Overexpression of hexokinase II increases insulin- and exercise-stimulated muscle glucose uptake in vivo

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Halseth, Amy E., Deanna P. Bracy, and David H. Wasserman. Overexpression of hexokinase II increases insu-
The hypothesis of this investigation was that glucose uptake would be increased in skeletal muscle of transgenic mice (TG) overexpressing hexokinase II (HK II) compared with their nontransgenic littermates (NTG) during euglycemic hyperinsulinemia and treadmill exercise. For insulin experiments, catheters were surgically implanted in the jugular vein and carotid artery for infusions and sampling, respectively. Conscious mice underwent experiments 5 days later in which 4 mU·kg⁻¹·min⁻¹ insulin and variable glucose (n = 7 TG and n = 7 NTG) or saline (n = 5 TG and n = 4 NTG) was infused for 140 min. Over the last 40 min of the experiments, 2-deoxy-
³H]glucose ([2-³H]DG) was infused, after which muscles were removed. For the exercise experiments, jugular vein cathers were surgically implanted. Five days later, mice received a bolus of [2-³H]DG and then remained sedentary (n = 6 TG and n = 8 NTG) or ran on a motorized treadmill (n = 12 TG and n = 8 NTG) for 30 min. TG and NTG had similar muscle [2-³H]DG 6-phosphate ([2-³H]DG-P) accumulation in the basal state (P > 0.05). In the hyperinsulinemic experiments, TG required ~25% more glucose to maintain euglycemia (P < 0.05), and muscle [2-³H]DG-P accumulation normalized to infused [2-³H]DG was similarly increased (P < 0.05). In the exercise experiments, muscle [2-³H]DG-P accumulation was significantly greater in TG than NTG (P < 0.05). In conclusion, we did not detect an effect of HK II overexpression on muscle [2-³H]DG-P accumulation under basal conditions. Hyperinsulinemia and exercise shift the control of muscle glucose uptake so that phosphorylation is a more important determinant of the rate of this process.

transgenic mice; glucose phosphorylation; 2-deoxyglucose

SKELETAL MUSCLE GLUCOSE uptake consists of three processes: delivery of glucose to the muscle, transport of glucose across the sarcolemma, and phosphorylation of that glucose intracellularly by hexokinase (HK). Delivery of glucose is determined by skeletal muscle blood flow and the diffusion distance between capillaries and the sarcolemma. Glucose transport occurs via facilitated diffusion by a member of the GLUT family. GLUT-1, which is expressed at low levels in skeletal muscle and is constitutively localized to the sarcolemma, is thought to mediate transport under basal conditions. When the rate of glucose uptake is stimulated by insulin or contraction, GLUT-4 is translocated to the sarcolemma from an intracellular location, thereby increasing the membrane permeability to glucose and the rate of transport (6, 29). Phosphorylation of glucose to yield glucose 6-phosphate (G-6-P) by HK (primarily HK II in skeletal muscle, Ref. 20) is essential to the process of glucose uptake because it serves to trap the glucose inside the cell and maintains the downhill glucose gradient from capillary to intracellular water. Although each of these processes has been studied in isolation, the role of each of these processes in the determination of the rate of muscle glucose uptake in vivo is not well understood.

