Expression of Glucokinase in Cultured Human Muscle Cells Confers Insulin-Independent and Glucose Concentration–Dependent Increases in Glucose Disposal and Storage

Susanna Baqué, Eulàlia Montell, Joan J. Guinovart, Christopher B. Newgard, and Anna M. Gómez-Foix

Insulin resistance, as is found in skeletal muscle of individuals with obesity and NIDDM, appears to involve a reduced capacity of the hormone to stimulate glucose uptake and/or phosphorylation. The glucose phosphorylation step, as catalyzed by hexokinase II, has been described as rate limiting for glucose disposal in muscle, but overexpression of this enzyme under control of a muscle-specific promoter in transgenic mice has had limited metabolic impact. In the current study, we investigated in a cultured muscle model whether expression of glucokinase, which in contrast to hexokinase II is not inhibited by glucose-6-phosphate (G-6-P), would have a pronounced metabolic impact. We used a recombinant adenovirus containing the cDNA-encoding rat liver glucokinase (AdCMV-GKL) to increase the glucose phosphorylating activity in cultured human muscle cells by fourfold. G-6-P levels increased in AdCMV-GKL–treated cells in a glucose concentration–dependent manner over the range of 1–30 mmol/l, whereas the much smaller increases in G-6-P in control cells were maximal at glucose concentrations <5 mmol/l. Further, cells expressing glucokinase accumulated 17 times more 2-deoxyglucose-6-phosphate than control cells. In AdCMV-GKL–treated cells, the time-dependent rise in G-6-P correlated with an increase in the activity ratio of glycogen synthase. AdCMV-GKL–treated cells also exhibited a 2.5- to 3-fold increase in glycogen content and a four- to fivefold increase in glycolytic flux, proportional to the increase in glucose phosphorylating capacity. All of these observations were made in the absence of insulin. Thus we concluded that expression of glucokinase in cultured human muscle cells results in proportional increases in insulin-independent glucose disposal, and that muscle glucose storage and utilization becomes controlled in a glucose concentration–dependent manner in AdCMV-GKL–treated cells. These results encourage testing whether delivery of glucokinase to muscle in vivo has an impact on glycemic control, which could be a method for circumventing the failure of insulin to stimulate glucose uptake and/or phosphorylation in muscle normally in insulin-resistant subjects. *Diabetes* **47:1392–1398, 1998**

Utilization of glucose by skeletal muscle cells requires its transport across the cell membrane via GLUT4 and GLUT1 and its phosphorylation by hexokinase II. The highly prevalent disorders of obesity and NIDDM are associated with insulin resistance, and there is increasing evidence that a critical component of this abnormality is the failure of insulin to stimulate glucose uptake and/or phosphorylation in skeletal muscle normally (1). This has led to intensive investigation of the relative importance of glucose transport versus glucose phosphorylation in regulation of glucose disposal in muscle cells. Kinetic studies with isolated rat myocytes have revealed that at low glucose levels and in the absence of insulin, no intracellular accumulation of glucose is observed, suggesting that glucose transport is the rate-limiting step under these conditions. As glucose and insulin concentrations rise, however, GLUT4 transporters are translocated to the cell surface, resulting in accumulation of intracellular glucose in excess of glucose-6-phosphate (G-6-P) and apparent saturation of hexokinase II (2). Similarly, overexpression of the insulin-independent glucose transporter GLUT1 in transgenic animals results in a fourfold increase in intracellular glucose concentration and enhanced glycogen accumulation, but no change in G-6-P levels in resected muscle samples (3).

Overexpression of hexokinase II in muscle of transgenic mice was shown (4) to have relatively modest metabolic effects. Thus a three- to eightfold overexpression of hexokinase II in striated muscle of transgenic mice had no effect on glucose tolerance, insulin, glycogen, or lactate levels, although it caused a 43% increase in G-6-P levels. In isolated muscle preparations, small effects of hexokinase II overexpression on insulin-stimulated glucose uptake were noted. Moreover, no differences in the in vivo glucose utilization index in gastrocnemius and gluteal muscle (5) were found in hexokinase II–overexpressing muscles in basal conditions, but significant increases were detected after a hyperglycemic-hyperinsulinemic clamp.

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AdCMV-GKL, cDNA-encoding rat liver glucokinase; DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; FBS, fetal bovine serum; FGF, fibroblast growth factor; G-6-P, glucose-6-phosphate; SSC, sodium chloride–sodium citrate.

