Transgenic Overexpression of Hexokinase II in Skeletal Muscle Does not Increase Glucose Disposal in Wild-Type or Glut1-Overexpressing Mice*

Running Title: Hexokinase-Overexpressing Transgenic Mice

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SUMMARY

Glut1 transgenic mice were bred with transgenic mice that overexpress hexokinase II in skeletal muscle in order to determine whether whole-body glucose disposal could be further augmented in mice overexpressing glucose transporters. Overexpression of hexokinase alone in skeletal muscle had no effect on glucose transport or metabolism in isolated muscles nor did it alter blood glucose levels or the rate of whole-body glucose disposal. Expression of the hexokinase transgene in the context of the Glut1 transgenic background did not alter glucose transport in isolated muscles but did cause additional increases in steady-state glucose-6-phosphate (3.2-fold) and glycogen (7.5-fold) levels compared to muscles that overexpress the Glut1 transporter alone. Surprisingly, however, these increases were not accompanied by a change in basal or insulin-stimulated whole-body glucose disposal in the doubly transgenic mice compared to Glut1 transgenic mice, probably due to an inhibition of de novo glycogen synthesis as a result of the high levels of steady-state glycogen in the muscles of doubly transgenic mice (430 µmol/g versus 10 µmol/g in wild-type mice). We conclude that the hexokinase gene may not be a good target for therapies designed to counteract insulin resistance or hyperglycemia.
INTRODUCTION

Transgenic mice that overexpress the Glut1 glucose transporter in skeletal muscle have been extensively characterized. Muscles isolated from these mice exhibit a dramatic increase in basal glucose transport and basal glucose metabolism (1,2). The Glut1-overexpressing mice are hypoglycemic relative to non-transgenic littermates and demonstrate an elevation in whole-body glucose disposal under both basal and euglycemic/hyperinsulinemic clamp conditions (1,3). These observations demonstrate that transport is rate-limiting for muscle glucose metabolism and for whole-body glucose disposal in normal mice, a finding that is consistent with a large body of additional experimental data that has accumulated over the past several decades (for a review, see (4)).

Surprisingly, however, muscles from the Glut1-overexpressing mice do not exhibit further increases in glucose transport after treatments that augment transport in normal muscles (insulin, hypoxia, electrically-induced contractions) (5), despite the fact that Glut4 translocates to the sarcolemma and transverse tubules normally in the transgenic muscles (6). These data suggest that the intrinsic activity of Glut4 can be subject to regulation in the plasma membrane domains of muscle fibers.

The dramatic elevation in basal glucose uptake in the muscles of Glut1-overexpressing mice results in the near equilibration of free glucose across the muscle fiber membranes, indicating that the phosphorylation of
glucose by hexokinase has become the rate-limiting step in glucose uptake in these muscles (2). This observation demonstrates that hexokinase catalyzes a secondary rate-limiting step in skeletal muscle glucose disposal that can become predominant if the transport step is artificially elevated. It therefore seemed reasonable that a further increase in muscle glucose uptake and whole-body glucose disposal might be induced in Glut1-overexpressing mice by increasing the expression of hexokinase in the muscles of these mice. This hypothesis has an important practical implication for therapies designed to counteract the insulin resistance and glucose intolerance associated with type 2 diabetes (4).

In order to test this hypothesis, Glut1-overexpressing mice were mated with mice that overexpress hexokinase II in skeletal muscle. Surprisingly, the overexpression of hexokinase II in the muscles of Glut1-overexpressing mice dramatically increased steady-state levels of glucose-6-phosphate and glycogen, but did not augment whole-body glucose disposal. Additionally, mice overexpressing hexokinase II alone exhibited no increase in muscle glucose uptake or whole-body glucose disposal compared to wild-type control mice. We conclude that increasing hexokinase activity in muscle is not likely to be an effective means of counteracting insulin resistance or glucose intolerance.
EXPERIMENTAL PROCEDURES

Materials - Purified porcine insulin (Iletin II) and human insulin (Humulin R U-100) were purchased from Eli Lilly and Co. Radioimmunoassay grade bovine serum albumin (BSA)\(^1\), and the nonradioactive forms of D-glucose, 2-deoxy-D-glucose (2-DG), and D-mannitol were obtained from Sigma. 2-Deoxy-D-\([1,2-\text{\(^3\)H}]\)glucose, \([5-\text{\(^3\)H}]\)glucose and \(\text{\(^3\)H}_2\text{O}\) were purchased from American Radiolabeled Chemicals. \([\text{U-}14\text{C}]\) Mannitol and HPLC-purified 3-[\text{\(^3\)H}]\)-glucose were obtained from DuPont-New England Nuclear.

