the model parameters to be estimated from our data are reduced to k_1 , f_{IS} , f_b , $[IS_g]$, and $[IC_g]$. Using these parameters, the cellular influx ($CI = V_g^G \times [IS_g]/(K_g^G + [IS_g])$), cellular efflux ($CE = V_g^G \times [IC_g]/(K_g^G + [IC_g])$), and phosphorylation rate ($PR = V^H \times [IC_g]/(K_g^H + [IC_g])$) of glucose can be determined.

In contrast to our previous study (Huang *et al* 2011) in which it was assumed that $f_b = 0$, in this study we considered the more general case that includes estimating f_b . In particular, during the early time frames, the blood radioactivity concentration $b(t)$ may be so high that $f_b \times b(t)$ in (4) cannot be neglected. The F -test (Landaw and DiStefano 1984) was used to determine if the improvement of fit was sufficient to warrant adding f_b as a parameter to be estimated.

In the current study, the model parameters were estimated by fitting the model output to the experimental data. An iteratively reweighted least-squares (IRLS) data fitting method was used, and the IRLS objection function (Muzic and Christian 2006) was minimized using the MATLAB 2010b (The Mathworks, Inc., Natick, MA) function, “fmincon,” with an interior-point method (Byrd *et al* 2000). The initial values and the lower and upper bounds of all the model parameters are summarized in Table 1. For both metabolic states, the Michaelis constant of GLUT4 for glucose (K_g^G) is set as 3.5 mmol/l (Olson and Pessin 1996). Similarly, the Michaelis constants of HK II for glucose (K_g^H) and 2FDG (K_{2FDG}^H) are set to 0.17 and 0.13 mmol/l, respectively (Muzi *et al* 2001). The Michaelis constants of GLUT4 for 2FDG (K_{2FDG}^G) and 6FDG (K_{6FDG}^G) are set to 14 and 10 mmol/l, respectively, based on transport measured in isolated adipocytes (Rodbell *et al* 1964) using (3-H) 3-O-methyl glucose (Chandramouli *et al* 1977) Biochemistry vol 16 pages 1151–1158). Analysis of the statistical significance was performed using the Student’s two-tailed *t*-test and a threshold of $p=0.05$.

3. Results

3.1 Metabolic conditions

Figure 2 shows the time courses of the mean plasma glucose concentration (top) and the infusion rates of glucose and somatostatin (bottom) during euglycemic ($n=13$) and hyperglycemic ($n=14$) clamps. Time zero refers to the point at which the tracer injection began. In general, it took the plasma glucose levels approximately 50 minutes to stabilize after the somatostatin infusion. Table 2 summarizes the general characteristics of all groups. Specifically, under euglycemic conditions, the plasma glucose concentrations measured in [^{18}F]6FDG and [^{18}F]2FDG studies, were 5.37 ± 0.35 (mean $\pm$ SD) and 6.02 ± 0.65 mmol/l, respectively. Under hyperglycemic conditions, the plasma glucose concentrations measured in [^{18}F]6FDG and [^{18}F]2FDG studies, were 21.74 ± 0.82 and 21.54 ± 1.03 mmol/l, respectively. For each condition, we found that the plasma insulin did not change significantly during the entire experiment. The insulin levels in hyperglycemic rats averaged ~2-fold higher than those in euglycemic rats during somatostatin infusion ($p<0.05$).

Table 2 shows the urinary radioactivity measured in the rats at time of euthanization after PET scanning. Compared to euglycemia, hyperglycemia caused a significant increase in the urinary excretion of [^{18}F]6FDG (0.19 ± 0.15 vs. 4.06 ± 1.26 MBq/ml; $p<0.05$) and a concordant increase in the glucose concentration of the urine. In addition, the urinary excretion of [^{18}F]2FDG is significantly higher in euglycemia than in hyperglycemia (5.54 ± 1.16 vs. 3.29 ± 0.81 MBq/ml; $p<0.05$).

3.2 Model analysis of the dynamic PET data

Compared to the model assuming that $f_b=0$, the more general model that estimates f_b , had a significant improvement in model fits ($p<0.01$), and a preliminary model simulation study performed on the computer analogous to that which we reported previously reported (Huang *et al* 2011) show that our model can provide reliable estimates of f_b , while achieving low bias ($<5\%$) and good precision ($<26\%$). Therefore, all of the results shown below were obtained from the model that estimates f_b . In addition, the model was fit to data from the 110-minute interval immediately following injection and during which the plasma glucose values were regarded as being stable.

