Fiber type-specific determinants of $V_{\text{max}}$ for insulin-stimulated muscle glucose uptake in vivo

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Petersen, Hilary Ann, Patrick T. Fueger, Deanna P. Bracy, David H. Wasserman, and Amy E. Halseth. Fiber type-specific determinants of $V_{\text{max}}$ for insulin-stimulated muscle glucose uptake in vivo. Am J Physiol Endocrinol Metab 284: E541–E548, 2003; 10.1152/ajpendo.00323.2002.—The aim of this study was to determine barriers limiting muscle glucose uptake (MGU) during increased glucose flux created by raising blood glucose in the presence of fixed insulin. The determinants of the maximal velocity ($V_{\text{max}}$) of MGU in muscles of different fiber types were defined. Conscious rats were studied during an 4-h insulin clamp with plasma glucose at 2.5, 5.5, and 8.5 mM. $[^{1-14}C]$mannitol and 3-O-methyl-$[^{3}H]$glucose ($[^{3}H]$MG) were infused to steady-state levels ($t = -180$ to 0 min). These isotope infusions were continued from 0 to 40 min with the addition of a 2-deoxy-$[^{3}H]$glucose ($[^{3}H]$DG) infusion. Muscles were excised at $t = 40$ min. Glucose metabolic rate ($R_g$) was calculated from muscle-phosphorylated $[^{3}H]$DG. $[^{1-14}C]$mannitol was used to determine extracellular (EC) H$_2$O. Glucose at the outer ($[G]_{\text{om}}$) and inner ($[G]_{\text{im}}$) sarcolemmal surfaces was determined by the ratio of $[^{3}H]$MG in intracellular to EC H$_2$O and muscle glucose. $R_g$ was comparable at the two higher glucose concentrations, suggesting that rates of uptake near $V_{\text{max}}$ were reached. In summary, by defining the relationship of arterial glucose to $[G]_{\text{om}}$ and $[G]_{\text{im}}$ in the presence of fixed hyperinsulinemia, it is concluded that 1) $V_{\text{max}}$ for MGU is limited by extracellular and intracellular barriers in type I fibers, as the sarcolemma is freely permeable to glucose; 2) $V_{\text{max}}$ is limited in muscles with predominantly type IIb fibers by extracellular resistance and transport resistance; and 3) limits to $R_g$ are determined by resistance at multiple steps and are better defined by distributed control rather than by a single rate-limiting step.

extracellular, intracellular water; glucose analogs; rat

MUSCLE GLUCOSE UPTAKE (MGU) requires three serial steps. These steps are delivery of glucose to the muscle, transport of glucose across the sarcolemma, and phosphorylation of glucose intracellularly. Because membrane permeability is low, transport is considered rate limiting for MGU in the basal state. This is not the case under all physiological conditions. Transport is increased during conditions such as hyperinsulinemia (16, 17, 36, 41) and exercise (16, 17, 21) to the point that delivery of glucose to muscle or glucose phosphorylation within muscle becomes a control point of greater significance. The limitations that define the maximal capacity of the muscle to take up glucose during physiological hyperinsulinemia ($V_{\text{max}}$) remain to be determined. It is important to understand the factors that determine the $V_{\text{max}}$ for insulin-stimulated MGU under normal conditions, because a reduction in this variable is a characteristic of insulin-resistant conditions (3).

Because the factors that determine the capacity for MGU involves the integration of many systems, it must be examined in vivo to be fully understood. We learned from our previous studies (18, 19) how barriers to MGU are affected by an increase in insulin. Here, we challenged the pathway for MGU by altering substrate (i.e., blood glucose) in the presence of fixed hyperinsulinemia. These studies utilized an isotopic technique that provides functional indexes of muscle glucose delivery, transport, and phosphorylation in the conscious, unstressed rat. The advantage of the rat model is that some muscles have a predominance of specific fiber types, allowing for effects of fiber type-specific changes to be assessed. The purpose of this study was to determine in vivo the muscle fiber type-specific barriers to insulin-stimulated glucose uptake at $V_{\text{max}}$.