Preparation of transgenic mice overexpressing GLUT1 and hexokinase II - Transgenic mice overexpressing human hexokinase II (HKII) in striated muscle (a kind gift from Dr. D.K. Granner) were constructed as described previously, using a transgene containing the rat muscle creatinine kinase promoter-enhancer coupled to the human HKII cDNA and an 850-base pair cassette (SVPA) containing the polyadenylation and splice site sequences of SV40 (\(^7\)). The construction of transgenic mice overexpressing the human GLUT1 glucose transporter was also described previously (\(^1\)). The minigene in this construct contains a 2.47-kilobase cDNA fragment encoding the human GLUT1 glucose transporter under the regulation of the 1.2-kilobase rat myosin light chain-2 promoter. Expression of the transgene is restricted to skeletal muscle and does not affect expression of the GLUT4 isoform. Male mice heterozygous for the GLUT1 transgene were bred with female mice heterozygous for the HKII transgene to produce mice of four separate genotypes: those carrying both transgenes, those carry one of the two, and those carrying none of the transgenes. The presence of the transgenes was determined by PCR analysis of genomic DNA obtained from tail biopsy and prepared using the
QiAmp tissue isolation kit (Qiagen, Valencia, CA). Sex-matched littermate mice between the ages of 2-4 months were used for all experiments.

Animal care and tissue preparation - Animals were housed in a room maintained at 23°C with a fixed 12 h light/dark cycle (lights on 6 AM to 6 PM) and given free access to Purina chow and water. On the morning of the experiment, animals were anesthetized with pentobarbital sodium (5 mg/100 g body weight by intraperitoneal injection). Extensor digitorum longus (EDL) muscles were excised and incubated for determination of 2-DG uptake or glucose utilization.

Measurement of 2-deoxyglucose uptake - EDL muscles were incubated for 30 min at 35°C in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol and 0.1% BSA in the absence or presence of 2 mU/ml porcine insulin. Next, muscles were transferred to KHB containing 40 mM mannitol, 0.1% BSA and 2 mU/ml insulin for 10 min to remove glucose from the extracellular space prior to measurement of 2-DG uptake as described previously (8). Briefly, muscles were incubated for 20 min in 1.5 ml KHB containing 1 mM 2-deoxy-D-[1,2-3H] glucose (1.5 µCi/mmolk), 39 mM [U-14C] mannitol (8.5 µCi/mmol), and 0.1% BSA. Insulin was added if it was present in previous incubations. The gas phase was 95% O2- 5% CO2 and the temperature was maintained at 29°C. Muscle extracellular space and intracellular 2-DG concentration (µmol · ml intracellular water−1 · 20 min−1) were determined as previously described (8).

Measurement of muscle glucose metabolism using 3H-glucose - In vitro rates of glucose utilization via glycolysis and glucose incorporation into glycogen were determined in skeletal muscle isolated from control and hexokinase-overexpressing
mice according to a modification (8) of previously described methods (9,10). EDL muscles were incubated for 3 hours at 35°C in 1 ml oxygenated KHB containing 2.25 µCi [5-3H]glucose under one of two conditions: either 8 mM glucose and 20 µU/ml porcine insulin or 24 mM glucose and 2 µU/ml insulin. Mannitol was added to the medium at a concentration sufficient to bring the total osmolality of glucose plus mannitol to 40 mOsm. Using this technique, it has been demonstrated previously that in isolated skeletal muscle, the sum of glucose utilization via glycolysis (measured as [3H] water formation from [5-3H]glucose) plus the amount of tritium retained in glycogen accounts for nearly all of the [5-3H]glucose transported into the cell (11).

