Compensation in Pancreatic β-Cell Function in Subjects With Glucokinase Mutations

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The relationship between the in vivo insulin secretory responsiveness of the pancreatic β-cell to glucose and the flux of glucose through the enzyme glucokinase was investigated in six subjects with heterozygous glucokinase mutations and in six matched control subjects. This was done by combining data published previously on the in vivo dose-response relationships between glucose and insulin secretion and on the in vitro enzymatic properties of wild-type and mutant forms of glucokinase. The flux of glucose through glucokinase (*GK flux*) in these subjects was estimated using a model based on the approximate Michaelis-Menten kinetics of wild-type and mutant forms of the enzyme. In two subjects with glucokinase mutations, which resulted in only a small reduction in enzymatic activity, the decrease in insulin secretion was directly proportional to the decrease in *GK flux* predicted using a Michaelis-Menten model for both mutant and wild-type glucokinase. However, in four subjects with glucokinase mutations, which resulted in severe reductions in enzymatic activity, insulin secretion was reduced compared with control subjects but less than predicted. This latter result implies the existence of a compensatory change in the β-cells of such subjects, which results in a relative increase in insulin secretory response. We propose modifications to the simple model relating glucose concentration and *GK flux*, including glucose-induced overexpression of the normal allele and a role of glucokinase regulatory protein. The modifications take into account the possibility that the degree of compensation may be directly related to the severity of the mutation. *Diabetes* 43:718–723, 1994

The glycolytic enzyme glucokinase catalyzes the phosphorylation of glucose in pancreatic β-cells and liver. Recent studies have shown that mutations in the gene encoding this key regulatory enzyme of glycolysis can cause an autosomal dominant form of diabetes with onset during childhood (1,2). It has been proposed that glucokinase is the β-cell glucose sensor and that the activity of this enzyme determines the insulin secretory response to glucose (3). Subjects with glucokinase mutations represent a unique opportunity to evaluate this hypothesis and to examine the effects of such mutations in vivo.

We have demonstrated recently (4) that in subjects with mutations in the glucokinase enzyme there is decreased responsiveness of the pancreatic β-cell to glucose and a shift in the glucose-insulin secretion rate dose-response curve downward and to the right. Also, a reduced ability to entrain the ultradian oscillations of insulin secretion with exogenous glucose has been observed in these subjects. These results support a key role for glucokinase in the regulation of insulin secretion and prompted us to examine the relationship between glucose flux through glucokinase (*GK flux*) and insulin secretion rate (ISR). If the glucokinase activity per se determines the insulin secretory response to glucose, a given glucose flux should uniquely define the ISR. The enzymatic properties of a number of the missense and nonsense mutations in glucokinase associated with diabetes have been characterized (5,6). These include the glucokinase mutations in the subjects in whom we have conducted clinical studies of insulin secretory response. This has allowed us to predict the *GK flux* at different glucose concentrations in subjects with glucokinase mutations and in normal control subjects. A comparison of in vivo ISRs and the predicted normalized *GK flux* in these two groups of subjects suggests the existence of compensatory changes in β-cell function in subjects with severe glucokinase mutations that result in a relative improvement in insulin secretion. We propose modified models that suggest a possible linear relationship between the severity of the mutation, as measured by the effect on V_{max} of glucokinase, and the degree of compensation.

RESEARCH DESIGN AND METHODS

This study uses experiments published recently on the in vivo dose-response relationships between glucose and insulin secretion (4) and on the in vitro enzymatic properties of wild-type and mutant forms of glucokinase (5). The dose-response relationships were obtained on two occasions in four male and two female subjects who are heterozygous for known glucokinase mutations, after an overnight fast and after priming with intravenous glucose for 42 h. The clinical profiles of these subjects are shown in Table 1. ISRs were calculated by deconvolution of peripheral C-peptide measurements using individually estimated C-peptide kinetic parameters (7). For further details about the experiments, we refer to the respective studies (4,5).

Modeling of glucose fluxes. The V_{max} and K_m for glucose of normal and mutant forms of human β-cell glucokinase have been determined by purifying the various mutant forms and the wild-type enzyme and expressing them in E. coli (5,6). The values for those mutations studied are listed in Table 1. Purified glucokinase obeys approximate Michaelis-Menten kinetics above a glucose concentration of 5 mM (10). The following assumptions were made to construct a simple model of *GK*

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GK flux, glucose flux through glucokinase; ISR, insulin secretion rate.