METHODS

Animal maintenance and surgical procedures. Male Sprague-Dawley rats were individually housed in cages at 23°C on a 0600–1800 light cycle. The rats were fed chow consisting of 65% carbohydrate, 11% fat, and 24% protein. Rat weight and food consumption were closely monitored, and a food consumption index (FCI: weight gained/food eaten) was calculated. The rats had free access to chow until 6:00 PM the evening before the study.

After reaching a weight of 250–300 g, rats were anesthetized with a intraperitoneal injection of ketamine, rompun, and acepromazine (50:5:1) and underwent surgical placement of polypropylene catheters (0.58 mm ID × 0.97 mm OD) using sterile procedures. A catheter was inserted in the left carotid artery and advanced so that its tip lay at the aortic arch. A second catheter was inserted in the right jugular vein

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was clamped at /H11011 [3H]DG infusion (900 mCi/min) was started. All isotope and placed in liquid N2 in ficortisol, and insulin. Rats were then anesthetized with pen-
samples were drawn for measurement of catecholamines, t was started at t = −180
range. These clamps provided a range of physiological glu-
7). The insulin infusion rate used was selected because it
min (Fig. 1). At t = 0 min, an infusion of deoxy-[2-3H]glucose ([3H]DG) infusion (900 mCi/min) was started. All isotope infusions were continued until the end of the study (t = 40 min). A 4.0 mU·kg⁻¹·min⁻¹ intravenous insulin infusion was started at t = −100 min, and arterial plasma glucose was clamped at −8.5 (n = 8), −5.5 (n = 7), or −2.5 mM (n = 7). The insulin infusion rate used was selected because it results in arterial concentrations in the upper physiological range. These clamps provided a range of physiological glucose concentrations (mild hyperglycemia to moderate hypoglycemia). The high-glucose clamp was chosen to create a blood glucose that would cause near-maximal MGU for a given insulin level so that the site that limits this process could be identified.

Small blood samples (50 μl) were taken every 10 min from t = −100 to 0 min to measure glucose. These measurements provided feedback on which the glucose infusion needed to clamp arterial glucose concentrations. Larger blood samples were taken at t = 0, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 30, and 40 min for tracer and glucose measurement. At t = 40 min, blood samples were drawn for measurement of catecholamines, cortisol, and insulin. Rats were then anesthetized with pentobarbital sodium, and soleus, gastrocnemius-plantaris, and white superficial vastus lateralis (SVL) muscles were excised and placed in liquid N2 in <1 min. The rat soleus consists of 89% type I and 11% type IIA muscle fibers by mass (1). As a postural muscle, it is associated with tonic activity and higher “basal” MGU (19). The rat SVL contains 0% type I, 1% type IIA, and 99% type IIB muscle fiber by mass (1). The gastrocnemius-plantaris complex (subsequently referred to as gastrocnemius) can be calculated from the data of Armstrong and Phelps (1) and is made up of 6% type I, 23% type IIA, and 71% type IIB muscle fibers by mass. Plasma and tissue samples were then stored at −70°C until analyses.

Analytical procedures. Plasma insulin was assessed via radioimmunoassay (32). Plasma glucose concentrations were measured via a glucose oxidase method with an automated glucose analyzer (Beckman Instruments, Fullerton, CA). Corticosterone levels were determined by competitive binding assay. Norepinephrine and epinephrine levels were measured via high-pressure liquid chromatography (31, 36, 44).

The measurement of the radioactivity of [14C]MN, [3H]MG, and [3H]DG in muscle and plasma was performed as has been described previously (19). Briefly, muscle and plasma samples were incubated with and without yeast hexokinase and treated with Ba(OH)₂ and ZnSO₄. Yeast hexokinase catalyzes the conversion of glucose to glucose-6-phosphate with a rate of 98% of the [2-H]DG is removed by the addition of Ba(OH)₂ and ZnSO₄, according to tests done in our laboratory. Tests in our laboratory also revealed that treatment of samples with yeast hexokinase resulted in the phosphorylation of ~25% of the [3H]MG (19). Because both muscle and plasma samples were similarly affected, this does not appreciably change the calculated value of the steady-state ratio of [3H]MG in intra-
cellular to extracellular water; therefore, no correction for this was necessary. Tissue radioactivity and glucose concentrations were measured in neutralized 0.5% perchloric acid extracts. Samples were double-label counted using a Packard Tri-Carb 2900TR liquid scintillation counter. [3H] and [14C] counting windows were defined on the basis of known spectra and correction for isotopic spillover and quenching based on relationships defined in our laboratory for the Packard Tri-Carb. Tissue glucose concentration is expressed as millimoles of muscle water, assuming a muscle water content of 0.75 ml/g (based on measurements performed in our laboratory).