Measurement of whole-body glucose disposal: euglycemic-hyperinsulinemic clamps - Clamp experiments were carried out as previously described (3,12) with the following modifications. After placement of the infusion catheter, an infusion of 3-[3H]-glucose at 0.04 µCi/min was begun for measurement of the rate of appearance of glucose, hepatic glucose production, and total body glucose utilization. The infusion was continued during a 75 min control period and 20 µl of blood was taken from the tail for determination of glucose specific activity at 60, 67.5 and 75 min. After 75 min, an infusion of insulin (regular human) was begun at a rate of 80 mU/kg/min and continued for at least 90 min. An infusion of dextrose (25%) was begun, and the infusion rate varied during the clamp period to maintain the blood glucose at approximately 160 mg/dl.
In all the clamps, the continuous infusion of 3-[3H]-glucose tracer was continued during the insulin infusion periods and, in addition, the tracer was added to the 25% dextrose infusion to approximate the glucose specific activity in the blood at the end of the control period. This approximation was based upon measurement of specific activity during identical conditions in the same type of mice in previous experiments. Blood samples for determination of specific activity were taken 20 and 10 min prior to and at the end of the experimental period. The glucose infusion rate was not changed for at least twenty minutes prior to the first determination of specific activity. Both the blood glucose and the glucose specific activity were in steady state during the control period and the clamp sampling periods. Blood for insulin measurement was obtained by cardiac puncture at the conclusion of the experiment.

Blood glucose was measured using 5 µl of whole blood in the Hemocue blood glucose meter (Mission Viejo, CA). Plasma insulin was measured by double-antibody radioimmunoassay using rat insulin standards (Eli Lilly). Specific activity of glucose in whole blood was determined by aqueous scintillation counting of 20 µl of blood deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). Aliquots of the resulting supernatant were dried at 70°C to remove tritiated water prior to resuspension and liquid scintillation counting. The rate of appearance of glucose ($R_a$), which equals the rate of total body glucose utilization ($R_d$) when the blood glucose is in steady state, was calculated by dividing the infusion rate of 3-
[3H]-glucose by the specific activity at the same time. Hepatic glucose production (HGP) was calculated by subtracting the cold glucose infusion rate from $R_a$.

**Measurement of hexokinase activity and GLUT1 glucose transporter content** - Total hexokinase activity and heat-stable hexokinase I activity in quadriceps muscle homogenates (7) were measured enzymatically (13). Hexokinase II activity was subsequently calculated by subtracting hexokinase I from total hexokinase activity. Glucose transporter protein levels were determined in muscle homogenates by immunoblotting, using rabbit polyclonal antibodies directed against the C terminus of either Glut4 (F349) or Glut1 (F350) followed by HRP-conjugated donkey anti-rabbit IgG. Antibody bound proteins were detected using ECL.

**Measurement of muscle metabolites** - Measurements of glycogen and glucose 6-phosphate were performed using gastrocnemius muscles clamp frozen in situ. Frozen muscles were homogenized in ice cold 0.3 M perchloric acid; one aliquot of this homogenate was analyzed for glycogen (14), while the remaining homogenate was centrifuged, and the resulting supernatant neutralized with buffer containing 0.4 M KCl, 2 N KOH, and 0.4 M imidazole. Glucose and glucose-6-phosphate (G-6-P) in this fraction were measured fluorometrically (15).

**Measurement of plasma metabolites**: Mice fed ad libitum were bled (~0.3 ml) from the tail. Plasma insulin concentrations were measured by double-antibody radioimmunoassays using human standards (Linco Research, Inc.). Blood glucose levels were measured using a Hemocue glucose analyzer (Hemocue, Mission Viejo, CA).
RESULTS

Overexpression of Hexokinase

Muscles were resected from littermate mice expressing either the Glut1 transgene, the hexokinase II transgene, both transgenes, or control non-transgenic littermates and analyzed for the expression of hexokinase I, hexokinase II, and total hexokinase activities. Figure 1 shows that the two groups of mice expressing the hexokinase II transgene exhibited similar (7.1 and 8.2-fold, respectively) increases in total hexokinase activity, due mostly to a specific increase in hexokinase II activity. The small increase in hexokinase I activity observed in these two groups is probably due to the fact that the assays do not quantitatively distinguish between the two isoforms. Glut1 transgenic mice exhibited a small increase in total hexokinase activity in muscle compared to their wild-type siblings. These data are consistent with previously published data concerning Glut1 (2) and hexokinase II (7)-overexpressing transgenic mice.

