Treatment of Type 2 Diabetes by Adenoviral-Mediated Overexpression of the Glucokinase Regulatory Protein

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The enzyme glucokinase (GK) plays a central role in glucose homeostasis. Hepatic GK activity is acutely controlled by the action of the GK regulatory protein (GKRP). In vitro evidence suggests that GKRP reversibly binds to GK and inhibits its activity; however, less is known about the in vivo function of GKRP. To further explore the physiological role of GKRP in vivo, we used an E1/E2a/E3-deficient adenoviral vector containing the cDNA encoding human GKRP (Av3hGKRP). High fat diet–induced diabetic mice were administered Av3hGKRP or a control vector lacking a transgene (Av3Null). Surprisingly, the Av3hGKRP-treated mice showed a significant improvement in glucose tolerance and had lower fasting blood glucose levels than Av3Null-treated mice. A coincident decrease in insulin levels indicated that the Av3hGKRP-treated mice had sharply improved insulin sensitivity. These mice also exhibited lower leptin levels, reduced body weight, and decreased liver GK activity. In vitro experiments indicated that GKRP was able to increase both GK protein and enzymatic activity levels, suggesting that another role for GKRP is to stabilize and/or protect GK. These data are the first to indicate the ability of GKRP to treat type 2 diabetes and therefore have significant implications for future therapies of this disease. Diabetes 50:1813–1820, 2001

Glucokinase (GK) is a high-Km hexokinase that plays a central role as the physiologic glucose sensor in pancreatic β-cells and catalyzes the first committed step in hepatic glucose metabolism (1,2). As such, it is critical to maintaining normal blood glucose and insulin levels in vivo. In fact, decreased GK enzymatic activity has been reported in patients with type 2 diabetes (3), and autosomal-dominant heterozygous mutations in the GK gene are associated with maturity-onset diabetes of the young (4,5). Transgenic animal studies have shown that even slight alterations in GK protein levels have profound effects on blood glucose homeostasis. Mice with increased hepatic GK activity showed lower fasting glucose and insulin levels and had improved glucose tolerance (6–8). Conversely, mice with only one copy of the GK gene (heterozygous knockout) showed increased fasting glucose levels and decreased glucose tolerance (9,10).

In the β-cell, GK activity is largely regulated by transcriptional mechanisms (1,2), whereas in the liver, GK activity is also acutely regulated by its binding to the GK regulatory protein (GKRP), localized within the nucleus (11–13). GKRP inhibits GK in an allosteric manner that is competitive with respect to glucose, and its activity is also modulated by phosphate esters of fructose. Fructose-6-phosphate binds to GKRP and greatly enhances its binding to and inhibition of GK, whereas fructose-1-phosphate promotes the dissociation of this complex (13,14). When released from GKRP, GK exits the nucleus due to the presence of a leucine-rich nuclear export signal sequence (15,16), where it can phosphorylate its substrate glucose.

Mutants of Xenopus GK with a reduced affinity for GKRP remain in the cytosol, even in conditions of low glucose (12). Thus, in hepatocytes, GK is sequestered by GKRP within the nucleus in an inactive state during conditions of low glucose (elevated fructose-6-phosphate) and is released by GKRP to the cytosol in an active state in the presence of high glucose (or by fructose-1-phosphate). This regulation provides a sensitive means of maintaining large pools of quickly activated GK that is needed for postprandial glycolysis without causing hypoglycemia. Due to its identified function to negatively regulate GK activity, GKRP has been postulated as a candidate type 2 diabetes gene; however, no mutations have yet been found in this gene in diabetic subjects (17).

The ability to use viral or nonviral technologies to transduce specific genes of known function into diseased or normal tissues provides for the basis of gene therapy. Gene therapy can be applied to illnesses of either genetic or environmental etiologies, and its technology also allows for in vivo experiments to explore the function of particular genes of interest. Among the most carefully studied gene therapy systems are adeno viral vectors that, when handled properly, are easy to produce, stable, and safe (18,19). These vectors have the fortuitous ability to transduce nondividing terminally differentiated cells and are highly hepatotropic. Although they are nonintegrating and...
thus likely to supply only transient expression, recently developed adenoval vector systems are capable of persisting in tissues in vivo for >6 months (20,21).