Calculations. The fraction of extracellular to total water space in biopsies (Fₑ) was calculated with [14C]MN as described previously (16). An index of MGU (Rₑ) was calculated from phosphorylated [3H]DG, the integrated plasma [3H]DG concentration over the infusion period, and the plasma glu-
cose concentration (23).

The steady-state ratio of [3H]MG concentration in intra-
cellular to extracellular water was determined to calculate the glucose concentration at the outer face of the sarcolemma ([G]ₒm) and the glucose concentration at the inner face of the sarcolemma ([G]ᵢm), described in detail previously (36) as have been their theoretical bases (8, 11, 16, 19, 29, 34, 36). The ratio of [3H]MG in intracellular and extracellular water (Sᵢ/Sₒ) is used to derive [G]ᵢm and [G]ₒm by the countertransport equation Sᵢ/Sₒ = (Kᵢm + [G]ᵢm)/[G]ᵢm + [G]ₒm, where Kᵢm is the Michaelis-Menten constant for glucose transport. A range of 2–5 mM has been reported for the Kᵢm of GLUT4 in vitro (15, 37). A Kᵢm of 2.6 mM was estimated in previous work using the same methodology as in the present studies (19) and was used. The use of different values for Kᵢm in calculations has a quantitative effect on the calculated results but not a qualitative effect (35).

The calculation described above defines the mathematical relationship between [G]ᵢm and [G]ₒm but not in the actual values. Because of the presence of glucose gradients, it is impossible to directly measure the true glucose concentration at the sarcolemma or anywhere in the interstitial or intra-

![Experimental protocols consisted of a 220-min 3-0-[3H]methyl-
ylglycose and [U-14C]mannotin infusion, an 80-min hyperinsulinemic glucose clamp at 1 of 3 concentrations, and a 40-min deoxy-[2-3H]glucose ([2-3H]DG) infusion. Values reported in tables and figures were derived from blood samples taken during the last 40 min of the experiment, and tissues were excised at the time that animals were killed.](http://ajpendo.physiology.org/issue/vol284/issue3/fig1.png)
cellular space. An approach for calculating limits for the average $[G]_{om}$ based on two theoretical glucose distributions was used (17, 19, 36). In the first calculation of $[G]_{om}$, $[G]_{im}$ is assumed to be localized to such a small volume of the intracellular water that it contributes only negligibly to the total muscle glucose mass (denoted by $\alpha$). The second approach for the calculation of the mean $[G]_{om}$ assumes that $[G]_{im}$ is distributed evenly throughout the intracellular water (denoted by $\beta$). Neither of these calculated values, or any other single value, will be identical to the actual $[G]_{om}$ at all places along the sarcolemma; however, the present approach allows the mean of these values within a known range to be expressed.

ANOVA for repeated measures was used for the statistical analysis of appropriate data. Specific comparisons between groups or doses were evaluated for significance through contrasts solved by univariate repeated measures. Trend analysis was performed using a two-way ANOVA to assess whether $[G]_{om}$ and $[G]_{im}$ were significantly increased with arterial plasma glucose. Slopes of these relationships were assessed using the individual data points for each animal. Differences between muscles were assessed using paired analysis. Ranges for each variable were compared by pooling data calculated using both $\alpha$- and $\beta$-distributions. Differences were considered significant at $P < 0.05$.

RESULTS

Arterial plasma glucose and hormone concentrations.

The average steady-state plasma glucose concentrations were not significantly different from the target glucose concentrations of 2.5, 5.5, and 8.5 mM (Fig. 2). Insulin levels were in the upper physiological range ($\sim$120 $\mu$U/ml) during all clamps, with no significant differences between groups (Fig. 2). Table 1 shows that the epinephrine, norepinephrine, and corticosterone levels were significantly increased during the 2.5 mM glucose clamp experiment compared with the 8.5 mM glucose clamp experiment ($P < 0.05$).