One approach that has been taken in recent years to gain insight into the control of glucose uptake has been the generation of transgenic mice that overexpress proteins that mediate the steps in this process. A number of groups have produced glucose transporter-overexpressing mice, and the results from these experiments have indicated that this intervention increases muscle glucose uptake under various conditions. Both GLUT-1- and GLUT-4-overexpressing mice have lower fasting and fed blood glucose concentrations than nontransgenic controls (18, 24, 25, 31). Basal rates of muscle glucose uptake are increased in GLUT-1 overexpressers but are not further increased by insulin (17, 18, 24), even though GLUT-4 levels are normal. GLUT-4 overexpressers typically show increased basal and insulin-stimulated glucose transport, decreased plasma glucose excursions during glucose tolerance tests (12, 16, 31), and increased whole body glucose disposal during euglycemic clamp experiments (17, 25, 30). Together, these experiments emphasize the importance of muscle glucose transport in determining the rate of basal and insulin-stimulated whole body and muscle glucose uptake. However, glucose transport may not be the sole site of control, as shown by data from HK II-overexpressing mice (3). Initial studies on these mice showed no differences between transgenic and nontransgenic mice in fasted or fed glucose or insulin, nor were there any differences in the glycemic profile when oral or intrave-
rous glucose tolerance tests were performed (3). In contrast, when the muscles were removed from the mice and the in vitro response to insulin and glucose was studied, transgenic animals did display greater insulin-stimulated 2-deoxyglucose uptake, suggesting that phosphorylation also is important in determining the rate of muscle glucose uptake. Therefore, to deter-
mine the role of glucose phosphorylation in the control of this process in vivo, euglycemic hyperinsulinemic clamp experiments and exercise experiments, in conjunction with muscle-specific measurements of glucose uptake with 2-deoxy-[³H]glucose ([2-³H]DG), were performed in mice overexpressing HK II in skeletal muscle (TG) and in nontransgenic control mice (NTG). We

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hypothesized that glucose phosphorylation would not be a site of control of muscle glucose uptake under basal conditions, but the stimulation of the rate of glucose transport by either of these interventions would result in glucose phosphorylation becoming an important site of control.

METHODS

Mouse maintenance, surgical, and experimental procedures. All procedures were preapproved by the Vanderbilt University Animal Care and Use Subcommittee. Male FVB/NJ mice that overexpress HK II in skeletal muscle were originally obtained from Dr. David E. Moller. The construct used to generate these animals contained the muscle creatine kinase promoter fused to the human HK II cDNA (22), as previously described (3). Expression of this construct results in up to an eightfold increase in HK activity, depending on the muscle. Subsequent generations of animals were bred at Vanderbilt University with wild-type female FVB/NJ mice obtained from Jackson Labs (Bar Harbor, ME). Mice were maintained in microisolator cages with their same gender littermates and fed standard rodent chow ad libitum. TG were distinguished from NTG with the polymerase chain reaction (PCR) on genomic DNA isolated from the tails of mice. PCR primers that flanked a 302 bp intron of the HK II gene were used. Because TG were generated with cDNA, the resulting PCR product differed in size between NTG and TG (NTG, 586 bp; TG, 284 bp).

Hyperinsulinemic-euglycemic clamp experiments. Insulin experiments were performed on mature mice (5–6 mo of age), as the larger size of these mice facilitated implantation of the carotid artery catheter. Mice were anesthetized with a mixture of rompun and ketamine (2 and 2.5 mg/mouse, respectively). The left common carotid artery was catheterized with a two-part catheter consisting of PE-10 (portion inserted in artery) and Silastic (0.025 in outer diameter (OD)). The right jugular vein was catheterized with a one-part Silastic catheter (0.025 in OD). The free ends of catheters were tunneled under the skin to the back of the neck, where they were attached via stainless steel connectors to lines made of Micro-Renathane (0.033 in OD), which were exteriorized and sealed with stainless steel plugs. Lines were heparinized daily with saline containing 200 U/ml of heparin and 5 mg/ml of ampicillin. Animal weight was monitored daily, and animals were used for experiments once they were within 2 g of presurgery body weight (5–7 days). Animals were housed individually after surgery.

