Limitations to exercise- and maximal insulin-stimulated muscle glucose uptake

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Halseth, Amy E., Deanna P. Bracy, and David H. Wasserman. Limitations to exercise- and maximal insulin-stimulated muscle glucose uptake. J. Appl. Physiol. 85(6): 2305–2313, 1998.—The hypothesis of this investigation was that insulin and muscle contraction, by increasing the rate of skeletal muscle glucose transport, would bias control so that glucose delivery to the sarcolemma (and t tubules) and phosphorylation of glucose intracellularly would exert more influence over glucose uptake. Because of the substantial increases in blood flow (and hence glucose delivery) that accompany exercise, we predicted that glucose phosphorylation would become more rate determining during exercise. The transsarcolemmal glucose gradient (TSGG; the glucose concentration difference across the membrane) is inversely related to the degree to which glucose transport determines the rate of glucose uptake. The TSGG was determined by using isotopic methods in conscious rats during euglycemic hyperinsulinemia [Ins; 20 mU/(kg·min); n = 7], during treadmill exercise (Ex, n = 6), and in sedentary, saline-infused rats (Bas, n = 13). Rats received primed, constant intravenous infusions of trace 3-O-[3H]methyl-d-glucose and [U-13C]mannitol. Then 2-deoxy-[3H]glucose was infused for the calculation of a glucose metabolic index (Rg). At the end of experiments, rats were anesthetized, and soleus muscles were excised. Total soleus glucose concentration and the steady-state ratio of intracellular to extracellular 3-O-[3H]methyl-d-glucose (which distributes on the basis of the TSGG) were used to calculate ranges of possible glucose concentrations ([G]) at the inner and outer sarcolemmal surfaces ([G]om and [G]im, respectively). Soleus Rg was increased in Ins and further increased in Ex. In Ins, total soleus glucose, [G]om, and the TSGG were decreased compared with Bas, while [G]im remained near 0. In Ex, total soleus glucose and [G]im were increased compared with Bas, and there was not a decrease in [G]om as was observed in Ins. In addition, accumulation of intracellular free 2-deoxy-[3H]glucose occurred in soleus in both Ex and Ins. Taken together, these data indicate that, in Ex, glucose phosphorylation becomes an important limitation to soleus glucose uptake. In Ins, both glucose delivery and glucose phosphorylation influence the rate of soleus glucose uptake more than under basal conditions.

glucose phosphorylation; glucose delivery; countertransport; 3-O-methyl-d-glucose; 2-deoxyxyglucose

GLUCOSE ENTRY into skeletal muscle is determined by membrane permeability to glucose and the transsarcolemmal glucose gradient (TSGG). Membrane permeability is a function of the number of glucose transporters in the sarcolemma [including t tubules (44)] and has been examined under a variety of conditions (39). The TSGG, defined as the difference in glucose concentration at the outer and inner surfaces of the plasma membrane, is determined by the balance of glucose delivery to the sarcolemma and removal of glucose from the inner face of the membrane by phosphorylation. If the TSGG is equal to 0, the sarcolemma is completely permeable to glucose, and pre- and posttransport steps will determine glucose flux. Therefore, glucose delivery, glucose transport, and glucose phosphorylation all may contribute to the control of glucose uptake under physiological or pathophysiological states. Discriminating among these closely coupled steps is difficult because neither the intracellular glucose concentration nor the TSGG can be measured directly. Total glucose in muscle has been measured both biochemically in muscle homogenates (24, 51) and on whole muscle by using NMR spectroscopy (4, 43). However, extrapolation of this value to an intracellular glucose concentration or value for the TSGG is complicated by the difficulty in measuring interstitial glucose concentrations, the presence of glucose gradients within the extracellular and intracellular water space, and compartmentation within the tissue. These technical limitations have made it difficult to understand the sites at which the rate of glucose uptake is controlled.

Although it is well known that increased insulin concentration and muscle contraction are the two primary physiological stimuli that increase skeletal muscle glucose uptake, the technical limitations discussed above have prevented a complete understanding of these processes. During hyperinsulinemia, the primary mechanism for increased glucose uptake is an increase in sarcolemmal permeability to glucose, whereas the exercise increment in glucose uptake occurs due to an increase in glucose transporter translocation as well as increased glucose delivery. We have previously demonstrated that submaximal increases in insulin result in a decrease of the TSGG in the soleus, which is at least in part caused by a fall in the interstitial glucose concentration (38). We hypothesized that the increased glucose delivery during exercise would better maintain the interstitial glucose concentration, even during high rates of glucose transport, shifting more control over the rate of glucose uptake to glucose phosphorylation. The aim of these experiments, therefore, was to investigate the maintenance of the TSGG in rat soleus during short-term, moderate-intensity treadmill exercise and compare it to the TSGG seen with a maximally effective insulin dose (21). An in vivo model, the chronically catheterized rat, was used so that the integrated systems involved in the regulation of muscle glucose delivery and uptake were intact. We were able to assess...
independently the TSGG and glucose uptake under these conditions by using isotopic techniques (38). In addition, comparison of muscle and plasma glucose concentration ratios with the muscle and plasma concentration ratios of a glucose analog allowed further assessment of alterations in the rate-determining components of glucose uptake in the soleus.

