Hexokinase II Overexpression Improves Exercise-Stimulated But Not Insulin-Stimulated Muscle Glucose Uptake in High-Fat–Fed C57BL/6J Mice

Patrick T. Fueger,[1] Deanna P. Bracy,[1,2] Carlo M. Malabanan,[2] R. Richard Pencek,[1] Daryl K. Granner,[1] and David H. Wasserman[1,2]

The aim of the present study was to determine the specific sites of impairment to muscle glucose uptake (MGU) in the insulin-resistant high-fat–fed, conscious C57BL/6J mouse. Wild type (WT) and hexokinase II overexpressing (HK^{Tg}) mice were fed either a standard diet or high-fat diet and studied at 4 months of age. A carotid artery and jugular veins had catheters chronically implanted for sampling and infusions, respectively, and mice were allowed to recovery for at least 5 days. Mice were fasted for 5 h and underwent a hyperinsulinemic-euglycemic clamp or saline infusion for 120 min. Separate groups of mice were studied during 30-min sedentary or treadmill exercise periods. A bolus of 2-deoxy[^{3}H]glucose was administered 25 min before the end of each study for determination of R_g, an index of tissue-specific glucose uptake. Fasting blood glucose was increased in high-fat compared with standard diet–fed WT (194 ± 4 vs. 171 ± 4 mg/dl) but not HK^{Tg} (179 ± 5 vs. 171 ± 3 mg/dl) mice. High-fat feeding created hyperinsulinemia in both WT and HK^{Tg} mice (58 ± 8 and 77 ± 15 μU/ml) compared with standard diet–fed mice (21 ± 2 and 20 ± 1 μU/ml). R_g was not affected by genotype or diet during either saline infusion or sedentary conditions. HK II overexpression augmented insulin-stimulated R_g in standard diet–fed but not high-fat–fed mice. Exercise-stimulated R_g was impaired by high-fat feeding in WT mice, but this impairment was largely rectified in HK^{Tg} mice. In conclusion, high-fat feeding impairs both insulin- and exercise-stimulated MGU, but only exercise-stimulated MGU was corrected by HK II overexpression. *Diabetes* 53:306–314, 2004

From the [1]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee; and the [2]Mouse Metabolic Phenotyping Center, Vanderbilt University School of Medicine, Nashville, Tennessee.

Address correspondence and reprint requests to Patrick T. Fueger, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232-0615. E-mail: patrick.fueger@vanderbilt.edu.

Received for publication 18 May 2003 and accepted in revised form 22 October 2003.

[2-^{3}H]DG, 2-deoxy[^{3}H]glucose; [2-^{3}H]DGP, 2-deoxy[2-^{3}H]glucose-6-phosphate; G6P, glucose-6-phosphate; GIR, glucose infusion rate; HK, hexokinase; HK II^{Tg}, hexokinase II transgene; MGU, muscle glucose uptake; NEFA, nonesterified fatty acid; OD, outer diameter; SVL, superficial vastus lateralis; WT, wild-type.

© 2004 by the American Diabetes Association.

Insulin resistance induced by high-fat feeding is characterized by a decrease in insulin-stimulated glucose disposal (1–3). The impaired glucose disposal is likely to be due to deficits in one or more of the steps required for skeletal muscle glucose uptake (MGU). Specifically, these steps are *1*) delivery of glucose to the muscle membrane, *2*) facilitated transport across the muscle membrane, and *3*) intracellular phosphorylation to glucose-6-phosphate (G6P) by a hexokinase (HK) isozyme. It is difficult, in the context of the whole animal, to elucidate which sites of the glucose uptake pathway are functionally altered by high-fat feeding. One approach is to alter protein levels by transgenic manipulation and measure the effect on glucose flux. Physiological stimuli can then be applied to better expose perturbations caused by a transgene. For example, overexpressing HK II in skeletal muscle increases glucose phosphorylation capacity and results in increased MGU in high-flux states created by insulin stimulation or exercise in standard diet–fed FVB/NJ mice but not under basal glucose flux conditions (4).

Transgenic manipulation can also isolate sites of impairment to MGU in insulin-resistant states, and high-flux states (e.g., insulin stimulation and exercise) can be used to amplify the signal resulting from such a deficit. In the present study, exercise and insulin were used in combination with HK II overexpression to determine the role of glucose phosphorylation in the impairment of MGU associated with the insulin resistance of the high-fat–fed C57BL/6J mouse (5,6). Previous investigations in the conscious rat have suggested that high-fat feeding leads to a functional impairment in muscle glucose phosphorylation (7). Therefore, it was hypothesized that impaired MGU resulting from high-fat feeding would be exposed in high-flux states and could be corrected by HK II overexpression. The unique surgical catheterization used in this study allows for these hypotheses to be tested in the conscious, unrestrained mouse. Determining the site(s) of impairment of MGU manifested by high-fat feeding will provide insight to the pathogenesis of insulin resistance and lead to the identification of potential therapeutic targets.

RESEARCH DESIGN AND METHODS

All procedures performed were approved by the Vanderbilt University Animal Care and Use Committee. Male FVB/NJ mice that selectively overexpress HK II in skeletal muscle were obtained from Dr. David E. Moller (8) and backcrossed onto the C57BL/6J background for at least five generations. The

HK II transgene is composed of the human HK II cDNA driven by the rat muscle creatine kinase promoter and yields a three-, five-, and sevenfold increase in HK activity in the soleus, gastrocnemius, and superficial vastus lateralis (SVL) muscles, respectively. This degree of overexpression is within the normal physiological range of HK activity in skeletal muscle (9,10). Male mice carrying the HK II transgene (HK^{Tg}) were subsequently bred with wild-type (WT) female mice. After a 3-week weaning period, littermates were separated by sex and maintained in microisolator cages. Genotyping for the HK II transgene was performed on genomic DNA obtained from a tail biopsy with the PCR as previously described (4). Mice were fed either a standard diet or a high-fat diet ad libitum and were studied at ~4 months of age. The high-fat diet contains 35.5% fat (Diet F3282; Bio-Serv, Frenchtown, NJ). This model of dietary-induced insulin resistance creates both fasting hyperglycemia and hyperinsulinemia and thus is a reasonable model for the human condition (5,6).

Surgical procedures. The surgical procedures were the same as those described previously (4,11,12). Mice were anesthetized with pentobarbital (70 mg/kg body wt). The left common carotid artery was catheterized for sampling with a two-part catheter consisting of PE-10 (inserted into the artery) and silastic (0.025 in outer diameter [OD]). The right jugular vein was catheterized for infusions with a 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 tubing made of Micro-Renathane (0.033 in OD), which were exteriorized and sealed with stainless steel plugs. Lines were flushed daily with ~50 μl of saline containing 200 units/ml heparin and 5 mg/ml ampicillin. Animals were individually housed after surgery, and body weight was recorded daily.

In vivo metabolic experiments. Metabolic experiments were performed as previously described (4) and were conducted after a postoperative recovery period of either ~5 days for hyperinsulinemic-euglycemic clamp experiments or ~7 days for exercise experiments. The recovery period was a sufficient time for body weight to be restored within 10% of presurgery body weight. For metabolic studies, conscious, unrestrained mice were placed in an ~1-l plastic container lined with bedding and fasted for 5 h. Approximately 1 h before an experiment, Micro-Renathane (0.033 in OD) tubing was connected to the catheter leads and infusion syringes. After a 1-h acclimation period, a baseline arterial blood sample (150 μl) was drawn for the measurement of arterial blood glucose, hematocrit, and plasma insulin and nonesterified fatty acids (NEFAs). The remaining erythrocytes were washed with 0.9% heparinized saline and reinfused.