The objective of this study was to explore the effect of increasing the hepatic levels of GKR protein on the diabetic phenotype of mice maintained on a high-fat diet. To do so, we have administered an adenoval vector expressing human GKR to these mice and assayed the fasting glucose, insulin, and leptin levels and the response to a glucose challenge. Surprisingly, we found this vector was able to correct the diabetic phenotype of these mice, and we have discussed the implications of these novel results.

**RESULTS**

To explore the physiological role of GKR in regulating glucose homeostasis in vivo, we used adenoval-mediated gene transfer to overexpress human GKR in the livers of diabetic mice. The human GKR cDNA was cloned into a replication-deficient adenoval vector (Av3hGKR) containing deletions of the viral E1, E2a, and E3 genes. This adenoviral vector was created by cre-lox–mediated recombination of pAvS6alx-hGKR with pS93, containing most of the adenoviral genome, and was purified by standard procedures (21,22). The adenoviral vector (Av3Null or Av3hGK, which lack a transgene and encode human liver GKR, respectively, were essentially as previously described (21,22), except that the Av3Null vector did not contain the Rous sarcoma virus (RSV) promoter and SV40 polyA signal.

In *vitro* experiments. Primary hepatocytes were isolated from male Sprague-Dawley rats as previously described (23). HepG2 cells (ATCC, Manassas, VA) or hepatocytes were incubated with the indicated adenoviral vectors Av3Null and Av3hGK, which lack a transgene and encode human liver GKR, respectively, were essentially as previously described (21,22), except that the Av3Null vector did not contain the Rous sarcoma virus (RSV) promoter and SV40 polyA signal.

**Assays.** Blood glucose concentrations were measured using a handheld glucometer (Bayer, Tarrytown, NY). Plasma insulin was measured using an enzyme-linked immunoassay kit from Crystal Chem (St. Louis, MO). Plasma samples were sent to an outside laboratory (AniLytics, Gaithersburg, MD) for analysis of glucose, triglycerides, free fatty acids, lactate, and alanine aminotransferase (ALT).

**GK activity measurements and Western analyses.** Protein extracts were prepared from liver as previously described after an initial tissue homogenization step (Kontes Duall tissue homogenizer; Kimble/Kontes, Vineland, N.J.) (27). The supernatant was assayed for GK activity using a method essentially as described previously (8), except that the assay buffer contained 100 mmol/l Tris-HCl, pH 7.4, 100 mmol/l KCl, 6 mmol/l MgCl2, 1 mmol/l diithiothreitol, 5 mmol/l ATP, 1 mmol/l thioNAD, 30 U/ml glucose-6-phosphate dehydrogenase, and 0.5 or 100 mmol/l glucose. GK activity was estimated as the differences in activity when samples were assayed at 100 mmol/l (GK plus hexokinase activity) and 0.5 mmol/l glucose (hexokinase activity) and is indicated as nmol · min⁻¹ · mg⁻¹ of protein. Western blot analyses were performed as previously described (27). Antibodies used were rabbit anti-GK (in-house affinity purified antibody against rat β-cell GK, or Santa Cruz H-88 [Santa Cruz Biotechnology, Santa Cruz, CA]) and goat anti-GKR (Santa Cruz sc-6340; Santa Cruz Biotechnology). Secondary antibodies were donkey anti-rabbit (Promega, Madison, WI) or donkey anti-goat (Santa Cruz sc-2020; Santa Cruz Biotechnology).

**Glycogen assay.** Liver samples were homogenized in 0.03 N HCl (to a final concentration of 0.5 g/ml). The homogenate (100 μl) was mixed with 400 μl of 1.25 N HCl and heated for 1 h at 100°C. Samples were centrifuged at 14,000 rpm and 10 μl supernatant was mixed with 1 ml glucose oxidase reagent (Sigma, St Louis, MO). After a 10-min incubation at 37°C, the absorbance was read at 505 nm. A standard curve using glycogen type III obtained from rabbit liver (Sigma) was also simultaneously analyzed to determine the final liver glycogen concentrations.

**Statistical analysis.** Results are reported as means ± SE. The comparison of different groups was performed using unpaired Student’s t test. Differences were considered statistically significant at *P* < 0.05.