**MATERIALS AND METHODS**

Animal maintenance and surgical procedures. Male Sprague-Dawley rats (Sasco, Omaha, NE) were individually housed at 23°C on a 0600–1800 light-dark cycle; they were allowed free access to water and a predetermined weight of chow (65% carbohydrate, 11% fat, 24% protein). The rats were housed under these conditions for ~1 wk, by which time their weights had reached 250–300 g. Also during this time, animals to be used in exercise experiments were familiarized with running on a motorized treadmill (2 sessions, 10 min each). During this period, the food conversion index (FCI; weight gained/food consumed) for each rat was calculated. Animals were anesthetized with a 50:5:1 vol/vol/vol mixture of ketamine, rompun, and acepromazine, and the left common carotid artery and the right jugular vein were catheterized with PE50. Catheters were exteriorized and secured at the back of the neck, filled with heparinized saline (150 U/ml), and sealed with a stainless steel plug. Immediately postsurgery, each animal received 40,000 U of penicillin G and 5 ml of sterile saline subcutaneously. After surgery, animal weights and food intake were monitored daily, and only animals in which presurgery weight and FCI were restored were used for experiments (~5 days).

On the morning of the experiment, catheters were flushed with heparinized saline (10 U/ml) and connected to Silastic tubing for infusions and sampling. All procedures were preapproved by the Vanderbilt University Animal Care and Use Subcommittee and followed the NIH Guidelines for the Care and Use of Laboratory Animals.

Experimental procedures. Figure 1 shows the experimental protocol. Food was removed from rats ~5 h before the beginning of a study. Rats were studied during a hyperinsulinemic clamp (Ins group, n = 7), or during moderate treadmill exercise (Ex group, n = 6), or in the basal state (Bas group, n = 13). At t = −100 min, an infusion of saline (Bas and Ex) or insulin (20.0 mU/kg·min) was begun. Also at t = −100 min, primed infusions of [U-14C]mannitol ([U-14C]MN; 3.5 µCi primer and 60 nCi/min infusion) and 3-0-[3H]methyl-D-glucose (3-O-[3H]MG; 25 µCi primer and 150 nCi/min infusion) were initiated. 3-O-[3H]MG is a substrate for glucose transport but is not further metabolized in skeletal muscle, whereas [U-14C]MN is restricted to the extracellular space and thus can be used to measure this space. From t = 0 to 20 min (Ex) or 40 min (Ins), a constant-rate infusion of 2-deoxy-[3H]glucose ([2−3H]DG; 900 nCi/min) was given. [2−3H]DG is transported into the cell and phosphorylated, yielding [2−3H]deoxyglucose-6-phosphate ([2−3H]DGP). All radioisotopes were obtained from New England Nuclear (Boston, MA). In Ex, the initiation of [2−3H]DG infusion coincided with the start of treadmill running (0.8 mi/h, 6% grade). This is a moderate-intensity workload that can be maintained by untrained rats for ~90 min (37). Under these experimental conditions, this workload was sustainable for the 20-min experimental period. Because of the different durations of the Ex and Ins experiments, Bas experiments were conducted in which the [2−3H]DG infusion period lasted either 20 (n = 6) or 40 (n = 7) min. Results from these two groups were not different; therefore, data were combined. Arterial blood samples (from 150 to 500 µl) for tracer or insulin analyses were taken at t = −100, −70, −40, −10, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 30, and 40 min (except when experiments were terminated after the 20-min sample). Washed erythrocytes from the experimental animal and whole blood, from a donor rat, were used to maintain hematocrit, but neither was infused after t = −10 min. At t = 20 or 40 min, the animal was anesthetized with pentobarbital sodium, and the soleus muscles were rapidly excised and frozen in liquid N2. In separate Bas experiments, in which the equilibration period was extended to 300 min, soleus muscle [U-14C]MN and 3-O-[3H]MG contents did not differ from those in the 120-min Bas experiments, indicating that steady-state conditions were attained. Gastrocnemius and superficial vastus lateralis muscles (muscles composed of fast-twitch oxidative and fast-twitch glycolytic fibers, respectively) were also obtained from rats. However, the 100-min isotope-equilibration period used in these experiments was not long enough to reach steady-state conditions in the gastrocnemius and superficial vastus lateralis muscles (38). Therefore, only data not dependent on 3-O-[3H]MG equilibration are presented.