Hyperinsulinemic-euglycemic clamp experiments. For hyperinsulinemic-euglycemic clamp and saline control experiments, an infusion of saline (n = 11 WT standard diet–fed; 11 HK^{Tg} standard diet–fed; 7 WT high-fat–fed; 7 HK^{Tg} high-fat–fed) or 4 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin (n = 14 WT standard diet–fed; 11 HK^{Tg} standard diet–fed; 10 WT high-fat–fed; 8 HK^{Tg} high-fat–fed) was begun at a rate of 1.375 μl/min at $t = -90$ min. Euglycemia was maintained during insulin experiments by frequently measuring arterial blood glucose (~5 μl; HemoCue, Mission Viejo, CA) and infusing 50% dextrose as necessary. Mice received saline-washed erythrocytes from a donor mouse as needed to prevent a marked fall in hematocrit (>5%). At $t = 0$ min, an arterial blood sample (150 μl) was obtained and processed as the baseline blood sample. At $t = 5$ min, a 12-μCi bolus of 2-deoxy[^{3}H]glucose ([2-^{3}H]DG) was administered to determine R_g, an index of tissue-specific glucose uptake. At t = 7, 10, 15, and 20 min, arterial blood (~50 μl) was sampled to determine arterial blood glucose and plasma [2-^{3}H]DG. At $t = 30$ min, a final arterial blood sample (150 μl) was withdrawn and processed as the baseline blood sample with the addition of the determination of plasma [2-^{3}H]DG, and mice were anesthetized with an infusion of sodium pentobarbital. The gastrocnemius (~6% type IIA, ~11% type IID, ~83% type IIB fibers), SVL (~3% type IIA, ~10 type IID, ~87% type IIB fibers), and soleus (~44% type I, ~51% type IIA, ~5% type IID fibers) muscles (13) were excised, immediately frozen in liquid nitrogen, and stored at −70°C until future tissue analysis.

Exercise experiments. For exercise and sedentary control experiments, mice were acclimated to treadmill running with a single 10-min bout of exercise (0.5–0.6 mph, 0% grade) ~5 days postsurgery. Experiments were performed 2 days after the treadmill acclimation trial. After the baseline arterial blood sampling, mice either remained sedentary (n = 9 WT standard diet–fed; 12 HK^{Tg} standard diet–fed; 7 WT high-fat–fed; 8 HK^{Tg} high-fat–fed) or ran (n = 9 WT standard diet–fed; 10 HK^{Tg} standard diet–fed; 8 WT high-fat–fed; 8 HK^{Tg} high-fat–fed) on a motorized treadmill (0.6 mph, 0% grade) for 30 min. At $t = 5$ min, a 12-μCi bolus of [2-^{3}H]DG was administered to determine R_g. At t = 10, 15, and 20 min, arterial blood (~50 μl) was sampled to determine blood glucose and plasma [2-^{3}H]DG. At $t = 30$ min, a final arterial blood sample (150 μl) was taken and processed as the baseline blood sample with the addition of the determination of plasma [2-^{3}H]DG, mice were anesthetized, and muscles were excised as described for the clamp studies.

TABLE 1
Baseline characteristics of all mice studied

	Standard diet		High-fat diet	
	WT	HK^{Tg}	WT	HK^{Tg}
n (male/female)	43 (24/19)	44 (22/22)	32 (15/17)	32 (17/15)
Body weight (g)	26 ± 1	25 ± 1	35 ± 1*	36 ± 1*
Glucose (mg/dl)	171 ± 4	171 ± 3	194 ± 4*	179 ± 5†
Insulin (μU/ml)	21 ± 2	20 ± 1	58 ± 8*	77 ± 15*
NEFA (mmol/l)	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1

*$P < 0.001$ vs. standard diet–fed mice; †$P < 0.001$ vs. high-fat–fed mice.

Processing of plasma and muscle samples. Immunoreactive insulin was assayed with a double-antibody method (14). NEFAs were measured spectrophotometrically by an enzymatic colorimetric assay (Wako NEFA C kit; Wako Chemicals, Richmond, VA). After deproteinization with barium hydroxide [$Ba(OH)_2$, 0.3 N] and zinc sulfate [$ZnSO_4$, 0.3 N], [2-^{3}H]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 [2-^{3}H]DG and [2-^{3}H]DG-6-phosphate ([2-^{3}H]DGP) radioactivity. A second aliquot was treated with $Ba(OH)_2$ and $ZnSO_4$ to remove [2-^{3}H]DGP and any tracer incorporated into glycogen (15) and then counted to determine [2-^{3}H]DG radioactivity. [2-^{3}H]DGP is the difference between the two aliquots. In all experiments, the accumulation of [2-^{3}H]DGP was normalized to tissue weight.

Muscle glycogen was determined by the method of Chan and Exton (16) on the contralateral gastrocnemius and SVL muscles. Soleus glycogen could not be determined because both of the small muscles were used to assay for [2-^{3}H]DG and [2-^{3}H]DGP. After deproteinization with 0.5% perchloric acid, tissue glucose and G6P were measured enzymatically (17) and expressed as millimoles per liter tissue water.

Calculations. R_g was calculated by the equation (18)

$$R_g = \frac{[2\text{-}^3\text{H}]\text{DGP}_{\text{tissue}}}{\text{AUC }[2\text{-}^3\text{H}]\text{DG}_{\text{plasma}}} \cdot \text{glucose}_{\text{plasma}} \quad (1)$$

where [2-^{3}H]DGP_{tissue} is the [2-^{3}H]DGP radioactivity in the muscle in dpm/g, AUC [2-^{3}H]DG_{plasma} is the area under the plasma [2-^{3}H]DG disappearance curve in $dpm \cdot ml^{-1} \cdot min^{-1}$, and $glucose_{plasma}$ is the average blood glucose in mmol/l during the experimental period.

Statistical analysis. Data are presented as mean ± SE. Differences between groups were determined by ANOVA. The significance level was set at $P < 0.05$.

RESULTS

Baseline descriptive characteristics for 5-h–fasted mice are reported in Table 1. HK II overexpression had no effect on body weight. High-fat feeding increased body weight by ~10 g in both WT and HK^{Tg} mice. Baseline arterial blood glucose, insulin, and NEFA concentrations were not influenced by HK II overexpression. High-fat feeding increased arterial blood glucose in WT mice and caused hyperinsulinemia. HK II overexpression protected mice against diet-induced fasting hyperglycemia but not hyperinsulinemia. NEFAs were not altered by high-fat feeding regardless of genotype.

Hyperinsulinemic-euglycemic clamp experiments. All measurements were made after 90 min of saline or insulin infusion to ensure steady-state conditions. Arterial blood glucose was not affected by high-fat feeding or HK II overexpression in saline-infused mice (Fig. 1*A*). Arterial blood glucose was similar in all groups during the hyperinsulinemic-euglycemic clamp experiments (Fig. 1*B*). In addition, there were no detectable differences in arterial blood glucose between saline- and insulin-infused mice.