**RESEARCH DESIGN AND METHODS**

Preparation of the recombinant adenoviruses. The 1.9-kb full-length cDNA encoding human GKR protein was polymerase chain reaction amplified from human liver QUICK-Clone cDNA (Clontech, Palo Alto, CA) with primers containing EcoRI and SalI cleavage sites (5′ primer: 5′-GAAGCTATGGCCAG-GCACAAAAAGGTGT-3′ and 3′ primer: 5′-GTCGACCTACTGAAGCTTGGCCGTCGACG-TCTAG-3′). The resulting product of polymerase chain reaction was then ligated into the TA-cloning vector, pCR2.1 (Invitrogen, Carlsbad, CA), and the correct sequence was confirmed. The human GKR cDNA fragment was excised from pCR2.1 with EcoRI-SalI, blunted-ended with Klenow (BLB, Gaithersburg, MD), and ligated into the EcoRI site of pAv996 (21,22) to form pAv996-hGKR. Finally, the 2.4-kb BamHI fragment from pAv66alx-hGKR was ligated into the BamHI site of pAv66alx to form pAv66alx-hGKR. The recombinant adenoval vector was created by cre-lox–mediated recombination of pAv66alx-hGKR with pS93, containing most of the adenoval genome, and was purified by standard procedures (21,22). The adenoval vectors Av3Null and Av3hGk, which lack a transgene and encode human liver GK, respectively, were essentially as previously described (21,22), except that the Av3Null vector did not contain the Rous sarcoma virus (RSV) promoter and SV40 polyA signal.

**In vitro experiments.** Primary hepatocytes were isolated from male Sprague-Dawley rats as previously described (23). HepG2 cells (ATCC, Manassas, VA) or hepatocytes were incubated with the indicated adenoval vectors for 2 h on a rocker in a 37°C incubator. Either 200 (Fig. 1) or 50 (Fig. 3) of the indicated viral particles/cell were used. Assays ([3H]O2 release and protein extraction) were performed on primary hepatocytes 48 h after viral transduction. Determination of [3H]O2 release from [2-3H]glucose (NEN, Boston, MA) was performed essentially as previously described (14,24). Protein lysates from HepG2 cells were prepared 72 h after viral transduction.

**Animal diet and adenoval treatment.** C57BL/6J male mice (3-4 weeks of age) were purchased from Jackson Laboratories (Bar Harbor, ME). All mice were housed in a pathogen free–barrier facility and were maintained on a 12-h light/dark cycle. These mice were maintained on either a high-fat diet (HF; 58% fat calories; D12309R) or a control diet (LF; 58% carbohydrate; D12451) (Research Diets, New Brunswick, NJ) for at least 4 months, after which diabetic mice were selected on the basis of elevated fasting blood glucose levels and impaired glucose tolerance (25). Mice were randomly divided into three treatment groups (Hanks’ balanced salt solution [HBSS], Av3Null, or Av3hGKR), such that each group had the same average fasting blood glucose levels. Adenoval vector administrations via tail vein injections and retro-orbital phlebotomy were performed as previously described (26). Mice received 12 × 10⁹ viral particles/animal in a 250-μl injection volume of HBSS. The diet treatment was continued after the vector administration until study termination.

**In vivo metabolic analyses.** An oral glucose tolerance test (OGTT) was performed 2 weeks before and 2 weeks after the vector treatment as follows. The mice were fasted for 14–16 h before receiving a glucose bolus (1 g/kg). Blood samples from conscious mice were then collected by retro-orbital phlebotomy at 0, 30, and 120 min for glucose and insulin measurements. The area-above-baseline area under the curve (AUC) was calculated such that differences in the fasting glucose values did not affect the AUC values. At 1 and 3 weeks after the vector treatment, the mice were fasted overnight, and blood samples were collected for glucose determination. At 3 weeks after the vector treatment, animals were killed by cervical dislocation, and the livers were weighed and harvested for analyses. Tissue sections were either fixed in formalin (for hematoxylin and eosin staining) or snap-frozen in liquid nitrogen. All animal procedures were conducted in accordance with principles and guidelines established by the Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act (GTI Humane Care and Use Manual, 1998).

**Statistical analysis.** The comparison of different groups was performed using unpaired Student’s t test. Differences were considered statistically significant at *P* < 0.05.
Glucose levels were equivalent. GKRP mice \((n = 11)\) were administered \(6 \times 10^{10}\) particles of Av3hGKRP plus \(6 \times 10^{10}\) particles of Av3Null, bringing the total viral dose to \(12 \times 10^{10}\) particles, a dosage consistent with our previous safety (data not shown) and toxicological profiles (21). Null mice \((n = 10)\) were administered \(12 \times 10^{10}\) particles of the Av3Null vector, and control mice \((n = 10)\) were administered with an equal volume of HBSS. The vectors were administered by intravenous (tail vein) injections that, due to the hepatotropic nature of adenoviral vectors, would allow near-complete transduction of hepatocytes preferentially to any other tissues (18,19). The age-matched LF mice \((n = 9)\) were also injected with HBSS (LF:HBSS) as a nondiabetic control.