Conscious 5-h-fasted mice were placed in 2,000-ml plastic beakers, and Micro-Renathane (0.033 in OD) tubing was connected to infusion syringes and the catheter leads. After the mice were allowed time to adapt to their surroundings (30 min), a baseline sample was drawn for measurement of hematocrit, glucose, and insulin. An infusion of saline (n = 5 TG and n = 4 NTG) or 4 nU·kg⁻¹·min⁻¹ of insulin (n = 7 TG and n = 7 NTG) was then begun (t = 0 min), and glucose (50 g/100 ml) was infused in insulin experiments as necessary to maintain euglycemia on the basis of feedback from frequent arterial glucose measurements (5–15 µl; HemoCue, Mission Viejo, CA). At t = 100 min, an infusion of [2-3H]IDG was begun and continued until t = 140 min (0.18 µCi/min). Infusion periods of 100 and 40 min were chosen on the basis of our previous experiments in the rat, because with these intervals whole body glucose disposal was constant during the 40-min [2-3H]IDG infusion period (21). During this period, larger arterial blood samples (50 µl) were drawn for the measurement of plasma [2-3H]IDG concentration. Total blood volume taken during experiments was ~400 µl; this resulted in a fall in hematocrit from ~38 to ~33%, which was similar in all groups. [2-3H]IDG is transported into cells and phosphorylated to yield [2-3H]DGP, which is trapped in muscle. At t = 140 min, animals were anesthetized with an intravenous infusion of pentobarbital sodium, and soleus (SOL), gastrocnemius (GAS), and superficial vastus lateralis (VAS) muscles were excised and rapidly frozen in liquid nitrogen. These muscles were chosen because they display different degrees of HK II overexpression (SOL, −3× overexpression; GAS, −5× overexpression; VAS, −7× overexpression).

Exercise experiments. Studies were performed on 5-h-fasted mice in the sedentary state (n = 6 TG and n = 8 NTG) and during exercise (n = 12 TG and n = 8 NTG). A separate group of exercised mice was fasted for 18 h (n = 6 TG and n = 6 NTG) before experiments to study muscle glucose uptake in a more glycogen-depleted state (30). Mice were maintained in an identical fashion to those used for hyperinsulinemic-euglycemic clamp experiments, except that younger mice were used (3 mo old), and during surgery, a catheter was implanted only in the jugular vein for infusion purposes. Three days postsurgery, all animals were acclimated to treadmill running with one 10-min bout of exercise (0.5–0.7 mph, 0% grade).

Exercise and corresponding sedentary control experiments were performed 5 days after surgery. After a 5- or 18-h fast, an intravenous bolus of [2-3H]IDG (12 µCi) was given. Animals then either remained sedentary or began running on a treadmill (0.7 mph, 0% grade). This work rate results in a rate of oxygen consumption that is ~85% of maximum in the mouse (8). After 30 min, animals were anesthetized with pentobarbital sodium, and capillary blood was sampled from a nick in the tail for measurement of blood glucose and plasma [2-3H]IDG concentration, and SOL, GAS, VAS, diaphragm (DIA), and brain were excised and frozen. DIA and brain served as controls for differences in the integrated plasma [2-3H]IDG concentration over the course of the experiments, as HK II activity is not higher in TG than NTG DIA, and HK II is not expressed to a significant degree in brain (23). This simplified the experiments by eliminating the need for arterial blood sampling during the exercise bout for subsequent tracer analysis.

Processing of plasma and muscle samples. Immunoreactive insulin was measured with a double antibody method (19). Plasma was deproteinized with barium hydroxide [Ba(OH)₂, 0.3%] and zinc sulfate (ZnSO₄, 0.3%), and [2-3H]IDG radioactivity was then determined by liquid scintillation counting (Beckman LS 5000TD, Beckman Instruments) with Ecolite+ as scintillant. Muscle samples were weighed, homogenized in 0.5% perchloric acid, and centrifuged, and supernatants were neutralized with KOH. One aliquot of homogenate was counted without further treatment to yield total muscle counts ([2-3H]IDG and [2-3H]DGP). A second aliquot of homogenate was treated with Ba(OH)₂ and ZnSO₄ to remove [2-3H]DGP and then was counted to yield [2-3H]IDG radioactivity. Although most [2-3H]IDG transported into the cell remains in the cell as [2-3H]DGP, it has recently been demonstrated that [2-3H]IDP can also serve as a substrate for glycogen synthesis in skeletal muscle (5, 32). As described previously (10), an advantage of the analytic approach utilized in these experiments is that any [2-3H]IDG incorporated into glycogen will be measured in the same fraction as free [2-3H]IDGP. This avoids the underestimation of [2-3H]IDP that occurs if ion exchange methods are used to separate [2-3H]IDG and [2-3H]IDP, because those methods do not account for [2-3H]IDP incorporated in glycogen (32).
Muscle glycogen concentration was determined with a standard method (2) on muscle isolated from separate groups of TG and NTG mice, fasted either 5 h or 18 h before sample collection. Briefly, samples were homogenized in 0.03 M HCl, heated at 80°C, and precipitated on chromatography paper in 66% ethanol. Glycogen was then hydrolyzed with amyloglucosi- dase in 0.04 M NaOAc, the resulting solution was lyophi- lized and reconstituted in 1/10 volume, and glucose concentra- tions were measured on an automated analyzer (Monarch Analyzer, Instrumentation Laboratory, Lexington, MA).