The glucose infusion rates (GIRs) required to maintain euglycemia averaged for the final 30 min of the experiment

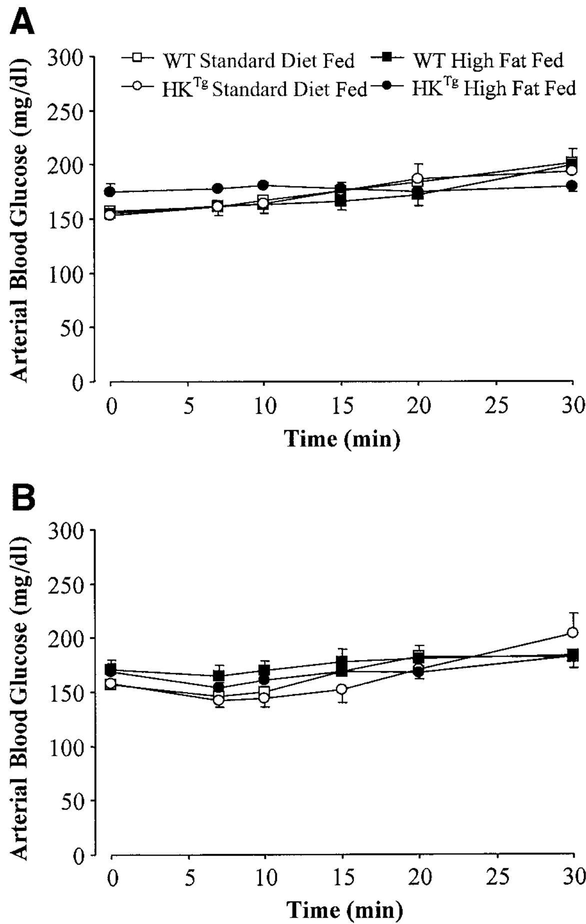

FIG. 1. Arterial blood glucose was measured during the last 30 min of a 120-min saline-infused (*A*) or hyperinsulinemic-euglycemic clamp (*B*) experiment in chronically catheterized, conscious mice. WT (□ and ■) or HK II^{Tg} (○ and ●) mice were fed either a standard diet (□ and ○) or a high-fat diet (■ and ●) up to 4 months of age and fasted for 5 h. Data are mean ± SE for 7–14 mice/group.

are reported in Fig. 2. GIR was increased by HK II overexpression in standard diet–fed mice, but this increase was not statistically significant (67 ± 8 vs. 58 ± 3 mg · kg^{-1} · min^{-1}; $P = 0.11$). High-fat feeding decreased GIR, and HK II overexpression was unable to correct the reduced GIR associated with high-fat feeding.

Disappearance of [2-^{3}H]DG from arterial plasma is shown in Fig. 3. Neither genotype nor diet significantly altered the disappearance of [2-^{3}H]DG in saline-infused mice. The disappearance of [2-^{3}H]DG was increased in insulin- compared with saline-infused mice irrespective of genotype or diet. Standard diet–fed HK^{Tg} mice had an accelerated [2-^{3}H]DG disappearance compared with standard diet–fed WT and high-fat–fed HK^{Tg} mice during insulin infusion.

R_g's for the gastrocnemius, SVL, and soleus muscles during saline and insulin infusions are shown in Fig. 4. There were no differences in R_g when saline alone was infused. R_g was significantly increased during insulin infusion in all muscles regardless of genotype or diet. HK II overexpression augmented the insulin-stimulated R_g in all muscles of standard diet–fed mice. High-fat feeding blunted insulin-stimulated R_g, and as with GIR, HK II overexpression was unable to correct the impaired response.

Muscle glycogen content was unaltered by HK II overexpression or high-fat feeding independently (Table 2). However, glycogen content was reduced in these muscles when HK II overexpression and high-fat feeding were combined. Muscle glucose was increased by high-fat feeding in the gastrocnemius of WT saline-infused mice, and this increase was normalized by HK II overexpression

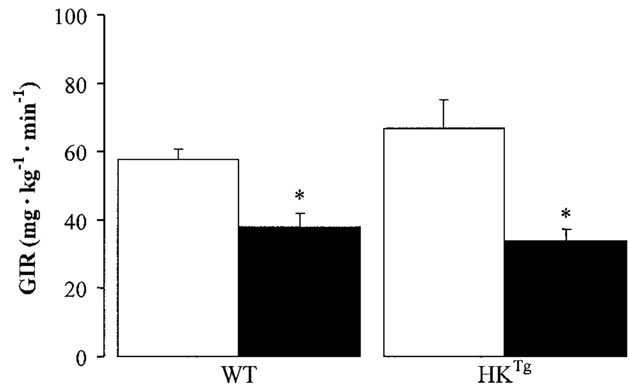

FIG. 2. Average GIR during the last 30 min of a 120-min hyperinsulinemic-euglycemic clamp experiment in chronically catheterized, conscious mice. WT or HK^{Tg} mice were fed either a standard diet (■) or a high-fat diet (□) up to 4 months of age. After a 5-h fast, 4 mU · kg^{-1} · min^{-1} insulin was infused and blood glucose was clamped at euglycemia by variable glucose infusion. The GIRs reported are the average values for the final 30 min of the experiment. Data are mean ± SE for 8–14 mice/group. *$P < 0.05$ vs. standard diet–fed mice.

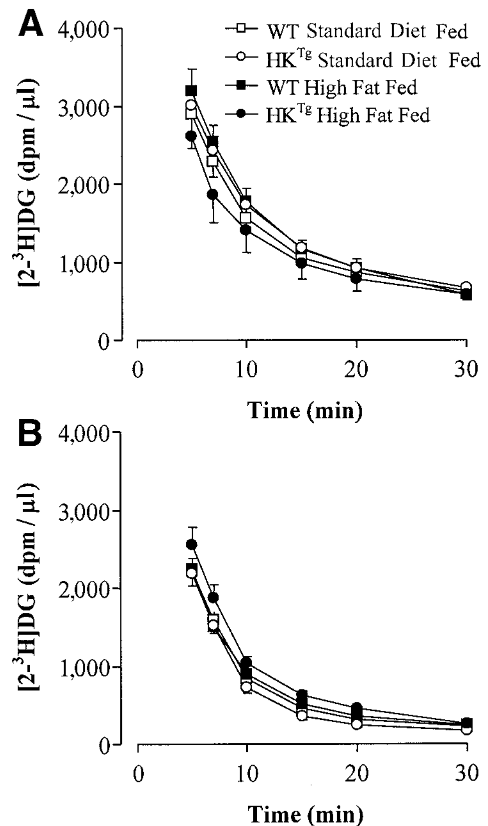

FIG. 3. Disappearance of [2-^{3}H]DG from the plasma during the last 25 min of a 120-min saline-infused (*A*) or hyperinsulinemic-euglycemic clamp (*B*) experiment in chronically catheterized, conscious mice. WT (□ and ■) or HK^{Tg} (○ and ●) mice were fed either a standard diet (□ and ○) or a high-fat diet (■ and ●) up to 4 months of age and fasted for 5 h. Arterial blood was obtained and measured for plasma [2-^{3}H]DG as described in RESEARCH DESIGN AND METHODS. Data are mean ± SE for 7–14 mice/group.