Fasting blood glucose levels were measured in the treated mice at weekly intervals after vector administration (Fig. 2). Null mice had lower fasting glucose levels than HBSS mice at all time points tested (although this may represent pre-existing differences between the groups). However, the GKRP mice showed a significant decrease in

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**FIG. 1.** Structure and function of the Av3hGKRP vector. \(A\): The schematic structure of Av3hGKRP. ITR and \(\phi\) represent the inverted terminal repeats and packaging signal, respectively. E1-, E2a-, and E3-represent the deletions of these genes from the adenovirus genome. The insertion of the human GKRP expression cassette (containing an RSV promoter and a SV40 poly[A] sequence) is indicated below. \(B\): Primary rat hepatocytes were transduced with 200 viral particles/cell of Av3hGKRP or Av3Null or remained untreated. Protein lysates were harvested and used for Western blot analysis using an antibody specific for GKRP 48 h after transduction. \(C\): Primary rat hepatocytes were transduced with 200 viral particles/cell of Av3hGKRP or remained untreated. A \(^{3}H\)O release assay for the rate of glucose phosphorylation was performed 48 h after viral transduction. Values are the means ± SE. \(^*\)A significant \((P < 0.00005)\) decrease in glucose turnover at 5 mmol/l glucose was seen in the Av3hGKRP-treated cells.
fasting glucose levels compared with both Null and HBSS mice at all times tested. By the end of the experiment, the blood glucose levels of the GKRP mice were statistically indistinguishable from the nondiabetic LF:HBSS group (104 vs. 102 mg/dl; *P < 0.82).

To assay the effect of increased hepatic GKRP expression on glucose tolerance, we performed an OGTT 2 weeks after vector administration. The HF:HBSS mice had a significantly impaired glucose tolerance (decrease in AUC) compared with their LF:HBSS counterparts (Table 1), further confirming the diabetic nature of these mice. Av3Null did not significantly improve glucose tolerance by itself, compared with the pretreatment values of either the HBSS (Table 1) or Null group (data not shown). However, Av3hGKRP treatment did result in an improvement in glucose tolerance that was significant compared with both the HBSS (Table 1) and GKRP groups’ pretreatment values (data not shown). In fact, the glucose tolerance curve for Av3hGKRP nearly overlapped with that of the nondiabetic LF:HBSS mice (data not shown).

Insulin resistance is a primary cause and diagnostic marker of type 2 diabetes. Therefore, we measured fasting plasma insulin levels 2 weeks after vector treatment. The HF mice displayed extreme hyperinsulinemia, consistent with an insulin-resistant phenotype (Table 1; compare HBSS and LF:HBSS). Av3Null was capable of decreasing insulin levels; however, Av3hGKRP lowered insulin levels significantly more so, to the levels seen in the LF:HBSS mice. This result was true at all points tested during the OGTT (data not shown).

To characterize the effect of Av3hGKRP treatment on hepatic function and fuel homeostasis, we assayed various parameters from fasting plasma samples taken 3 weeks after vector administration (Table 1). Elevated plasma levels of the liver enzyme ALT was observed in mice treated with either Av3Null or Av3hGKRP (Table 1). This result was expected, because adenoviral transduction often results in liver toxicity (18,19,28). An increased ability to metabolize glucose in the liver has previously been shown to result in disturbances in energy storage and

![FIG. 2. Fasting blood glucose values in vector-treated mice. Blood samples were collected from overnight-fasted mice at 1 week before or 1, 2, or 3 weeks after vector administration, and whole-blood glucose values determined. HBSS, Null, and GKRP represent HF mice administered HBSS (n = 10), Av3Null (n = 10), and Av3hGKRP (n = 11), respectively. LF: HBSS represents chow-fed mice administered HBSS (n = 9). Values are the mean ± SE. Significant differences from Null mice are indicated as follows: *P < 0.0005; **P < 0.0001.](image-url)
E. SLOSBERG AND ASSOCIATES