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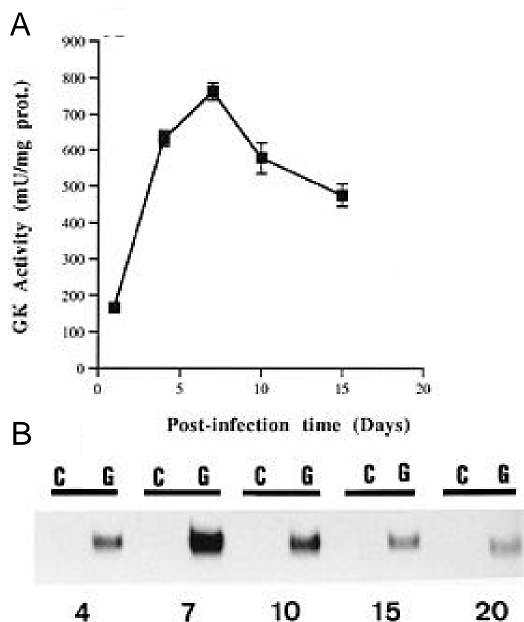

FIG. 1. Expression of glucokinase in cultured human muscle cells. *A*: Glucokinase (GK) activity was measured as the difference in glucose phosphorylating activity at 100 and 0.5 mmol/l glucose. The phosphorylating activity in the presence of 0.5 mmol/l glucose was 67 ± 9 mU/mg protein in cells exposed to control virus and 65 ± 4 in cells exposed to AdCMV-GKL virus (■). Data are means ± SE of four independent experiments performed in duplicate. *B*: Northern blot analysis of glucokinase mRNA in cells exposed to control virus (C) or AdCMV-GKL virus (G) at various days after viral treatment. The blot shown is representative of two experiments, each performed in duplicate.

We have previously shown that large increases in G-6-P concentration, glycolytic flux, and glycogen synthesis are observed on overexpression of an alternate hexokinase isoform, hexokinase IV or glucokinase, in isolated hepatocytes (6,7). Glucokinase differs from the other members of the hexokinase gene family in that it has a much lower affinity for glucose and is not subject to product inhibition by G-6-P (8). Interestingly, hepatocytes with overexpressed hexokinase I (an isoform similar to hexokinase II in terms of its high affinity for glucose and sensitivity to G-6-P inhibition) exhibit no enhancement in glycogen synthesis and a lesser increase in glycolytic flux compared with liver cells engineered for glucokinase overexpression (6,7). These findings raise the possibility that the modest metabolic impact of overexpressed hexokinase II in muscle cells may be due to the enzyme's exquisite sensitivity to inhibition by G-6-P, and that expression of the G-6-P–insensitive glucokinase enzyme in muscle cells could represent a potent method for enhancing glucose disposal. To test this hypothesis, we used the recombinant adenovirus system to express glucokinase efficiently in cultured human muscle cells. We found that this maneuver resulted in large increases in G-6-P levels, glycogen synthesis, and glycolytic flux that were proportional to the media glucose concentration, suggesting that expression of glucokinase in muscle may represent a means for enhancing glucose utilization in muscle in response to high glucose concentrations.

RESEARCH DESIGN AND METHODS

Human muscle primary cultures. Human muscle cultures were initiated from satellite cells of muscle biopsies of patients considered free of muscle disease after all diagnostic studies were reported. Aneural muscle cultures were established in monolayers according to the explant–re-explantation technique, as previously described (9). The cultures were grown in a Dulbecco's modified Eagle's medium (DMEM)/M-199 medium (3:1) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), 10 μg/ml insulin (Sigma, St. Louis, MO), 20 mmol/l glutamine, 25 ng/ml fibroblast growth factor (FGF), 10 ng/ml epidermal growth factor (EGF) (Becton Dickinson, Franklin Lakes, NY), and an antibiotic-antimycotic mixture. Immediately after myoblast fusion, cells were rinsed with Hank's balanced salt solution and the medium was replaced by a medium devoid of factors FGF, EGF, and glutamine. The medium was switched to fresh medium every other day. Experiments were performed using muscle cultures established from seven different patients' muscle biopsies.

Treatment of cells with cDNA-encoding rat liver glucokinase virus. A recombinant adenovirus containing the rat liver glucokinase cDNA has been previously described (10). As a control virus we used a recombinant adenovirus with no foreign cDNA. The recombinant viruses were amplified in 293 cells, and viral stocks of 5–50 $\times$ 10^7 plaque-forming units/ml were prepared in 10% FBS/DMEM. Gene delivery into primary cultured human muscle fibers was performed by exposing 14-day-old fibers, induced to fuse by removal of growth factors for 1 h to virus (AdCMV-GKL) including cDNA-encoding rat liver glucokinase at a multiplicity of infection of 10.