Processing of blood and muscle samples. Plasma glucose concentrations were measured by the glucose oxidase method by using an automated glucose analyzer (Beckman Instruments, Fullerton, CA), and immunoreactive insulin was measured by using a double-antibody method (32). Total plasma radioactivity ([U-14C]MN, 3-O-[3H]MG, and [2−3H]DG) was determined on resulting supernatants after deproteinization with barium hydroxide [Ba(OH)2, 0.3 N], and zinc sulfate (ZnSO4, 0.3 N) followed by centrifugation. Radioactivity was determined by dual-label scintillation counting (Beckman LS 5000TD, Beckman Instruments) with the use of Ecollite(-) scintillation fluid (ICN, Irvine, CA). To separate plasma 3-O-[3H]MG and [2−3H]DG radioactivity, samples were treated with Ba(OH)2 and ZnSO4 incubated for 30 min at room temperature with a solution that contained (as final concentrations) 2.5 mg/ml yeast hexokinase (21 U/mg solid), 100 mM KCl, 40 mM Tris·Cl, 20 mM MgCl2, 4 mM EDTA (pH 8.1), and 10 mM ATP (pH 8.1) and then retreated with Ba(OH)2 and

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**Fig. 1.** Experimental protocol used for analysis of glucose uptake in conscious rats under basal conditions (Bas; n = 13), during a hyperinsulinemic clamp (Ins; n = 7), and during exercise (Ex; n = 6). [2−3H]DG, 2-deoxy-[3H]glucose. Arrow, time at which animals were anesthetized and muscles were removed.
Muscle samples were homogenized in 0.5% perchloric acid, centrifuged, and neutralized with KOH. One aliquot of homogenate was counted without further treatment to yield total muscle counts \((\text{U-14C})\text{MN}\) and \((\text{3-O-[\text{3H}]MG})\). A second aliquot of homogenate was treated with Ba(OH)\(_2\) and ZnSO\(_4\) to remove \((2-\text{H})\text{DG}\) and then counted to yield \((\text{U-14C})\text{MN}\) and \((\text{3-}\text{O-[\text{3H}]MG})\). A second aliquot of homogenate was incubated with yeast hexokinase and ATP (as described for plasma samples), treated with Ba(OH)\(_2\) and ZnSO\(_4\) to remove \((2-\text{H})\text{DG}\), and then counted to yield \((\text{U-14C})\text{MN}\) and \((\text{3-}\text{O-[\text{3H}]MG})\) radioactivity. A third aliquot of homogenate was incubated with \((\text{2-3H})\text{DG}\) (anion-exchange chromatography), \((\text{2-3H})\text{DGP}\) synthesis in skeletal muscle (5, 48). In the method generally used to separate phosphorylated and nonphosphorylated \((2-\text{H})\text{DG}\) (anion-exchange chromatography), \((2-\text{H})\text{DG}\) that has been incorporated into glycogen elutes from the column in the same fraction as nonphosphorylated \((2-\text{H})\text{DG}\); this results in an overestimation of this radioactive metabolite (48).

An advantage of the analytic approach utilized in these experiments is that any \((2-\text{H})\text{DG}\) incorporated into glycogen precipitates glycogen both in muscle homogenates and in a glycogen standard. Because \((\text{U-14C})\text{MN}\) is unaffected by analytic methods, radioactivity in the \(14\text{C}\) counting window provided an internal control for each plasma and muscle sample. Tissue glucose was measured after deproteinization with 0.5% perchloric acid by an enzymatic method (27) and is expressed as millimoles per liter muscle water.

Calculations. The fraction of extracellular to total water space in biopsies was calculated by the equation

\[
F_e = \frac{([\text{U-14C}]\text{MN})_i}{([\text{U-14C}]\text{MN})_o}
\]

(1)

where \(F_e\) is the fraction of the tissue water that is extracellular, and the subscripts \(m\) and \(p\) refer to muscle and arterial plasma, respectively. Because mannitol is not metabolized by muscle, \(([\text{U-14C}]\text{MN})_i\) equals the interstitial \((\text{U-14C})\text{MN}\) concentration.

