Phosphorylation Barriers to Skeletal and Cardiac Muscle Glucose Uptakes in High-Fat–Fed Mice

Studies in Mice With a 50% Reduction of Hexokinase II

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OBJECTIVE—Muscle glucose uptake (MGU) is regulated by glucose delivery to, transport into, and phosphorylation within muscle. The aim of this study was to determine the role of limitations in glucose phosphorylation in the control of MGU during either physiological insulin stimulation (4 mU · kg^{-1} · min^{-1}) or exercise with chow or high-fat feeding.

RESEARCH DESIGN AND METHODS—C57BL/6J mice with (HK$^{+/-}$) and without (WT) a 50% hexokinase (HK) II deletion were fed chow or high-fat diets and studied at 4 months of age during a 120-min insulin clamp or 30 min of treadmill exercise (n = 8–10 mice/group). 2-deoxy[^{3}H]glucose was used to measure R_g, an index of MGU.

RESULTS—Body weight and fasting arterial glucose were increased by high-fat feeding and partial HK II knockout (HK$^{+/-}$). Both high-fat feeding and partial HK II knockout independently created fasting hyperinsulinemia, a response that was increased synergistically with combined high-fat feeding and HK II knockout. Whole-body insulin action was suppressed by ~25% with either high-fat feeding or partial HK II knockout alone but by >50% when the two were combined. Insulin-stimulated R_g was modestly impaired by high-fat feeding and partial HK II knockout independently (~15–20%) but markedly reduced by the two together (~40–50%). Exercise-stimulated R_g was reduced by ~50% with high-fat feeding and partial HK II knockout alone and was not attenuated further by combining the two.

CONCLUSIONS—In summary, impairments in whole-body metabolism and MGU due to high-fat feeding and partial HK II knockout combined during insulin stimulation are additive. In contrast, combining high-fat feeding and partial HK II knockout during exercise causes no greater impairment in MGU than the two manipulations independently. This suggests that MGU is impaired during exercise by high-fat feeding due to, in large part, a limitation in glucose phosphorylation. Together, these studies show that the high-fat–fed mouse is characterized by defects at multiple steps of the MGU system that are precipitated by different physiological conditions. ***Diabetes*** **56:2476–2484, 2007**

The importance of limitations in muscle glucose phosphorylation capacity in insulin resistance is a point of contention. In rodent muscle, glucose phosphorylation to glucose-6-phosphate is catalyzed primarily by the hexokinase (HK) II isozyme. HK II content may be normal (1) or reduced (2) in insulin-resistant states, depending on the muscle fiber type composition. Regardless, glucose phosphorylation capacity is affected due to allosteric inhibition of HK II by acyl-CoA and glucose-6-phosphate or compartmentation of HK II, its allosteric regulators, and its substrates (i.e., glucose and ATP). Insulin stimulation and exercise can be used to expose functional deficits in glucose phosphorylation capacity that may otherwise be undetectable (3). Indeed, we previously used such a strategy in conscious mice overexpressing HK II to provide evidence that glucose phosphorylation is, in fact, impaired by high-fat feeding (4). This previous study showed that HK II overexpression does not cause a unique phenotype in the basal state and is unable to correct impairments in insulin-stimulated muscle glucose uptake (MGU) due to high-fat feeding but largely corrects impairments in exercise-stimulated MGU.

The aims of this study were to determine whether diminished glucose phosphorylation capacity induced by a genetic 50% reduction in HK II *1*) impairs insulin-stimulated MGU in chow-fed mice, *2*) worsens the insulin resistance of high-fat–fed mice, and *3*) further attenuates exercise-stimulated MGU observed with high-fat–feeding and accelerates the onset of fatigue. To this end, mice with (HK$^{+/-}$) and without (WT) a 50% HK II deletion fed either a standard chow or a high-fat diet were studied during insulin clamps and exercise. One premise underlying these studies was that if limitations to glucose phosphorylation were a cause of impaired MGU resulting from high-fat feeding, then removal of 50% of HK II protein should have little additional effect when combined with high-fat feeding. Reciprocally, additivity would predict that the mechanism of impairment created by genetic and dietary factors are likely to be distinct. It was hypothesized that HK II reduction in high-fat fed mice would further impair MGU during an insulin clamp but not during exercise. Furthermore, it was hypothesized that a reduction in HK II

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[2-^{3}H]DG, 2-deoxy[^{3}H]glucose; [2-^{3}H]DGP, 2-deoxy[^{3}H]glucose-6-phosphate; GIR, glucose infusion rate; HK, hexokinase; ISI, insulin sensitivity index; MGU, muscle glucose uptake; NEFA, nonesterified fatty acid.

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TABLE 1
Cardiovascular parameters of conscious C57BL/6J mice

	WT mice		$HK^{+/-}$ mice	
	Chow fed	High-fat fed	Chow fed	High-fat fed
Heart rate (bpm)	694 ± 12	711 ± 7	700 ± 5	695 ± 18
SBP (mmHg)	109 ± 4	113 ± 5	120 ± 3*	115 ± 4
LV_{mass} (mg)	92 ± 5	109 ± 5†	97 ± 4	115 ± 8†
FS (%)	54 ± 1	53 ± 2	57 ± 1*	53 ± 2
IVSd (mm)	0.91 ± 0.01	0.98 ± 0.02†	0.93 ± 0.01	0.97 ± 0.02†
LVIDd (mm)	3.12 ± 0.07	3.19 ± 0.06	3.02 ± 0.05	3.16 ± 0.10
LVPWd (mm)	0.90 ± 0.02	1.04 ± 0.02†	0.97 ± 0.03*	1.01 ± 0.03
IVSs (mm)	1.69 ± 0.03	1.70 ± 0.04	1.78 ± 0.04	1.73 ± 0.05
LVIDs (mm)	1.44 ± 0.06	1.52 ± 0.07	1.45 ± 0.04	1.57 ± 0.05
LVPWs (mm)	1.34 ± 0.04	1.52 ± 0.03†	1.45 ± 0.04*	1.57 ± 0.05†

Data are means ± SEM. n = 8–10 per group. FS, fractional shortening; IVSd, interventricular septal thickness in diastole; IVSs, interventricular septal thickness in systole; LV_{mass}, left ventricular mass; LVIDd, LV end-diastolic dimension; LVIDs, LV end-systolic dimension; LVPWd, LV posterior wall thickness in diastole; LVPWs, LV posterior wall thickness in systole; SBP, systolic blood pressure. *$P < 0.05$ vs. WT mice; †$P < 0.05$ vs. chow-fed mice.

would not affect exercise endurance, a functional consequence of metabolic dysregulation, in high-fat fed mice.