Calculations. In insulin and corresponding basal control experiments, [2-3H]DGP accumulation in muscle was calculated in three ways: 1) as the uncorrected counts (dpm/mg tissue), 2) normalized between animals with the integrated [2-3H]DG concentration of the plasma during the 40-min [2-3H]DG infusion period, and 3) normalized between ani- mals with the [2-3H]DGP concentration of the infusate. In exercise and corresponding sedentary control experiments, [2-3H]DGP accumulation in muscle was normalized between animals with [2-3H]DGP in brain, as these animals were not fitted with an arterial catheter to allow for plasma sampling. In each case, a correction factor was determined for each animal by dividing either the integrated plasma [2-3H]DG concentration, infusate [2-3H]DG concentration, or brain [2-3H]DGP content in that animal by the average for all animals. The accumulation of [2-3H]DGP in each muscle of that animal was then divided by the appropriate correction factor.

Statistical analysis. Differences between groups were deter- mined with Student’s t-test or repeated-measures ANOVA followed by contrasts as a post hoc test. The significance level was set at P < 0.05.

RESULTS

Hyperinsulinemic clamp experiments. All groups con- sisted of mixtures of male and female mice, and there were no differences between groups in body weight (data not shown). Analysis of the data did not display differences between the responses of male and female mice; therefore, data were combined. Preexperiment blood glucose (NTG, 141 ± 5 mg/dl; TG, 140 ± 6 mg/dl) and insulin concentrations (NTG, 15.5 ± 3.6 µU/ml; TG, 20.4 ± 3.2 µU/ml) did not differ between groups. Shown in Table 1 are the muscle [2-3H]DGP contents after the basal experiments, which did not differ between TG and NTG in any muscle studied. There were also no differences in muscle [2-3H]DGP accumulation between TG and NTG under basal conditions if data are normalized for the infused [2-3H]DG concentration (NTG: 289,300 ± 9,250 dpm/µl infusate; TG: 286,105 ± 5,550 dpm/µl infusate) or the integrated plasma [2-3H]DG concentrations over the course of the experi- ments (NTG: 21,680 ± 2,314 dpm·min⁻¹·µl⁻¹; TG: 23,014 ± 4,786 dpm·min⁻¹·µl⁻¹), as shown in Table 2.

During the hyperinsulinemic-euglycemic clamp experi- ments, there were no differences in insulin concentration (NTG, 73 ± 15 µU/ml; TG, 58 ± 15 µU/ml) or blood glucose concentration between TG and NTG (Fig. 1). The glucose infusion rate necessary to main-

<table>
<thead>
<tr>
<th>Muscle</th>
<th>NTG</th>
<th>TG</th>
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<tr>
<td>SOL</td>
<td>559 ± 255</td>
<td>826 ± 243</td>
</tr>
<tr>
<td>GAS</td>
<td>95 ± 33</td>
<td>98 ± 12</td>
</tr>
<tr>
<td>VAS</td>
<td>68 ± 22</td>
<td>80 ± 25</td>
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</table>

Table 1. Muscle [2-3H]DGP content following basal and hyperinsulinemic-euglycemic experiments in TG and NTG