Soleus glucose uptake was assessed by using the glucose metabolic index \(R_g\) (21), which is dependent on the muscle metabolic index \(K_m\) in calculations has no qualitative effect on the result.

\[
R_g = \frac{[\text{(2-3H)DG}]_m}{[(2-\text{H})\text{DG}]_o \int_0^t [\text{(2-3H)DG}]_p \, dt} \cdot [\text{G}]_m
\]

(2)

where \(t = 20\) or 40 min (depending on experiment length) and \([\text{G}]_m\) is the average arterial plasma glucose concentration from \(t = 0\) to 20 or 40 min. The application of 2-DG to muscle water concentration at the sarcolemma or anywhere in the interstitial or intracellular space. This presents a major problem, which in the past has been largely ignored, in interpreting muscle glucose measurements. We have developed a novel approach for calculating limits for the average \([\text{G}]_m\) on the basis of two extreme conditions. In the first calculation of \([\text{G}]_m\), \([\text{G}]_m\) 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 with superscript \(a\)). The second approach for the calculation of the mean \([\text{G}]_m\) assumes that \([\text{G}]_m\) is distributed evenly throughout the intracellular water (denoted with superscript \(b\)). \([\text{G}]_m\) was calculated as

\[
[G]_m^a = [G]_m/F_e
\]

(5)

where \([G]_m\) is the muscle glucose concentration. \([G]_m^b\) was then calculated from Eq. 4. \([G]_m^b\) was calculated as

\[
[G]_m^b = [G]_m - [G]_m^a \cdot (1 - F_e)/F_e
\]

(6)

The solution to Eq. 6 can be substituted into Eq. 4 and then solved for \([G]_m^b\).

A range of 2-5 mM has been reported for the \(K_m\) of GLUT-4 in vitro (39). A value of 3 mM was used in these calculations because it yielded a value for \([G]_m\) of \(-02\) under Bas conditions, which are generally assumed to exist (e.g., 4, 11, 23), and it approximates estimates obtained from muscle venous drainage in vivo (10, 52). The impact of \(K_m\) on the data has been discussed previously (38); briefly, using different values for \(K_m\) in calculations has no qualitative effect on the resulting data, and the impact of using different values of \(K_m\) in the calculations decreases as \(S_i/S_o\) increases. It is notable that the \(K_m\) for muscle glucose transport is unchanged by insulin stimulation or contraction (14, 35, 42). TSGG\(_a\) and TSGG\(_b\) are calculated by using the values of \([G]_m^a\) and \([G]_m^b\) and \([G]_m^a\) and \([G]_m^b\), respectively

\[
\text{TSGG} = [G]_m^b - [G]_m^a
\]

(7)

Statistical analyses. Statistical significance for variables with only one value was determined with one-factor ANOVA,
using Fisher’s protected least significant difference as a post hoc test. For comparison of ranges defined by α- and β-bounds-aries ([G]_im, [G]_om, and TSGG), one-factor ANOVA was used to compare sets of data consisting of both α- and β-values for each group. Also presented in the text, when appropriate, are P values for between-group comparisons (one-factor ANOVA) made between the extremes of the ranges instead of the entire ranges. We took this statistical approach so as not to exclude the possibility that either the α- or β-calculation is more representative of the actual distribution of glucose. Differences were considered statistically significant at P < 0.05. Data are expressed as means ± SE.

RESULTS

Animal characteristics. As shown in Table 1, there was no difference between groups in body weight or in arterial plasma glucose concentration during the experiments. Insulin levels were elevated ~30-fold by the infusion of 20 mU/(kg·min) of insulin, and they decreased ~75% compared with Bas by the end of the 20-min exercise bout. Glucose was infused at a rate of 206 ± 12 µmol/(kg·min) in Ins.

Muscle glucose concentration and R_g. Muscle glucose concentrations in the soleus, gastrocnemius, and superficial vastus lateralis are shown in Table 2. In all three muscles, glucose concentration was significantly decreased compared with Bas in Ins, and the glucose concentration was significantly increased compared with Bas in Ex. Also shown in Table 2 are values for R_g, a measurement of the rate of [2-3H]DGP accumulation. R_g was significantly increased compared with Bas in Ins and Ex in all three muscles. The increment in R_g was greater in Ex than in Ins in soleus and gastrocnemius, whereas R_g was similar in Ins and Ex in the superficial vastus lateralis.