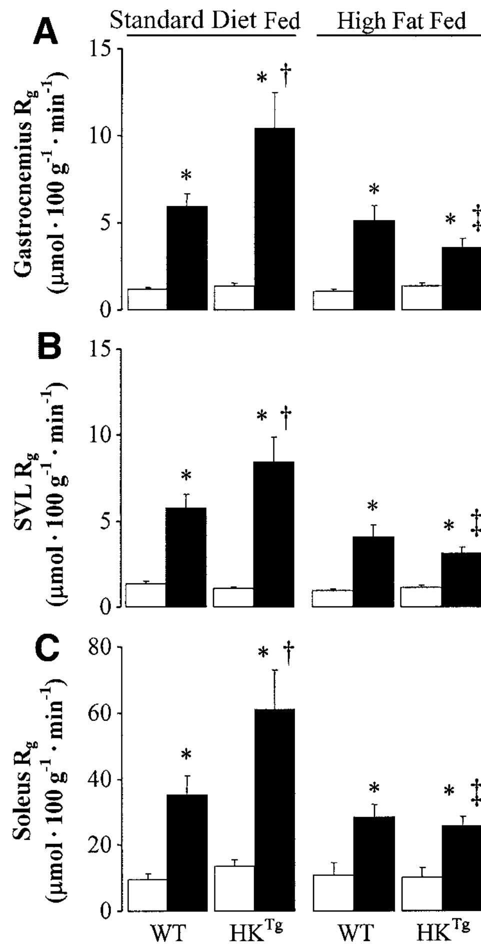

FIG. 4. ***R_g*, a tissue-specific index of glucose uptake, was measured for the gastrocnemius (*A*), SVL (*B*), and soleus (*C*) muscles during the last 30 min of a 120-min saline-infused (□) or hyperinsulinemic-euglycemic clamp (■) experiment in chronically catheterized, conscious mice. WT or HK^{Tg} mice were fed either a standard diet or a high-fat diet up to 4 months of age and fasted for 5 h. Data are mean ± SE for 7–14 mice/group. **P* < 0.05 vs. saline condition; †*P* < 0.05 vs. WT standard diet fed; ‡*P* < 0.05 vs. HK^{Tg} standard diet fed.**

(Table 3). Stimulation by insulin led to a fall in muscle glucose in the gastrocnemius and SVL of high-fat–fed WT mice, raising the possibility of impaired glucose delivery in muscles that consist of type II fibers. No significant differences in muscle G6P were detected between groups (Table 4).

Exercise experiments. Arterial blood glucose concentrations during sedentary and exercise experiments are shown in Fig. 5. There were no differences in arterial blood glucose between standard diet–fed WT and HK^{Tg} and high-fat–fed HK^{Tg} mice under sedentary conditions (Fig. 5*A*). The elevated arterial blood glucose observed in sedentary, high-fat–fed WT mice waned such that by the end of the study, concentrations were no longer greater than standard diet–fed mice. The relative hyperglycemia observed in high-fat–fed WT mice, however, persisted throughout exercise (Fig. 5*B*). There was a general trend for arterial blood glucose to rise to a plateau by 20 min of exercise in all groups.

Disappearance of [2-^{3}H]DG from arterial plasma is shown in Fig. 6. Genotype and diet did not alter [2-^{3}H]DG disappearance in sedentary mice (Fig. 6*A*). HK II overexpression augmented [2-^{3}H]DG disappearance during exercise in both standard diet– and high-fat–fed mice (Fig. 6*B*).

R_g's for soleus, gastrocnemius, and SVL during sedentary and exercise conditions are shown in Fig. 7. There were no differences in sedentary R_g between any of the groups. Exercise significantly increased R_g in the gastrocnemius and SVL regardless of genotype and diet. Exercise was able to increase R_g only in the soleus muscle of fed HK^{Tg} mice. The lack of an increase in soleus R_g of WT mice during exercise is due to the high tonic activity of this muscle. High-fat feeding blunted the exercise-stimulated R_g in the gastrocnemius and the SVL of WT mice. In addition, the exercising soleus R_g was reduced in high-fat–fed WT mice compared with standard diet–fed controls. The impairment of exercise-stimulated R_g as a result of high-fat feeding was markedly improved in the gastrocnemius and SVL muscles of HK^{Tg} mice. In addition, exercise-stimulated R_g in the gastrocnemius and SVL muscles was significantly increased in high-fat–fed HK^{Tg} compared with high-fat–fed WT mice. However, the attenuated exercise-stimulated R_g in the soleus as a result of high-fat feeding was not corrected by HK II overexpression.

Exercise led to a significant fall in glycogen content in the gastrocnemius and SVL of standard diet–fed WT mice and in the gastrocnemius of standard diet–fed HK^{Tg} mice (Table 2). Glycogen content in the gastrocnemius was greater in high-fat–fed compared with standard diet–fed WT mice after exercise. Muscle glucose was lowered by HK II overexpression in the gastrocnemius of sedentary standard diet–fed mice, the soleus of sedentary high-fat–fed mice, and the SVL of exercising high-fat–fed mice (Table 3). Exercise increased muscle G6P in the gastrocnemius of high-fat–fed WT mice and SVL of high-fat–fed HK^{Tg} mice (Table 4). HK II overexpression increased G6P in the gastrocnemius of sedentary, high-fat–fed mice and in the soleus of both sedentary and exercising, high-fat–fed mice.

DISCUSSION

MGU consists of three serial steps: the delivery of glucose from the blood to the muscle membrane, the transport across the muscle membrane, and the phosphorylation to G6P by an HK. As with other metabolic pathways (19,20), the regulation of MGU is distributed and may be altered by physiological conditions at more than one step. Likewise,

TABLE 2
Muscle glycogen (mg/g) of mice from all experiments

	Standard diet		High-fat diet	
	WT	HK^{Tg}	WT	HK^{Tg}
Gastrocnemius				
Saline	3.6 ± 0.2	3.4 ± 0.3	4.1 ± 0.4	3.0 ± 0.2*
Insulin	4.0 ± 0.3	3.7 ± 0.2	3.8 ± 0.3	3.0 ± 0.3*
Sedentary	2.8 ± 0.2	2.8 ± 0.2	3.1 ± 0.2	3.0 ± 0.3
Exercise	1.9 ± 0.2†	2.1 ± 0.2†	2.7 ± 0.3‡	2.6 ± 0.3
SVL				
Saline	3.6 ± 0.5	3.1 ± 0.2	3.2 ± 0.2	2.6 ± 0.2*
Insulin	4.3 ± 0.4	4.1 ± 0.3	3.4 ± 0.3	2.6 ± 0.3*
Sedentary	2.8 ± 0.2	2.2 ± 0.3	2.8 ± 0.2	2.8 ± 0.3
Exercise	1.9 ± 0.3†	1.7 ± 0.3	2.5 ± 0.3	2.2 ± 0.3

**P* < 0.05 vs. standard diet–fed HK^{Tg} and high-fat–fed WT mice; †*P* < 0.05 vs. sedentary within genotype; ‡*P* < 0.05 vs. standard diet–fed WT mice.