Data are means ± SE; n = 4, 5, 7, and 7 for basal nontransgenic controls (NTG), basal transgenic (HKII overexpressing mice; TG), insulin-stimulated NTG, and insulin-stimulated TG, respectively. SOL, GAS, VAS, soleus, gastrocnemius, and superficial vastus lateralis muscles, respectively. *Significantly greater 2-deoxy-[3H]glucose 6-phosphate ([2-3H]DGP) accumulation (dpm/mg tissue) in TG than NTG (P < 0.05).

final 10 min, infusion rates were not different between TG and NTG. Shown in Table 1 is the muscle [2-3H]DGP content (not normalized) after the hyperinsulinemic-euglycemic experiments, which was significantly greater (P < 0.05) in TG than in NTG in SOL, GAS, and VAS. There were no significant differences between NTG and TG in infusate [2-3H]DG concentration (NTG: 264,325 ± 10,410 dpm/µl infusate; TG: 248,520 ± 11,515 dpm/µl infusate) or integrated plasma [2-3H]DG concentration (NTG: 14,170 ± 1,248 dpm·min⁻¹·µl⁻¹; TG: 14,869 ± 1,555 dpm·min⁻¹·µl⁻¹). Table 2 shows [2-3H]DGP accumulation in TG expressed relative to NTG. In SOL, there was greater [2-3H]DGP accumulation in TG than NTG regardless of how data were normalized. For both GAS and VAS, [2-3H]DGP accumulation not normalized and [2-3H]DGP accumulation normalized to infusate were greater in TG than NTG (P < 0.05). When integrated plasma [2-3H]DG concentration was used to normalize muscle [2-3H]DGP content between groups, [2-3H]DGP accumulation was still greater in TG in SOL.

Table 2. [2-3H]DGP accumulation during basal and hyperinsulinemic-euglycemic experiments in TG expressed relative to NTG

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<tr>
<td>SOL</td>
<td>1.48 ± 0.44</td>
<td>0.99 ± 0.40</td>
<td>0.96 ± 0.28</td>
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<tr>
<td>GAS</td>
<td>1.03 ± 0.13</td>
<td>1.10 ± 0.22</td>
<td>1.06 ± 0.12</td>
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<tr>
<td>VAS</td>
<td>1.17 ± 0.37</td>
<td>1.25 ± 0.40</td>
<td>1.19 ± 0.35</td>
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Insulin stimulated

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<tr>
<td>SOL</td>
<td>1.38 ± 0.13*</td>
<td>1.38 ± 0.14*</td>
<td>1.52 ± 0.22*</td>
</tr>
<tr>
<td>GAS</td>
<td>1.31 ± 0.13*</td>
<td>1.13 ± 0.12*</td>
<td>1.35 ± 0.18*</td>
</tr>
<tr>
<td>VAS</td>
<td>1.44 ± 0.21*</td>
<td>1.30 ± 0.14*</td>
<td>1.57 ± 0.31*</td>
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</table>

Data are means ± SE. [2-3H]DGP accumulation in TG is expressed relative to NTG (n = 7). [2-3H]DGPbaseline is derived from data in Table 2. Data in [2-3H]DGP AUC column are normalized for area under plasma [2-3H]DG curves. Data in [2-3H]DGPfinal column are normalized for [2-3H]DG infusion concentration. See CALCULATIONS for details. *Significantly greater [2-3H]DG accumulation in TG than NTG (P < 0.05) normalized in identical manner.
but was no longer significantly greater in TG than NTG in GAS (P = 0.27) or VAS (P = 0.09).

Exercise experiments. As was the case for the hyperinsulinemic clamp experiments, male and female mice were used for exercise experiments, and responses were not different depending on gender. There were no differences between groups in body weight in 5-h-fasted mice, whereas the 18-h-fasted groups tended to be 2–3 g lighter because of the overnight fast (data not shown). As shown in Table 3, at the end of experiments, blood glucose concentration was not significantly different between sedentary TG and NTG from 20 to 130 min during experiments (P < 0.05–0.005).

