Hexokinase II partial knockout impairs exercise-stimulated glucose uptake in oxidative muscles of mice

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Submitted 28 April 2003; accepted in final form 8 July 2003

Fueger, Patrick T., Sami Heikkinen, Deanna P. Bracy, Carlo M. Malabanan, R. Richard Pencek, Markku Laakso, and David H. Wasserman. Hexokinase II partial knockout impairs exercise-stimulated glucose uptake in oxidative muscles of mice. Am J Physiol Endocrinol Metab 285: E958–E963, 2003. First published July 15, 2003; 10.1152/ajpendo.00190.2003.—Muscle glucose uptake (MGU) is distributively controlled by three serial steps: delivery of glucose to the muscle membrane, transport across the muscle membrane, and intracellular phosphorylation to glucose 6-phosphate by hexokinase (HK). During states of high glucose fluxes such as moderate exercise, the HK activity is of increased importance, since augmented muscle perfusion increases glucose delivery, and increased GLUT4 at the cell membrane increases glucose transport. Because HK II overexpression augments exercise-stimulated MGU, it was hypothesized that a reduction in HK II activity would impair exercise-stimulated MGU and that the magnitude of this impairment would be greatest in tissues with the largest glucose requirement. To this end, mice with a HK II partial knockout (HKII+/−) were compared with their wild-type controls (WT) littermates during either sedentary or moderate exercise periods. Rg, an index of glucose metabolism, was measured using 2-deoxy-[3H]glucose. No differences in glucose uptake in oxidative muscles of mice were detected between sedentary groups. In conclusion, MGU is impaired by reductions in HK II activity during exercise, a physiological condition characterized by 10.220.32.247 on April 7, 2017 http://ajpendo.physiology.org/ Downloaded from

MUSCLE GLUCOSE UPTAKE (MGU) is distributively controlled by three serial steps: delivery of glucose to the muscle membrane, transport across the muscle membrane, and intracellular phosphorylation to glucose 6-phosphate (G-6-P). The relative importance of each step in controlling MGU is dependent on the physiological milieu and can be elucidated by transgenic manipulation of proteins contained in the MGU pathway. For example, work in GLUT1 (14) and GLUT4 (13, 32) transgenic mice has shown that overexpression of either glucose transporter increases MGU in resting, fasted mice. In contrast, hexokinase (HK) II overexpression has no effect during resting conditions but augments MGU during exercise (7, 10). This suggests that, during exercise, the onus of control shifts so that glucose phosphorylation plays a greater role.

It has been proposed that, during states of high glucose fluxes such as moderate exercise, the HK activity becomes increasingly important, because augmented muscle perfusion increases glucose delivery, and increased GLUT4 at the cell membrane increases glucose transport (33). The dependence on HK activity is physiologically relevant, since overexpression of HK II in FVB/NJ (4, 10) and C57Bl/6J (7) mice increases both exercise- and insulin-stimulated MGU in vivo by increasing the capacity to phosphorylate glucose. Because some models of insulin resistance are characterized by a reduction in the activity of this enzyme (11, 21, 28, 30, 31), it is important to determine whether reductions in HK protein content, and therefore activity, would impair the increased flux through the MGU pathway during moderate exercise. To test this, Ukk01 (a mixture of BALB/c and DBA/2 strains) mice with an HK II partial knockout (16) were compared during exercise with their wild-type (WT) control littermates. It was hypothesized that a reduction in HK II activity would impair exercise-stimulated MGU and that this impairment is critically dependent on metabolic activity and associated glucose flux.

MATERIALS AND METHODS

Mouse maintenance and genotyping. All procedures performed were approved by the Vanderbilt University Animal Care and Use Committee. Male Ukk01 (a mixture of BALB/c and DBA/2 strains) mice containing a partial deletion to the HK II gene (HKII+/−) that results in a 50% reduction in HK II

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activity in heart and skeletal muscle, as well as in adipose tissue, but does not change HK I activity (16) were bred to WT females. Full HK II knockout results in early embryonic lethality. After a 3-wk weaning period, littermates were separated by sex and maintained in microisolator cages. Genotyping for the WT and disrupted alleles was performed as previously described (16) with the polymerase chain reaction on genomic DNA obtained from a tail biopsy and isolated by a DNeasy Tissue Kit (Qiagen, Valencia, CA). Mice were fed standard chow ad libitum and were studied at ~4 mo of age.