TABLE 1
Clinical characteristics of patients and control subjects

	Sex (M/F)	Age (years)	BMI (kg/m^2)	Fasting glucose (mM)	Fasting insulin (pM)	Pedigree	Mutation	V_{max} (U/mg)	K_m glucose (mM)
Patients									
GK2	M	18	19.0	7.2	41	F8	E279X	—	—
GK3	F	33	18.8	6.4	21	F331	V182M	49 ± 6	70 ± 9
GK4	F	48	24.6	6.7	62	F51	E300Q	100 ± 8	20 ± 1.2
GK5	M	21	21.2	6.8	48	F51	E300Q	100 ± 8	20 ± 1.2
GK6	M	45	25.0	6.8	49	F390	G261R	≤0.46	2.5 ± 2.1
GK7	M	18	23.1	6.4	59	F422	V203A	0.5 ± 0.04	100 ± 20
Subjects with mutations		30.5 ± 5.6	22.0 ± 1.1	6.7 ± 0.1	46.7 ± 6.0			—	—
Control subjects		29.0 ± 5.0	22.5 ± 1.4	5.0 ± 0.02	54.7 ± 6.9		Wild-type (wt)	100 ± 8	8 ± 2

Data are means ± SE. Pedigree data are from Froguel et al. (1) and V_m and K_m data are from Gidh-Jain et al. (5). V_{max} units are specific activity of glucokinase ($\mu mol \cdot min^{-1} \cdot mg^{-1}$ enzyme). One control subject (labeled GK1) was an unaffected sibling of GK2.

flux: *1*) Each allele is expressed to equal levels and therefore will contribute to total glucokinase activity in proportion to the relative activity of its product (i.e., wild-type or mutant enzyme); and *2*) the total glucokinase activity A can be described by the sum of two approximate Michaelis-Menten terms using the individual values for K_m and V_{max} determined in vitro.

$$A = \frac{V_{max\ wt} \times [Glu]}{K_{m\ wt} + [Glu]} + \frac{V_{max\ gk} \times [Glu]}{K_{m\ gk} + [Glu]} \quad (1)$$

where subscripts *wt* and *gk* denote wild-type and mutant glucokinase, respectively, and [*Glu*] denotes glucose concentration. Total glucokinase activity determines the *GK flux*, and it is assumed that the flux is directly proportional to the activity. The normalized *GK flux* is therefore defined as

$$GK\,flux = \frac{0.5 \times [Glu]}{K_{m\ wt} + [Glu]} + \frac{0.5 \times V_{max\ gk} \times [Glu]}{V_{max\ wt} \times (K_{m\ gk} + [Glu])} \quad (2)$$

To obtain values of *GK flux* between 0 and 1, each term in equation 1 has been divided by $2 \times V_{max\ wt}$. With this representation, the flux is predicted to be half normal in a subject with one normal allele and one mutant allele producing an inactive enzyme. The relationship between *GK flux* and ISR was then plotted for each individual subject. Under the assumption that the *GK flux* uniquely determines the ISR, this representation should yield curves that are superimposable in all 12 subjects.

RESULTS

Relationship between plasma glucose levels and ISR. The individual glucose-ISR dose-response curves are shown in Fig. 1 for each subject with a glucokinase mutation and the matched control subjects. The dose-response curves in the subjects with mutations are consistently shifted downward and to the right compared with their control subjects. In all subjects, priming with glucose for 42 h resulted in a shift in the dose-response curve upward and to the left.

Relationship between glucose concentration and *GK flux*. Figure 2 shows the predicted *GK flux* as a function of the glucose concentration, calculated by equation 2 for a subject with two wild-type alleles and for subjects with different mutations. Using this approach, the E279X, G261R, and V203A mutations all have similar effects on glucose flux, because they result in the synthesis of a truncated protein or virtually inactive enzyme (Table 1). The V182M and E300Q mutations are predicted to have intermediate effects on glucose flux, because these mutations retain partial biological activity.