RESEARCH DESIGN AND METHODS

Mouse maintenance and genotyping. Procedures were approved by the Vanderbilt University Animal Care and Use Committee. Male Ukko1 (a mixture of BALB/c and DBA/2 strains) mice containing a partial deletion to the HK II gene ($HK^{+/-}$) that results in a 50% reduction in HK II activity in heart, skeletal muscle, and adipose tissue, but does not change HK I activity (5), were backcrossed onto the C57BL/6J background for at least five generations (6). At 3 weeks of age, littermates were separated by sex and maintained in microisolator cages. Genotyping was performed as previously described (5) with the PCR on genomic DNA obtained from a tail biopsy and isolated by a

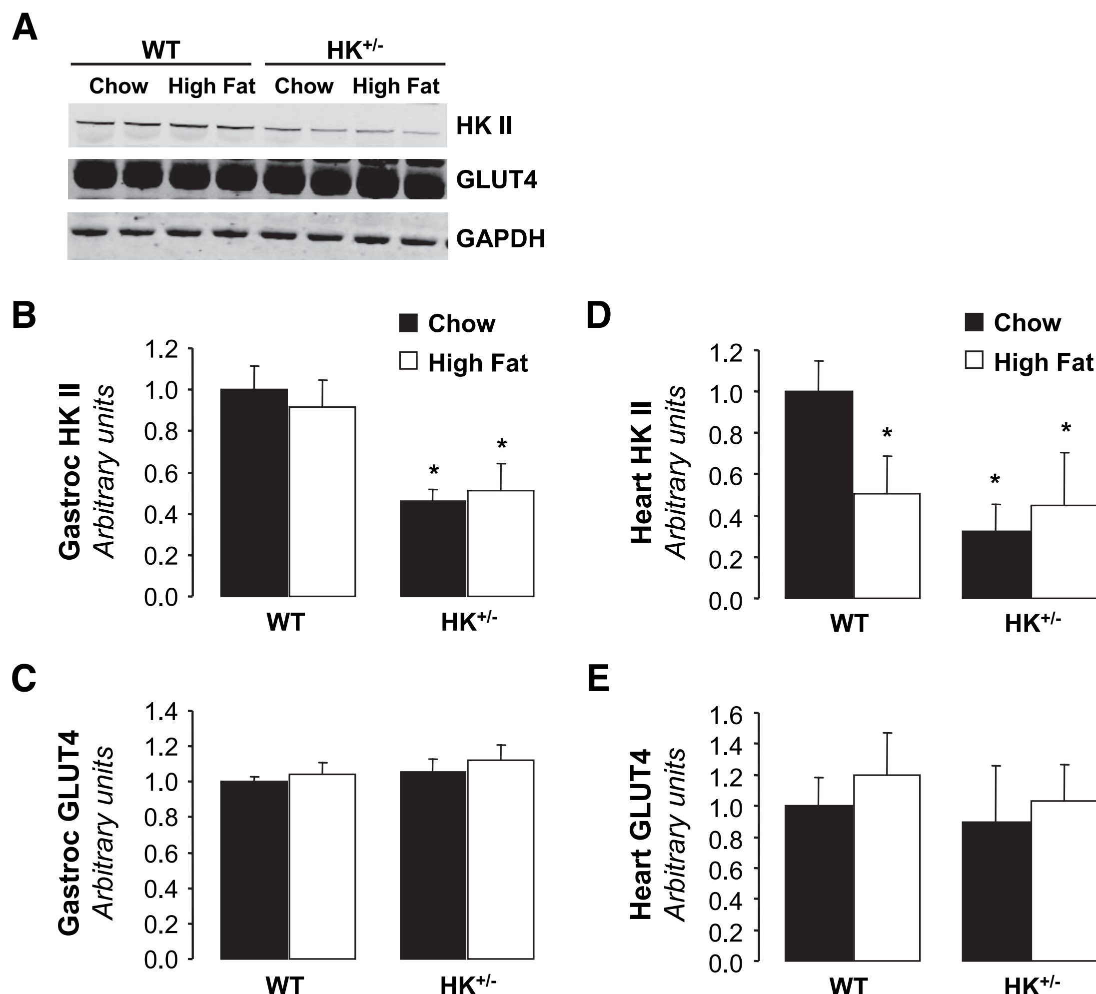

FIG. 1. Total muscle HK II and GLUT4 content. Immunoblotting was performed to measure total HK II (*B* and *D*) and GLUT4 (*C* and *E*) protein content in the gastrocnemius (*B* and *C*) and cardiac (*D* and *E*) muscles of WT mice or $HK^{+/-}$ mice fed either a chow or a high-fat diet. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serves as an internal control. Representative blots for the gastrocnemius are shown in *A*. Data are means ± SEM for 4–8 mice/group. *$P < 0.05$ vs. WT chow-fed mice.