Soleus S_i/S_o and the TSGG. Soleus S_i/S_o was 0.54 ± 0.07 in Bas (Fig. 2). As shown in Fig. 3, the calculated range for [G]_om in Bas was between 4.2 ± 0.3 mM ([G]_im) and 3.6 ± 0.5 mM ([G]_om). Glucose concentration at the inner face of the membrane did not differ significantly from 0 in Bas (0.9 ± 0.4 mM for [G]_im and 0.3 ± 0.2 mM for [G]_om). The calculated TSGG^α and TSGG^β were 3.3 ± 0.5 mM and 3.4 ± 0.6 mM, respectively (Fig. 3).

S_i/S_o was significantly increased in Ex to 0.80 ± 0.05 (Fig. 2). Comparison of data in Ex and Bas emphasize the importance of considering glucose distribution in intracellular and extracellular water when calculating [G]_im, [G]_om, and the TSGG. Although there are significant differences between groups, these are in some cases dependent on whether α- or β-distributions exist. The full range of Ex [G]_im bounded by 7.6 ± 1.6 mM ([G]_im) and 3.3 ± 0.8 mM ([G]_om) was not different from that of Bas (Fig. 3). However, if the glucose distribution in Ex is better represented by the α-assumptions, then [G]_om is significantly higher in Ex than Bas (P < 0.01). The range for Ex [G]_om was increased above the Bas range, with values for [G]_im and [G]_om of 5.2 ± 1.1 and 1.8 ± 0.4 mM, respectively (P < 0.01). The TSGG in Ex was the range bounded by 2.4 ± 0.8 mM (TSGG^α) and 1.5 ± 0.5 mM (TSGG^β) and was significantly decreased compared with Bas (P < 0.05; Fig. 3). If, however, instead of comparing the ranges of TSGG, one assumes that the actual TSGG value is TSGG^α, the TSGG is not decreased compared with Bas (P = 0.18).

Ins was also characterized by a significant increase in S_i/S_o compared with Bas, to a value of 0.81 ± 0.05 (Fig. 2). However, in contrast to the Ex situation, the range for [G]_om in Ins was decreased compared with the Bas range (P < 0.01), with [G]_om equal to 2.2 ± 0.5 mM and [G]_om equal to 1.3 ± 0.2 mM, as shown in Fig. 3.

Table 1. Body weight, arterial plasma glucose concentration, insulin concentration, and glucose infusion rate during experiments

<table>
<thead>
<tr>
<th></th>
<th>Weight, g</th>
<th>Plasma Glucose, mM</th>
<th>Insulin, µU/m</th>
<th>GIR, µmol/(kg·min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bas</td>
<td>302 ± 9</td>
<td>7.6 ± 0.2</td>
<td>29 ± 4</td>
<td></td>
</tr>
<tr>
<td>Ins</td>
<td>299 ± 5</td>
<td>7.5 ± 0.3</td>
<td>839 ± 16*</td>
<td>206 ± 12*</td>
</tr>
<tr>
<td>Ex</td>
<td>299 ± 11</td>
<td>8.0 ± 0.3</td>
<td>6.2 ± 2†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 13, 7, and 6 in basal (Bas), hyperinsulinemia (Ins), and exercise (Ex) groups, respectively. GIR, glucose infusion rate. *Significant differences from Bas. †Significant differences between Ins and Ex.

Table 2. Muscle glucose concentration and muscle glucose uptake index in soleus, gastrocnemius, and superficial vastus lateralis

<table>
<thead>
<tr>
<th></th>
<th>Muscle Glucose, mmol/l</th>
<th>R_g, µmol/(100 g·min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soleus</td>
<td>Gastrocnemius</td>
</tr>
<tr>
<td>Bas</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Ins</td>
<td>0.7 ± 0.2*</td>
<td>0.5 ± 0.1*</td>
</tr>
<tr>
<td>Ex</td>
<td>2.2 ± 0.5†</td>
<td>1.8 ± 0.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 13, 7 and 6 in Bas, Ins, and Ex groups, respectively. R_g, muscle glucose uptake; vastus, superficial vastus lateralis. *Significant differences from Bas. †Significant differences between Ins and Ex.
cannot be determined how the [2-3H]DG is distributed within the total muscle water. However, the fact that the muscle-to-plasma [2-3H]DG ratio is greater than $F_e$ in Ins (in 2 muscles) and Ex (in all 3 muscles) means there must be an accumulation of intracellular [2-3H]DG. Also shown in Table 3 is the muscle glucose-to-plasma glucose ratio, which displayed the same pattern in each muscle: a significant decrease from Bas in Ins, and a significant increase from Bas in Ex. If the muscle [2-3H]DG-to-plasma [2-3H]DG ratio exceeds the muscle glucose-to-plasma glucose ratio, then discrimination occurs between the two substrates such that metabolism of glucose is favored over metabolism of [2-3H]DG. Comparison of the glucose and [2-3H]DG data shows that in soleus, the muscle glucose-to-plasma glucose ratio is lower than that for [2-3H]DG in Bas, Ins, and Ex. In gastrocnemius and superficial vastus lateralis, the muscle glucose-to-plasma glucose ratio is lower than that for [2-3H]DG in Ins and Ex. This suggests that, even in Bas, glucose is preferentially utilized by soleus. This preference becomes stronger in soleus (and becomes detectable in fast-twitch muscle) when the rate of glucose uptake is stimulated, as in Ins and Ex.