Surgical procedures. The surgical procedures utilized were similar to those described previously (10, 27, 29). Mice were anesthetized with pentobarbital sodium (70 mg/kg body wt). The left common carotid artery was catheterized for sampling of arterial blood with a two-part catheter consisting of PE-10 (inserted into the artery) and Silastic (0.025 in. OD). The right jugular vein was catheterized for infusions with a Silastic catheter (0.025 in. OD). The free ends of the 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 kept patent by flushing each daily with 10–40 μl of saline containing 200 U/ml heparin and 5 mg/ml ampicillin. Animals were individually housed after surgery, and body weight was recorded daily. After an ~5-day period during which body weight was restored (within 10% of presurgery body weight) mice were acclimated to treadmill running with a single 10-min bout of exercise (0.5–0.6 mph, 0% grade). Experiments were performed 2 days after the treadmill acclimation trial.

Experimental procedures. Conscious mice were placed in a 1-liter plastic container lined with bedding and fasted for 5 h on the day of the experiment. Approximately 1 h before an experiment, Micro-Renathane (0.033 in. OD) tubing (22 cm long) was connected to the catheter leads, and mice were placed on an enclosed treadmill and allowed to acclimate to the new environment. At t = 0 min, a baseline blood sample (150 μl) was drawn for the measurement of blood glucose (HemoCue, Mission Viejo, CA), hematocrit, and plasma insulin and nonesterified fatty acids (NEFAs). The remaining red blood cells were washed once with 0.9% saline containing 10 U/ml heparin and reinfused. The mice either remained sedentary (n = 8 WT, 11 HK+/−) or ran (n = 11 WT, 11 HK+/−) on the treadmill for 30 min at 0.6 mph with a 0% grade. The selected work intensity is ~80% of maximal oxygen consumption (6). At t = 5 min, a 12-μCi bolus of 2-deoxy-[3H]glucose ([2-3H]G; New England Nuclear, Boston, MA) was administered to determine arterial blood glucose and plasma [2-3H]DG accumulation. At t = 10, 15, and 20 min, ~50 μl of blood were sampled to determine arterial blood glucose and plasma [2-3H]DG. At t = 30 min, a 150-μl blood sample was withdrawn to determine arterial blood glucose, hematocrit, plasma insulin, [2-3H]DG, and NEFAs, and mice were anesthetized with an arterial infusion of pentobarbital sodium. The heart and soleus (~44% type I, ~51% type IIA, ~5% type IIB fibers), gastrocnemius (~6% type IIA, ~11% type IID, ~83% type IIB fibers), and superficial vastus lateralis (SVL; ~3% type IIA, ~10% type IIB, ~87% type IIB fibers) skeletal muscles (1) were excised, immediately frozen in liquid nitrogen, and stored at −70° C until future tissue analysis.

Processing of plasma and muscle samples. Immunoreactive insulin was assayed with a double-antibody method (26). NEFAs were measured spectrophotometrically by an enzymatic colorimetric assay (Wako NEFA C kit; Wako Chemicals, Richmond, VA). After deproteinization with Ba(OH)2 (0.3 N) and ZnSO4 (0.3 N), [2-3H]DG radioactivity of plasma was determined by liquid scintillation counting (Packard TRI-CARB 2900TR, Packard, Meriden, CT) with Ultima Gold (Packard) as scintillant. Muscle samples were weighed and homogenized in 0.5% perchloric acid. Homogenates were centrifuged and neutralized with KOH. One aliquot was counted directly to determine total radioactivity ([2-3H]DG and [2-3H]DG 6-phosphate [DGP]). A second aliquot was treated with Ba(OH)2 and ZnSO4 to remove [2-3H]DG and any tracer incorporated into glycogen and then counted to determine [2-3H]DG radioactivity (24). [2-3H]DG is the difference between the two aliquots. In all experiments the accumulation of [2-3H]DG was normalized to tissue weight.