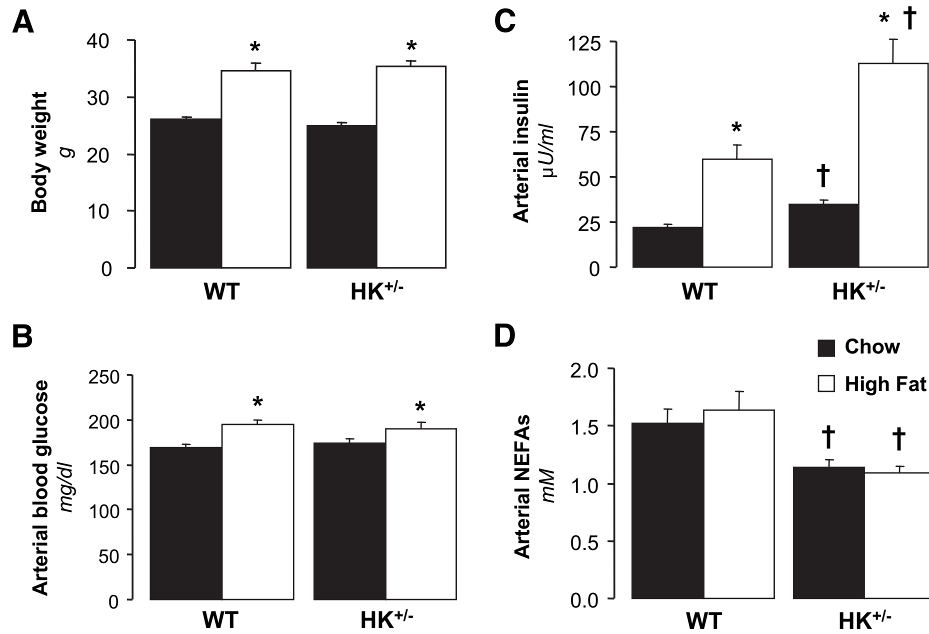

FIG. 2. Metabolic parameters of chronically catheterized, 5-h–fasted C57/BL6J mice. WT or HK$^{+/-}$ mice fed either a chow or a high-fat diet had catheters surgically implanted in a carotid artery and jugular vein and were allowed to recover for at least 5 days. Body weight was measured (*A*), and mice were fasted for 5 h. Arterial blood was obtained and measured for blood glucose (*B*), insulin (*C*), and NEFAs (*D*). Data are means ± SEM for 9–12 mice/group. *$P < 0.05$ vs. chow-fed mice; †$P < 0.05$ vs. WT mice.

DNeasy tissue kit (Qiagen, Valencia, CA). Upon weaning at 3 weeks of age, mice were fed a standard chow or a high-fat diet ad libitum and were studied at ~4 months of age. The high-fat diet contained 35.5% fat (Diet F3282; Bio-Serv, Frenchtown, NJ), whereas the chow diet contained 5.5% fat (5001 Laboratory Rodent Diet; Purina, Richmond, IN).

Immunoblotting. Total GLUT4 and HKII protein were determined in homogenates of gastrocnemius and cardiac muscles. A total of 20 μg protein was resolved on 4–12% Bis-Tris SDS-PAGE gels, followed by electrophoretic transfer to polyvinylidine fluoride membranes. Membranes were blocked with 1× milk buffer (Chemicon; Temecula, CA) for 15 min, probed with rabbit anti-GLUT4 (1:1,000; Abcam) overnight at 4°C, and then incubated with anti-rabbit horseradish peroxidase (1:20,000; Pierce, Rockford, IL) for 1 h at 23°C. Membranes were exposed to chemiluminescent substrate and imaged using the VersaDoc imaging system (Bio-Rad; Hercules, CA). Membranes were stripped with a Re-Blot Western blot recycling kit (Chemicon), probed with rabbit anti-HKII (1:1,000; Chemicon), incubated with anti-rabbit horseradish peroxidase (1:20,000), and developed as before. To confirm equal protein loading and transfer, membranes were stripped and reprobed with monoclonal anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:4,000; Abcam) and then incubated with anti-mouse horseradish peroxidase (1:20,000). All antibodies were diluted in 1% polyvinylpyrrolidone in Tris-buffered saline, and membranes were washed between antibody incubations three times with Tris-buffered saline for 10 min. Densitometry was performed using Quantity One Analysis Software (Bio-Rad).

Echocardiography and blood pressure measurement. At 16 weeks of age, transthoracic echocardiograms were performed on resting conscious mice using a 15-MHz transducer (Sonos 5500 system; Agilent) as previously described (7,8). In addition, systolic blood pressure was measured in conscious mice using tail-cuff plethysmography (9). Echocardiographic and blood pressure measurements were made twice and thrice, respectively, to account for acclimation.

Surgical procedures. Surgical procedures were similar to those described previously (10,11). Briefly, mice of either sex were anesthetized with pentobarbitol (70 mg/kg body wt) and the left common carotid artery and the right jugular vein catheterized for sampling and infusions, respectively. Free catheter ends 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 OD), which were exteriorized and sealed with stainless steel plugs. Lines were kept patent by flushing daily with 10–40 μl saline containing 200 units/ml heparin and 5 mg/ml ampicillin. Animals were housed individually after surgery, and body weight was recorded daily.

Hyperinsulinemic-euglycemic clamp and saline control experiments. Experiments were performed on 5-h fasted mice as previously described (4,12–14). For hyperinsulinemic-euglycemic clamp and saline control experiments, 4 mU · kg^{-1} · min^{-1} insulin or an infusion of saline, respectively, was begun at a rate of 1.375 μl/min at $t = -90$ min. Both experiments were conducted in the same environment (e.g., dimensions of the experimental chamber and catheter connections); therefore, data from the saline-infusion experiments serve as basal controls for clamp experiments. Euglycemia was maintained during clamps by frequent measurement of 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 fall in hematocrit of >5%. At $t = 0$ min, arterial blood (150 μl) was obtained and processed for baseline analyses. 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 glucose and [2-^{3}H]DG. At $t = 30$ min, a final arterial sample (170 μl) was drawn and processed in the same way as the baseline blood sample but with added blood removal for determination of plasma [2-^{3}H]DG. Mice were anesthetized with a pentobarbital infusion. The gastrocnemius and heart muscles were excised, frozen in liquid nitrogen, and stored at −70 C.

Exercise and sedentary control experiments. Experiments were performed on 5-h–fasted mice as previously described (4,13,14). Approximately 1 h before an experiment, mice were placed on a treadmill to acclimate them to the changed environment. At $t = 0$ min, a baseline arterial blood sample (150 μl) was drawn for the measurement of glucose, hematocrit, insulin, and nonesterified fatty acids (NEFAs). To prevent a fall in hematocrit, the remaining erythrocytes were washed once with 0.9% saline containing 10 units/ml heparin and reinfused. The mice either remained sedentary or ran on a treadmill to 30 min at 16.7 m/min with a 0% grade ($n = 9–11$ for each experimental group and genotype). Both experiments were conducted in the same environment (e.g., dimensions of the experimental chamber on the treadmill and catheter connections); therefore, data from sedentary experiments serve as basal controls for exercise experiments. At $t = 5$ min, a 12-μCi bolus of [2-^{3}H]DG (New England Nuclear, Boston, MA) was administered to determine R_g. At $t = 10$, 15, and 20 min, ~50 μl arterial blood was sampled to determine glucose and [2-^{3}H]DG. At $t = 30$ min, 150 μl arterial blood was withdrawn to determine glucose, hematocrit, insulin, [2-^{3}H]DG, and NEFAs, and mice were anesthetized with an arterial pentobarbital infusion. The gastrocnemius and heart muscles were excised, frozen in liquid nitrogen, and stored at −70 C.