Relationship between *GK flux* and ISR. If it is assumed that glucokinase activity per se determines the insulin secretory response to glucose, a given glucose flux should uniquely define the ISR. Figure 3 shows the individual relationships between normalized *GK flux* and ISR (see equation 2 above). Each panel shows the curves in a single subject with a glucokinase mutation and in the correspond-

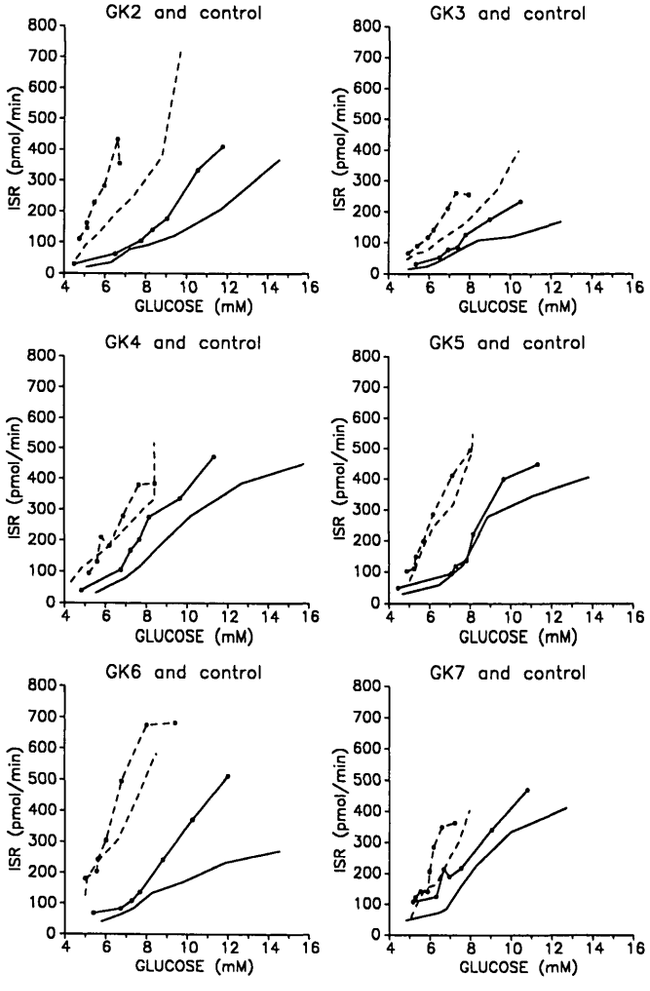

FIG. 1. In vivo dose-response relationships between glucose concentration and ISR obtained in the six subjects with glucokinase mutations and in their matched control subjects. Each panel shows the individual basal dose-response curves obtained in each subject with a glucokinase mutation at baseline (———) and post glucose priming (●——●) and in the corresponding control subject at baseline (- - - -) and post glucose priming (●----●). See Table 1 and Fig. 2 for details about the nature of each mutation.

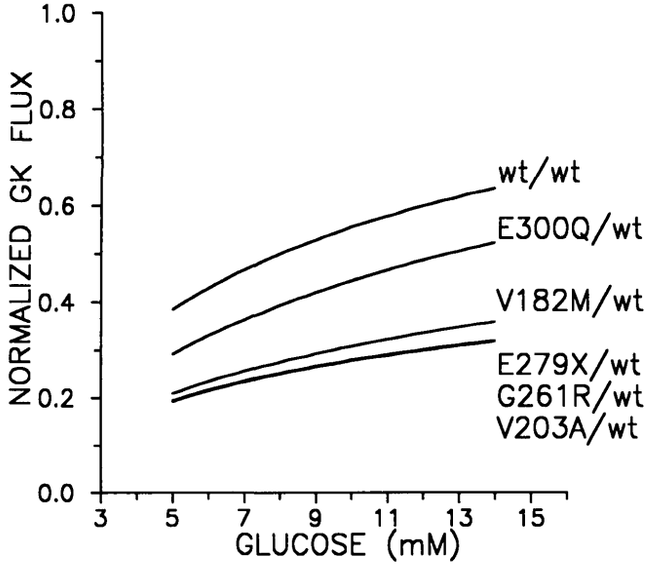

FIG. 2. The *GK flux* plotted as a function of the glucose concentration using equation 2 and the individual kinetic parameters (5) listed in Table 1.