Glucose infusion rate. The glucose infusion rates (Fig. 3) increased significantly with glucose clamp concentrations. The increase was most sensitive between 2.5 and 5.5 mM glucose clamp concentrations (increment of 126 $\mu$mol·kg$^{-1}$·min$^{-1}$). The increment in glucose infusion rate was only $38$ $\mu$mol·kg$^{-1}$·min$^{-1}$ between 5.5 and 8.5 mM glucose clamps, indicating that whole body glucose kinetics were approaching $V_{\text{max}}$.

Skeletal muscle $R_g$. $R_g$ (Fig. 3) was $6.0 \pm 0.6, 30.2 \pm 3.8,$ and $26.7 \pm 2.6$ $\mu$mol·kg$^{-1}$·min$^{-1}$ in soleus; $5.6 \pm 1.7, 10.3 \pm 0.8,$ and $12.3 \pm 1.4$ $\mu$mol·100 g$^{-1}$·min$^{-1}$ in gastrocnemius; and $3.2 \pm 0.6, 10.7 \pm 1.3,$ and $11.4 \pm 2.1$ $\mu$mol·100 g$^{-1}$·min$^{-1}$ in SVL at 2.5, 5.5, and 8.5 mM glucose clamps, respectively. $R_g$ was consistently twofold higher in soleus compared with gastrocnemius and SVL at all glucose clamp levels. There was no significant difference between $R_g$ at the two highest glucose clamp concentrations. The plateau seen at the two higher glucose clamps suggest that $V_{\text{max}}$ for MGU had been obtained or was approached in each muscle.

Table 1. Arterial plasma norepinephrine, epinephrine, and cortisol concentrations

<table>
<thead>
<tr>
<th>Glucose Clamp, mM</th>
<th>Norepinephrine, pg/ml</th>
<th>Epinephrine, pg/ml</th>
<th>Cortisol, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>577 ± 10</td>
<td>2,882 ± 663</td>
<td>422 ± 41</td>
</tr>
<tr>
<td>5.5</td>
<td>424 ± 88</td>
<td>530 ± 272</td>
<td>201 ± 50</td>
</tr>
<tr>
<td>8.5</td>
<td>330 ± 56</td>
<td>111 ± 41</td>
<td>259 ± 57</td>
</tr>
</tbody>
</table>

Data are means ± SE; $n = 7$ at 2.5 and 5.5 mM glucose clamps, and $n = 8$ at 8.5 mM. *Significantly different from concentrations at 2.5 mM glucose clamp ($P < 0.05$–0.005).

Fig. 2. Arterial insulin (top) and plasma glucose (bottom) during hyperinsulinemic (4 mU·kg$^{-1}$·min$^{-1}$) clamps at 1 of 3 glucose levels. Glucose concentrations were targeted. Data are means ± SE. Differences in glucose were significant between groups ($P < 0.01$).

Fig. 3. The relationship between glucose infusion rate and arterial plasma glucose (top) and between glucose metabolic index ($R_g$) and arterial plasma glucose (bottom) during hyperinsulinemic (4 mU·kg$^{-1}$·min$^{-1}$) clamps. *Rates significantly increased from those seen at the 2.5 mM glucose clamp ($P < 0.05$). SVL, superficial vastus lateralis.
Table 2. Glucose concentration and $F_e$ in gastrocnemius, soleus, and SVL

<table>
<thead>
<tr>
<th>Glucose Clamp, mM</th>
<th>Gastrocnemius</th>
<th>Soleus</th>
<th>SVL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.48 ± 0.07</td>
<td>0.37 ± 0.12</td>
<td>0.50 ± 0.09</td>
</tr>
<tr>
<td>5.5</td>
<td>0.73 ± 0.15</td>
<td>0.84 ± 0.10*</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>8.5</td>
<td>0.94 ± 0.04*</td>
<td>1.78 ± 0.21*</td>
<td>0.72 ± 0.16</td>
</tr>
</tbody>
</table>

$F_e$ values are in bold. Data are means ± SE; $n = 7$ at 2.5 and 5.5 mM glucose clamps, and $n = 8$ at 8.5 mM. $*P < 0.05$.