Muscle glycogen was determined by the method of Chan and Exton (3) on the heart and contralateral gastrocnemius and SVL muscles. Soleus glycogen was not determined, because both of the small muscles were used for the radioactivity assay. Glycogen breakdown in response to exercise was calculated as the average glycogen concentration for sedentary mice within a genotype minus the individual postexercise glycogen concentrations. After deproteinization with 0.5% perchloric acid, tissue glucose and G-6-P were measured enzymatically (22) and expressed as millimoles per liter of tissue water.

Calculations. Rg was calculated by the method of Kraegen et al. (20) with the following equation

\[
R_g = \frac{[2-3H]DGP_{\text{muscle}}}{\text{AUC}[2-3H]DGP_{\text{plasma}}} \cdot \frac{\text{glucose}_{\text{plasma}}}{\text{glucose}_{\text{plasma}}}
\]

where [2-3H]DGP_{muscle} is the [2-3H]DG radioactivity in the muscle in dpm/g, AUC [2-3H]DGP_{plasma} is the area under the plasma [2-3H]DG disappearance curve in dpm·ml⁻¹·min, and glucoseplasma is the average blood glucose in millimoles per liter during the experimental period.

Statistical analysis. Data are presented as means ± SE. Differences between groups were determined by unpaired t-tests or ANOVA when appropriate. The level of significance was set at P < 0.05.

RESULTS

Baseline characteristics. Baseline characteristics in 5-h-fasted WT and HK+/− mice are shown in Table 1. There were no differences in body weight, fasting arterial blood glucose, plasma insulin concentration, and NEFAs between experimental groups within a genotype; therefore, baseline characteristics were pooled. Partial HK II knockout did not significantly alter body weight, blood glucose, plasma insulin concentration, or NEFAs.

Sedentary experiments. Arterial blood glucose remained stable throughout the 30-min sedentary period

| Table 1. Baseline characteristics in 5-h-fasted Ukkol mice |
|-----------------|-----------------|
| **WT**          | **HK+/−**       |
| n (male/female) | 18 (9/9)        | 16 (9/7)      |
| Body weight, g  | 26 ± 1          | 28 ± 1        |
| Glucose, mg/dl  | 155 ± 5         | 163 ± 6       |
| Insulin, μU/ml  | 23 ± 3          | 21 ± 2        |
| NEFA, mM        | 1.7 ± 0.1       | 1.6 ± 0.1     |

Data are means ± SE. WT, wild type; HK+/−, hexokinase II partial knockout. NEFA, nonesterified fatty acids.
and was not altered by partial HK II knockout in the 5-h-fasted mice (Fig. 1A). Neither the disappearance of [2-3H]DG from the plasma (Fig. 2A) nor Rg values in the heart, soleus, gastrocnemius, and SVL (Table 2) of sedentary mice were affected by reductions in HK II activity. Likewise, glycogen content in the heart, gastrocnemius, and SVL muscles of sedentary mice was the same between genotypes (Table 3). Plasma insulin and NEFA concentrations remained relatively stable throughout the sedentary condition and were not affected by genotype (Table 4). Muscle glucose and G-6-P were also not changed by partial HK II knockout in sedentary mice, with the exception of a reduction in soleus G-6-P in HK+/− compared with WT mice (Table 5).