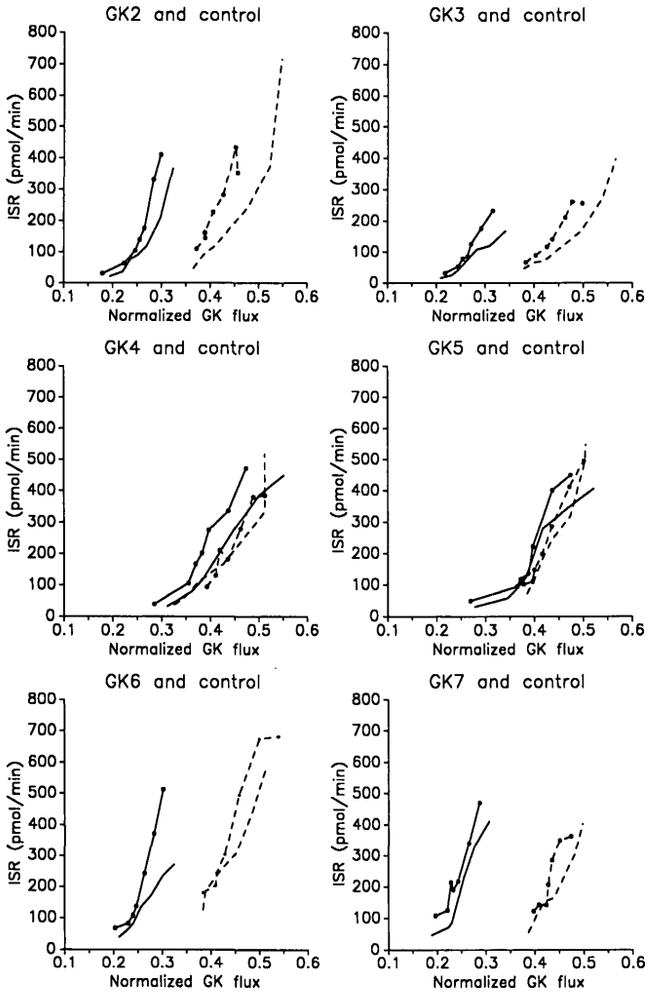

FIG. 3. Relationships between in vivo ISR rate (obtained in six subjects with glucokinase mutations and in six matched control subjects) and the *GK flux* calculated by equation 2. Each panel shows the individual basal dose-response curves obtained in each subject with a glucokinase mutation at baseline (———) and post glucose priming (●——●) and in the corresponding control subject at baseline (– – – –) and post glucose priming (●----●). See Table 1 and Fig. 2 for details about the nature of each mutation.

ing control subject. In the two subjects (GK4 and GK5) who had the relatively mild E300Q mutation, the curves are virtually superimposable on those of the control subjects. This result suggests that equation 2 provides a reasonable approximation of *GK flux* in the pancreatic β-cell. In contrast, the curves are consistently shifted to the left in the remaining four subjects who had mutations that are predicted to cause a substantial decrease in cellular glucokinase activity. Thus, these subjects secreted more insulin at each level of *GK flux* than their matched control subjects. This increased relative sensitivity to glucose implies the existence of a compensatory change in β-cell function in subjects with severe glucokinase mutations.

Sensitivity of glucose flux to variations in total glucokinase expression and K_m. The molecular mechanism(s) leading to this compensatory increase in the responsiveness of the pancreatic β-cells to glucose in subjects with severe glucokinase mutations is unknown. Models designed to explore the compensatory mechanisms must take into account that these heterozygous subjects express one allele that encodes a normal protein and a second allele encoding a protein with reduced or no activity, depending on the nature of the mutation. Thus, molecular mechanisms that increase glucokinase activity, such as increased expression, could result in increased glucose flux and account for the apparent increase in the responsiveness of the β-cell to glucose, as noted in Fig. 3. β-cell glucokinase gene expression is believed to be upregulated by glucose (11,12) and chronically elevated glucose levels could thus affect the amount of glucokinase in the β-cells in these subjects. For example, if in a particular subject there is a 50% compensatory increase in total expression, equation 2 should be multiplied by 1.5 for this particular subject. Similarly, a given factorial change in glucokinase affinity for glucose can be modeled by multiplication of K_m by the appropriate factor.