Examples of TACs (○) and model fits (–) for [^{18}F]6FDG PET for skeletal muscle during euglycemic (panel a) and hyperglycemic (panel b) states, are shown in Figure 3. Following [^{18}F]6FDG injection, the tissue radioactivity increased over time under euglycemic conditions, although they peaked and subsequently decreased after 20 min under hyperglycemic conditions. Figures 3(c) and 3(d) show the model-estimated radioactivity

concentration for each compartment under euglycemic (panel a) and hyperglycemic (panel b) conditions, respectively. The washout of interstitial [^{18}F]6FDG ($\text{IS}_{6\text{FDG}}$) was faster in hyperglycemia than in euglycemia, although the accumulation of intracellular [^{18}F]6FDG ($\text{IC}_{6\text{FDG}}$) was slower in hyperglycemia than in euglycemia.

Figure 4 is analogous to Figure 3 with the difference being that Figure 4 shows [^{18}F]2FDG data and model fits, with the latter including phosphorylated tracer as [^{18}F]2FDG which, unlike [^{18}F]6FDG, is phosphorylatable. In terms of curve shapes, both conditions had similar TACs, i.e. the differences between euglycemia and hyperglycemia were less distinct with [^{18}F]2FDG than with [^{18}F]6FDG. However, the model-estimated radioactivity concentrations for each compartment shown in Figures 4(c) and 4(d) for euglycemic (panel a) and hyperglycemic (panel b) states, differed with there being higher intracellular [^{18}F]2FDG ($\text{IC}_{2\text{FDG}}$) and lower intracellular [^{18}F]2FDG-6-P ($\text{IP}_{2\text{FDG-6-P}}$) in hyperglycemia than in euglycemia.

The parameter estimates of our model are summarized in Table 3. For k_1 and f_b , the mean values did not differ significantly irrespective of either the tracers or the physiological state. In addition, both the [^{18}F]6FDG and the [^{18}F]2FDG data show that hyperglycemia resulted in significant increases in f_{IS} , $[\text{IS}_g]$ and $[\text{IC}_g]$ ($p < 0.05$). In the euglycemic studies, $[\text{IS}_g]$ estimated using [^{18}F]6FDG (4.61 ± 0.39 mmol/l) was slightly lower than that estimated using [^{18}F]2FDG (5.22 ± 0.53 mmol/l, $p < 0.05$). In the hyperglycemic studies, $[\text{IC}_g]$ estimated using [^{18}F]6FDG (7.90 ± 1.52 mmol/l) was significantly higher than that estimated using [^{18}F]2FDG (5.41 ± 2.09 mmol/l, $p < 0.05$).

As seen in Figure 5, both the [^{18}F]6FDG and the [^{18}F]2FDG data show that hyperglycemia caused a significant increase in the maximal glucose transport rate (V^G) ($p < 0.05$). However, the maximal phosphorylation rate (V^H) was similar irrespective of the tracers and the physiological states. Similarly, hyperglycemia caused significant increases in cellular influx (CI) and cellular efflux (CE) ($p < 0.05$), although there was no increase in the phosphorylation rate (PR) (figure 6). More importantly, the estimates of V^G , V^H , CI, CE, and PR obtained from both tracers were similar.

4. Discussion

As shown in Table 2, insulin secretion was not abolished by somatostatin in the hyperglycemic animals, however, the insulin levels seen during the hyperglycemic clamping did not exceed the normal basal insulin levels of 2–5 ng/ml (Rossetti *et al* 1990) and they did not rise during the experiment. Nonetheless, a two-fold increment in insulin levels would be expected to increase the rate of glucose transport by as much as 20–40% (Hager *et al* 1991, Dimitriadis *et al* 1997).