Table 3. Blood glucose and plasma [2-3H]DG concentrations after 30-min sedentary period or 30 min of treadmill exercise in TG and NTG

<table>
<thead>
<tr>
<th>Blood [Glucose], mg/dl</th>
<th>Plasma [2-3H]DG, dpm/µl</th>
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<tr>
<td><strong>Sedentary</strong></td>
<td></td>
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<tr>
<td>NTG</td>
<td>129 ± 6</td>
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<tr>
<td>TG</td>
<td>110 ± 11</td>
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<tr>
<td><strong>Exercise (5 h fasted)</strong></td>
<td></td>
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<tr>
<td>NTG</td>
<td>126 ± 22</td>
</tr>
<tr>
<td>TG</td>
<td>111 ± 6*</td>
</tr>
<tr>
<td><strong>Exercise (18 h fasted)</strong></td>
<td></td>
</tr>
<tr>
<td>NTG</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>TG</td>
<td>102 ± 11</td>
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</table>

Data are means ± SE. Plasma [2-3H]DG concentrations are normalized for [2-3H]DG concentration of infusate. *Value significantly different from corresponding NTG (P < 0.01–0.05).
VAS, muscles in which muscle glycogen concentrations were unchanged between the 5-h- and 18-h-fasted states, greater \([2-3^H]DGP\) accumulation was observed in TG than in NTG (NTG GAS: 1,800 ± 214 dpm/mg; TG GAS: 3,614 ± 636 dpm/mg; NTG VAS: 809 ± 191 dpm/mg; TG VAS: 1,727 ± 218 dpm/mg; \(P < 0.05\)), but the increase in TG compared with NTG was similar to that during exercise in the 5-h-fasted state. DIA \([2-3^H]DGP\) accumulation was not significantly different between TG and NTG (NTG: 5,095 ± 1,082 dpm/mg; TG: 7,323 ± 1,073 dpm/mg).

**DISCUSSION**

These results demonstrate that in conscious, chronically catheterized mice, endogenous HK activity appears to be sufficient to phosphorylate glucose that enters the cell in the basal state; therefore, this step does not appear to play a limiting role in determining the rate of muscle glucose uptake under this condition. During moderate-intensity exercise, the overexpression of HK II in skeletal muscle results in increased muscle glucose uptake, as assessed by \([2-3^H]DGP\) accumulation normalized to \([2-3^H]DGP\) in brain. During hyperinsulinemic euglycemia, HK II overexpression resulted in significant alterations in whole body glucose metabolism, as the glucose infusion rate needed to maintain euglycemia was significantly increased in TG, presumably due to increased glucose uptake by skeletal muscle. When \([2-3^H]DGP\) accumulation was used to assess muscle glucose uptake directly, the results generally supported the finding that glucose phosphorylation can be a significant site of resistance to insulin-stimulated skeletal muscle glucose uptake.

It was surprising that the manner in which muscle \([2-3^H]DGP\) accumulation was normalized between animals affected the magnitude of the difference in \([2-3^H]DGP\) accumulation between TG and NTG. In the SOL, \([2-3^H]DGP\) accumulation was higher in TG than in NTG under hyperinsulinemic-euglycemic conditions independent of the manner in which uptake was normalized. In the GAS and VAS, however, when the integrated plasma \([2-3^H]DG\) value was used to normalize uptake between animals in these experiments, \([2-3^H]DGP\) accumulation was no longer significantly greater in TG than in NTG. Because glucose clearance was higher in TG than in NTG and more \([2-3^H]DGP\) was present in muscle, one would predict a smaller integrated plasma \([2-3^H]DG\) value in TG at identical \([2-3^H]DG\) infusion rates. Instead, this value was similar in TG and NTG. One explanation for this could be that nonmuscle tissue in TG utilized \([2-3^H]DG\) at a lower rate, but because the glucose infusion rate was higher in TG, this seems unlikely. The \([2-3^H]DG\) infusion period coincided with the interval during which the glucose infusion rate was declining in TG, which may have lessened the difference between groups to the degree that we were no longer able to detect a difference. It may also be that the small sample volume and infrequent measurements of plasma \([2-3^H]DG\) concentrations...