Northern blot analysis of RNA. Total RNA was isolated from human myocytes by a single-step guanidium thiocyanate-phenol-chloroform method, fractionated by electrophoresis in 1.5% agarose gels, and transferred to nitrocellulose membranes. Hybridizations with antisense [^{32}P]-labeled riboprobes derived from a 1.03 kb *EcoRI*-*SphI* fragment of the rat liver glucokinase cDNA (10), a 1.3 kb *AccI*-*HindIII* fragment of the rabbit muscle glycogen phosphorylase cDNA (11), or a 2.72 kb *NotI*-*SalI* fragment of the human muscle glycogen synthase cDNA (12) were performed at 65°C in the presence of 50% formamide. Filters were washed at 65°C to a final stringency of 0.1 $\times$ sodium chloride–sodium citrate (SSC) (1 $\times$ SSC = 0.15 mol/l NaCl, 0.015 mol/l sodium citrate) and 0.1% sodium lauryl sulfate, and subjected to autoradiography.

Enzyme activity assays. To measure enzyme activities, extracts were prepared by scraping cell monolayers in a buffer consisting of 10 mmol/l Tris-HCl (pH 7.0), 600 mmol/l sucrose, 150 mmol/l KF, 15 mmol/l EDTA, 15 mmol/l 2-mercaptoethanol, 10 μg/ml leupeptin, 1 mmol/l benzamidine, and 1 mmol/l phenylmethylsulfonyl fluoride, and then sonicating them. Homogenates were centrifuged at 10,000*g* for 15 min; the resulting supernatants were used for the determination of enzyme activities. Protein concentration was measured with Bio-Rad protein assay reagent (Bio-Rad, Richmond, CA). Glucokinase activity was measured as described (13) as the difference of the glucose phosphorylating activity in the presence of 100 and 0.5 mmol/l glucose. Glycogen phosphorylase activity was determined by the incorporation of [U-^{14}C]glucose 1-phosphate into glycogen in the absence or presence of the allosteric activator AMP (5 mmol/l), and glycogen synthase activity was measured in the absence or presence of 10 mmol/l G-6-P, as described (14).

Metabolite determinations. To perform metabolic studies, cells were treated with AdCMV-GKL or control virus and cultured for 7 days. Before beginning the experiment, cells were switched to a medium devoid of insulin and FBS for 18 h and then deprived of glucose for 2 h. This condition was considered the basal state. For measuring glycogen content, cell monolayers were scraped into 30% KOH, and homogenates were boiled for 15 min and centrifuged at 5,000*g* for 15 min. An aliquot of the supernatants was used for the measurement of protein concentration. Supernatants were spotted onto Whatman 31-ET papers (Whatman, Springfield Mill, U.K.) and glycogen was precipitated by immersing the papers in ice-cold 66% ethanol. Dried papers containing precipitated glycogen were incubated with α-amyloglucosidase, and free glucose was measured enzymatically (GlucoQuant; Boehringer Mannheim, Mannheim, Germany) in a Cobas-Bio autoanalyzer. G-6-P or 2-deoxyglucose-6-phosphate was measured enzymatically in neutralized $HClO_4$ extracts. L-Lactate concentrations in the incubation medium were measured enzymatically.

RESULTS

Adenovirus-mediated expression of glucokinase in cultured human muscle cells. In a previous study, we demonstrated that application of a recombinant adenovirus containing the β-galactosidase gene to cultured muscle cells results in gene transfer with an efficiency >90% (15). We have therefore adopted this approach for expression of glucokinase. As shown in Fig. 1*B*, glucokinase mRNA is undetectable in control muscle preparations, but appears within 4 days of treatment with the AdCMV-GKL virus. Glucokinase transcript levels are maximal 7 days after viral treatment, with diminished but still persistent expression at 15 days. The increase in glucokinase mRNA is correlated with an increase

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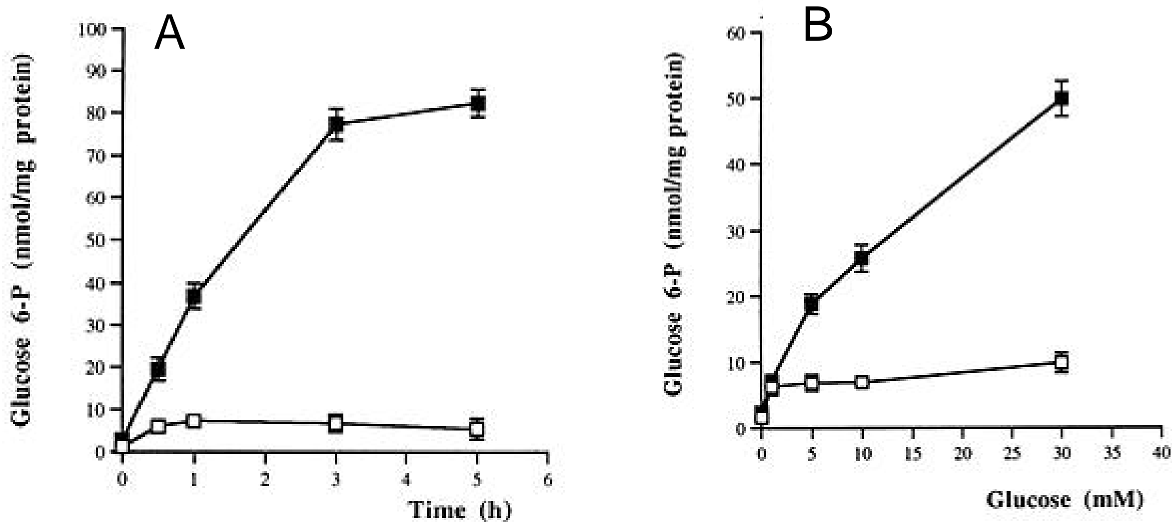