Table 2 shows that urinary excretion of [^{18}F]6FDG is negligible in euglycemia; this is consistent with our prior study results indicating that [^{18}F]6FDG is reabsorbed by the kidneys more like glucose than is [^{18}F]2FDG (Landau *et al* 2007). Actually, similar to the way in which hyperglycemia causes glycosuria, we observed that urinary [^{18}F]6FDG is higher in hyperglycemia than in euglycemia ($p < 0.05$). Therefore, we speculate that the renal excretion of [^{18}F]6FDG reduced plasma [^{18}F]6FDG and therefore resulted in lower interstitial and intracellular [^{18}F]6FDG in skeletal muscle during hyperglycemia compared with that during euglycemia. It is also likely that the lower intracellular [^{18}F]6FDG is caused by a hyperglycemia-induced increase in the competition between glucose and [^{18}F]6FDG for cellular entry. It is significant that our model predictions shown in Figure 3 are consistent with these premises.

Under euglycemic conditions, as hexokinase phosphorylates [^{18}F]2FDG essentially as fast as it is transported, the intracellular concentration of [^{18}F]2FDG, but not of [^{18}F]2FDG-6-phosphate (2FDG-6-P), is very low. However, in contrast to euglycemia, hyperglycemia leads to more competition between glucose and [^{18}F]2FDG for phosphorylation by hexokinase. Therefore, we speculate that [^{18}F]2FDG-6-P would accumulate more slowly in hyperglycemia than in euglycemia. This speculation also suggests that there is increased intracellular [^{18}F]2FDG in hyperglycemia. Once again, the model predictions shown in Figure 4 support these assumptions. Table 2 also shows that euglycemia has high urinary [^{18}F]2FDG activity which is consistent with the fact that [^{18}F]2FDG is not reabsorbed well by the kidneys, especially when there is hyperglycemia (Moran *et al* 1999).

As shown in Figures 4(a) and 4(b), the [^{18}F]2FDG data acquired under euglycemic and hyperglycemic conditions have similarly shaped TACs. However, as shown in Figures 3(a) and 3(b), the [^{18}F]6FDG data acquired under euglycemic and hyperglycemic conditions have distinctly shaped TACs. These results suggest that [^{18}F]6FDG may be preferable to [^{18}F]2FDG for characterizing the differences between euglycemia and hyperglycemia.

In Table 3, our model predicted significant increases in f_{IS} , $[\text{IS}_g]$, and $[\text{IC}_g]$ in response to hyperglycemic, hypoinsulinemic vs. euglycemic, and hypoinsulinemic conditions. The increase of f_{IS} may be due to the hyperglycemia-induced osmotic flux of water from the intracellular to the interstitial space. In contrast to the NMR method (Cline *et al* 1998) that showed a small amount of intracellular glucose (~ 0.3 mmol/l) in rat muscle under hyperglycemic, hypoinsulinemic conditions, our method predicted an appreciable amount of intracellular glucose (Table 3) under similar conditions. It is unclear whether this divergent finding is caused by methodological differences, although the degree of increase of $[\text{IC}_g]$ predicted by our model is similar to that observed under similar conditions in humans, as determined using biochemical assay of biopsy samples (Katz *et al* 1991).

Table 3 shows that $[\text{IS}_g]$ obtained using [^{18}F]2FDG was slightly higher than that obtained using [^{18}F]6FDG in euglycemia but not in hyperglycemia. This may be a consequence of the slightly higher $[\text{P}_g]$ with [^{18}F]2FDG than with [^{18}F]6FDG ($p < 0.05$, Table 2) in euglycemia but not in hyperglycemia. We also found that both [^{18}F]2FDG and [^{18}F]6FDG provided similar estimates of $[\text{IS}_g]$ in hyperglycemia (Table 3); however, $[\text{IC}_g]$ obtained using [^{18}F]2FDG data was significantly lower than that obtained using [^{18}F]6FDG ($p < 0.05$). We speculate that there is an underestimation of $[\text{IC}_g]$ with [^{18}F]2FDG. Indeed, our computer simulations made to assess the theoretical precision of parameter estimates revealed that parameter estimates of $[\text{IC}_g]$ using [^{18}F]2FDG under hyperglycemic conditions, tended to have a negative bias and poor precision, neither of which was observed with [^{18}F]6FDG. Although the true $[\text{IC}_g]$ is not known, the pattern seen in our experimental data is consistent with the simulation in that the precision is the poorest with [^{18}F]2FDG in hyperglycemia (Table 3).