Skeletal muscle glucose concentrations, extracellular water space, and $S_i/S_o$. The total glucose concentration in soleus homogenates rose significantly with increasing glucose clamp levels (Table 2). The total glucose concentration in gastrocnemius was significantly higher at the 8.5 mM glucose clamp compared with the 2.5 mM glucose clamp. There were no significant differences in SVL glucose concentration between different glucose clamp experiments. Soleus glucose concentrations were approximately twofold higher than that in the other two muscles at the 8.5 mM glucose clamp level.

$F_e$ for soleus and gastrocnemius showed no significant difference between glucose clamp levels (Table 2). The $F_e$ in SVL was slightly higher at the lowest glucose clamp concentration. Differences between soleus and SVL were significant at all glucose clamp levels and between the soleus and gastrocnemius at the two highest glucose clamp levels.

$S_i/S_o$ was not significantly different from 1.0 at any glucose clamp level in soleus (1.07 ± 0.10, 1.09 ± 0.16, and 1.06 ± 0.12 at 2.5, 5.5, and 8.5 mM, respectively). $S_i/S_o$ fell gradually with increasing glucose clamp levels in gastrocnemius (0.82 ± 0.09, 0.73 ± 0.11, and 0.59 ± 0.06 at 2.5, 5.5, and 8.5 mM, respectively) and SVL (0.85 ± 0.09, 0.78 ± 0.09, and 0.74 ± 0.08 at 2.5, 5.5, and 8.5 mM, respectively). This decrease was significant in gastrocnemius ($P < 0.05$).

Muscle $[G]_{om}$ and $[G]_{im}$. Because it is based on the premise that intracellular glucose is localized to such a small space that it contributes only negligibly to the total muscle glucose mass, the $\alpha$-distribution gives $[G]_{om}$ and $[G]_{im}$ values that are greater than those of the $\beta$-distribution, which assumes that glucose is distributed evenly throughout the intracellular water.

Figures 4, 5, and 6 show $[G]_{om}$ and $[G]_{im}$ plotted against steady-state glucose clamp levels for soleus, gastrocnemius, and SVL, respectively. Arterial blood glucose concentration for each clamp protocol (line of identity) is included for reference. A slope of $< 1$ for the relationship between $[G]_{om}$ and arterial glucose suggests that capillary perfusion and extracellular glucose diffusion is a significant barrier to insulin-stimulated MGU. A slope of the relationship of $[G]_{im}$ to arterial glucose that is greater than the slope of that of $[G]_{im}$ and arterial glucose would indicate that the sarcolemma is a barrier to MGU. A slope of the relationship of $[G]_{im}$ to arterial glucose that is $> 0$ would be indicative of a barrier at glucose phosphorylation. It is important to note that the relationships of the glucose concentrations in arterial blood and inner and outer surfaces of the sarcolemma were the same regardless of whether $\alpha$- or $\beta$-distribution was assumed. The slopes of $[G]_{om}$ and $[G]_{im}$ vs. glucose clamp concentration were essentially the same in soleus, equaling $0.63 ± 0.12$ and $0.68 ± 0.19$ with $\alpha$-distribution and $0.23 ± 0.09$ and $0.23 ± 0.06$ with $\beta$-distribution. Similar $[G]_{om}$ and $[G]_{im}$ concentrations at each glucose clamp concentration suggest that glucose is equilibrated across the sarcolemma and that transport is not rate limiting in the soleus, which is predominantly type I muscle, even at a rate of insulin-stimulated glucose utilization at or near $V_{max}$. Instead, the limitations are in the capacity to deliver glucose to the muscle and phosphorylate it in muscle.