To investigate the effects of alterations in total glucokinase expression or K_m on the relationship between ISR and *GK flux*, two families of curves were drawn using *1*) different degrees of a compensatory increase in glucokinase expression and *2*) different degrees of a factorial change in K_m. Because the results in Fig. 3 are similar for the four most severe mutations, the procedures are demonstrated with the data from one representative example.

Variations in total expression. To model the effect of changes in glucokinase expression on *GK flux*, the insulin secretion data from the subject with the E279X mutation were plotted against the *GK flux*, which was modified by a factor ranging between 1 and 2. This corresponds to a 0 to 100% increase in glucokinase expression and yields a maximal normalized *GK flux* between 0.5 and 1. For comparison, this figure also shows the curves in the corresponding control subject assuming no increase in expression. If a relatively modest increase in total expression of the normal allele by 40–60% in the subject with the E279X mutation is assumed, the curves relating ISR and *GK flux* are superimposable in the subject and the matched control subject. Thus, a small relative increase in glucokinase or activity could account for the increased relative sensitivity to glucose of subjects with severe glucokinase mutations. This procedure was performed in all subjects with glucokinase

mutations to estimate the degree of compensation present. We thus propose an alternative equation for *GK flux*:

$$GK\,flux = \alpha\left(\frac{0.5 \times [Glu]}{K_{m\,wt} + [Glu]} + \frac{0.5 \times V_{max\,gk} \times [Glu]}{V_{max\,wt} \times (K_{m\,gk} + [Glu])}\right) \quad (3)$$

This equation is obtained by multiplication of equation 1 by a factor α, which measures the degree of compensation. For α = 1, there is no compensation; α = 1.5, for instance, denotes a 50% increase in glucokinase expression.

For each subject with a glucokinase mutation, the approximate value of α was determined from a family of curves relating ISR and *GK flux* at different values of α as illustrated in Fig. 4*A*. As can be seen in Fig. 4*B*, a linear relationship appears between the degree of compensation α and $V_{max\,gk}$. Thus, the degree of compensation may be directly related to the severity of the mutation.

Variations in K_m. The effects of varying K_m on *GK flux* are shown in Fig. 5*A*. Here, the insulin secretion data in the control subject are plotted against the *GK flux* as calculated when K_m is modified by a factor ranging between 1 and 2. For comparison, the baseline curve in the subject with the E279X mutation that was obtained using equation 2 and the nonmodified values of V_{max} and K_m is also shown. This figure demonstrates that a 60–70% increase in the in vivo K_m of glucokinase in normal subjects compared with subjects with glucokinase mutations could also explain the apparent increase in glucose sensitivity of ISR in subjects with glucokinase mutations.

β-cell glucokinase activity is inhibited in the presence of the glucokinase regulatory protein (13). This inhibition appears as an increase in the K_m for glucose rather than a decrease in V_{max}, and the degree of inhibition is different for wild-type and mutant forms of glucokinase (E. Van Schaftingen, S.J.P., unpublished observations). If β represents the degree of inhibition by glucokinase regulatory protein and is the same for the mutant and wild-type enzyme in a particular subject, an alternative modification of the original equation 2 for *GK flux* is

$$GK\,flux = \frac{0.5 \times [Glu]}{\beta \times K_{m\,wt} + [Glu]} + \frac{0.5 \times V_{max\,gk} \times [Glu]}{V_{max\,wt} \times (\beta \times K_{m\,gk} + [Glu])} \quad (4)$$

The values of K_m and V_{max} for the wild-type and mutant forms of the enzyme were determined for the purified enzymes in the absence of glucokinase regulatory protein. Thus, the in vivo K_m may be higher. The value of β = 1 would indicate no inhibition by regulatory protein of wild-type or mutant glucokinase. Choosing the value of β = 2 to represent the degree of inhibition in all subjects with only wild-type glucokinase (i.e., control subjects), the procedure illustrated in Fig. 5*A* was used to estimate the value of β for which the data obtained in each subject with a glucokinase mutation are approximated with the data obtained in the matched control subject. Figure 5*B* shows the estimated value of β plotted against V_{max} of the corresponding mutation. As in Fig. 4*B*, a linear relationship has been observed between the degree of inhibition β, which glucokinase regulatory protein may exert on the enzyme, and $V_{max\,gk}$. Thus, a possible decreased inhibition by regulatory protein in the subjects with mutations may be directly related to the severity of the mutation.