As described above, the value of $K_{2\text{FDG}}^G$ is set to 14 mM and that of $K_{6\text{FDG}}^G$ to 10 mM. This is based on our recent measurements of the Michaelis constant for GLUT4 using isolated rat adipose cells. Using these two values, we showed that model analyses of the [^{18}F]6FDG and [^{18}F]2FDG data agree in the estimates of V^G , V^H , CI, CE, and PR. However, the Michaelis constants obtained in cell systems may not be exactly the same as those obtained in intact animals. Based on our previous study (Huang *et al* 2011), changing the Michaelis constants has less than proportional effects on estimates of V^G , V^H , CI, CE, and PR and negligible effects on estimates of k_1 , $[\text{IS}_g]$, $[\text{IC}_g]$, and f_{IS} . Therefore, slight differences in the values of Michaelis constants would not change the relative relationship between the euglycemic and hyperglycemic groups. This has also been shown in other studies (O'Doherty *et al* 1998, Halseth *et al* 2001).

As seen in Figures 5 and 6, our method shows increases in the maximal velocities of glucose transport as well as in the cellular influx and efflux of glucose during hyperglycemia compared with euglycemia. This finding is consistent with the results reported by Galante *et al* (1995) and Kawano *et al* (1999). Specifically, hyperglycemia results in a nearly four-fold increase in the cellular influx of glucose and which can be attributed not only to increased V^G but also to mass-action. However, due to the somatostatin infusion, as insulin secretion is inhibited, glucose phosphorylation under hyperglycemic conditions is not enhanced (Katz *et al* 1991). This may be why our method shows no significant increase in either the maximal velocities of glucose phosphorylation or the glucose phosphorylation rate (Figures 5 and 6) during hyperglycemia compared with those during euglycemia ($p < 0.05$). Finally, comparison of the derived rates of glucose transport and glucose phosphorylation using [^{18}F]6FDG and [^{18}F]2FDG, shows that the rates of each step are comparable using either tracer, and this is a novel finding of our study. In other words, our model appears to be able to estimate the rate of transport using [^{18}F]2FDG and the rate of phosphorylation using [^{18}F]6FDG.

Ideally each arterial blood sample would be centrifuged so that the activity in plasma (and fraction of blood activity due to activity in plasma) could be determined. In practice we were limited by the blood volume that we could withdraw from the rat. Consequently we implicitly assumed that the plasma to blood activity was constant. Indeed, this approximation has been commonly used (Van den Brom CE *et al* 2009; Su *et al* 2009). If the fraction actually changed over time, our parameter estimates could be impacted. We expect the significance of such a putative impact to be minor given that differences in plasma glucose concentration caused physiologically plausible differences in parameter estimates.

5. Conclusions

Using information obtained from either the [^{18}F]2FDG or the [^{18}F]6FDG PET data, our model resolves questions regarding the delivery, transport, and phosphorylation of glucose in rat skeletal muscle. A significant finding of this study is that in the physiologic states examined, the model provides a reasonable estimate of the rate of glucose transport using [^{18}F]2FDG (a phosphorylatable tracer) and can also estimate the rate of phosphorylation using [^{18}F]6FDG (a non-phosphorylatable tracer). Finally, our model also successfully detects the glucose-induced increase in glucose transport in skeletal muscle, a finding that has not been previously identified using the modeling of dynamic PET imaging.

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Figure 1.

The diagram of the compartmental model used to describe the delivery, transport, and phosphorylation of the glucose analog in skeletal muscle.

Figure 2.

Time courses of the mean plasma glucose concentration (top) and the infusion rate of glucose and somatostatin (bottom) during euglycemic (n=13) and hyperglycemic (n=14) clamps. Values are expressed as mean $\pm$ SD. Time zero refers to the point at which tracer injection began.

Figure 3.

Time-activity curves (○) and model fits (—) of skeletal muscle during [^{18}F]6FDG PET scans acquired under (a) euglycemic and (b) hyperglycemic conditions. The model-estimated compartmental concentrations show that the intracellular [^{18}F]6FDG (IC_{6FDG}) is higher under (c) euglycemic than under (d) hyperglycemic conditions. Clearance of the interstitial [^{18}F]6FDG (IS_{6FDG}) is faster under hyperglycemic than under euglycemic conditions.

Figure 4.