<table>
<thead>
<tr>
<th>SOL</th>
<th>GAS</th>
<th>VAS</th>
<th>DIA</th>
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<tr>
<td>NTG</td>
<td>12.2 ± 2.6</td>
<td>13.2 ± 1.5</td>
<td>13.6 ± 1.9</td>
</tr>
<tr>
<td>TG</td>
<td>10.8 ± 2.5</td>
<td>9.8 ± 1.2</td>
<td>13.1 ± 1.6</td>
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<tr>
<td>NTG</td>
<td>6.8 ± 1.0*</td>
<td>9.6 ± 1.6</td>
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<tr>
<td>TG</td>
<td>5.5 ± 0.6*</td>
<td>8.9 ± 1.4</td>
<td>11.0 ± 2.0</td>
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*Significantly different from corresponding 5-h-fasted group \((P < 0.05)\). There were no significant differences between muscle glycogen concentrations \((\mu mol·glucose·equivalents·1·g·wet·wt.·1)\) in TG and NTG.

**Table 4. Muscle glycogen concentration in SOL, GAS, VAS, and DIA after a 5- or 18-h fast in TG and NTG**
In vivo muscle glucose uptake in HK II-overexpressing mice.

Muscle glucose uptake during exercise in 5-h-fasted mice was ~40% greater in TG than NTG GAS and ~80% greater in TG than NTG VAS, the two muscles studied with the highest degree of HK II overexpression (~5-fold and ~7-fold, respectively). In SOL, which overexpresses HK II approximately threefold, uptake in TG and NTG was similar. In none of these muscles was the increment in HK II overexpression matched by a comparable increment in [2-3H]DGP accumulation. Because HK II is so sensitive to inhibition by its reaction product, G-6-P (inhibition constant = 0.16 mM, Refs. 1 and 9), we hypothesized that high G-6-P concentrations formed from exercise-induced glycogenolysis (14) may inhibit both the endogenous and the transgenically expressed HK II. To address this, a group of mice were exercised after a fast that reduced muscle glycogen in the SOL by ~50%, an intervention that has been shown to blunt the contraction-induced increase in muscle G-6-P (II). Under these conditions, the multiples of increase in [2-3H]DGP accumulation in TG compared with NTG in SOL (~2- to 3-fold) nearly reached the degree of HK II overexpression (~3-fold). In the muscles in which glycogen depletion was not observed, the increment in muscle glucose uptake in TG compared with NTG was similar in 5- and 18-h-fasted mice. There is an explanation besides glycogenolysis-derived G-6-P inhibition of HK, however. During exercise in a more glycogen-depleted state, glucose transporter translocation may be increased. Consistent with this are the greater increases in GLUT-4 translo-

One reason that glucose transport is frequently assumed to be the only rate-limiting step in muscle glucose uptake is because of the inability of investigators to measure free intracellular glucose (4, 13). There are a number of significant problems with estimates of intracellular glucose that severely limit conclusions drawn from these measurements. To obtain a value for intracellular glucose, total muscle glucose must first be measured either biochemically or, more recently, with 

Even if a positive value is obtained for the mass of glucose present in the intracellular space, it is still necessary to know the intracellular volume of distribution of glucose to determine the glucose concentration. The glucose concentrations that would actually be of interest for determining the roles of glucose transport and phosphorylation are those at the outer and inner surfaces of the sarcolemma, which cannot be determined from glucose measurements alone. Therefore, studies of the rate-limiting step for muscle glucose uptake on the basis of the calculation of "intracellular glucose" (4, 13, 28, 33) must be evaluated critically.

Data acquired with different techniques strongly support the concept that the control of the insulin-stimulated rate of muscle glucose uptake is distributed, not restricted to a single rate-determining step. Experiments performed with glucose countertransport and biopsy glucose measurements during moderate hyperinsulinemia in the rat (21) support the hypothesis that glucose transport is the primary determinant of glucose uptake during basal conditions. With progressive increases in insulin, the control exerted by transport is decreased such that at slightly supraphysiological insulin concentrations, the glucose gradient across the sarcolemma is essentially zero, indicating that the rate of glucose uptake is being controlled by the pre- and posttransport steps (21).