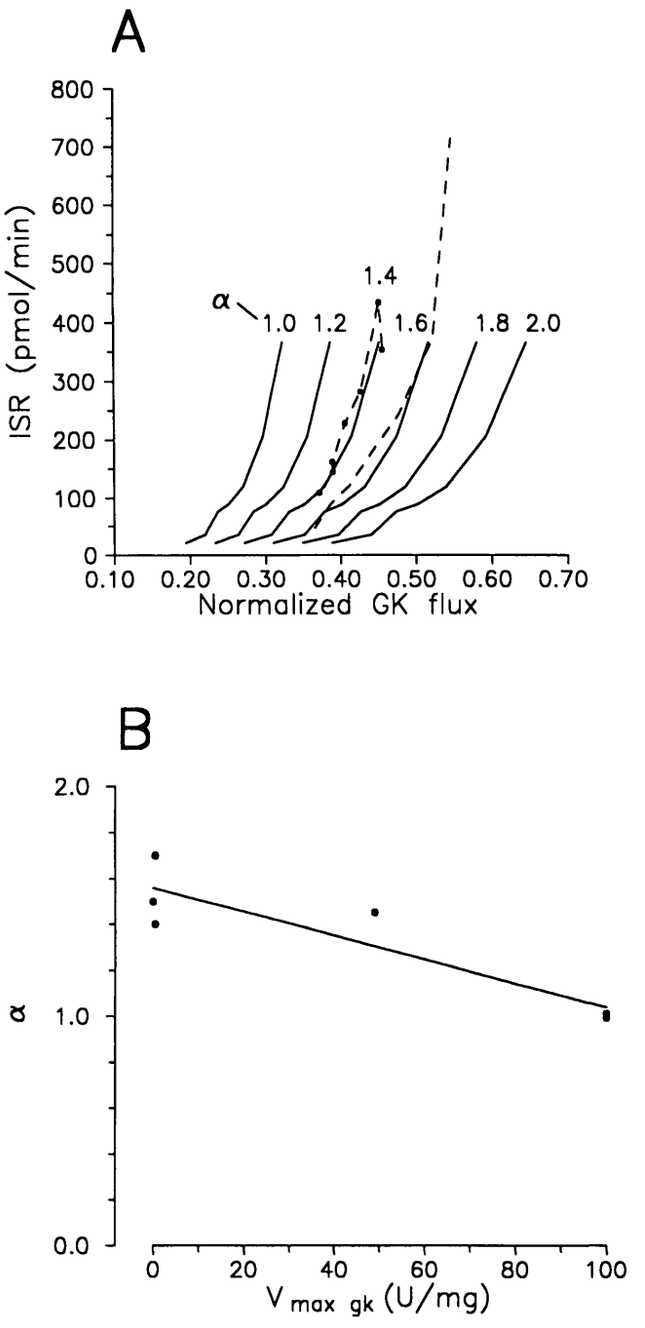

FIG. 4. *A*: Sensitivity of the ISR-*GK flux* curves to changes in total glucokinase expression. Subjects GK2 with E279X mutation at baseline (———) and at different factorial increases in glucokinase expression (denoted α). Matched control subject at baseline (– – – –) and post glucose priming (●----●). *B*: Relationship between the degree of compensation (α) plotted as a function of V_{max} of the mutant enzyme. These α values must be invoked in each subject with a glucokinase mutation to explain the data with equation 3.

Variations in both expression and K_m. Equations 3 and 4 can be combined to produce a general model of *GK flux* that takes into account the possibility that increased total glucokinase expression and changes in K_m both play a role in producing the compensatory β-cell response. Further, if we allow for the possibilities that *1*) there may be differences in the regulation of expression of each allele (e.g., promoter mutations that affect transcription and/or effects of elevated glucose levels on gene expression) and *2*) the affinity for glucose may be different for wild-type and various mutant