Processing of plasma and muscle samples. Immunoreactive insulin was assayed with a double-antibody method (15). NEFAs were measured spectrophotometrically by an enzymatic assay (Wako NEFA C kit; Wako Chemicals, Richmond, VA). Following deproteinization with $Ba(OH)_2$ (0.3 N) and $ZnSO_4$ (0.3 N), plasma [2-^{3}H]DG radioactivity was determined by liquid scintillation counting.

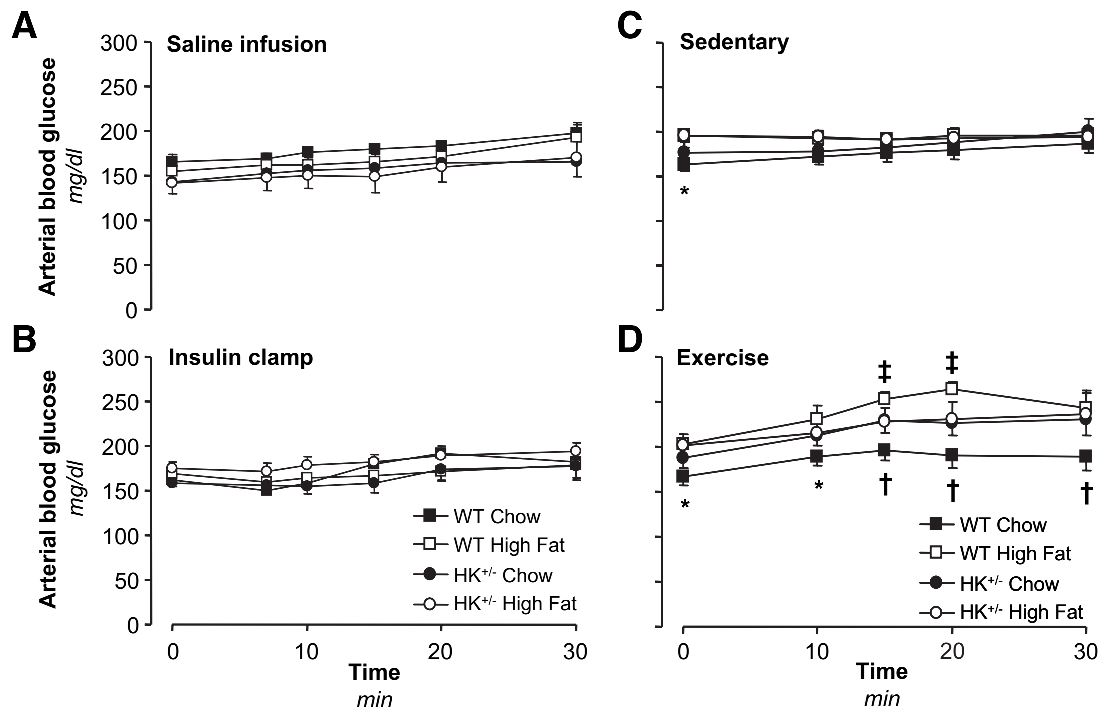

FIG. 3. Arterial blood glucose concentration during experimental conditions. WT or HK$^{+/-}$ mice fed either a chow or a high-fat diet had catheters surgically implanted in a carotid artery and jugular vein and were allowed to recover for at least 5 days. Arterial blood glucose was measured during the last 30 min of a 120-min saline infusion (*A*) or hyperinsulinemic-euglycemic clamp (*B*) or during a 30-min sedentary (*C*) or exercise (*D*) period. Data are means ± SEM for 9–12 mice/group. *$P < 0.05$ vs. WT high-fat–fed mice; †$P < 0.05$ vs. all other groups; ‡$P < 0.05$ vs. all other groups.

Muscle was 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-deoxy[^{3}H]glucose-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. Radioactivity in [2-^{3}H]DG was then determined. [2-^{3}H]DGP was the difference between the two aliquots. Muscle glycogen was measured according to the method of Chan and Exton (16).

Capillary density was determined in 5-μm sections of paraffin-embedded gastrocnemius muscles following immunohistochemical detection of CD-31 (PECAM-1 [platelet endothelial cell adhesion molecule-1]) in endothelial cells. Endogenous peroxidase was quenched with 0.03% hydrogen peroxide, and samples were treated with diluted rabbit serum before primary antibody addition. Slides were incubated with goat anti–CD-31/PECAM-1 (1:400, Santa Cruz Biotechnology) for 45 min. The Vectastain ABC Elite (Vector Laboratories) System and DAB+ (Dako Cytomation) was used to produce visible staining. Slides were lightly counterstained with Mayer's hematoxylin, dehydrated, and coverslipped. Capillaries in three visible fields were counted and averaged for each muscle.

Endurance capacity. In separate groups of animals, exercise endurance was measured as previously described (6). Briefly, mice were placed in an enclosed treadmill (Columbus Instruments) and allowed to acclimate to their surroundings for 45 min. Mice were then run at 20 m/min until they were no longer able to keep pace with the treadmill. Mice were encouraged to run as long as possible with the use of an electric grid placed at the end of the treadmill (1.5 mA, 200-ms pulses, 4 Hz). Mice were defined as exhausted if they remained on the shock grid for 5 continuous seconds.

Calculations and statistical analysis. The insulin sensitivity index (ISI) was calculated by dividing the average glucose infusion rate (GIR) during the last 30 min of an insulin clamp by the insulin concentration over the same time interval. R_g was calculated as previously described (4,14,17). To ascertain the effects of dietary and/or genetic manipulations, R_g data are expressed relative to chow-fed WT mice. Gastrocnemius R_g for chow-fed WT mice during saline, insulin clamp, sedentary, and exercise experiments were 1.1 ± 0.1, 6.6 ± 0.8, 2.6 ± 0.5, and 24.4 ± 4.7 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$, respectively. Heart R_g for chow-fed WT mice during saline, insulin clamp, sedentary, and exercise experiments were 29.9 ± 11.5, 147.0 ± 12.6, 23.3 ± 6.3, and 39.8 ± 8.3 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$, respectively. Data are presented as means ± SEM. Differences between groups were determined by ANOVA or repeated-measures ANOVA for time course data followed by a Tukey's post hoc test. The significance level was set at $P < 0.05$.

RESULTS

Baseline characteristics of mouse models. HK II protein was decreased in HK$^{+/-}$ mice by 50% in the gastrocnemius and heart muscles compared with WT mice (Fig. 1). While high-fat feeding did not alter gastrocnemius HK II content, it did lead to a reduction in heart HK II content, albeit only in WT mice. Neither a partial HK II knockout nor high-fat feeding altered total GLUT4 content.