Time-activity curves (○) and model fits (—) of skeletal muscle during $[^{18}F]2FDG$ PET scans acquired under (a) euglycemic and (b) hyperglycemic conditions. The model-estimated compartmental concentrations show that the intracellular $[^{18}F]2FDG$ (IC_{2FDG}) is lower under (c) euglycemic than under (d) hyperglycemic conditions, but that the intracellular phosphorylated $[^{18}F]2FDG$ ($IP_{2FDG-6-P}$) is higher under euglycemic than under hyperglycemic conditions.

Figure 5.

Estimates of the maximal velocities of transport (V^G) and phosphorylation (V^H) obtained from [¹⁸F]6FDG and [¹⁸F]2FDG PET scans acquired under euglycemic and hyperglycemic conditions. Values are expressed as mean ± SD. * $p < 0.05$ vs. euglycemia.

Figure 6.

Estimates of the cellular influx (CI) and efflux (CE) of glucose and the phosphorylation rate (PR) obtained from $[^{18}\text{F}]6\text{FDG}$ and $[^{18}\text{F}]2\text{FDG}$ PET scans acquired under euglycemic (a) and hyperglycemic (b) conditions. Values are expressed as mean $\pm$ SD. * $p < 0.05$ vs. euglycemia.

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Table 1

Initial values and bounds for parameter estimation

Parameter	k_1 (min^{-1})	$[IS_g]$ (mmol/l)	$[IC_g]$ (mmol/l)	f_{IS} (unitless)	f_b (unitless)
Euglycemic conditions					
Initial value	0.01	$0.8 \times [P_g]$	0.1	0.15	0.01
Upper bound	0.5	$[P_g]$	1	0.6	0.04
Lower bound	0.001	1	0.001	0.1	0
Hyperglycemic conditions					
Initial value	0.01	$0.8 \times [P_g]$	1	0.15	0.01
Upper bound	0.5	$[P_g]$	10	0.6	0.04
Lower bound	0.001	10	0.1	0.1	0

Table 2

General characteristics of each study.

Conditions Tracers	Euglycemia		Hyperglycemia	
	[¹⁸ F]6FDG	[¹⁸ F]2FDG	[¹⁸ F]6FDG	[¹⁸ F]2FDG
No. of rats	6	7	7	7
Injection dose (MBq)	32.5±4.0	37.4±1.7	34.0±5.8	38.9±2.3
Body mass (g)	235±17	225±14	238±21	228±14
P _g (mmol/l)	5.37±0.35	6.02±0.65 ^b	21.74±0.82	21.54±1.03
Glucose infusion rate (μl/min)	~0	~0	10.59±0.44	7.86±0.69 ⁺
Urinary radioactivity concentration (MBq/ml) at 120 minutes	0.19±0.15	5.54±1.16	4.06±1.26 ^a	3.29±0.81
Insulin concentration (ng/ml) before somatostatin-glucose infusions	0.25±0.19		0.30±0.21	
at 10 min after tracer injection	0.17±0.12		0.44±0.23 ^a	
at 60 min after tracer injection	0.15±0.13		0.42±0.23 ^a	
at 120 min after tracer injection	0.18±0.13		0.37±0.18 ^a	

^a p<0.05 vs. euglycemia and^b p<0.05 vs. [¹⁸F]6FDG. Values are means±SD.

Table 3

Parameter estimates of our model

Conditions Tracers	Euglycemia		Hyperglycemia	
	[¹⁸ F]6FDG	[¹⁸ F]2FDG	[¹⁸ F]6FDG	[¹⁸ F]2FDG
Parameter estimates				
k ₁ (min ⁻¹)	0.09±0.03	0.08±0.03	0.08±0.02	0.07±0.02
f _{IS} (unitless)	0.35±0.06	0.37±0.05	0.43±0.05 ^a	0.45±0.05 ^a
f _b (unitless)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
[IS _g] (mmol/l)	4.61±0.39	5.22±0.53 ⁺	20.98±0.73 ^a	20.43±1.08 ^a
[IC _g] (mmol/l)	0.47±0.23	0.43±0.08	7.90±1.52 ^a	5.41±2.09 ^{a, b}

^ap<0.05 vs. euglycemia and^bp<0.05 vs. [¹⁸F]6FDG. Values are means±SD.