TABLE 3
Muscle glucose (mmol/l) of mice from all experiments

	Standard diet		High-fat diet	
	WT	HK^{Tg}	WT	HK^{Tg}
Gastrocnemius				
Saline	0.61 ± 0.08	0.59 ± 0.10	0.86 ± 0.09*	0.48 ± 0.13†
Insulin	0.55 ± 0.09	0.55 ± 0.11	0.39 ± 0.12‡	0.31 ± 0.08
Sedentary	0.82 ± 0.14	0.47 ± 0.10*	0.89 ± 0.08	0.61 ± 0.10
Exercise	0.65 ± 0.22	0.66 ± 0.22	0.98 ± 0.22	0.57 ± 0.10
SVL				
Saline	0.64 ± 0.10	0.43 ± 0.08	0.64 ± 0.06	0.42 ± 0.11
Insulin	0.37 ± 0.05‡	0.33 ± 0.07	0.33 ± 0.07‡	0.30 ± 0.09
Sedentary	0.72 ± 0.16	0.55 ± 0.13	0.79 ± 0.17	0.64 ± 0.09
Exercise	0.98 ± 0.27	0.57 ± 0.15	1.08 ± 0.21	0.50 ± 0.09†
Soleus				
Saline	1.20 ± 0.13	0.90 ± 0.17	1.01 ± 0.16	1.07 ± 0.24
Insulin	1.03 ± 0.09	0.99 ± 0.29	0.86 ± 0.22	0.54 ± 0.15
Sedentary	1.17 ± 0.27	1.12 ± 0.31	1.37 ± 0.21	0.79 ± 0.08†
Exercise	1.48 ± 0.38	1.34 ± 0.28	2.01 ± 0.42	1.06 ± 0.19

*$P < 0.05$ vs. standard diet–fed WT mice; †$P < 0.05$ vs. high-fat–fed WT mice; ‡$P < 0.05$ vs. saline within genotype.

dysregulation, resulting in impaired MGU, may occur at multiple steps of the glucose uptake pathway. The aim of the present study was to determine, in conscious, unrestrained C57BL/6J mice, the functional sites of impairment created by high-fat feeding and, more specifically, to determine whether a diet-induced form of insulin resistance creates a functional impairment to glucose phosphorylation. To this end, glucose fluxes (i.e., R_g's) were measured during two independent physiological conditions characterized by high fluxes (i.e., stimulation by insulin or exercise) in both standard diet– and high-fat–fed mice with and without HK II selectively overexpressed in skeletal muscle. Here we show that high-fat feeding results in a functional impairment in glucose phosphorylation capacity in conscious, unrestrained C57BL/6J mice. It is also apparent from these studies that diminished glucose phosphorylation capacity is not the only deficit contributing to high-fat feeding–induced impairments in MGU in vivo.

High-fat feeding resulted in insulin resistance, as evidenced by the ~30-mg/dl increase in fasting arterial blood glucose, the more than doubling of fasting plasma insulin concentration, and the marked reduction in exogenous glucose required to maintain euglycemia during insulin infusion. Whereas it has been demonstrated that high-fat feeding elicits deficits in glucose transport (21–25), less is known regarding high-fat feeding–induced deficits to steps both proximal and distal to glucose transport. Functional impairments to steps distal to glucose transport (e.g., glucose phosphorylation) can occur as a result of high-fat feeding (26) or accumulation of intracellular lipids (27). A recent report demonstrated that overexpression of glucokinase, an HK isozyme that is not inhibited by G6P, in skeletal muscle prevented glucose intolerance and insulin resistance in high-fat–fed mice (28). Our laboratory has provided evidence from the high-fat–fed conscious rat using a modeling approach (7) that diet-induced insulin resistance can be manifested by impairments to both delivery of glucose to the muscle and intracellular phosphorylation to G6P. More recently it was shown that the

TABLE 4
Muscle G6P (mmol/l) of mice from all experiments

	Standard diet		High-fat diet	
	WT	HK^{Tg}	WT	HK^{Tg}
Gastrocnemius				
Saline	1.05 ± 0.19	0.90 ± 0.15	0.89 ± 0.19	0.50 ± 0.11
Insulin	0.89 ± 0.17	0.75 ± 0.13	0.69 ± 0.13	0.84 ± 0.17
Sedentary	0.64 ± 0.13	0.88 ± 0.19	0.48 ± 0.09	0.81 ± 0.16‡
Exercise	0.90 ± 0.20	1.35 ± 0.29	0.88 ± 0.16†	1.08 ± 0.18
SVL				
Saline	1.11 ± 0.20	1.09 ± 0.16	1.33 ± 0.14	0.82 ± 0.19
Insulin	1.06 ± 0.14	0.95 ± 0.11	1.03 ± 0.24	0.99 ± 0.15
Sedentary	0.87 ± 0.08	0.92 ± 0.13	0.79 ± 0.07	0.75 ± 0.16
Exercise	0.99 ± 0.16	1.32 ± 0.24	1.19 ± 0.27	1.58 ± 0.17†
Soleus				
Saline	0.39 ± 0.08	0.41 ± 0.07	0.42 ± 0.11	0.25 ± 0.07
Insulin	0.48 ± 0.10	0.55 ± 0.12	0.60 ± 0.09	0.56 ± 0.17
Sedentary	0.27 ± 0.06	0.40 ± 0.09	0.28 ± 0.05	0.51 ± 0.05‡
Exercise	0.26 ± 0.06	0.52 ± 0.08*	0.28 ± 0.05	0.46 ± 0.07‡

*$P < 0.05$ vs. standard diet–fed WT mice; †$P < 0.05$ vs. sedentary within genotype; ‡$P < 0.05$ vs. high-fat–fed WT mice.

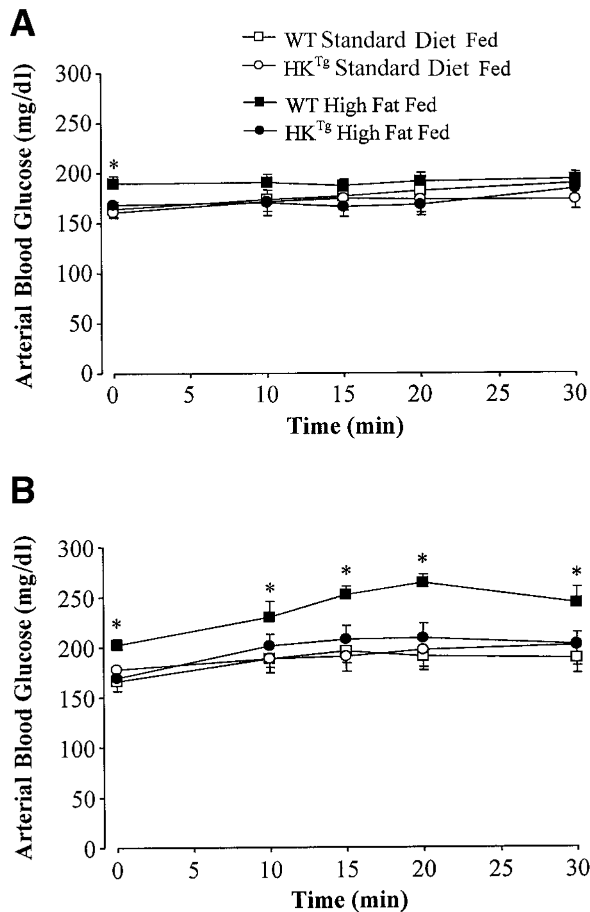

FIG. 5. Arterial blood glucose was measured during a 30-min sedentary (*A*) or exercise (*B*) experiment in chronically catheterized, conscious mice. WT (□ and ■) or HK^{Tg} (○ and ●) mice were fed either a standard diet (□ and ○) or a high-fat diet (■ and ●) up to 4 months of age and fasted for 5 h. Data are mean ± SE for 7–12 mice/group. *$P < 0.05$ vs. remaining groups.

ability of insulin to increase bulk blood flow and capillary recruitment and thus glucose delivery is impaired in the obese Zucker rat, another animal model of insulin resistance (29). A recent study by Kim et al. (30) reported impaired glucose disposal in high-fat–fed rats despite unaltered GLUT4 content or HK activity, again emphasizing the possibility of impaired glucose delivery. The current study provides further direct and indirect evidence that multiple steps of MGU are impaired in a diet-induced model of insulin resistance as HK II overexpression rescued impaired MGU during exercise but neither in the basal state nor during insulin stimulation.