Characterization of Mice Overexpressing Hexokinase Alone

In order to determine whether overexpression of hexokinase II induced an alteration in glucose transport activity, 2-deoxyglucose uptake measurements were performed on isolated extensor digitorum longus muscles. Figure 2 shows that neither basal nor insulin-stimulated glucose transport was significantly altered in hexokinase II-overexpressing muscles.
Similar results were observed in isolated soleus muscles (data not shown). These results suggest that overexpression of hexokinase II in muscle did not directly or indirectly impact on glucose transporter expression, regulation, or activity. Immunoblot analysis confirmed that neither Glut1 nor Glut4 protein levels were altered in the skeletal muscle of hexokinase overexpressing mice relative to wild-type littermates, nor were Glut1 or Glut4 protein levels altered in Glut1 x Hex mice relative to Glut1 mice (data not shown).

In order to determine whether hexokinase overexpression by itself can augment muscle glucose metabolite levels, steady-state glucose-6-phosphate and glycogen levels were measured in muscles clamp frozen in situ from the four groups of mice. Figure 3 shows that neither metabolite was altered in the muscles of mice overexpressing hexokinase II compared to wild-type littermates. This is consistent with the observation that transport, not phosphorylation, is rate-limiting for glucose metabolism in normal mouse muscle.

A previous study with hexokinase-overexpressing mice suggested that hexokinase may become rate-limiting or partially rate-limiting for glucose metabolism in skeletal muscle under conditions of hyperinsulinemia and/or hyperglycemia (7), but direct evidence in the form of glucose tracer analysis in isolated muscles was not provided. In order to address this issue, the metabolism of [5-3H] glucose was examined in muscles isolated from control
and hexokinase II-overexpressing mice. Figure 4 shows that under both basal (8 mM glucose, 20 µU/ml insulin) and hyperglycemic/hyperinsulinemic (24 mM glucose, 2 mU/ml insulin) conditions, neither glycolytic nor glycogenic utilization of glucose was increased in muscles from hexokinase II transgenic mice compared to wild-type littermates. These data are in complete agreement with the data presented above on steady-state levels of glucose metabolites and further confirm the rate-limiting nature of the transport step in normal muscle.

In order to determine the effect of the hexokinase transgene on whole-body glucose homeostasis, blood glucose and whole-body glucose disposal measurements were conducted. Figure 5 shows that expression of the hexokinase II transgene alone had no significant effect on fed blood glucose levels compared to wild-type controls. Figure 6 shows that the hexokinase transgene alone had no significant effect on whole-body glucose disposal (Rd) in either the basal state or during a hyperinsulinemic/euglycemic clamp.

Characterization of Mice Overexpressing Both Hexokinase and Glut1

Steady-state Glucose-6-phosphate levels were increased 2.4-fold and glycogen was increased 5.4-fold in muscles from Glut1 transgenic mice (Figure 3). Increasing hexokinase activity in muscles that also overexpress Glut1 caused a further dramatic elevation in glucose-6-phosphate (7.6-fold compared to wild-type and 3.2-fold compared to Glut1-overexpressing
muscles) and glycogen (40.5-fold compared to wild-type and 7.5-fold compared to Glut1-overexpressing muscles). These observations are completely consistent with the observation that phosphorylation is rate-limiting for glucose uptake in the muscles of the Glut1 transgenic mice (2).

The massive overexpression of glycogen in muscles of the doubly transgenic animals resulted in a dramatic disruption in the normal ultrastructure of striated muscle (see Figure 7). Myofibrils in the doubly transgenic mice exhibited greater electron density compared to mice overexpressing hexokinase alone, which exhibited a normal muscle architecture, and the myofibril bundles were disrupted by numerous glycogen deposits, some of them very large in size. Despite their grossly abnormal muscle architecture, the doubly transgenic mice did not display any obvious change in mobility or behavior compared to their non-transgenic siblings.