**Table 2**
The effect of Av3hGKRP on body and liver weights, glycogen content, and GK activity

<table>
<thead>
<tr>
<th></th>
<th>LF:HBSS</th>
<th>HBSS</th>
<th>Null</th>
<th>GKRPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>20.7 ± 0.7a†</td>
<td>47.9 ± 0.5</td>
<td>45.9 ± 1.4</td>
<td>40.1 ± 1.0a†</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.30 ± 0.0a†</td>
<td>1.99 ± 0.1</td>
<td>2.77 ± 0.2a</td>
<td>2.03 ± 0.1</td>
</tr>
<tr>
<td>Liver/body (%)</td>
<td>4.4 ± 0.1a†</td>
<td>4.1 ± 0.3</td>
<td>6.0 ± 0.3a†</td>
<td>5.0 ± 0.2a†</td>
</tr>
<tr>
<td>Glycogen (mg/g)</td>
<td>5.9 ± 1.6a†</td>
<td>10.6 ± 0.7</td>
<td>19.9 ± 1.6a†</td>
<td>13.7 ± 1.3</td>
</tr>
<tr>
<td>GK activity nmol · min⁻¹ · mg⁻¹</td>
<td>4.24 ± 0.85a†</td>
<td>7.29 ± 0.70</td>
<td>6.79 ± 0.47</td>
<td>5.22 ± 0.27a†</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Significant differences (P < 0.05) from HBSS and Null mice are indicated by * and †, respectively. HBSS, Null, and GKRPM represent high fat–fed mice administered HBSS (n = 10), Av3Null (n = 10), and Av3hGKRP (n = 11), respectively. LF:HBSS represents mice fed standard laboratory diet administered HBSS (n = 9).

**Discussion**

In this study, we have detailed the first gene therapy use of GKRPM for diabetes. Type 2 diabetes in humans is a complex polygenic disease with strong environmental underpinnings, the most noteworthy of which is obesity. Therefore, we used a murine model in which diabetes was induced by high-fat feeding, because it most accurately mimicked the etiology of the human disease. We used an E1/E2a/E3-deficient adenoviral vector containing the cDNA encoding human GKRPM (Av3hGKRP), which produced high levels of functional GKRPM protein in vitro (Fig. 1) (32). Diabetic mice were administered either Av3hGKRP, Av3Null (a control vector), or the saline carrier HBSS.

Because the documented function of GKRPM is to inhibit GK activity under low-glucose conditions, we expected to see a more diabetic phenotype in mice administered Av3hGKRP. However, mice overexpressing GKRPM unexpectedly exhibited a decrease in fasting blood glucose levels similar to that seen in nondiabetic mice (Fig. 2). GKRPM mice also had sharply reduced insulin levels (Table
revealing their normalized insulin sensitivity. Furthermore, they showed improved glucose tolerance during an OGTT (Table 1). In fact, by these measures, Av3hGKRP treatment completely corrected the diabetic phenotype of the animals and was capable of maintaining this effect for the duration of the 3-week study. Based on our experience with related adenoviral vectors (21,22,26), it is expected that Av3hGKRP treatment should be effective for substantially longer periods of time. The beneficial effects of GKRP overexpression were actually quite similar to those found after administration of an adenovirus expressing GK to diabetic mice (data not shown) (22,30). In the GKRP mice, there was also a trend toward higher hepatic glycogen levels compared with HBSS mice, which is consistent with the decreased or increased glycogen levels seen in GKRP knockout (33) and GK transgenic mice (6), respectively. However, the increase observed in our studies may in part be an artifact of supplementing the Av3hGKRP viral dose with Av3Null, which itself can increase glycogen content (Table 2). Further studies will be needed to clarify this result.

Numerous studies have reported a role for GKRP in sequestering GK in the hepatocyte nucleus in an enzymatically inactive state under metabolically quiescent conditions (low glucose) (11,12,15,16,32,34). However, in our in vitro studies, we found that HepG2 cells simultaneously transduced with Av3hGKRP and an adenoviral vector expressing human GK (Av3hGK) had significantly elevated GK protein and activity levels compared with cells transduced with Av3hGK alone (data not shown) (22,30). In the GKRP mice, there was also a trend toward higher hepatic glycogen levels compared with HBSS mice, which is consistent with the decreased or increased glycogen levels seen in GKRP knockout (33) and GK transgenic mice (6), respectively. However, the increase observed in our studies may in part be an artifact of supplementing the Av3hGKRP viral dose with Av3Null, which itself can increase glycogen content (Table 2). Further studies will be needed to clarify this result.