FIG. 2. Effect of glucokinase expression on G-6-P accumulation. Control cells (□) or cells treated with AdCMV-GKL virus (■) that had been incubated for 2 h in the absence of glucose were incubated with 30 mmol/l glucose and collected at the indicated times for G-6-P determination (*A*) or incubated for 2 h in the presence of the indicated concentrations of glucose (*B*). Data are means ± SE of three independent experiments performed in duplicate.

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in total glucose phosphorylating capacity, with the maximal fourfold increase observed at the 7-day time point (Fig. 1*A*).

G-6-P levels increase in glucokinase-expressing muscle cells. To investigate the impact of glucokinase expression on G-6-P accumulation, control or AdCMV-GKL–treated human muscle cells were cultured for 7 days to allow maximal glucokinase expression. The time course of G-6-P accumulation was then determined by switching the cells from a medium devoid of glucose to one containing 30 mmol/l glucose for varying times. As shown in Fig. 2*A*, control cells exhibited a time-dependent increase in G-6-P levels, but reached a plateau 4.5-fold above the basal value after only 1 h of incubation (1.2 ± 0.2 [basal] vs. 7.2 ± 1.0 nmol/mg protein [at 1 h]). In contrast, in AdCMV-GKL–treated cells, G-6-P levels increased in a near linear fashion for 3 h after the switch to high glucose, and were sustained at the maximal value of more than 25-fold above baseline up to 5 h (2.8 ± 0.6 [baseline] vs. 82 ± 3 [at 5 h] nmol/mg protein). Note that in cells incubated in the absence of glucose, a difference of about twofold in G-6-P levels was observed between control and AdCMV-GKL–treated cells (1.2 vs. 2.8). In cells incubated for 2 h at varying glucose concentrations, G-6-P was shown to accumulate in a glucose concentration–dependent fashion (Fig. 2*B*). Levels were similar at 1 mmol/l glucose in AdCMV-GKL–treated cells (7.2 ± 1.0) relative to controls (6.3 ± 1.2), whereas G-6-P concentrations were 2.7-, 3.7-, and 5-fold higher at 5, 10, and 30 mmol/l glucose in AdCMV-GKL–treated cells compared with controls, respectively.

Glucokinase expression enhances 2-deoxyglucose uptake into human muscle cells. To evaluate the impact of glucokinase expression on the capacity for glucose transport and phosphorylation in muscle cells, we incubated control and AdCMV-GKL–treated cells with the glucose analog 2-deoxyglucose, which is transported into cells and phosphorylated but not further metabolized. Exposure of AdCMV-GKL–treated cells to 5 mmol/l 2-deoxyglucose for 1 h resulted in accumulation of 2-deoxyglucose-6-phosphate to a level 17 times higher than in control cells (190 ± 5 [AdCMV-GKL–treated cells] vs. 10 ± 1 [controls] nmol hexose-6-phosphate/mg protein). The difference between the maximal levels of 2-deoxyglucose-6-phosphate (190 nmol/mg) and G-6-P (80 nmol/mg) attained in muscle cells treated with AdCMV-GKL was likely due to the active metabolism of the latter intermediate compared with 2-deoxyglucose-6-phosphate.

