COUNTERREGULATORY RESPONSES TO HYPOGLYCEMIA IN PATIENTS WITH GLUCOKINASE GENE MUTATIONS

E. GUENAT (1), G. SEEMATTER (1), J. PHILIPPE (2), E. TEMLER (3), E. JEQUIER (1), L. TAPPY (1)

SUMMARY - The glucokinase gene is expressed not only in pancreatic β cells and in the liver, but also in pancreatic α cells, and in some cells of the central nervous system. A decreased glucokinase activity in the latter cell types may interfere with counterregulatory responses to hypoglycemia. In order to assess functional consequences of glucokinase mutations, counterregulatory hormones secretion and glucose production (6,6–2 H glucose) were monitored during an hyperinsulinemic clamp at about 2.4 pmol.kg⁻¹.min⁻¹ insulin with progressive hypoglycemia in 7 maturity onset diabetes of the young (MODY2) type 2 patients, 5 patients with type 2 diabetes, and 13 healthy subjects. Basal glucose concentrations were significantly higher in MODY2 patients (7.6 ± 0.4 mmol.l⁻¹) and type 2 diabetic patients (12.4 ± 2.3 mmol.l⁻¹) than in healthy subjects (5.3 ± 0.1 mmol.l⁻¹, p < 0.01) but counterregulatory hormones concentrations were identical. Insulin-mediated glucose disposal and suppression of endogenous glucose production at euglycemia were unchanged in MODY2 patients, but were blunted in type 2 diabetes. During progressive hypoglycemia, the glycemic thresholds of MODY2 patients for increasing glucose production (6.0 ± 0.4 mmol.l⁻¹) and for glucagon stimulation (4.5 ± 0.4 mmol.l⁻¹) were higher than those of healthy subjects and type 2 diabetic patients (3.9 ± 0.1 and 4.1 ± 0.1 mmol.l⁻¹ respectively for glucose production and 3.7 ± 0.1 and 3.5 ± 0.1 mmol.l⁻¹ for glucagon stimulation, p < 0.02 in both cases). These results indicate that counterregulatory responses to hypoglycemia are activated at a higher plasma glucose concentration in MODY2 patients. This may be secondary to decreased glucokinase activity in hypothalamic neuronal cells, or to alterations of glucose sensing in pancreatic α cells and liver cells.

Key-words: glucagon, endogenous glucose production, growth hormone, cortisol, epinephrine, MODY2.

RÉSUMÉ - Réponses des hormones de la contre-régulation à l’hypoglycémie chez les patients porteurs d’une mutation du gène de la glucokinase.

Le gène codant pour l’enzyme glucokinase est exprimé non seulement dans les cellules β des îlots de Langerhans et dans le foie, mais aussi dans les cellules α des îlots et dans certaines cellules du système nerveux central. Les conséquences d’une baisse d’activité de la glucokinase dans les cellules α des îlots et les cellules du système nerveux pourraient être une altération de la contrerégulation à l’hypoglycémie. Pour évaluer cette hypothèse, nous avons étudié 7 patients avec un MODY2 secondaire à une mutation de la glucokinase, 5 patients diabétiques de type 2 et 13 sujets sains au cours d’un clamp hyperinsulinémique (2,4 pmol.kg⁻¹.min⁻¹ insuline) avec baisse progressive de la glycémie. Les glycémies basales étaient élevées chez les patients MODY2 (7,6 ± 0,4 mmol.l⁻¹) et chez les patients diabétiques de type 2 (12,4 ± 2,3 mmol.l⁻¹) par rapport aux sujets sains (5,3 ± 0,1 mmol.l⁻¹). Les concentrations plasmatiques des hormones de contrerégulation n’étaient cependant pas différentes dans les trois groupes. Le seuil glycémique de déclenchement d’une augmentation de la production endogène de glucose (6,6 H glucose) était augmenté chez les patients MODY2 (5,0 ± 0,4 mmol.l⁻¹) par rapport aux sujets sains (3,9 ± 0,1 mmol.l⁻¹). Le seuil glycémique de sécrétion du glucagon était aussi augmenté chez les patients MODY2 (4,5 ± 0,4 mmol.l⁻¹), vs 3,5 ± 0,1 mmol.l⁻¹ chez les diabétiques de type 2 et 3,7 ± 0,1 mmol.l⁻¹ chez les sujets sains, p < 0,02). Ces résultats indiquent qu’une mutation de la glucokinase augmente le seuil glycémique auquel une réponse de contrerégulation se déclenche. Ceci peut être dû à une baisse d’activité de la glucokinase dans certaines cellules hypothalamiques, ainsi que dans les cellules α des îlots de Langerhans et les hépatocytes.