A second possibility is that although the in vivo overexpression of GKRP leads to a decrease in overall GK activity, this activity may be applied in a more efficient manner toward metabolizing blood glucose. The subcellular compartmentalization by scaffolding proteins of enzymes or signaling proteins into clusters is often used as a means of increasing system efficiencies (40,41). In this...
case, even decreased levels of overall GK activity can be applied temporally and spatially in more appropriate ways to increase the effective hepatic GK activity, similar to how increased levels of the scaffold protein targeting to glycogen (PTG) results in improved glucose tolerance (42). Finally, a possible role for the GK-associated phosphatase (GKAP) in regulating GK activity (43), as well as potentially unidentified functions of GKRP, may further explain the discrepancy between our in vivo and in vitro results.

To our surprise, we observed a beneficial effect of Av3Null treatment on glucose tolerance when compared with HBSS treatment (Table 1). Although gene therapies for diabetes using adenoaviral vectors have been reported, the control vectors used in these studies contained transgenes (i.e., β-gal) and were not compared with untreated or saline-treated control groups (29,42,44–46). Therefore, we know of no other reports suggesting an influence of adenoviruses or adenoaviral vectors on glucose tolerance or on the expression or function of hepatic proteins involved in glucose metabolism. The adenoaviral vector (Av3) used in these studies would be expected to express several adenoaviral backbone genes (i.e., E4, L1) within the transduced cell. The resulting proteins, and/or the viral capsid proteins necessary for transduction, may directly affect host cell functions or may induce an immune response against the transduced cells (18,19,28). In fact, a preliminary gene expression analysis using a RNA hybridization array chip (Incyte Pharmaceuticals, Fremont, CA) has shown >20 genes differentially regulated (of ~10,000 tested) in response to treatment with Av3Null (compared with HBSS; data not shown). Furthermore, these mice had increased liver function test values (i.e., ALT), liver glycogen contents, and liver weight (Tables 1 and 2). In subsequent studies, it may be possible to minimize these effects by using ‘gutless’ adenoaviral vectors, which would only express the transgene (20,47).

Several gene therapies for type 2 diabetes have been studied with mixed success, including insulin, GK, and PTG (29,42,48). Most insulin gene therapies suffer from constitutive or inappropriate timing of insulin production, which has the potential for causing hypoglycemia, and would also be ineffective for the insulin-resistant patient (48). GK, insulin, and PTG gene therapies also have the potential to cause hypoglycemic episodes, and GK overexpression has resulted in the deleterious accumulation of lipids within the liver and bloodstream (42). The fundamental problem with overexpressing these transgenes is that they deregulate metabolic pathways. In contrast, by overexpressing GKRP, there is the potential to increase GK activity in a controlled manner only when it is needed. Av3hGKRP treatment did not cause hypoglycemia in diabetic (Table 1) or normal mice (data not shown) nor did it result in hepatic steatosis (data not shown) or increased plasma triglyceride or free fatty acid levels (Table 1). The only unanticipated side effect we noted with Av3hGKRP treatment was weight loss, which could be an added benefit to the largely obese type 2 diabetic population.

Our study and the two recent reports of mice deficient in GKRP showing decreased levels of GK protein (33,35), provide support for a novel level of GK regulation: protein stability. The manipulation of a protein’s half-life is a common means of signal transduction and directly controlling biochemical/metabolic pathways (49). Several pharmaceutical entities (small molecule compounds) designed to manipulate the half-life of specific proteins are currently under development (50,51). Our findings suggest the validity of such an approach with respect to GK. However, any approach to increase GK activity by interfering with the binding of GKRP to GK risks worsening the disease it seeks to alleviate (diabetes), because prolonged disruption may result in decreased hepatic GK levels. In summary, we have developed a novel therapeutic approach for type 2 diabetes, GKRP gene therapy, which we have shown to be quite effective in correcting the phenotype of diabetic mice. Our studies have also provided insight into the physiological role of GKRP. In the future, it would be of interest to determine whether an adenovirus expressing both GKRP and GK would show a synergistic ability to treat diabetes without the deleterious effects (hepatic steatosis and dyslipidemia) that may result from GK overexpression alone.

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