In the gastrocnemius and SVL, both of which consist of predominantly type IIb fibers, $[G]_{om}$ rose with increasing glucose clamp concentration, whereas $[G]_{im}$ was essentially unchanged. The slopes of gastrocnemius $[G]_{om}$ vs. arterial glucose clamp concentration were $0.57 ± 0.13$ and $0.40 ± 0.16$ for the $\alpha$- and $\beta$-distributions, respectively. The slopes of gastrocnemius $[G]_{im}$ vs. arterial glucose clamp concentration were not significantly different from 0 ($0.11 ± 0.10$ and $0.05 ± 0.03$, respectively, assuming $\alpha$- and $\beta$-distributions) regardless of assumptions pertaining to glucose distribution. In the SVL, the slopes of $[G]_{om}$ vs. arterial glucose clamp concentration were $0.42 ± 0.12$ and $0.37 ± 0.12$. The Fe in SVL was slightly higher at the lowest glucose clamp concentrations. Differences between soleus and gastrocnemius at the two high-glucose clamp concentration. Differences between soleus and gastrocnemius at the two high-glucose clamp concentration. Differences between soleus and gastrocnemius at the two high-glucose clamp concentration. Differences between soleus and gastrocnemius at the two high-glucose clamp concentration.
0.23 ± 0.09 assuming α- and β-distributions, respectively. The slopes of gastrocnemius [G]_{im} vs. arterial glucose clamp concentration were not significantly different from zero (0.10 ± 0.14 and 0.02 ± 0.03, respectively, assuming α- and β-distributions) regardless of assumptions pertaining to glucose distribution. These data suggest that muscle glucose delivery and membrane transport, but not phosphorylation, are barriers to insulin-stimulated glucose uptake throughout a physiological range of glucose concentrations, including those that elicit rates of uptake at or near V_{max} in muscle that contains a large percentage of type IIb fibers.

**DISCUSSION**

Barriers to insulin-stimulated MGU were defined in the present study by relating increments in arterial glucose to changes in the “intermediates” of the glucose uptake pathway (i.e., [G]_{om} and [G]_{im}) at a fixed hyperinsulinemia. The highest arterial glucose clamp level was sufficient to elicit an MGU that was at or near the V_{max} of this process. The premise is that the greatest barrier to MGU will have the steepest glucose gradient across it. The drop in glucose concentration from artery to muscle surface (arterial glucose − [G]_{im}) is a reflection of the capillary bed/extracellular diffusion barrier to glucose uptake, whereas the drop in concentration across the sarcolemma ([G]_{om} − [G]_{im}) is a reflection of the transport barrier to glucose uptake. Because [G]_{im} is the substrate for the irreversible, intracellular metabolism of glucose, its value alone is an index of the importance of the phosphorylation barrier to glucose uptake. This approach assumes that glucose fluxes through the steps that comprise MGU are similar in a steady state. The effect of increasing substrate availability on limitations to MGU is evident from the relationships of [G]_{om} and [G]_{im} to arterial glucose (Figs. 4–6). Changes in [G]_{om} were significantly damped in relation to a given change in arterial glucose, clearly demonstrating vascular/extracellular resistance to MGU in all fiber types. Changes in [G]_{im} in relation to changes in arterial glucose were the same as those for [G]_{om} in type I muscle fibers, indicating that membrane transport does not create an added barrier to MGU under the conditions of the present study. In muscles with a high percentage of type IIb fibers, [G]_{im} did not increase, as did [G]_{om}, with increasing arterial glucose. This indicates that, in contrast to type I fibers, the membranes of muscles consisting predominantly of type IIb fibers are barriers to MGU. The fact that [G]_{im} rose with increasing arterial glucose in soleus is indicative of a barrier at glucose phosphorylation in muscle consisting predominantly of type I fibers. Such a barrier was not evident in muscle consisting of a predominance of type IIb fibers, as [G]_{im} did not rise with increasing arterial glucose. The relative association among [G]_{om}, [G]_{im}, and arterial glucose was independent of whether the α-distribution (glucose confined to negligible intracellular volume) or β-distribution (glucose evenly distributed intracellularly) of intracellular glucose was assumed.

Previous work (36) showed that the sarcolemma of type I muscle fibers is freely permeable to glucose at the insulin infusion rate used in these studies (4
mU·kg$^{-1}$·min$^{-1}$). The equivalent relationships of $[G]_{om}$ and $[G]_{im}$, to arterial glucose in Fig. 4 extend this finding by demonstrating that the sarcolemma of the soleus does not become a site of resistance to MGU during physiological hyperinsulinemia at glucose concentrations that create a range of glucose fluxes that extend from $\sim 20\%$ of $V_{max}$ to $V_{max}$. Limitations to insulin-stimulated MGU at rates of uptake that approach $V_{max}$ in type I fibers of the soleus are those factors required to sustain the transsarcolemmal glucose gradient (i.e., glucose delivery to muscle and intracellular glucose phosphorylation). The transsarcolemmal glucose gradient in muscle containing a preponderance of type IIb fibers was consistently greater than zero at all glucose concentrations despite the hyperinsulinemia, indicating that the sarcolemma was a significant barrier to MGU at all glucose concentrations. These findings are consistent with a study that showed that GLUT4 protein was closely coupled to glucose uptake in rat muscle consisting of type II fibers but was disassociated from glucose uptake in muscle consisting of type I fibers (42). One reason for this could be that the greater number of GLUT4 transporters in type I fibers (30) creates a situation wherein membrane transport is not a limitation even at high rates of insulin-stimulated glucose uptake.