Consistent with previously published results (3,12), Glut1-overexpressing mice exhibited increases in basal whole-body glucose disposal and insulin-stimulated whole-body glucose disposal under euglycemic/hyperinsulinemic clamp conditions compared to wild-type littermates (Figure 6). Because overexpression of hexokinase in the context of Glut1 overexpression dramatically increased steady-state levels of muscle metabolites compared to overexpression of Glut1 alone (see Figure 3), it was expected that whole-body glucose disposal would be markedly elevated in the Glut1 x Hex mice relative to the Glut1 mice. Surprisingly, however, no
significant difference was observed in basal or insulin-stimulated whole-body glucose disposal in Glut1 x Hex mice compared to Glut1 mice.

**DISCUSSION**

The data presented in this study are consistent with a large body of previous experimental data indicating that, under most conditions and in most skeletal muscle types, transport of glucose is rate-limiting for glucose uptake and metabolism (4). However, it is possible that in some skeletal muscle types, i.e., those expressing a relatively high Glut4/hexokinase II ratio, the phosphorylation step may become partially rate-limiting for overall glucose metabolism under certain conditions (16).

One unanticipated finding of this study is that the hexokinase transgene did not further augment whole-body glucose disposal in mice that also overexpress Glut1 in muscle. The observation that intracellular free glucose is dramatically elevated in the muscle of Glut1 transgenic mice (2) indicates that the hexokinase step is rate-limiting for glucose metabolism in Glut1-overexpressing muscles. Consistent with this interpretation is the finding that the muscles of doubly transgenic mice exhibited elevated steady-state glucose-6-phosphate and glycogen levels relative to muscles overexpressing Glut1 alone (Figure 3), implying an increased flux through the glycogenic pathway mediated by the hexokinase transgene. Why then are the elevated steady-state metabolite levels in doubly transgenic muscles
not associated with a further increase in whole-body glucose disposal relative to mice that only carry the Glut1 transgene? There are several possible explanations, one being that the grossly elevated steady-state glycogen level caused an inhibition of \textit{de novo} glycogen synthesis in muscles of the doubly transgenic mice (17). Support for this interpretation is provided by the observation that muscles of Glut1 transgenic mice with a 5-fold elevation in steady-state glycogen levels relative to control muscles exhibit a 50% reduction in the proportion of glycogen synthase in the active form (2). Inhibition of flux through the glycogenic pathway due to the very high glycogen levels probably contributed to the grossly elevated glucose-6-phosphate levels in the muscles of mice carrying both transgenes. Because glycogen synthesis is the major pathway for the disposal of glucose in muscle under hyperinsulinemic conditions (18), this could explain why the doubly transgenic mice do not exhibit a further increase in whole-body glucose disposal during the euglycemic/hyperinsulinemic clamp procedure.

Our data are in apparent disagreement with data published previously on hexokinase II transgenic mice. Chang and colleagues (7) reported that hexokinase II-overexpressing extensor digitorum longus and soleus muscles exhibited increased 2-deoxyglucose uptake relative to control muscles, especially in the presence of pharmacologic concentrations of insulin. We observed no increase in 2-deoxyglucose uptake in either muscle type in the presence or absence of insulin (Figure 2). The data of Chang and colleagues
(7) imply that hexokinase overexpression augmented the activity and/or expression of endogenous glucose transporters in skeletal muscle, because 2-deoxyglucose uptake reflects transport activity and not hexokinase activity under the conditions employed in these experiments (19). This is also definitively demonstrated by our uptake data where several-fold increases in hexokinase activity had no effect on the rate of 2-deoxyglucose uptake (see Figure 2). However, since hexokinase activity appears to be rate-limiting for glucose uptake in the muscles of Glut1-overexpressing mice, one might expect to observe an increase in 2-deoxyglucose uptake after transgenic augmentation of hexokinase activity in these muscles. The explanation for the inability of hexokinase to increase 2-deoxyglucose uptake in Glut1-overexpressing mice lies in the lack of feedback inhibition of hexokinase activity by 2-deoxyglucose-6-P. Even at a concentration of 30 mM, 2-deoxyglucose-6-P fails to inhibit hexokinase activity in muscle homogenates, whereas glucose-6-P inhibits hexokinase activity by 80% at a concentration of 500 μM (19). Thus, in situ, with an ambient blood glucose concentration of ~8 mM and an intracellular glucose-6-P concentration of ~0.8 mM (see Figure 3), hexokinase activity was severely inhibited and was rate-limiting for glucose uptake in the muscles of Glut1-overexpressing mice. However, when muscles were removed and then incubated under conditions used to measure 2-deoxyglucose uptake (no glucose and 1 mM 2-deoxyglucose), the lack of feedback inhibition restored hexokinase activity to the point where transport
once again became rate-limiting for uptake. Thus, 2-deoxyglucose uptake in isolated tissues continues to reflect only the rate of glucose transport in the muscles of Glut1-overexpressing mice, even though hexokinase is rate-limiting for glucose uptake in muscle in situ.