One reason that glucose transport is frequently assumed to be the only rate-limiting step in muscle glucose uptake is because of the inability of investigators to measure free intracellular glucose (4, 13). There are a number of significant problems with estimates of intracellular glucose that severely limit conclusions drawn from these measurements. To obtain a value for intracellular glucose, total muscle glucose must first be measured either biochemically or, more recently, with 

As this value is the sum of intracellular and extracellular glucose, the extracellular glucose must be subtracted from the total, which requires knowledge of the concentration of glucose in the extracellular water. In theory, an improvement has been made to this technique by using a microdialysis-derived average value for the extracellular glucose concentration (4); however, this method introduces other sources of error (e.g., the recovery of the microdialysis probe, and the assumption that the microdialysis-measured glucose concentration reflects that in all extracellular fluid). The implication of these errors is not trivial. Because the calculation of intracellular glucose involves the subtraction of one relatively large number (extracellular glucose) from another (total glucose) to yield a small number (intracellular glucose), relatively small errors in one of the large numbers have dramatic effects on the calculated "intracellular" concentration.

Because glucose delivery is not accomplished by a single protein, analogous experiments to investigate the role of the third potential controlling element of muscle glucose uptake, glucose delivery, cannot be performed. Second, these genetically manipulated mice have overexpressed the proteins of interest through their entire development and life span, and it is possible that secondary adaptations have occurred that result in a phenotype that differs from that observed in nontransgenic mice.

Data acquired with different techniques strongly support the concept that the control of the insulin-stimulated rate of muscle glucose uptake is distributed, not restricted to a single rate-determining step. Experiments performed with glucose countertransport and biopsy glucose measurements during moderate hyperinsulinemia in the rat (21) support the hypothesis that glucose transport is the primary determinant of glucose uptake during basal conditions. With progressive increases in insulin, the control exerted by transport is decreased such that at slightly supraphysiological insulin concentrations, the glucose gradient across the sarcolemma is essentially zero, indicating that the rate of glucose uptake is being controlled by the pre- and posttransport steps (21).
tion during exercise in humans in untrained compared with trained muscle (27) and at later time points during an exercise bout (15), both of which are associated with lower muscle glycogen concentrations. In rat skeletal muscle, although glucose transport is higher during contraction in the glycogen-depleted state, the increment in glucose uptake is greater than that for transport, indicating that release of inhibition of glucose phosphorylation by G-6-P is the primary means by which glycogen depletion increases the rate of glucose uptake (11). We cannot, however, exclude that some other fasting-associated factor was involved in the differences seen in 18-h compared with 5-h-fasted mice.

Measurements of total muscle glucose have been useful in the examination of the control of muscle glucose uptake during moderate-intensity exercise. Muscle glucose content rises steeply compared with basal during the first few minutes of exercise (14, 15), then declines slightly over the course of the exercise bout, but still remains elevated over the basal muscle glucose concentration (10, 14, 15, 27). The elevation in muscle glucose concentration is likely due in part to the increased muscle perfusion and decreased fractional extraction of glucose observed during moderate-intensity exercise (26), but it also shows that there is some degree of accumulation of intracellular glucose during exercise. Therefore, the finding that increased expression of HK II in mouse skeletal muscle increases exercise-stimulated muscle glucose uptake is consistent with the shifting of the control of muscle glucose uptake to posttransport steps.

In conclusion, data presented here indicate that during hyperinsulinenic euglycemia and moderate-intensity treadmill exercise, control of the process of muscle glucose uptake is shifted so that glucose phosphorylation exerts more control over this process. During exercise, this may be in part due to inhibition of HK by its reaction product, G-6-P, as evidenced by the greater increase in muscle glucose uptake in TG in a more glycogen-depleted state. In contrast, we were unable to demonstrate that glucose phosphorylation is a significant site of control of glucose uptake under basal conditions in normal animals. These results emphasize that the control of skeletal muscle glucose uptake is distributed over several processes, and a single “rate-limiting step” therefore does not exist.

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