In non–insulin-resistant states, different physiological stimuli that increase glucose fluxes can be used in an attempt to differentially expose the barriers to MGU. Under basal conditions, glucose transport is the primary rate-controlling step of glucose uptake (31–33). However, upon stimulation by insulin (34,35) or exercise (36–38), GLUT4 translocates to the sarcolemma, making it highly permeable to glucose. GLUT4 translocation functionally lowers the barrier to glucose transport and, in effect, shifts the onus of control to glucose delivery and phosphorylation. In agreement with previous work in standard diet–fed FVB/NJ mice (4), glucose phosphorylation is a significant barrier to insulin- and exercise-stimulated MGU as transgenic overexpression of HK II selectively in skeletal muscle was able to augment both insulin- and exercise-stimulated R_g in the muscles of conscious, unrestrained, standard diet–fed C57BL/6J mice (see Figs. 4 and 7). It is important to note that under conditions of high resistance to glucose transport (e.g., saline infusion and sedentary protocols), HK II overexpression had no effect on R_g, providing further support for glucose transport's important role in controlling MGU under basal, sedentary conditions.

It is interesting that the same transgene resulted in subtle phenotypic differences between the C57BL/6J and FVB/NJ mouse strains. For example, in the previous report, HK II overexpression augmented GIR and thus whole-body glucose disposal by ~25%. Here, GIR was only increased by ~15 in HK^{Tg} mice ($P = 0.11$). Whereas HK II overexpression augmented indexes of insulin-stimulated MGU in both mouse strains, the effectiveness of the transgene was increased in the C57BL/6J mice. Differences in physiological parameters between mouse strains are common; thus, one needs to exercise caution when comparing the phenotypes created by genetic manipulations.

High-fat feeding yielded a dramatic reduction in insulin-stimulated MGU that was not improved by HK II overexpression. This observation can be accounted for by one of three possibilities. The first possibility is that glucose phosphorylation during insulin stimulation is not impaired in skeletal muscles of mice, and thus HK II overexpression would not improve insulin-stimulated R_g. The second

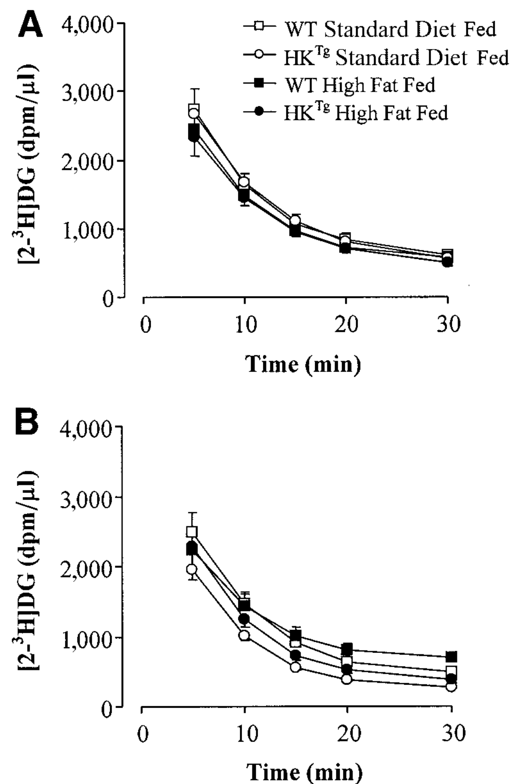

FIG. 6. Disappearance of [2-^{3}H]DG from the plasma during a 30-min sedentary (*A*) or exercise (*B*) experiment in chronically catheterized, conscious mice. WT (□ and ■) or HK^{Tg} (○ and ●) mice were fed either a standard diet (□ and ○) or a high-fat diet (■ and ●) up to 4 months of age and fasted for 5 h. Arterial blood was obtained and measured for plasma [2-^{3}H]DG as described in RESEARCH DESIGN AND METHODS. Data are mean ± SE for 7–14 mice/group.

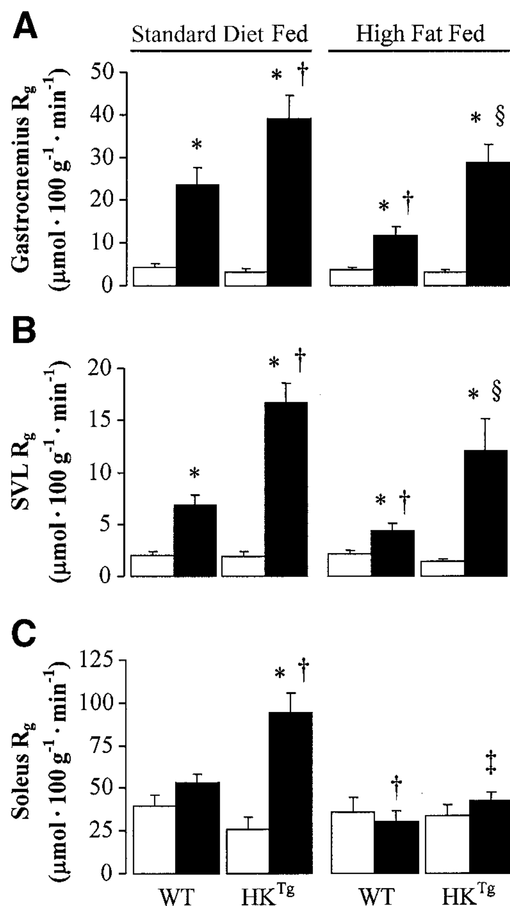

FIG. 7. ***R_g*****, a tissue-specific index of glucose uptake, was measured for the gastrocnemius (*****A*****), SVL (*****B*****), and soleus (*****C*****) muscles during a 30-min sedentary (□) or exercise (■) experiment in chronically catheterized, conscious mice. WT or HK^{Tg} mice were fed either a standard diet or a high-fat diet up to 4 months of age and fasted for 5 h. Data are mean ± SE for 7–12 mice/group. ****P*** **< 0.05 vs. sedentary condition; †*****P*** **< 0.05 vs. WT standard diet. ‡*****P*** **< 0.05 vs. HK^{Tg} standard diet. §*****P*** **< 0.05 vs. WT high-fat fed.**

possibility is that insulin-stimulated translocation of GLUT4 is impaired by high-fat feeding, and, therefore, HK II overexpression alone would not improve insulin-stimulated R_g. The third possibility is that high-fat feeding impairs the function of all steps proximal to glucose phosphorylation during insulin stimulation, masking a functional impairment in glucose phosphorylation. Work from our laboratory using an independent method to assess functional deficits to the steps that compose MGU and another rodent model of insulin resistance, the high-fat–fed rat, suggest the latter (7). The data presented here cannot directly distinguish between potential deficits in glucose delivery and membrane transport.

Monogenic forms of insulin resistance have been created in mice by disrupting nitric oxide synthase (39) and GLUT4 (40,41). These genetic manipulations perturb skeletal muscle glucose delivery or transport and independently impair MGU. In addition, a reduction in HK II activity can impair MGU in an alternative stimulated physiological condition such as exercise (42). Because independent manipulations to each step of the MGU pathway can impair MGU, it is possible that insulin resistance created by high-fat feeding results from an impairment in any of the steps individually or in combination.