**DISCUSSION**

Using a combination of blood and tissue sampling and isotopic techniques, we demonstrated that the importance of the steps involved in the control of skeletal muscle glucose uptake in the soleus is different under basal conditions than during maximal insulin stimulation and exercise, conditions characterized by

![Fig. 3. Soleus glucose concentration ranges (see Calculations) at the outer (top) and inner (middle) sarcolemmal surfaces, and transsarcolemmal glucose gradient (bottom) determined in Bas (n = 13), Ins (n = 7), and Ex (n = 6). Bars are limited by group means ± SE. *Significant differences between ranges.](image)
an increase in glucose flux. Moreover, control is different under maximal insulin conditions compared with exercise in soleus. In Bas, [GI]m is significantly lower than the arterial plasma glucose concentration, and [GI]m is essentially 0, which indicates that glucose delivery and glucose transport are the primary determinants of soleus glucose uptake. In Ins, there was a decrease in the total soleus glucose and the calculated [GI]m, even though arterial glucose was clamped at euglycemia, suggesting that glucose delivery to the muscle is a more important limitation to glucose flux than in Bas. Although [GI]m was not increased in Ins above Bas, there was an increase in intracellular [2-3H]DG, which, for reasons described later, indicates that glucose phosphorylation also may be a site of increased resistance to glucose uptake during hyperinsulinemia than under basal conditions. In Ex, the total soleus glucose concentration was increased, and [GI]m was at least maintained ([GI]m) and perhaps increased ([GI]m) compared with that in Bas. This suggests that glucose delivery to the sarcolemma was sufficient to maintain the high rates of glucose transport. [GI]m and intracellular [2-3H]DG were increased in Ex; this suggests a limitation at glucose phosphorylation in soleus. An accumulation of intracellular [2-3H]DG was also observed in the gastrocnemius and superficial vastus lateralis in Ex; this indicates that phosphorylation is also a site of resistance to glucose uptake during exercise in fast-twitch muscle.

In these experiments, the Ex-induced increments in soleus and gastrocnemius Rg were greater than that for Ins; these results are in agreement with other in vivo data (22). This is inconsistent with measurements made in the isolated soleus (16, 28), where maximal insulin-stimulated glucose transport exceeds contraction-stimulated transport, although in the perfused hindlimb it appears that soleus glucose transport is increased to a similar degree by insulin and contraction (40). It is important to note that different patterns in the relative effects of contraction vs. insulin are observed for different muscles (16, 50). For example, in the extensor digitorum longus, similar increases in maximal insulin- and contraction-stimulated muscle glucose transport are observed in vitro (16, 50). The discrepancy between soleus in vitro findings and in vivo findings reported here and previously (22) demonstrates the functional importance of the increase in glucose delivery for the exercise-induced increment in glucose uptake, as isolated muscle contracted in vitro is not exposed to hemodynamic alterations that accompany exercise in vivo. Treadmill running at the velocity used in this investigation has been shown to increase blood flow in the rat soleus approximately fourfold above resting conditions (20, 26). The perfused capillary surface area during exercise has been estimated to increase approximately four- to tenfold (8). This increase has a major influence on the interstitial diffusion distance of glucose, as a fourfold increase in capillary surface area corresponds to a 50% decrease in diffusion distance in muscle (8). The result of these hemodynamic alterations during exercise is that, despite the large increase in glucose transport and utilization, the average interstitial glucose concentration does not decrease compared with Bas and may even exceed that of Bas. Studies that used microdialysis to determine the interstitial glucose concentration during dynamic exercise in humans (D. A. MacLean, J. Bangsbo, and B. Saltin, unpublished observations) and electrically stimulated contraction in rats (31) have indicated that the glucose gradient between arterial plasma and interstitial water is narrowed during exercise compared with during resting conditions. The maintenance of the extracellular glucose concentration during exercise, in conjunction with the direct effects on muscle glucose transport, allows glucose uptake to increase to a far greater extent in the soleus and gastrocnemius than during exposure to even a maximally effective insulin concentration.