Glucokinase expression increases glycogen deposition in human muscle cells. We next evaluated whether the large increase in glucose transport and phosphorylation observed in response to AdCMV-GKL treatment was manifest in increased glycogen deposition. AdCMV-GKL and control cells were cultured in the absence of glucose for 2 h and then switched back to 30 mmol/l glucose for varying times. Control cells exhibited a time-dependent accumulation of glycogen that reached a plateau after 1 h (Fig. 3*A*). Cells treated with AdCMV-GKL had more than double the basal level of glycogen as controls and a much higher glycogenic capacity. Higher basal glycogen after depletion of glucose for 2 h provides a likely explanation for the increased basal G-6-P levels noted in Fig. 2, since glycogenolysis is stimulated in glucose-deprived cells. Although AdCMV-GKL–treated cells also reached a plateau within the first hour of exposure to 30 mmol/l glucose, the increment in glycogen was much larger than in controls (400 µg glycogen synthesized in the first hour in AdCMV-GKL–treated cells vs. 80 µg in controls). Further, the maximal level attained in AdCMV-GKL–treated cells of 800 µg/mg cellular protein was approximately threefold greater than in control cells. The dependence of glycogen synthesis on glucose concentration was also determined (Fig. 3*B*). In control cells, a significant increment in glycogen accumulation was noted as glucose was raised from 0 to 1 mmol/l, followed by a small rise at higher concentrations (230 ± 18 vs. 302 ± 20 µg glucose/mg protein at 1 and 30 mmol/l, respectively). AdCMV-GKL–treated cells exhibited a larger rise in glycogen content as glucose was raised from 0 to 1

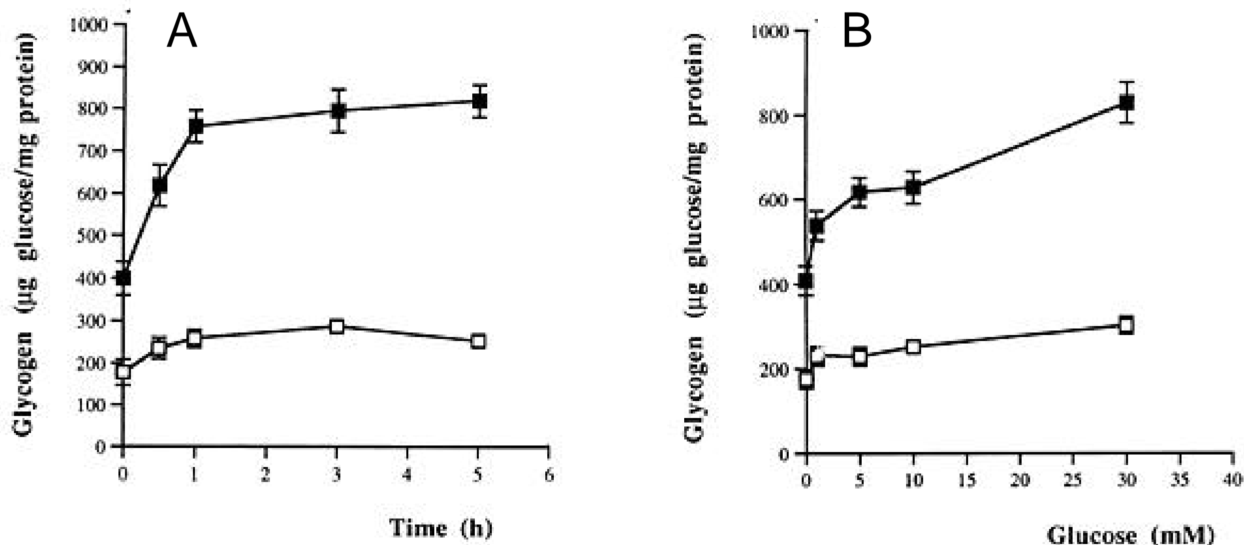

FIG. 3. Glycogen levels in muscle cells expressing liver glucokinase. Cells exposed to control (□) or AdCMV-GKL (■) virus were incubated for 2 h in a medium devoid of glucose and then incubated with 30 mmol/l glucose for different times (*A*) or incubated for 2 h with the concentrations of glucose indicated (*B*). Data are means ± SE of three independent experiments performed in duplicate.

mmol/l, and then a further significant and glucose concentration–dependent increase in glycogen over the range of 1–30 mmol/l glucose. Levels of glycogen in AdCMV-GKL–treated cells were higher than in control cells at all concentrations of glucose studied, and were increased by a maximum of 2.7-fold at 30 mmol/l glucose.

Regulation of glycogen synthase by expression of glucokinase. We previously demonstrated that glucokinase overexpression in hepatocytes causes a potent activation of glycogen synthase without affecting glycogen phosphorylase activity (7). Because muscle and liver express different isoforms of glycogen synthase and phosphorylase (12,16), we sought to determine whether glucokinase expression activates glycogenesis in muscle by a similar mechanism as that described for liver cells. Different from our observations in hepatocytes, AdCMV-GKL–treated human muscle cells exhibited an ~50% increase in total glycogen synthase activity relative to control cells at all time points studied from day 5 to 15 after AdCMV-GKL treatment (Fig. 4*A*). This increase in total glycogen synthase activity was not due to increased glycogen synthase mRNA levels (Fig. 4*B*), suggesting that overexpressed glucokinase exerted its regulatory effect at a posttranscriptional level. Similar to glucokinase-overexpressing hepatocytes, the glycogen synthase activity ratio (the ratio of glycogen synthase activity in the absence versus the presence of its allosteric activator G-6-P, which is a measurement of the activation state) was increased in muscle cells treated with AdCMV-GKL relative to control cells. As shown in Fig. 5, the activity ratio in cells incubated in the absence of glucose before switching to 30 mmol/l glucose