In contrast to stimulation by insulin, the marked impairment in exercise-stimulated MGU created by high-fat feeding in WT mice was improved by HK II overexpression in the gastrocnemius and SVL. A primary difference between stimulation by exercise versus insulin is that exercise results in a massive hyperemia. The result of the hyperemia is that the barrier to glucose delivery is lowered. Therefore, these results in conjunction with those from the hyperinsulinemic-euglycemic clamp experiments suggest that high-fat feeding increases the resistance to MGU at sites upstream of glucose phosphorylation (i.e., glucose delivery and membrane transport). However, when the barriers to both glucose transport and delivery are minimized during exercise as a result of hyperemia and contraction-stimulated GLUT4 translocation, an impairment in glucose phosphorylation that can be improved by HK II overexpression is exposed. In agreement with the glucose flux data, after high-fat feeding, G6P concentrations were lower in two of the muscles studied in WT compared with HK^{Tg} mice (see Table 4), further suggesting a functional impairment in glucose phosphorylation.

Control of metabolic pathways is more sensitively assessed using flux measurements as opposed to concentrations of metabolites alone (43). Therefore, it can be difficult to draw conclusions solely from the concentrations of glucose and G6P in tissue homogenates. Several factors complicate the interpretation of tissue glucose and G6P measurements from crude homogenates, including cellular compartmentation, bidirectionality of metabolic substrate flux, and breakdown during the tissue excision process. In addition, flux through a metabolic pathway may be increased without commensurate increases in metabolic intermediates. These factors underscore the value of in vivo flux measurements. Although the [2-^{3}H]DG method is free from concerns associated with measuring metabolites, there are still issues that one must consider (e.g., analog discrimination, tracer recycling). Glucose and [2-^{3}H]DG have slightly different affinities for GLUTs and HKs. A correction factor, the lumped constant, can be applied to adjust for analog discrimination. The lumped constant for 2-deoxyglucose has been determined to be ~1.2 in human muscle in a variety of conditions (44–46) and ~0.9 in rat muscle (47,48). Thus, in rodent muscle, R_g determined from [2-^{3}H]DG accumulation may slightly underestimate MGU. Nevertheless, the measures of R_g provide the most sensitive means for studying the regulation of tissue-specific glucose uptake in vivo and the impact that dietary manipulations have on it.

In conclusion, high-fat feeding impairs both insulin- and exercise-stimulated MGU, but only exercise-stimulated MGU is increased by HK II overexpression. These findings indicate that *1*) the impairment in MGU during insulin stimulation in high-fat–fed mice is due to functional deficits in glucose delivery and/or membrane transport and *2*) deficits in glucose phosphorylation can best be exposed when the barriers to glucose delivery and transport are lowered such as they are during stimulation by exercise. Just as the regulation of MGU can be distributed among glucose delivery, transport, and phosphorylation, dysregulation can occur at multiple steps. Therefore, the most successful treatment strategies for correcting impairments

in MGU will involve targeting multiple if not all of the steps of MGU.

ACKNOWLEDGMENTS

We acknowledge the Department of Veterans Affairs and the National Institute of Diabetes and Digestive and Kidney Diseases for Grants R01 DK-54902 (to D.H.W.), U24-DK-59637 (to D.H.W.), and DK-20593 (to D.K.G. for the Vanderbilt Diabetes Research and Training Center).

We thank Wanda Snead and Greg Poffenberger of the Vanderbilt Hormone Assay Core for performing the insulin assays.