Baseline characteristics in 5-h fasted WT and HK$^{+/-}$ mice fed either chow or high-fat diet are shown in Fig. 2. Partial HK II knockout did not alter body weight or blood glucose concentration in chow-fed mice; it did, however, increase plasma insulin concentrations and decrease plasma NEFA concentrations.

High-fat feeding increased body weight and arterial glucose and insulin concentrations in both genotypes. High-fat feeding did not, however, alter NEFAs in either genotype. While HK II partial knockout did not exacerbate the hyperglycemia created by high-fat feeding, high-fat feeding and partial HK II knockout together resulted in a synergistic increase in fasting insulin.

Partial HK II knockout increased systolic blood pressure and fractional shortening without alterations in heart rate or left ventricular mass (Table 1). High-fat feeding increased left ventricular mass, indicating cardiac hypertrophy, but did not alter heart rate, systolic blood pressure, or fractional shortening. Neither partial HK II knockout nor high-fat feeding altered capillary density in the gastrocnemius muscle (467 ± 72, 418 ± 34, 574 ± 125, and 516 ± 70 capillaries/mm^2 for WT chow-fed, WT high-fat–fed, HK$^{+/-}$ chow-fed, and HK$^{+/-}$ high-fat–fed mice, respectively).

Arterial blood glucose levels during the last 30 min of the saline infusion control period for insulin clamps and

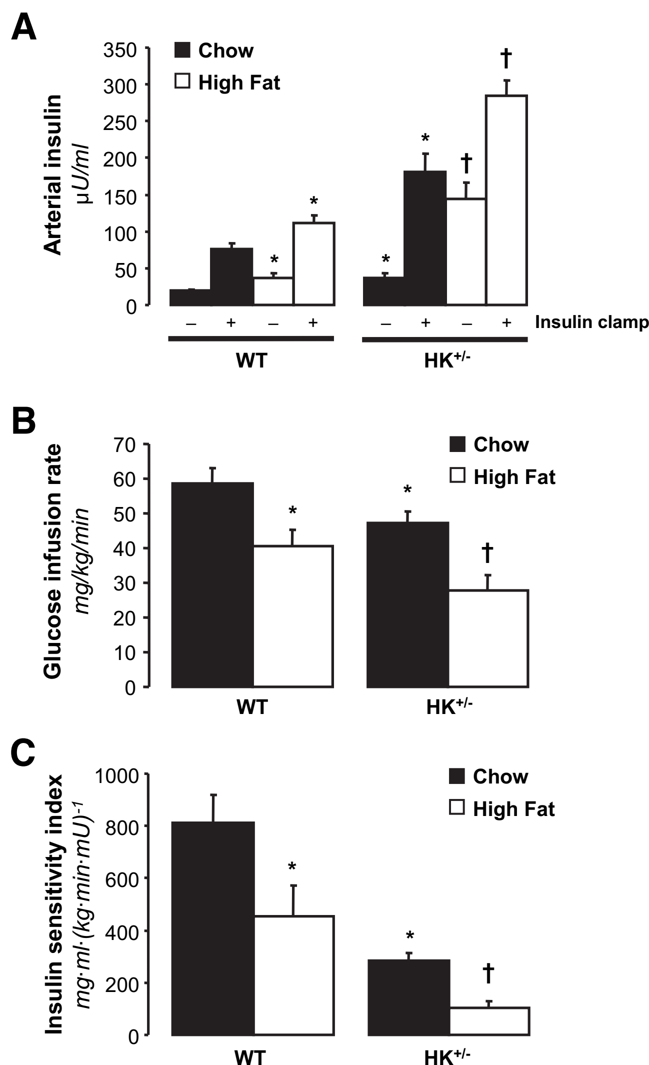

FIG. 4. Insulin concentration, GIR, and ISI during a hyperinsulinemic-euglycemic clamp. WT or $HK^{+/-}$ mice fed either a chow or a high-fat diet had catheters surgically implanted in a carotid artery and jugular vein and were allowed to recover for at least 5 days. After a 5-h fast, insulin was infused at 4 mU · kg^{-1} · min^{-1} and arterial blood glucose maintained by infusing glucose into a jugular vein. Average arterial plasma insulin concentrations during the experiment are shown (*A*). The average GIRs over the final 30 min of the 120-min hyperinsulinemic-euglycemic clamp (*B*) and the ISI (*C*) are reported. Data are means ± SEM for 9–12 mice/group. *$P < 0.05$ vs. WT chow-fed mice; †$P < 0.05$ vs. all other groups.

the last 30-min sedentary control period for exercise studies are shown in Fig. 3*A* and *C*. There were no differences in blood glucose levels between groups.

Insulin-stimulated metabolism. Arterial blood glucose was not different between groups during the saline-infusion control (basal) or hyperinsulinemic-euglycemic clamp experiments (Fig. 3*A* and *B*). The differences in insulin concentration observed in the basal condition remained during the insulin clamp (Fig. 4*A*). During the insulin clamp, NEFAs were reduced by ~50% compared with saline infusion in all groups (0.7 ± 0.1, 0.8 ± 0.2, 0.5 ± 0.1, and 0.6 ± 0.1 mmol/l for WT chow-fed, WT high-fat–fed, $HK^{+/-}$ chow-fed, and $HK^{+/-}$ high-fat–fed mice, respectively). The GIR was reduced in chow-fed $HK^{+/-}$ mice (Fig. 4*B*). High-fat feeding in WT mice decreased GIRs to the same extent as rates observed in chow-fed $HK^{+/-}$ mice. Further, GIR was lower in high-fat–fed $HK^{+/-}$ mice than in their WT littermates who were also fed a high-fat diet. Because of the differences in the plasma insulin concentrations during the clamp, ISI was calculated. As with GIR, the ISI was lower in chow-fed $HK^{+/-}$ mice than in their WT littermates, and high-fat feeding in WT mice decreased ISI to a level comparable with that observed in chow-fed $HK^{+/-}$ mice (Fig. 4*C*). The combination of high-fat feeding and partial HK II knockout dramatically decreased ISI in comparison with all other groups.