A

	Control	AdCMV-GKL
Glycogen synthase	7.4±0.2	11.5±0.4
Glycogen phosphorylase	87±5	89±8

B

C GK

GLYCOGEN SYNTHASE

GLYCOGEN PHOSPHORYLASE

FIG. 4. Glycogen synthase and glycogen phosphorylase expression. *A:* Glycogen synthase and glycogen phosphorylase activity were measured in cultured muscle cells 8 days after treatment with control (C) or AdCMV-GKL (GK) virus. Enzyme activity is expressed as mU/mg protein. Data are means ± SE of three independent experiments performed in duplicate. *B*: Total RNA from control (C) or AdCMV-GKL–transduced (GK) human muscle cells was used for Northern blot analysis of glycogen synthase and glycogen phosphorylase mRNA levels. The blots shown are representative of two experiments, each performed in duplicate. Band intensities quantified by densitometry were glycogen synthase (C, 100; G, 98 ± 12), glycogen phosphorylase (C, 100; G, 118 ± 14).

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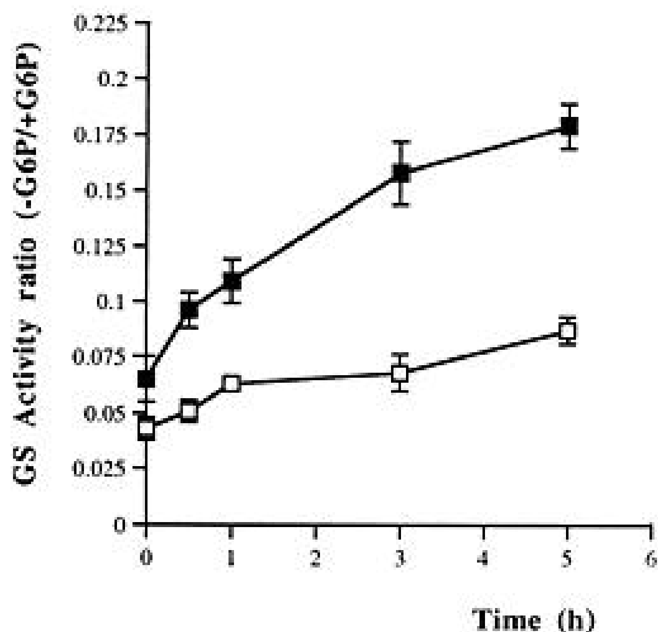

FIG. 5. Effect of glucokinase expression on glycogen synthase activity ratio. Cells exposed to control (□) or AdCMV-GKL (■) virus were incubated for 2 h in a medium devoid of glucose and then switched to a medium containing 30 mmol/l glucose and further incubated for the indicated times. Data are means ± SE of four independent experiments performed in duplicate.

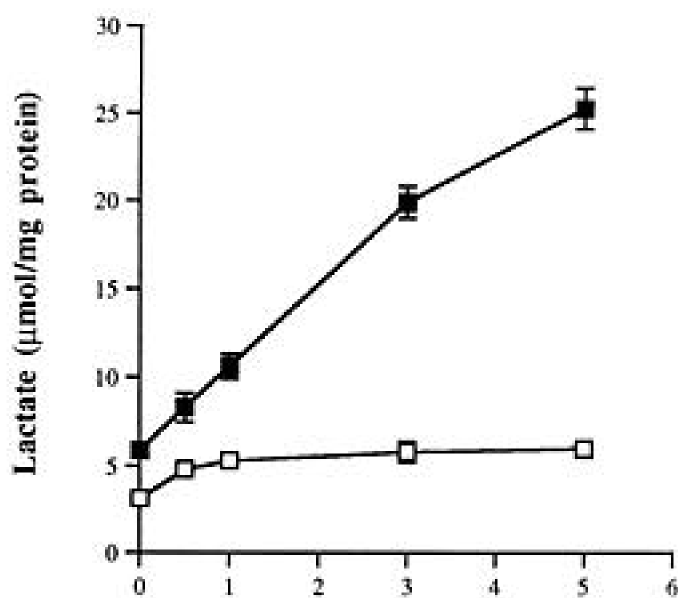

FIG. 6. Lactate release. Cultured muscle cells treated with control (□) or AdCMV-GKL (■) virus were incubated for 2 h without glucose and then switched to a medium containing 30 mmol/l glucose; aliquots of the medium were taken for determination of lactate at the indicated times. Data are means ± SE of three independent experiments performed in duplicate.