REFERENCES

1. Storlien LH, Baur LA, Kriketos AD, Pan DA, Cooney GJ, Jenkins AB, Calvert GD, Campbell LV: Dietary fats and insulin action. *Diabetologia* 39:621–631, 1996
2. Han D, Hansen P, Host H, Holloszy J: Insulin resistance of muscle glucose transport in rats fed a high-fat diet: a reevaluation. *Diabetes* 46:1761–1767, 1997
3. Kraegen EW, Cooney GJ, Ye JM, Thompson AL, Furler SM: The role of lipids in the pathogenesis of muscle insulin resistance and beta cell failure in type II diabetes and obesity. *Exp Clin Endocrinol Diabetes* 109 (Suppl. 2):S189–S201, 2001
4. Halseth AE, Bracy DP, Wasserman DH: Overexpression of hexokinase II increases insulin- and exercise-stimulated muscle glucose uptake in vivo. *Am J Physiol* 276:E70–E77, 1999
5. Burcelin R, Crivelli V, Dacosta A, Roy-Tirelli A, Thorens B: Heterogeneous metabolic adaptation of C57BL/6J mice to high-fat diet. *Am J Physiol Endocrinol Metab* 282:E834–E842, 2002
6. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN: Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* 37:1163–1167, 1988
7. Halseth AE, Bracy DP, Wasserman DH: Limitations to basal and insulin-stimulated skeletal muscle glucose uptake in the high-fat-fed rat. *Am J Physiol Endocrinol Metab* 279:E1064–E1071, 2000
8. Chang PY, Jensen J, Printz RL, Granner DK, Ivy JL, Moller DE: Overexpression of hexokinase II in transgenic mice. Evidence that increased phosphorylation augments muscle glucose uptake. *J Biol Chem* 271: 14834–14839, 1996
9. Vogt C, Ardehali H, Iozzo P, Yki-Jarvinen H, Koval J, Maezono K, Pendergrass M, Printz R, Granner D, DeFronzo R, Mandarino L: Regulation of hexokinase II expression in human skeletal muscle in vivo. *Metabolism* 49:814–818, 2000
10. Koval JA, DeFronzo RA, O'Doherty RM, Printz R, Ardehali H, Granner DK, Mandarino LJ: Regulation of hexokinase II activity and expression in human muscle by moderate exercise. *Am J Physiol* 274:E304–E308, 1998
11. Rottman JN, Bracy D, Malabanan C, Yue Z, Clanton J, Wasserman DH: Contrasting effects of exercise and NOS inhibition on tissue-specific fatty acid and glucose uptake in mice. *Am J Physiol Endocrinol Metab* 283:E116–E123, 2002
12. Niswender KD, Shiota M, Postic C, Cherrington AD, Magnuson MA: Effects of increased glucokinase gene copy number on glucose homeostasis and hepatic glucose metabolism. *J Biol Chem* 272:22570–22575, 1997
13. Allen DL, Harrison BC, Sartorius C, Byrnes WC, Leinwand LA: Mutation of the IIB myosin heavy chain gene results in muscle fiber loss and compensatory hypertrophy. *Am J Physiol Cell Physiol* 280:C637–C645, 2001
14. Morgan CR, Lazarow A: Immunoassay of pancreatic and plasma insulin following alloxan injection of rats. *Diabetes* 14:669–671, 1965
15. Meszaros K, Bagby GJ, Lang CH, Spitzer JJ: Increased uptake and phosphorylation of 2-deoxyglucose by skeletal muscles in endotoxin-treated rats. *Am J Physiol* 253:E33–E39, 1987
16. Chan TM, Exton JH: A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. *Anal Biochem* 71:96–105, 1976
17. Lloyd B, Burrin J, Smythe P, Alberti KG: Enzymic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol, and 3-hydroxybutyrate. *Clin Chem* 24:1724–1729, 1978
18. Kraegen EW, James DE, Jenkins AB, Chisholm DJ: Dose-response curves for in vivo insulin sensitivity in individual tissues in rats. *Am J Physiol* 248:E353–E362, 1985
19. Westerhoff HV, Groen AK, Wanders RJ: Modern theories of metabolic control and their applications. *Biosci Rep* 4:1–22, 1984
20. Fell DA, Thomas S: Physiological control of metabolic flux: the requirement for multisite modulation. *Biochem J* 311:35–39, 1995
21. Garvey WT, Maianu L, Zhu JH, Brechtel-Hook G, Wallace P, Baron AD: Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. *J Clin Invest* 101:2377–2386, 1998
22. Zierath JR, He L, Guma A, Odegoard Wahlstrom E, Klip A, Wallberg-Henriksson H: Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. *Diabetologia* 39:1180–1189, 1996
23. Ryder JW, Yang J, Galuska D, Rincon J, Bjornholm M, Krook A, Lund S, Pedersen O, Wallberg-Henriksson H, Zierath JR, Holman GD: Use of a novel impermeable biotinylated photolabeling reagent to assess insulin- and hypoxia-stimulated cell surface GLUT4 content in skeletal muscle from type 2 diabetic patients. *Diabetes* 49:647–654, 2000
24. Tremblay F, Lavigne C, Jacques H, Marette A: Defective insulin-induced GLUT4 translocation in skeletal muscle of high fat-fed rats is associated with alterations in both Akt/protein kinase B and atypical protein kinase C (zeta/lambda) activities. *Diabetes* 50:1901–1910, 2001
25. Hansen PA, Han DH, Marshall BA, Nolte LA, Chen MM, Mueckler M, Holloszy JO: A high fat diet impairs stimulation of glucose transport in muscle. Functional evaluation of potential mechanisms. *J Biol Chem* 273:26157–26163, 1998
26. Furler SM, Oakes ND, Watkinson AL, Kraegen EW: A high-fat diet influences insulin-stimulated posttransport muscle glucose metabolism in rats. *Metabolism* 46:1101–1106, 1997
27. Thompson AL, Cooney GJ: Acyl-CoA inhibition of hexokinase in rat and human skeletal muscle is a potential mechanism of lipid-induced insulin resistance. *Diabetes* 49:1761–1765, 2000
28. Otaegui PJ, Ferre T, Riu E, Bosch F: Prevention of obesity and insulin resistance by glucokinase expression in skeletal muscle of transgenic mice. *FASEB J* 17:2097–2099, 2003
29. Wallis MG, Wheatley CM, Rattigan S, Barrett EJ, Clark AD, Clark MG: Insulin-mediated hemodynamic changes are impaired in muscle of Zucker obese rats. *Diabetes* 51:3492–3498, 2002
30. Kim CH, Youn JH, Park JY, Hong SK, Park KS, Park SW, Suh KI, Lee KU: Effects of high-fat diet and exercise training on intracellular glucose metabolism in rats. *Am J Physiol Endocrinol Metab* 278:E977–E984, 2000
31. Marshall BA, Ren JM, Johnson DW, Gibbs EM, Lillquist JS, Soeller WC, Holloszy JO, Mueckler M: Germline manipulation of glucose homeostasis via alteration of glucose transporter levels in skeletal muscle. *J Biol Chem* 268:18442–18445, 1993
32. Gulve EA, Ren JM, Marshall BA, Gao J, Hansen PA, Holloszy JO, Mueckler M: Glucose transport activity in skeletal muscles from transgenic mice overexpressing GLUT1: increased basal transport is associated with a defective response to diverse stimuli that activate GLUT4. *J Biol Chem* 269:18366–18370, 1994
33. Ren JM, Marshall BA, Gulve EA, Gao J, Johnson DW, Holloszy JO, Mueckler M: Evidence from transgenic mice that glucose transport is rate-limiting for glycogen deposition and glycolysis in skeletal muscle. *J Biol Chem* 268:16113–16115, 1993
34. Cushman SW, Wardzala LJ: Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell: apparent translocation of intracellular transport systems to the plasma membrane. *J Biol Chem* 255:4758–4762, 1980
35. Suzuki K, Kono T: Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proc Natl Acad Sci U S A* 77:2542–2545, 1980
36. Ploug T, Galbo H, Ohkuwa T, Tranum-Jensen J, Vinten J: Kinetics of glucose transport in rat skeletal muscle membrane vesicles: effects of insulin and contractions. *Am J Physiol* 262:E700–E711, 1992
37. Etgen GJ Jr, Memon AR, Thompson GA Jr, Ivy JL: Insulin- and contraction-stimulated translocation of GTP-binding proteins and GLUT4 protein in skeletal muscle. *J Biol Chem* 268:20164–20169, 1993
38. Brozinick JT Jr, Etgen GJ Jr, Yaspelkis BB 3rd, Ivy JL: The effects of muscle contraction and insulin on glucose-transporter translocation in rat skeletal muscle. *Biochem J* 297:539–545, 1994
39. Shankar RR, Wu Y, Shen HQ, Zhu JS, Baron AD: Mice with gene disruption of both endothelial and neuronal nitric oxide synthase exhibit insulin resistance. *Diabetes* 49:684–687, 2000
40. Stenbit AE, Tsao TS, Li J, Burcelin R, Geenen DL, Factor SM, Houseknecht K, Katz EB, Charron MJ: GLUT4 heterozygous knockout mice develop muscle insulin resistance and diabetes. *Nat Med* 3:1096–1101, 1997
41. Katz EB, Stenbit AE, Hatton K, DePinho R, Charron MJ: Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. *Nature* 377:151–155, 1995
42. Fueger PT, Heikkinen S, Bracy DP, Malabanan CM, Pencek RR, Laakso M,

Wasserman DH: Hexokinase II partial knockout impairs exercise-stimulated glucose uptake in oxidative muscles of mice. *Am J Physiol Endocrinol Metab* 285:E958–E963, 2003
43. Stephanopoulos G: Metabolic fluxes and metabolic engineering. *Metab Eng* 1:1–11, 1999
44. Utriainen T, Lovisatti S, Makimattila S, Bertoldo A, Weintraub S, DeFronzo R, Cobelli C, Yki-Jarvinen H: Direct measurement of the lumped constant for 2-deoxy-[1-(14)C]glucose in vivo in human skeletal muscle. *Am J Physiol Endocrinol Metab* 279:E228–E233, 2000
45. Peltoniemi P, Lonnroth P, Laine H, Oikonen V, Tolvanen T, Gronroos T, Strindberg L, Knuuti J, Nuutila P: Lumped constant for [(18)F]fluorodeoxyglucose in skeletal muscles of obese and nonobese humans. *Am J Physiol Endocrinol Metab* 279:E1122–E1130, 2000
46. Kelley DE, Williams KV, Price JC, Goodpaster B: Determination of the lumped constant for [18F] fluorodeoxyglucose in human skeletal muscle. *J Nucl Med* 40:1798–1804, 1999
47. Burnol AF, Ferre P, Leturque A, Girard J: Effect of insulin on in vivo glucose utilization in individual tissues of anesthetized lactating rats. *Am J Physiol* 252:E183–E188, 1987
48. Ferre P, Leturque A, Burnol AF, Penicaud L, Girard J: A method to quantify glucose utilization in vivo in skeletal muscle and white adipose tissue of the anaesthetized rat. *Biochem J* 228:103–110, 1985