In contrast to Ex, during Ins, there was a significant fall in the glucose concentration at the outer face of the sarcolemma ([GI]om) in soleus and a fall in total muscle glucose in all three muscles. Total muscle glucose has been previously reported either to fall or to be unchanged in rat (38, 51) and human (23, 41, 46) skeletal muscle in response to hyperinsulinemia. Studies using microdialysis in an attempt to estimate changes in the average interstitial glucose concentration have reported findings consistent with those reported here. A fall in dialysate glucose concentration of nearly 50% has been reported in rat skeletal muscle when the insulin-infusion rate was increased from 8 to 20 mU/ (kg·min) (17). These data suggest that, during euglycemic hyperinsulinemia, glucose delivery is not sufficient to maintain the extracellular portion of the TSGG. Insulin has been reported to increase skeletal muscle blood flow and capillary perfusion (see Ref. 2 for review). It does not appear that these potential hemodynamic shifts are sufficient to maintain the extracellular glucose concentration when the membrane permeability is increased to the degree observed during hyperinsulinemia.

Recently, 13C-NMR has been used to measure total muscle glucose and calculate the intracellular glucose concentration in human (43) and rat (4) skeletal muscle with the goal of differentiating control between transport and phosphorylation, such as has been done many times by using muscle biopsies (23, 38, 41, 46, 51). The NMR approach is an improvement over biopsy measurements of muscle glucose (provided that the experimental protocol can accommodate mass infusions of enriched glucose) in that tissue need not be excised. However, it is not a conceptual advance in the difficult problem of calculating intracellular glucose from total glucose values. The assumptions required to determine intracellular glucose from NMR-measured muscle glucose are exactly the same as those for biochemically determined muscle glucose in biopsy samples.

Another interesting finding of these experiments was that in both Ex and Ins, there was an increase in the soleus and gastrocnemius concentration of [2-3H]DG relative to the plasma concentration. In superficial vastus lateralis, accumulation of muscle [2-3H]DG was
observed in Ex. It is clear from the demonstration that the muscle [2-3H]DG-to-plasma [2-3H]DG ratio exceeded the fraction of muscle water that is extracellular (Fw) that intracellular [2-3H]DG was increased. Although the presence of intracellular free [2-3H]DG has been previously reported under conditions of hyperinsulinemia (18) and exercise (49), the method we used to separate [2-3H]DG and [2-3H]DGP [precipitation with Ba(OH)₂ and ZnSO₄ instead of anion-exchange chromatography] does not overestimate [2-3H]DG, as described in MATERIALS AND METHODS. Also presented here is a comparison of the muscle [2-3H]DG and glucose concentration. The rationale for this comparison is that the muscle-to-plasma [2-3H]DG ratio will be less than or equal to the muscle glucose-to-plasma glucose ratio unless glucose is preferentially utilized, in which case [2-3H]DG in muscle will accumulate disproportionately to muscle glucose. What we found was that in soleus, the muscle-to-plasma [2-3H]DG ratio exceeded that for glucose in Bas, and that difference was increased by Ex and Ins; this indicates that glucose is favored as a substrate. This same phenomenon was observed in gastrocnemius and superficial vastus lateralis in Ex and Ins. In studies of glucose metabolism that use [2-3H]DG, a correction factor is often employed to account for differences in the affinity of the components of the glucose uptake system for glucose and [2-3H]DG, which is referred to as a lumped constant (LC) (12, 47).

Investigators working on skeletal muscle glucose uptake have for the most part assumed that LC approximates 1 (e.g., Ref. 21), on the basis of data obtained in vitro (12) although there also are data that suggest that the LC for isolated skeletal muscle is closer to 0.5 (30) or have at least assumed that the LC is unchanged by various conditions such as exercise (19, 22, 34), hyperinsulinemia (21), or insulin resistance (25). However, investigators working on cardiac muscle, which expresses the same glucose transporter and hexokinase proteins as skeletal muscle, have used changes in the LC that occur under different conditions to indicate alterations in the processes that limit the rate of glucose uptake (36, 45). The rationale for this is that glucose and [2-3H]DG delivery to the muscle should be identical, glucose transport does not have a strong preference for glucose or [2-3H]DG (1, 6), and phosphorylation by hexokinase II favors glucose, especially when that hexokinase is associated with mitochondria (3, 45). Because glucose uptake consists of processes arranged in series, changes in the LC of the entire system reflect shifts in the influence of the steps that control the rate of glucose uptake. Relevant to this, studies in the perfused, working rat heart have shown that increasing the perfusate insulin concentration decreases the LC (36, 45), suggesting that glucose phosphorylation plays a more important role in the control of glucose uptake than under basal conditions. Our data suggest that this is exactly what is occurring in skeletal muscle.