(basal activity) was higher in the AdCMV-GKL–treated cells than in controls, an effect that may have been related to higher G-6-P levels under these conditions. After switching cells to 30 mmol/l glucose, both AdCMV-GKL–treated and control cells exhibited a time-dependent increase in the glycogen synthase activity ratio, but this increase was more pronounced in the glucokinase-expressing cells. Thus by 3–5 h after exposure to high glucose, the glycogen synthase activity ratio was two times higher in AdCMV-GKL–treated cells than in controls. Total glycogen phosphorylase activity and glycogen phosphorylase mRNA levels were the same in AdCMV-GKL and control muscle cells (Fig. 4). The lack of effect of glucokinase expression on glycogen phosphorylase activity was consistent with our prior observations in the hepatocyte system.

Effect of glucokinase expression on glycolytic flux in muscle cells. The impact of glucokinase expression in muscle cells on glycolytic flux was evaluated by the accumulation of lactate in the culture medium as a function of time of exposure to 30 mmol/l glucose (Fig. 6). In control cells, lactate concentration increased in the first hour after exposure to high glucose, but then reached a plateau. In AdCMV-GKL–treated cells, in contrast, lactate accumulated in a linear fashion for 3 h hours after the switch to high glucose, and then continued to increase at a slower rate between 3 and 5 h. At 5 h, AdCMV-GKL–treated cells had accumulated five times more lactate in the medium than control cells. Note that the time dependence of lactate accumulation in muscle cells incubated at high glucose paralleled the pattern for G-6-P (compare Figs. 2 and 6)
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DISCUSSION

Because skeletal muscle occupies more mass than any other tissue in mammals, deficient glucose disposal in this organ makes a major contribution to perturbation of fuel homeostasis in diabetic individuals. Impaired muscle glucose disposal in NIDDM is thought to occur as a consequence of the development of insulin resistance, and nuclear magnetic resonance studies by Rothman et al. (17) in humans have suggested that the primary site of deficient action of the hormone in muscle may be at the level of promoting glucose uptake and/or phosphorylation. Insulin functions to promote glucose uptake in muscle by stimulating translocation of the GLUT4 transporter, and to increase glucose phosphorylating capacity via stimulation of transcription of the hexokinase II gene and by promoting translocation of the enzyme from the cytosol to the mitochondria, thereby increasing its activity (18,19). These findings have led to active investigation of the relative importance of glucose transport versus its phosphorylation in determining the rate of glucose disposal in skeletal muscle. Based on kinetic studies in normal and transgenic animals, the consensus seems to be that glucose transport is limiting for muscle glucose uptake at low glucose concentrations and in the absence of insulin, but that at physiological glucose concentrations and in the presence of insulin, the capacity for glucose transport exceeds the capacity for glucose phosphorylation.

In light of the foregoing model, overexpression of hexokinase II in skeletal muscle of transgenic mice increased in vivo glucose utilization in gastrocnemius and gluteal muscle only after a hyperglycemic-hyperinsulinemic clamp (5), whereas in incubated muscle the major increases in the rate of glucose uptake were observed after maximal stimulation with insulin (4). However, overexpression of hexokinase II failed to impact glucose homeostasis (4). The metabolic impact of overexpression of hexokinase II in muscle might be self-limiting due to the exquisite sensitivity of the

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enzyme to G-6-P inhibition (20); thus expression of glucokinase, an enzyme that is highly insensitive to G-6-P regulation, might uncover the full control strength of the glucose phosphorylation step in this tissue. As a first approach to evaluating the impact of expressing glucokinase in muscle, we delivered glucokinase to isolated human muscle cells via the same recombinant adenovirus previously used for studies in isolated islet and hepatocyte studies (6,7). We found that expression of glucokinase caused large enhancement in glucose disposal, reflected in increases in G-6-P levels, glycogen synthesis and accumulation, and glycolytic flux in human muscle cells.