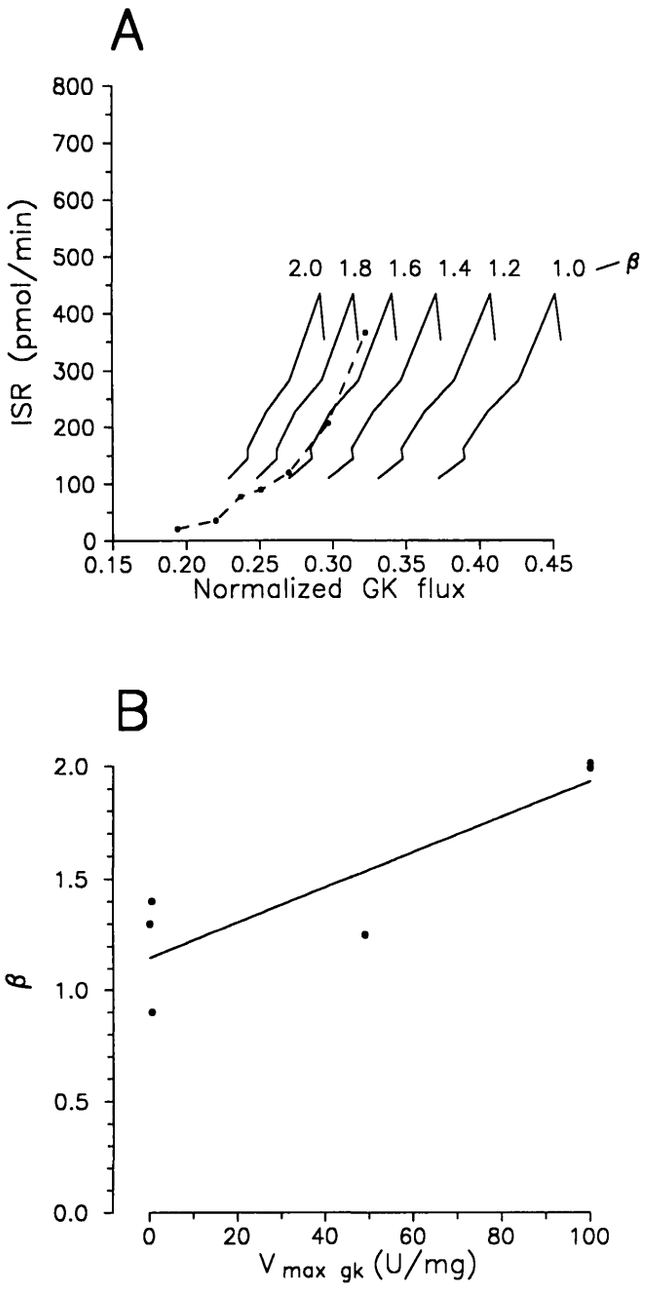

FIG. 5. *A*: **Sensitivity of the ISR-*GK flux* curves to factorial changes in K_m. Subject GK2 with E279X mutation at baseline (●----●). Corresponding control subject post glucose priming (———) at different factorial increases in K_m (denoted β).** *B*: **Relationship between the factorial change in K_m (β) plotted as a function of V_{max} of the mutant enzyme. If β = 2 is assumed for subjects with no mutations, the plotted β values must be invoked in each subject with a glucokinase mutation to explain the data with equation 4.**

forms of glucokinase because of differences in response of the mutant to regulatory protein as well as to changes in the mutants' K_m for glucose per se, we can write

$$GK\,flux = \frac{\alpha_1 \times [Glu]}{\beta_1 K_{m\,wt} + [Glu]} + \frac{\alpha_2 \times V_{max\,gk} \times [Glu]}{V_{max\,wt} \times (\beta_2 K_{m\,gk} + [Glu])} \quad (5)$$

In this formulation, $\alpha_1 = \alpha_2 = 0.5$ corresponds to no compensatory increase in expression and $\beta_1 = \beta_2 = 1$ corresponds to no inhibition by glucokinase regulatory protein. Different values of α_1 and α_2 and of β_1 and β_2 may play a role in subjects in whom the mutation leads to the production of a partially active enzyme (e.g., the V182M mutation). Because this formulation contains four unknown parameters, we shall not attempt to fit the data to this model, but merely remark that many different combinations of α_1, α_2, β_1, and β_2 will be able to account for the observed results.

DISCUSSION

We have investigated the insulin secretory defects observed in subjects with mutations in the gene encoding for glucokinase. The study of subjects with glucokinase mutations offers an opportunity to examine the role of glucokinase in the regulation of insulin secretion in vivo and the relationship between the ISR and the *GK flux* over the physiological glucose concentration range.