The ability to sustain a glucose gradient across the sarcolemma is dependent on the ability to deliver glucose to the muscle and phosphorylate glucose once it is within the cell. Muscle perfusion/extracellular diffusion was a determinant of $V_{max}$ to MGU in all muscles. The barrier role of the extracellular space is reflected by the fact that $[G]_{om}$ did not increase proportionally to the increment in arterial glucose in any of the muscles studied. $[G]_{im}$ rose by only $\sim 40\%$–$60\%$ of the increment in arterial glucose assuming the $\alpha$-distribution and by only $\sim 20\%$–$40\%$ assuming the $\beta$-distribution. This is consistent with the work of Baron (2). The difference between the increment in arterial glucose and the increments in $[G]_{om}$ was similar in muscles, regardless of predominant fiber type. Because, however, the glucose flux rate ($R_{g}$) in type I fibers of the soleus was so much higher, the extracellular barrier to muscle glucose uptake must be much less. This is consistent with the greater vascularization and the associated shorter extracellular diffusion distances of muscles comprised of slow-twitch fibers (39).

The second determinant of the glucose gradient across the sarcolemma, the effectiveness with which glucose is phosphorylated after it crosses the sarcolemma, showed considerable fiber type dependence. The barrier role of glucose phosphorylation in the soleus was evident by the significant increment in $[G]_{im}$ that occurred when glucose concentration approached $V_{max}$. In contrast to the soleus, phosphorylation was not a major site of resistance to MGU in the gastrocnemius and SVL, which are both composed primarily of type IIb fibers, at $V_{max}$. Type I muscle fibers of the soleus have $\sim 30\%$ more hexokinase activity than the gastrocnemius and $\sim 80\%$ more hexokinase activity than the SVL (35). The difference in glucose uptake in soleus is even higher than in gastrocnemius and SVL ($\sim 240\%$ higher; Fig. 3). Thus, although hexokinase activity is higher in type I fibers, rates of glucose delivery and transport into this muscle are even greater. It is not surprising, when one considers these factors, that phosphorylation is a greater barrier in soleus.

The advantage of glucose flux measurements in vivo is that a true functional measure of glucoregulation is obtained, inclusive of all circulatory and neural factors. At the lowest plasma glucose concentration (2.5 mM), epinephrine, norepinephrine, and cortisol were increased due to hypoglycemic stimulation. It is possible that epinephrine in particular could have acutely attenuated MGU and affected its determinants. The transsarcolemmal glucose gradient and $[G]_{im}$ were not increased at the lowest glucose concentration, indicating that neither transport nor phosphorylation was markedly impaired by the counterregulatory response in any of the muscles studied. This may not reflect the absence of physiological regulation so much as the manifestation of offsetting physiological effects. Epinephrine has been shown to reduce insulin-stimulated glucose transport by inhibiting the intrinsic activity of the transporter (20) and phosphorylation by causing a buildup of glucose 6-phosphate (6). $[G]_{om}$ did not fall disproportionately to circulating glucose at the lowest glucose clamp concentration, so it is unlikely that extracellular barriers are increased relatively more compared with euglycemic levels. These studies suggest that the decrease in MGU during hypoglycemia is primarily a result of the decreased arterial glucose concentration and the associated mass action effect.