Two important insights emerge from our data. First, all of the measurements reported herein were carried out in the absence of insulin. It therefore appears that the capacity for glucose transport in muscle cells is sufficient to support a large enhancement in glucose disposal when glucose phosphorylating activity is increased, even in the absence of the hormone. Upon expression of glucokinase, the modulating role of hexokinase II may be superseded, thereby "unmasking" the additional transport capacity of the system. Consistent with this notion, we found that control cells that contain a normal complement of hexokinase II exhibit a saturable capacity for G-6-P accumulation, reaching a maximum level of accumulation of the nonmetabolizable analog 2-deoxyglucose-6-phosphate of 10 nmol/mg protein. In contrast, muscle cells expressing glucokinase can raise G-6-P concentration in proportion to glucose availability and accumulate 17 times more 2-deoxyglucose-6-phosphate than controls. Caution must be taken, however, in extrapolating these results to the in vivo setting because isolated myocytes may have a higher glucose transport capacity than intact skeletal muscle. Cultured muscle cells differ in many respects from muscle in vivo; specifically, expression of GLUT4 is impaired and so is the stimulation of glucose utilization by insulin. We presume that the increase in glucose uptake and disposal caused by glucokinase expression in the absence of insulin is mediated by increased flux through the glucose transporters residing in the plasma membrane. It remains to be determined whether expression of glucokinase in muscle of intact animals will have the same profound impact on glucose disposal and storage as it does in isolated cells.

A second important finding is that muscle cells that express glucokinase accumulate glycogen and produce lactate in direct proportion to the medium glucose concentration, in contrast to control cells, which reach a plateau of metabolic activity at low glucose. The most likely explanation for this relates to the kinetic features of the glucokinase enzyme. Glucokinase has a K_m for glucose of ~8 mmol/l, and this property and its insensitivity to regulation by G-6-P allows the enzyme to control glucose flux in proportion to the circulating glucose concentration in tissues in which it is normally expressed, such as liver and islets (21–23). In contrast, hexokinases I, II, and III have a K_m for glucose in the range of 10–100 µmol/l (19). Thus the plateau of metabolic activity observed in control muscle cells is likely explained by the fact that hexokinase II is operating at maximal velocity at glucose concentrations of 1 mmol/l. Remarkably, muscle cells engineered for glucokinase expression gain the capacity to increase glucose disposal in proportion to glucose concentrations in the range of 1–20 mmol/l, consistent with the well-described regulation of glucokinase enzyme activity that occurs over the same range (19,20). This is the first example that we know of in which expression of glucokinase has conferred regulation of metabolic flux at the high range of glucose concentrations in cells not normally responsive over this range. The expression of glucokinase in insulinoma cells that have high low-K_m hexokinase activity and naturally elevated rates of glycolysis at low glucose concentrations does not shift the dosage-response curve to the right (22). However, the opposite maneuver of overexpression of hexokinase I can enhance glycolytic flux and insulin secretion at low glucose concentrations (24,25). It is interesting that expression of glucokinase also appears to supersede the kinetic features of the glucose transporter isoforms expressed in muscle cells, since both GLUT1 and GLUT4 are known to have a very high affinity (low K_m) for glucose (26,27). However, the ultimate test that glucose transport may be superseded by the increased glucose phosphorylation capacity requires glucokinase delivery to muscle in vivo.

Our study also provides support for a critical link between G-6-P levels and activation of glycogen synthesis (28). Consistent with our previous studies in isolated rat hepatocytes (7), we showed here a tight correlation between the rise in G-6-P and glycogen synthase activation state in AdCMV-GKL–treated muscle cells. It is interesting that the large increase in glycogen synthesis in glucokinase-expressing cells occurred in the absence of added insulin, further indicating that activation of glycogen synthase via accumulation of G-6-P is sufficient to activate glycogenesis. It should be noted, however, that although glycogen synthase activity and G-6-P levels rose in parallel for 3 h in AdCMV-GKL–treated cells after application of high glucose (Figs. 2 and 5), glycogen synthesis reached a plateau after only 1 h (Fig. 3*A*), suggesting that the capacity for glycogen accumulation is saturable in muscle cells. It is noteworthy that lactate production shows a similar time-dependent increase as the level of G-6-P increases, suggesting that once glycogen synthesis is saturated, the excess of G-6-P will be diverted towards glycolysis.

The implication of our study is that muscle cells that express glucokinase could bypass the requirement for insulin regulation of glucose disposal. However, it is important to emphasize that it remains to be determined whether the glucose transport step is more limiting in intact skeletal muscle in vivo than it is in the isolated human muscle cells used in this study. This question can be answered only by overexpression of glucokinase in skeletal muscle of intact animals. Possible approaches include generation of transgenic mice expressing glucokinase in skeletal muscle, but germline manipulation for delivery of the glucokinase gene to muscle of larger animals is much less efficient. Approaches for delivery of genes to the skeletal muscle bed, such as direct injection of naked DNA (29) or adenovirus vectors (30), although having intrinsic limitations, may provide information about the feasibility of this genetic manipulation for altering muscle glucose disposal. Nevertheless, our findings suggest that it will be of interest to test the metabolic impact of expressed glucokinase in muscle of intact mice, and provide encouragement for generation of transgenic mice and/or improvement in vector technology.

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