Exercise experiments. HK+/− mice had normal exercise tolerance during the 30-min bout of running at 0.6 mph compared with their WT littermates of the Ukko1 strain. Arterial blood glucose did not significantly change throughout the 30 min of exercise and was not affected by genotype (Fig. 1B). Insulin concentrations were lower at the end of exercise compared with the onset of exercise in both genotypes. NEFAs were not significantly altered by exercise in either genotype (Table 4). Disappearance of [2-3H]DG from the plasma was the same between WT and HK+/− mice (Fig. 2B). The increase in Rg due to exercise is shown in Fig. 3. In the tissues with high oxidative capacity and the greatest glucose flux (heart and soleus), partial ablation of HK II decreased the exercise-stimulated component of Rg. In contrast, the exercise-stimulated component of Rg was not significantly blunted in the less oxidative tissues characterized by lower glucose fluxes (gastrocnemius and SVL muscles) in HK+/− compared with WT mice. In the heart, soleus, and gastrocnemius, exercise led to a reduction in muscle glucose in both genotypes (Table 5). G-6-P levels remained largely unchanged after exercise. As in sedentary mice, the soleus of exercised HK+/− mice had a lower G-6-P level compared with WT mice. Glycogen breakdown during exercise was significantly increased in the gastrocnemius but not heart or SVL muscles of HK+/− compared with WT mice (Fig. 4).

**DISCUSSION**

The aim of the present study was to determine whether a reduction in HK activity would impair MGU during a state of increased glucose flux such as moderate exercise. Alterations in glucose metabolism at rest were not expected, since studies utilizing GLUT1-
and GLUT4-overexpressing mice have demonstrated that glucose transport exerts the most control in regulating MGU under basal, resting conditions (13, 14, 32). In addition, there was no previous evidence of glucose intolerance in sedentary HK\textsuperscript{+/−} mice despite the 50% reduction in tissue HK II activity (16). However, because overexpression of HK II augments exercise-stimulated MGU in C57B/6J (7, 10) and FVB/NJ (10) mice, it was hypothesized that a 50% reduction in HK II activity created by a heterozygous knockout would impair exercise-stimulated MGU. Impairment in MGU of HK\textsuperscript{+/−} mice was only observed in muscles with high oxidative capacity and accelerated glucose flux (e.g., the heart and soleus muscles). This is consistent with previous reports that show that glucose phosphorylation, assessed using a technique requiring three isotopic glucose analogs, is the primary barrier to MGU under basal, resting conditions (13, 14, 15, 17). 

A striking observation is that the correlation between the oxidative capacity of a muscle (5, 8, 15, 17) and the magnitude to which MGU was stimulated by exercise is ablated by a reduction in glucose phosphorylation capacity (see Fig. 3). The exercised HK\textsuperscript{+/−} mice also have a lower G-6-P concentration in the soleus muscle compared with their WT controls. Although the other muscles studied tend to show impairments in MGU during exercise, the differences did not reach statistical significance in the present study. Other physiological or pharmacological interventions that would result in a greater glucose flux in these muscles might reveal an impairment in MGU. Interestingly, in the gastrocnemius there was a marked increase in glycogen breakdown associated with exercise, and a similar trend was observed in the SVL. When the MGU and glycogen breakdown data are considered together, it appears that, in the less oxidative muscles, MGU is slightly impaired, thereby shifting the preference for glucose substrate from an intracellular pool (i.e., glycogen). Perhaps lowering the glycogen pool by a more extended fast (5-h fast was used in this study) might unmask a more pronounced impairment in MGU during exercise by lowering muscle G-6-P (10).

In the heart, MGU is impaired in the HK\textsuperscript{+/−} mice compared with WT controls, yet there are no differences in glycogen utilization during exercise. If one assumes that the work performed is equal between the HK\textsuperscript{+/−} and WT mice, then the deficits in glucose uptake must be compensated by an increase in lactate or free fatty acid utilization. In a similar manner, the accelerated glycogen breakdown in the gastrocnemius and SVL muscles of HK\textsuperscript{+/−} mice exceeds the attenuation of exercise-stimulated R\textsubscript{ex} in this case, a decrease in fatty acid utilization or metabolic efficiency could account for the difference. Further studies utilizing lactate and free fatty acid tracers will be needed to determine the method of compensation.