If indeed the hexokinase-overexpressing mice studied by Chang and colleagues (7) also exhibited an increase in the activity of an endogenous glucose transporter, then that would explain other differences between their data and ours. For example, Chang and colleagues (7) reported a small but significant increase in glucose-6-phosphate levels in gastrocnemius muscles isolated from hexokinase transgenic mice after a bolus injection of glucose and insulin, but we observed no such increase under steady-state conditions. The increase they observed may in fact have been due to an increase in glucose transport activity in their mice. In a different collaborative study (20), some of the same investigators reported that hexokinase-overexpressing mice exhibited increases in the glucose utilization index of gluteal and gastrocnemius muscles under hyperglycemic/hyperinsulinemic clamp conditions. It is possible that this was also due to an increase in the activity of an endogenous glucose transporter isoform expressed in the skeletal muscles of the hexokinase transgenic mice. In support of this suggestion, mice that overexpressed both Glut4 and hexokinase II had lower rates of muscle glucose utilization than mice overexpressing the Glut4 transgene alone (20), and these differences in the in situ glucose utilization rates
paralleled the differences in muscle 2-deoxglucose uptake data, i.e., muscles overexpressing both hexokinase and Glut4 exhibited lower rates of 2-deoxyglucose uptake in the presence of insulin than muscles overexpressing Glut4 alone. Why mice that overexpress both Glut4 and hexokinase might have lower glucose uptake rates than mice that overexpress hexokinase alone remains unclear, but this observation is consistent with a dominant role for transport as compared to phosphorylation in determining the rate of muscle glucose disposal, and supports the hypothesis that increases in metabolism observed in the hexokinase-overexpressing muscles may in fact have been due to an increase in endogenous glucose transport activity rather than the increase in hexokinase activity.

The hexokinase transgenic mice used in our study were derived from the line originally constructed by Chang and colleagues (7). One difference is that we cross-bred the original hexokinase line (in a FVB/NJ background) with a different hybrid strain of mice that overexpress Glut1 (C57BL/6 x SJL), so that all four mouse genotypes studied were in a FVB/NJ x (C57BL/6 x SJL) background. This may account for some of the differences observed by Chang and colleagues (7) and by us. Regardless, our data on hexokinase-overexpressing mice as well as the data described previously by others, when analyzed in the present context, are consistent with a dominant if not exclusive role for glucose transport in determining the rate of muscle glucose metabolism and whole-body glucose disposal in mice. This conclusion is in
agreement with a recent elegant study employing NMR that directly measured the concentration of free glucose and glucose-6-phosphate in human skeletal muscle (21). This study demonstrated that glucose transport, as opposed to phosphorylation, is rate-limiting for muscle glucose disposal under hyperglycemic/hyperinsulinemic conditions in normal subjects and in type 2 diabetics.
FIGURE LEGENDS

FIG. 1. Hexokinase activity in quadriceps muscles from control and transgenic mice. Total, type I, and type II hexokinase activities were determined on muscle homogenates from wild-type mice or mice overexpressing Glut1 (Glut1), hexokinase II (Hex), or both transgenes (Glut1 x Hex) as described in Experimental Procedures. The values represent the mean ± SE for 4-6 individual muscles per group. *P < .001 for Hex or Glut1 x Hex versus wild-type or Glut1.

FIG. 2. Glucose transport activity of muscles from wild-type and transgenic mice. Extensor digitorum longus muscles were excised from wild-type mice or mice overexpressing Glut1 (Glut1), hexokinase II (Hex), or both transgenes (Glut1 x Hex). Muscles were subjected to [3H]-2-deoxyglucose uptake assays as described in Experimental Procedures. The values represent the mean ± SE for 8-10 individual muscles per group. *P < .001 for Glut1 or Glut1 x Hex versus wild-type or Hex.