During the saline infusion, gastrocnemius R_g was not different between groups (Fig. 5*A*). A partial HK II knockout decreased cardiac R_g during the saline infusion (Fig. 6*A*). Neither a partial HK II knockout nor high-fat feeding significantly decreased insulin-stimulated R_g in gastrocnemius (Fig. 5*B*). However, the combination of genetic and diet perturbations significantly decreased skeletal muscle insulin-stimulated R_g. In contrast, cardiac insulin-stimulated R_g was decreased by either partial HK II knockout or high-fat feeding alone, and this decrease was exacerbated by the combination of the two (Fig. 6*B*). The attenuation of R_g was marked in high-fat–fed mice with partial HK II knockout despite higher insulin levels.

Gastrocnemius glycogen was generally lower in mice with a partial HK II knockout, whereas cardiac glycogen was higher in mice with a partial HK II knockout (Table 2). Cardiac, but not gastrocnemius, glycogen increased dramatically during the insulin clamp.

Exercise metabolism and endurance capacity. Arterial blood glucose did not significantly change throughout the 30 min of exercise in chow-fed WT mice (Fig. 3*D*). High-fat feeding, a partial HK II knockout, and both together resulted in exercise-induced hyperglycemia such that by 15 min of exercise, glucose was greater than in chow-fed WT mice. Despite the exercise-induced hyperglycemia, insulin concentrations fell in chow-fed $HK^{+/-}$ and high-fat–fed mice of both genotypes (data not shown). However, high-fat–fed mice of either genotype still had higher insulin concentrations. While plasma NEFAs remained stable throughout exercise in chow-fed WT mice, they fell in the other groups (data not shown).

Sedentary R_g was unaltered by diet or genotype in gastrocnemius but was elevated by partial HK II knockout in cardiac muscle (Figs. 5*C* and 6*C*). Exercise-stimulated R_g was blunted by high-fat feeding in gastrocnemius of WT mice (Fig. 5*D*). A partial HK II knockout also severely impaired gastrocnemius R_g in chow-fed exercised mice. In fact, it was reduced to the same rate as that seen in high-fat–fed WT mice. High-fat feeding did not further impair gastrocnemius R_g in exercised $HK^{+/-}$ mice. High-fat feeding dramatically increased cardiac exercise–stimulated R_g (Fig. 6*D*). However, this diet-induced increase was severely dampened in mice with partial HK II knockout. High-fat feeding, partial HK II knockout, and both together decreased endurance capacity, as measured by the time until exhaustion (Fig. 7). Overall, the effects on endurance time closely paralleled those of exercise-stimulated R_g in gastrocnemius.

Gastrocnemius glycogen was lower in all mice following exercise compared with that in sedentary mice with the exception of WT high-fat–fed mice (Table 2). As before, gastrocnemius glycogen was generally lower in mice with a partial HK II knockout, but this observation did not hold true with cardiac glycogen.

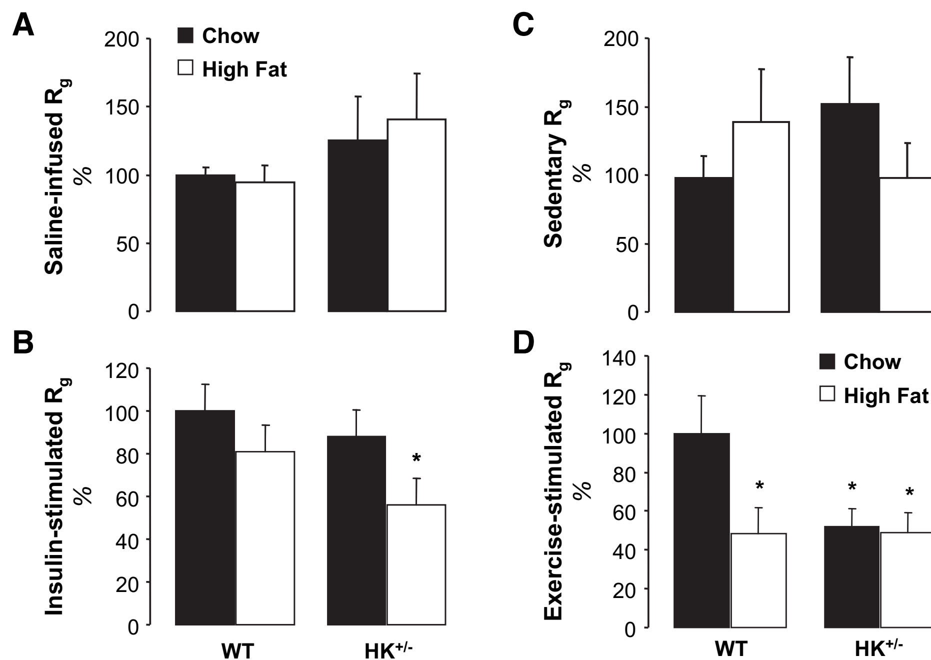

FIG. 5. Glucose uptake into the gastrocnemius muscle during insulin stimulation and exercise. Gastrocnemius R_g, an index of muscle glucose uptake, was measured in conscious, freely moving WT or $HK^{+/-}$ mice fed either a chow or a high-fat diet during a control saline infusion (*A*), a hyperinsulinemic-euglycemic clamp (*B*), a sedentary state (*C*), and exercise (*D*). Data are expressed relative to the values obtained for WT chow-fed mice and are means ± SEM for 9–12 mice/group. Gastrocnemius R_g for chow-fed WT mice during saline, insulin clamp, sedentary, and exercise experiments were 1.1 ± 0.1, 6.6 ± 0.8, 2.6 ± 0.5, and 24.4 ± 4.7 μmol · 100 g^{-1} · min^{-1}, respectively. *P < 0.05 vs. WT chow-fed mice.