The data presented here show that glucose phosphorylation is more influential in controlling the rate of muscle glucose uptake in Ex. Although intracellular free glucose cannot be measured directly, our isotopic data in the soleus showed an increase in glucose at the inner sarcolemmal surface, and we were able to demonstrate an apparent increase in the discrimination between glucose and [2-3H]DG during Ex in all three muscles. For glucose phosphorylation to play an important role in the determination of the rate of glucose uptake, the rate of inward glucose transport must exceed the muscle phosphorylation capacity. Whether this is because the transport rate is greater than the maximal hexokinase activity or because of the high sensitivity of hexokinase to inhibition by glucose-6-phosphate (G-6-P) (inhibition constant (Ki) = 0.16 mM (15)) is unknown. It has been demonstrated previously that during moderate-intensity exercise, there is an approximately twofold increase in G-6-P, at least during the first 40 min of exercise (24); this increase is thought to be due to rapid glycogenolysis. This issue is difficult to resolve because of problems in relating properties of an isolated enzyme to G-6-P measurements on tissue biopsies, especially since the lability of the glycogen pool makes measurement of G-6-P concentration very difficult, and compartmentalization of hexokinase and/or G-6-P within the cell may dramatically alter the effective concentrations of G-6-P at the enzyme.

Under the maximal insulin conditions used here, glucose transport is increased to the extent that pre- and posttransport processes limit the rate of glucose uptake in soleus. The results from measurements of glucose countertransport and muscle glucose suggest that glucose delivery becomes a greater factor, as the total muscle glucose concentration was decreased compared with Bas and [Glum] was not different from 0, consistent with the findings of other investigators (4, 51). The [2-3H]DG data indicate that glucose phosphorylation becomes more important, based on the increase in discrimination between metabolism of [2-3H]DG and glucose. Both glucose delivery and glucose phosphorylation may become more important factors under these conditions of maximal insulin stimulation. Phosphorylation may exert more control over the rate of glucose uptake, but if there is less mass flux through the system because glucose delivery cannot keep pace with utilization, intracellular glucose will not rise. If this hyperinsulinemia is able to increase membrane glucose permeability to a sufficient degree, the pre- and posttransport phenomena, by determining the TSGG, could both be important loci for determining the rate of glucose uptake.

In these studies, isotopic steady state was not attained in the 100-min equilibration period in the gastrocnemius and superficial vastus lateralis (38). Analysis of muscle glucose concentrations in these muscles revealed a similar response to that observed in soleus in muscle glucose concentration: a fall compared with Bas in Ins, and an increase above Bas in Ex. Also, apparent discrimination occurred between [2-3H]DG and glucose in Ins and Ex in the gastrocnemius and superficial vastus lateralis. This suggests, at least in a qualitative sense, that in fast-twitch as well as slow-twitch muscle,
pre- and posttransport processes may limit maximal insulin-stimulated muscle glucose uptake. The fold increase in \( R_g \) above Bas was much greater in gastrocnemius and superficial vastus lateralis than in the soleus in response to both Ins and Ex. This is most likely due to the fact that the soleus, as a postural muscle, is activated to some degree even under basal conditions. The responses to Ins and Ex were similar in gastrocnemius and soleus in that the Ex \( R_g \) was greater than that in Ins, while Ins and Ex \( R_g \) were similar in the superficial vastus lateralis. Whether maximal insulin and exercise result in similar increments in sarcomeral glucose transporter content in these two fast-twitch muscles has not yet been determined.

In conclusion, stimuli that increase glucose transport make pretransport (glucose delivery) and posttransport (glucose phosphorylation) steps more important in the control of the rate of glucose uptake in skeletal muscle. With maximal insulin, both glucose delivery and phosphorylation become more important in determining the rate of glucose uptake than under basal conditions. During moderate-intensity exercise, the cardiovascular alterations that accompany exercise (increased muscle blood flow and increased capillary perfusion) appear to be sufficient to maintain glucose delivery, allowing for much higher rates of glucose uptake than during maximal insulin in the soleus. Under these conditions, glucose phosphorylation plays a greater role in influencing the rate of muscle glucose uptake. These results emphasize the importance of considering glucose delivery and intracellular metabolism when considering the regulation of skeletal muscle glucose uptake.

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