Total soleus and gastrocnemius glucose concentrations rose significantly with arterial glucose concentrations, whereas total SVL glucose concentration was not significantly affected by the clamp glucose concentration. The increase in glucose concentration in soleus was most marked, as it obtained levels approximately twofold greater than the two muscles comprised predominantly of type IIb muscle fibers. It is not surprising that soleus glucose concentration more closely paralleled arterial glucose concentration, because a greater fraction of soleus water volume is extracellular (Table 2). Although measurements of total muscle glucose are informative in and of themselves, they alone do not provide insight into glucose concentration in the muscle intracellular space. Estimates of intracellular glucose concentration require that interstitial glucose be known. Use of blood glucose concentrations in place of interstitial glucose (46) leads to erroneous estimates, as muscle interstitial glucose concentration is well below arterial or venous plasma concentrations (25). Glucose concentration gradients may also exist within the intracellular space as physical barriers, and spatial glucose gradients may compartmentalize glucose. This is supported by evidence that glucose transporters (10, 12, 27, 28, 30) and hexokinases (4, 22, 24, 26, 40, 43) are localized to specific regions within the skeletal muscle cell. The uncertainties created by extracellular and intracellular glucose gradients are circumvented.
with the use of [3H]MG. Because 3-O-MG is not metabolized, it is homogenous within the contiguous extracellular and intracellular spaces when it is at equilibrium in the blood.

The modeling approach used in this study allows for estimation of intracellular glucose concentrations. Three markedly different modeling approaches have been conducted utilizing data from rats (13, 45) or humans (5) in addition to the model used here. The results from all of these studies indicate that factors other than transport are significant barriers to insulin-stimulated glucose uptake. The model used here is based on principles of glucose countertransport, a concept that has been applied to study diverse model systems (7, 9, 11, 14, 29, 33, 34, 38). The linking of the transmembrane glucose distribution to 3-O-MG countertransport assumes that 1) glucose and 3-O-MG share the same transport system; 2) the reaction between carrier and sugar is rapid compared with carrier mobility; 3) the relative affinity of each sugar for the transport proteins is the same on the extracellular and intracellular sides of the plasma membrane; and 4) carrier mobility is independent of whether or not the transporter is bound to either sugar. The validity of these assumptions has been discussed in detail previously (11, 36).

A surprising finding in these studies is that $F_e$ rose significantly with decreasing glycemia in the SVL, which consists of type IIB (99%) fibers, and there was a trend for such an increase in the gastrocnemius, which also consists of type IIB (71%) fibers, albeit somewhat less than the SVL. The reason for this rise in $F_e$ specifically in type I muscle fibers has not, to our knowledge, been described. It is unlikely that the trend of a reduced $F_e$ at higher glucose levels is due to a general systemic alteration of either physiological or technical origin, since no such effect was seen in the type I soleus muscle. It is possible that there is relative swelling of type IIB muscle cells at high compared with low glucose concentrations. One could postulate that this might be due to increased Na$^+$-K$^+$-ATPase and/or increased glycogen storage. Again, there is no immediate explanation for the fiber type heterogeneity of this response. It may be significant that the increased $F_e$ at the lower glucose clamp corresponds to an increased counterregulatory response. One could speculate that a reduction in cell volume is a consequence of the resultant hormonal changes that occur with hypoglycemia, with different fiber type endocrine sensitivities causing the discrepancy in type I, type IIA, and type IIB fibers. Although the blood glucose-related changes in $F_e$ do not alter the calculations of $G_{im}$, $G_{om}$, and $G_{im}$ to the extent that they affect the conclusions of these studies, it may reflect an as yet undefined response to a decrement in blood glucose.

Skeletal muscle comprises ~50% of total body mass and exhibits the greatest increases in glucose uptake in response to insulin. Knowledge of the regulation of skeletal muscle glucose uptake, therefore, is a prerequisite to understanding normal and pathophysiological whole body glucose uptake. These studies provide insight into the barriers that limit MGU during increased glucose flux created by raising blood glucose concentration in the presence of fixed insulin (~120 µU/ml) by showing that 1) MGU is at or near $V_{max}$, with only modest hyperglycemia in muscle consisting predominantly of type I fibers (soleus) and type IIb fibers (gastrocnemius and SVL); 2) membranes of type I fibers are freely permeable to glucose at $V_{max}$, suggesting that extracellular (glucose delivery to muscle) and intracellular (glucose phosphorylation) resistances limit MGU; and 3) $V_{max}$ is limited in muscles that consist predominantly of type IIb fibers by extracellular resistance and to a lesser extent transport resistance, whereas intracellular resistance plays a relatively small role. In conclusion, the mechanisms that limit MGU at $V_{max}$ are complex and may be determined at multiple sites that are critically dependent on fiber type.

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REFERENCES