DISCUSSION

We previously reported that mice with a 50% reduction in HK II content have a phenotype at rest that is largely indistinguishable from WT littermates but that impaired exercise-induced increase in MGU was observed in oxidative muscles of mice on a mixed genetic background (14). In the present study, we backcrossed mice with the disrupted HK II gene onto the C57BL/6J background to study the effect of this genetic manipulation in combination with high-fat feeding. The high-fat–fed C57BL/6J mice are obese and insulin resistant compared with chow-fed mice of the same strain (18,19). Here we show that a quantitative genetic reduction of HK II predisposes mice to a greater insulin resistance due to high-fat diet. A 50% HK II knockout and high-fat feeding independently resulted in modest insulin resistance states that were most evident by increased 5-h fasting insulin concentrations and a diminished rate of glucose disposal during the insulin clamp using a physiological insulin dose. However, when mice with half the normal complement of HK II were fed a high-fat diet, fasting insulin was fivefold greater than in WT mice fed a chow diet. Moreover, insulin resistance became marked because the combined effects of reduced HK II and high-fat feeding on whole-body glucose disposal, insulin sensitivity, and heart and skeletal muscle R_g responses were additive. The additive nature of this interaction in conjunction with an effect of high-fat feeding on HK II protein level is consistent with the notion that muscle glucose delivery and/or transport, and not glucose phosphorylation, is/are the major causes of dietary insulin resistance in the mouse. There are, however, two alternate interpretations. One cannot strictly rule out that high-fat feeding may influence the compartmentation of HK during the insulin clamp. If this does occur, the additive effect of diet and genotype on glucose uptake could, in fact, result due to further impairment of glucose phosphorylation. It is also possible that high-fat diet influences the availability of allosteric regulators of HK II, leading to further impairment of glucose phosphorylation.

The results presented here build upon our previous findings demonstrating that a genetic manipulation that resulted in overexpression of HK II did not improve insulin action in high-fat–fed mice (4). High-fat feeding reduced HK II protein content in the heart but not gastrocnemius. These studies provide further support that a functional deficit in glucose phosphorylation capacity is present with high-fat feeding even in the presence of normal HK II protein content. This may be due to the increased acyl-CoA (20) or HK II compartmentation (21,22).

When the mice performed moderate exercise, plasma insulin and NEFA concentrations generally declined. In contrast, arterial blood glucose remained constant in chow-fed WT mice but increased at the onset of exercise and reached a plateau by 15 min of the 30-min protocol in mice fed a high-fat diet with or without a partial HK II deletion, such that blood glucose was greater in these groups. During exercise, the massive hyperemia (23,24) and translocation of GLUT4 from the cytoplasm to sarcolemma (25–27) leads to a robust increase in MGU. The result of these two exercise-stimulated events is that glucose phosphorylation capacity becomes increasingly important in determining MGU (10,13,14,28). Despite the relative hyperglycemia and hyperinsulinemia in $HK^{+/-}$ mice fed a chow or a high-fat diet, the exercise-induced increase in gastrocnemius R_g was reduced by 50% of the response seen in WT chow-fed mice. In contrast to insulin stimulation, the combination of high-fat feeding and a 50% partial HK II knockout had no greater effect than either variable alone. This suggests that partial HK II knockout and high-fat feeding impair exercise-stimulated R_g through a common mechanism, i.e., glucose phosphorylation. This

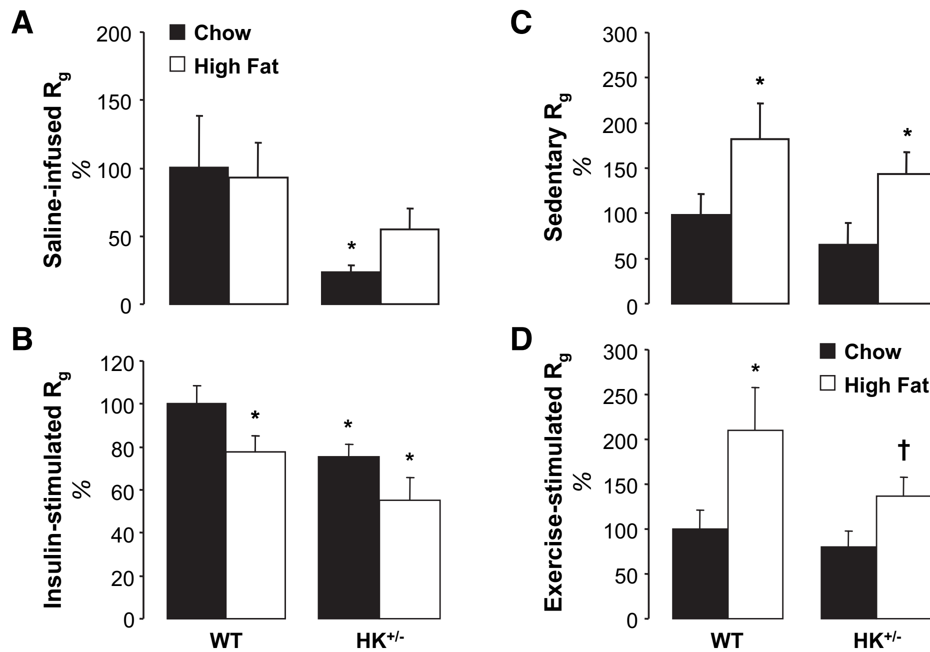

FIG. 6. Glucose uptake into the heart during insulin stimulation and exercise. Heart R_g, an index of muscle glucose uptake, was measured in conscious, freely moving WT or HK$^{+/-}$ mice fed either a chow or a high-fat diet during a control saline-infusion (*A*), a hyperinsulinemic-euglycemic clamp (*B*), a sedentary state (*C*), and exercise (*D*). Data are expressed relative to the values obtained for WT chow-fed mice and are means ± SEM for 9–12 mice/group. Heart R_g for chow-fed WT mice during saline, insulin clamp, sedentary, and exercise experiments was 29.9 ± 11.5, 147.0 ± 12.6, 23.3 ± 6.3, and 39.8 ± 8.3 μmol · 100 g^{-1} · min^{-1}, respectively. *P < 0.05 vs. WT chow-fed mice; †P < 0.05 vs. all other groups.

result is again consistent with a previous study where we showed that HK II overexpression in muscle virtually corrects the reduction in exercise-stimulated R_g seen with high-fat feeding (4).

High-fat feeding and a partial HK II knockout both independently impaired endurance capacity. The combination of the two manipulations did not further reduce endurance. Interestingly, the premature fatigue was closely correlated with exercise-stimulated MGU, and thus endurance capacity and MGU may be linked in the mouse. We previously reported that skeletal muscle HK II content, exercise-stimulated MGU, and exercise endurance capacity are closely correlated (6). Here we show that high-fat feeding, which impairs exercise-stimulated MGU by 50%, is associated with a 40% reduction in endurance time in the mouse. Mice with a partial HK II knockout also have slightly less skeletal muscle glycogen than their WT littermates, and this might contribute to their premature fatigue. In a similar manner, high-fat–fed WT mice are slightly resistant to skeletal muscle glycogenolysis; this inability to efficiently utilize glycogen as a fuel for contractions might thus promote fatigue. Therefore, the combination of impairments in MGU and limitations in glycogen metabolism created by a partial HK II knockout and/or high-fat feeding likely leads to accelerated fatigue.