Despite impairments in MGU in the soleus and heart and the tendency toward an impairment in the gastrocnemius and SVL created by a 50% reduction in HK II activity, the HK\textsuperscript{+/−} mice had normal exercise tolerance within the context of the present study. One could speculate that impairments in MGU would lead to poor exercise tolerance. This was not the case, however, in

Table 3. Muscle glycogen from 5-h-fasted, sedentary Ukko 1 mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>HK\textsuperscript{+/−}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>4.82 ± 1.03</td>
<td>3.94 ± 0.94</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>14.45 ± 1.78</td>
<td>13.99 ± 1.07</td>
</tr>
<tr>
<td>SVL</td>
<td>13.74 ± 1.59</td>
<td>12.36 ± 1.10</td>
</tr>
</tbody>
</table>

Data are means ± SE in μmol glucosyl units/g muscle for 8–11 mice/group.

Table 4. Plasma insulin and NEFA concentrations from all mice studied

<table>
<thead>
<tr>
<th></th>
<th>WT 0 min</th>
<th>WT 30 min</th>
<th>HK\textsuperscript{+/−} 0 min</th>
<th>HK\textsuperscript{+/−} 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, μU/ml</td>
<td>17 ± 3</td>
<td>23 ± 5</td>
<td>20 ± 2</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>Sedentary Exercised</td>
<td>26 ± 5</td>
<td>15 ± 2*</td>
<td>22 ± 3</td>
<td>15 ± 4*</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>1.51 ± 0.08</td>
<td>1.22 ± 0.15</td>
<td>1.46 ± 0.17</td>
<td>1.19 ± 0.11</td>
</tr>
<tr>
<td>Sedentary Exercised</td>
<td>1.79 ± 0.18</td>
<td>1.64 ± 0.11</td>
<td>1.65 ± 0.14</td>
<td>1.33 ± 0.12</td>
</tr>
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Data are means ± SE for 8–11 mice/group. \*P < 0.05 vs. sedentary mice.
the Ukko1 strain at the duration and intensity of exercise used in this study.

Interestingly, the disappearance of [2-3H]DG from the plasma was not altered in exercising HK$^{+/−}$ mice despite impairments in Rg from the working muscle. This paradox can be explained by two possibilities. First, the disappearance of the tracer from the plasma reflects a whole body process. Tracer uptake into other organs such as the brain and liver would not be expected to be altered in HK$^{+/−}$ mice; therefore, it would be difficult to detect an impaired tracer clearance. Second, the impairment in MGU was significantly manifested only in a subset of skeletal muscle and cardiac muscle. The gastrocnemius and SVL may be more representative of the total muscle mass; hence, an impaired tracer clearance might be observed only if these muscles had marked reductions in exercise-stimulated Rg.

The mismatch between the whole body tracer clearance and muscle-specific Rg is of importance when relating the data presented here to those in patients with type 2 diabetes and/or insulin resistance. Pendergrass et al. (28) have reported a reduction in HK activity in patients with type 2 diabetes compared with healthy controls. Thus one might expect to see similar attenuations in exercise-stimulated MGU in patients with diabetes as we have reported here in HK$^{+/−}$ mice. That, however, has not been demonstrated (2, 18, 19, 23, 25). Direct comparison of those results with the present results is impossible because patients with type 2 diabetes generally have greater glucose and insulin concentrations compared with healthy controls. Elevated insulin and glycemia may compensate for decreased HK activity, thereby masking an impairment in MGU.

In conclusion, MGU is impaired by reductions in HK activity during a physiological condition characterized by high glucose flux such as exercise. This impairment is critically dependent on the overall metabolic rate of the tissue and perhaps oxidative capacity. We propose that because glucose delivery and transport are amplified during moderate exercise, it can be used as a provocative tool to diagnose functional impairments to intracellular glucose phosphorylation in glucose-intolerant or insulin-resistant states.
We thank Wanda Snead of the Vanderbilt Hormone Assay Core for performing the insulin assays.

DISCLOSURES

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants R01 DK-54902 and U24 DK-58637.

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