FIG. 3. Steady-state metabolite levels in muscles from wild-type and transgenic mice. Gastrocnemius muscles from wild-type mice or mice overexpressing Glut1 (Glut1), hexokinase II (Hex), or both transgenes (Glut1 x Hex) were clamp frozen in situ, resected, homogenized, and then used to measure steady-state concentrations of glucose-6-phosphate (A) and glycogen.
(B) as described in Experimental Procedures. The values given represent the mean ± SE for 8-10 individual muscles per group. *P < .001 for Glut1 x Hex versus all other groups; **P < .01 for Glut1 versus wild-type or Hex.

FIG. 4. **Rates of glycolytic and glycogenic glucose utilization in muscles from control and hexokinase transgenic mice.** Extensor digitorum longus muscles from wild-type and hexokinase (Hex) transgenic mice were resected, incubated in the presence of [5-3H] glucose and either 8 mM cold glucose, 20μU/ml insulin (Low Gluc/Ins) or 24 mM cold glucose, 2 mU/ml insulin (High Gluc/Ins). The amounts of the isotope incorporated into H2O (A) or glycogen (B) were then determined as described in Experimental Procedures. Values represent the mean ± SE for 6-8 individual muscles per group.

FIG. 5. **Blood glucose levels in control and transgenic mice.** Blood was obtained from the tail veins of freely feeding wild-type mice or mice expressing the hexokinase transgene (Hex), the Glut1 transgene (Glut1) or both transgenes (Glut1 x Hex). Glucose concentrations were determined as described in Experimental Procedures. Values given represent mean ± SE for 11-22 animals per group. * P < 0.001 for Glut1 or Glut1 x Hex versus wild-type or Hex.
FIG. 6. **Rates of whole-body glucose disposal in control and transgenic mice.** Wild-type mice or mice carrying the hexokinase transgene (Hex), the Glut1 transgene (Glut1) or both transgenes (Glut1 x Hex) were subjected to a euglycemic/hyperinsulinemic clamp procedure along with the infusion of [3H]glucose to ascertain the rate of whole-body glucose disposal under basal and hyperinsulinemic conditions (see Experimental Procedures). Values given represent the mean ± SE for 4 animals per group. * P < 0.01 for Glut1 or Glut1 x Hex versus wild-type or Hex; ** P < 0.05 for Glut1 versus wild-type or Hex.

FIG. 7. **Ultrastructure of muscle from transgenic mice.** Extensor digitorum longus muscles were resected from mice overexpressing hexokinase (Hex) or both Glut1 and hexokinase (Glut1 x Hex) and then processed for electron microscopy as described previously (22). Cross sections through the muscle fibers are shown. Muscles from Hex mice exhibit a normal ultrastructure, whereas muscles from Glut1 x Hex mice display an abnormal architecture due to the large increase in glycogen content. Note the unusually high electron density of the myofibrils in the Glut1 x Hex muscle and the large and small glycogen deposits interrupting the bundles of myofibrils. Mi, mitochondria; F, myofibrils; G, glycogen. The small arrows point to small glycogen deposits within myofibril bundles.
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    C226-C232


Figure 1
Figure 2

2-Deoxyglucose Uptake (µmol/ml/20 min)

Wild Type

Hex

Glut1

Glut1 x Hex

Basal

Insulin

Figure 2
Figure 3
Figure 4

A. \[^{3}\text{H}2\text{O}\] Production

- Wild-type
- Hex

\(\mu\text{mol/g/h}\)

Low Gluc/Ins: 
- Wild-type: 6
- Hex: 14

High Gluc/Ins: 
- Wild-type: 12
- Hex: 14

B. Glycogen Synthesis

- Wild-type
- Hex

\(\mu\text{mol glucose/g/h}\)

Low Gluc/Ins: 
- Wild-type: 1
- Hex: 3

High Gluc/Ins: 
- Wild-type: 6
- Hex: 6

Figure 4
Wild Type

Glut1 x Hex

Hex

Glut1

Wild Type

Fed Blood Glucose (mg/dl)

100 110 120 130 140 150 160 170

Figure 5
Figure 6

- **Glut1 x Hex**
- **Hex**
- **Glut1**
- **Wild-Type**

Rd (mg/kg/min)

- Insulin
- Basal

* P < 0.05
** P < 0.01