We have previously shown that glucose phosphorylation is a critical determinant of insulin-stimulated MGU (4,29,30), just as it is with exercise-stimulated MGU (13,31). Thus, we hypothesized that decreasing HK II protein would create a monogenic form of insulin resistance. This was in fact the case as HK$^{+/-}$ mice had fasting hyperinsulinemia and decreased GIR during a hyperinsulinemic-euglycemic clamp, independent of di-

TABLE 2
Muscle glycogen following physiological conditions in C57BL/6J mice

	WT mice		HK$^{+/-}$ mice	
	Chow fed	High-fat fed	Chow fed	High-fat fed
Gastrocnemius glycogen (mg/g)				
Saline infusion	3.3 ± 0.2	3.9 ± 0.3*	2.1 ± 0.3†	2.0 ± 0.3†
Insulin clamp	3.9 ± 0.2‡	3.8 ± 0.3	2.9 ± 0.4†	2.5 ± 0.3†
Sedentary	2.8 ± 0.2	3.0 ± 0.3	1.8 ± 0.2†	2.6 ± 0.2
Exercise	1.9 ± 0.2‡	2.6 ± 0.3*	1.2 ± 0.2†‡	1.5 ± 0.2†‡
Heart glycogen (mg/g)				
Saline infusion	0.6 ± 0.1	0.8 ± 0.2	1.1 ± 0.1†	2.0 ± 0.4†‡
Insulin clamp	2.0 ± 0.4‡	2.0 ± 0.3‡	2.5 ± 0.4‡	2.4 ± 0.3
Sedentary	0.9 ± 0.3	0.6 ± 0.1	0.8 ± 0.2	1.3 ± 0.2*†
Exercise	0.3 ± 0.1‡	0.4 ± 0.1	0.7 ± 0.1†	0.9 ± 0.2*‡

Data are means ± SEM. n = 8–10 per group. *P < 0.05 vs. chow-fed mice; †P < 0.05 vs. WT mice; ‡P < 0.05 vs. control mice (i.e., saline infusion for insulin clamp and sedentary for exercise).

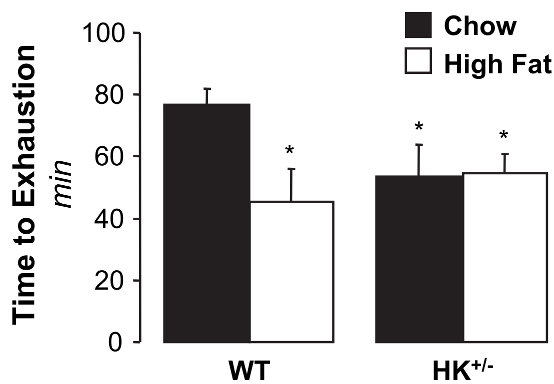

FIG. 7. Endurance capacity is impaired by partial HK II knockout or high-fat feeding. WT or $HK^{+/-}$ mice fed either a chow or a high-fat diet were run at 20 m/min on a motorized treadmill until exhaustion to determine exercise endurance capacity. Data shown are means ± SEM for 9–12 mice/group. **P **< 0.05 vs. WT chow-fed mice.

etary conditions. Interestingly, the form of insulin resistance created by a partial HK II knockout was not manifested by decreases in skeletal muscle R_g during insulin stimulation. It was, however, associated with decreases in cardiac R_g, suggesting that the heart might be an important tissue to consider when dissecting out the cause of whole-body insulin resistance. Strategies to increase muscle HK activity, such as exercise training, may be an important component of combating dietary-induced insulin resistance, as decreases in glucose phosphorylation capacity might worsen the prognosis of patients with metabolic syndrome.

High-fat feeding provoked a cardiovascular phenotype. Glucose uptake was increased during both rest and exercise in hypertrophic hearts of high-fat–fed mice, despite a 50% reduction in cardiac HK II content. The rise in exercise-stimulated glucose uptake was, however, dampened in mice lacking one HK II allele. These results suggest that the control of glucose uptake exerted by glucose phosphorylation during exercise is different between the heart and skeletal muscle. In contrast to exercise, insulin-stimulated glucose uptake was lower in hearts of high-fat–fed mice compared with that in WT littermates. Further, a loss of one HK II allele also led to a decrease in insulin-stimulated glucose uptake. These results closely match the qualitative decreases in cardiac HK II content created by high-fat feeding or a partial HK II knockout. Unlike exercise, insulin stimulation leads to a dramatic fall in the heart's preferred substrate, NEFA, thereby forcing the heart to utilize glucose as a substrate. The end result is that the lower glucose phosphorylation capacity in high-fat–fed or $HK^{+/-}$ mice limits cardiac glucose uptake.

High-fat feeding and commensurate weight gain leads to an increase in cardiac mass. It was of interest to determine whether a reduction in the heart's ability to phosphorylate glucose resulting in decreased cardiac glucose uptake would limit cardiac remodeling following high-fat feeding. The high-fat feeding maneuver used here increased left ventricular mass but did not alter heart rate or systolic blood pressure. Interestingly, $HK^{+/-}$ mice had elevated systolic blood pressure compared with WT mice, but this degree of hypertension did not lead to compensatory hypertrophy, suggesting that a reduction in glucose phosphorylation capacity might limit cardiac remodeling. Further, the reduction in HK II content altered cardiac function, as evidenced by increased fractional shortening.

In summary, to ascertain the significance of the glucose phosphorylation barrier to diet-induced insulin resistance, mice absent one HK II allele were fed chow or high-fat diets. High-fat feeding in WT mice impaired the insulin- and exercise-stimulated increase in glucose kinetics. High-fat feeding in mice with a partial HK II deletion did not further impair exercise-stimulated MGU but resulted in a greatly exaggerated state of insulin resistance. The latter was evident at both the muscle and whole-body levels. These results demonstrate that glucose phosphorylation is an important barrier to MGU during exercise in high-fat–fed mice. Consistent with earlier studies in HK II overexpressing mice, diet-induced insulin resistance does not, on the other hand, appear to be due to an increased glucose phosphorylation barrier, since the effects of a reduction in HK II add to the effects of high-fat feeding. These data show that the impact of a quantitative reduction (50%) in a protein involved in glucose phosphorylation contributes to the insulin-resistant state resulting from an environmental factor (i.e., diet).

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