The *GK flux* was estimated from a simple model (equation 2) assuming that *1*) each allele is expressed to equal levels and therefore will contribute to total glucokinase activity in proportion to the relative activity of the products of each allele (i.e., wild-type or mutant enzyme) and *2*) the total glucokinase activity can be described by the sum of two approximate Michaelis-Menten terms using the individual values for K_m and V_{max} determined in vitro. Graphic representations relating the ISR and normalized *GK flux* (equation 2) revealed that the curves of two subjects who had a mild mutation and their matched control subjects were superimposable. This result is consistent with the defect in insulin secretion being a consequence of a simple gene dosage effect (i.e., reduced cellular glucokinase activity because of the expression of a mutant enzyme having decreased activity). In contrast, the remaining four subjects who had more severe mutations exhibited a greater ISR than was predicted from the estimated *GK flux*. Thus, these results imply the existence of additional compensatory mechanism(s) in some subjects.

In subjects with glucokinase mutations as well as in control subjects, priming with glucose for 42 h before obtaining the dose-response curve caused the *GK flux*-ISR curve to be shifted upward and to the left (4) as shown in Fig. 3. During the 42-h glucose priming period, the mean plasma glucose concentration in the control subjects was almost identical to that of the subjects with glucokinase mutations under baseline (non-priming) conditions (6.8 ± 0.2 vs. 6.7 ± 0.1 mM). Because glucose in some studies, although not in others (14), appears to regulate the activity of glucokinase in pancreatic β-cells (11,12), it could be argued that the curves obtained at baseline in the subjects with glucokinase mutations should be compared with the curves obtained post glucose priming in the control subjects. The discrepancy between the curves is indeed smaller when this comparison is made (Fig. 3). Note, however, that this only accounts for a proportion of the observed shift. The subjects with glucokinase mutations have obviously been subjected to mildly elevated glucose levels much longer than 42 h, and it is possible that longer-term glucose priming in the control subjects would further reduce or eliminate the discrepancy. However, we observed no correlation between the fasting glucose concentration in the glucokinase subjects and the severity of the mutation: the two subjects who had the mildest mutation and whose curves were not shifted had fasting glucose levels identical to the mean of the group. Thus, the elevation in glucose levels per se is unlikely the entire explanation for the observed discrepancies.

We have investigated how the degree of compensation differs for the various mutations and have proposed two models that can account for the results. In one model, the compensation was assumed to be mediated through a relative increase in glucokinase expression in subjects with severe glucokinase mutations. In the second model, the compensation was assumed to be mediated through differential inhibition of wild-type glucokinase by the glucokinase regulatory protein in normal subjects and those with severe glucokinase mutations. We are unable to distinguish between these two models based on the results of our in vivo studies of subjects with glucokinase mutations. Although we prefer the model in which cellular glucokinase activity is increased as a result of increased glucokinase expression because such a mechanism could account for the similar effects of missense, nonsense, and splicing mutations on insulin secretion, differential regulation of glucokinase activity by its regulatory protein is also reasonable. For example, the inhibitory effect on liver glucokinase activity exerted by glucokinase regulatory protein is strongly catalyzed by fructose-6-phosphate (13). These effects have been demonstrated in vitro at relatively low levels of both glucokinase regulatory protein and fructose-6-phosphate, which suggests that this effect may also occur in vivo. If glucokinase activity is decreased as in subjects with a severe mutation, the intra-islet levels of fructose-6-phosphate would be expected to be reduced to a similar degree, which could cause a reduction in the inhibition of glucokinase activity by glucokinase regulatory protein. If such differences in fructose-6-phosphate occur in vivo, the K_m for glucose of the wild-type allele may be lower in the subjects with glucokinase mutations than in the control subjects. Another potential source of compensation could be increased expression of hexokinase that, because of its low K_m for glucose, would not be able to sense changes in glucose but might contribute to an overall increase in glucose flux. Although it is difficult to address these possibilities directly in humans, it may be possible to do so in animal models of glucokinase-deficient diabetes that could be produced using gene knock-out technology (15).

In conclusion, the pancreatic β-cells in subjects with severe glucokinase mutations are able to compensate and improve the insulin secretory response to glucose. A better understanding of this phenomenon may lead to therapeutic approaches for ameliorating β-cell dysfunction in diabetic subjects.

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