Mots-clés : glucagon, production endogène de glucose, hormone de croissance, cortisol,adrénaline, MODY2.
In healthy subjects, plasma glucose concentrations have to be maintained within tight limits throughout the day to prevent unwanted hypoglycemia or hyperglycemia. This goal is reached through balanced secretions of insulin and counterregulatory hormones (glucagon, epinephrine, cortisol, growth hormone) which are exquisitely sensitive to changes in glycemia. Defective insulin secretion, insulin resistance, and/or increased counterregulatory hormone secretion can result in the development of hyperglycemia or diabetes mellitus [1, 2]. There is also evidence that the central nervous system (CNS) plays an important role in coordinating hormones secretion and metabolic responses, and that dysfunction of this integrative glucoregulatory function of the CNS can lead to the development of diabetes in animals [3].

Glucokinase (Hexokinase IV) catalyzes the phosphorylation of glucose to glucose-6-phosphate. In contrast with other isoforms of hexokinase, it is characterized by a high Km for glucose and by the absence of inhibition by its product glucose-6-phosphate [4]. These unique properties confer a glucose sensing ability to tissues where glucokinase is expressed [5].

Glucokinase is expressed in pancreatic β and α cells and in the liver [4, 6]. In pancreatic β cells, it is essential to couple insulin secretion to ambient glucose levels [5, 7]. In liver cells, glucokinase plays a role in glucose-induced suppression of glucose production [8] and post-prandial hepatic glycogen synthesis [9]. Its role in pancreatic α cells remains unknown, although it can be expected to trigger an inhibition of glucagon secretion during hyperglycemia as well as its stimulation during hypoglycemia. There is also evidence that glucokinase is expressed in cells of the CNS [10] where it may participate in glucose sensing and integrated control of glucose homeostasis. Of particular interest, the pancreatic isoform of glucokinase is expressed in the ventromedial hypothalamus, a brain area involved in counterregulatory responses to hypoglycemia [11]. In contrast with most CNS cells, which express hexokinase I, the intracellular glucose metabolism of these glucokinase-expressing cells can be expected to vary according to extracellular glucose concentration throughout the physiological range.

Maturity-Onset Diabetess of the Young type 2 (MODY2) patients with glucokinase gene mutations [12] have impaired glucose-induced insulin secretion [7], impaired inhibition of hepatic glucose output by hyperglycemia [8] and decreased post-prandial liver glycogen synthesis [9], indicating decreased glucose-phosphorylating activity in pancreatic β cells and liver cells. Whether glucokinase gene mutation also affects glucokinase activity in the CNS and what are the possible consequences of such decreased activity remain unexplored. An altered glucokinase activity in the CNS could interfere with the integrated counterregulatory responses to hypoglycemia. We therefore compared the counterregulatory response to graded hypoglycemia in MODY2 patients with glucokinase gene mutations, in type 2 diabetic patients and in a group of healthy human subjects of similar age and anthropometric characteristics. We hypothesized that MODY2 patients would have a higher glycemic threshold for counterregulatory responses to hypoglycemia than healthy subjects.

### METHODS

**Patients** – Seven MODY2 patients (3 males, 4 females), 5 patients with type 2 diabetes, and 13 healthy subjects (7 males, 6 females) were recruited to participate in this study. Their characteristics are shown in Table I. Mean HbA1c concentrations were not different in MODY2 patients (6.7 ± (SD) 0.4%) and in type 2 diabetic patients (7.3 ± (SD) 0.8). Mutations of glucokinase were detected by the analysis of single-strand conformational polymorphism following polymerase chain reaction amplification of the glucokinase gene. Products exhibiting polymorphisms were sequenced and the gene mutations are described in Table I.

The experimental protocol had been approved by the ethical committee of Lausanne University School of Medicine, and every participant provided an informed, written consent. Oral antidiabetic agents were discontinued 3 days before the study and insulin treatment the day before the study.

**Experimental protocol** – The experiments began between 7 am and 8 am. Subjects had been fasting since 10 pm the day before.

At their arrival at the metabolic laboratory, subjects were weighed and their height was measured. Thereafter they took place in a bed where they rested quietly during the whole experiment while watching video movies. One cannula was inserted into an antecubital vein of the left arm for infusion of insulin, glucose, and 6,6-2 H glucose. A second cannula was inserted into a wrist vein of the right arm for blood sampling. The right hand was placed in a thermostabilized box heated at 50 °C to achieve partial arterialization of venous blood. In MODY2 and type 2 diabetic patients, a bolus of 12 µmol.kg⁻¹ 6,6-2 H glucose (Masstrace, Worcester, MA) and a primed-continuous infusion of insulin (2.4 pmol.kg⁻¹ min⁻¹) were started at time 0 min [13], and variable infusion of 20% dextrose was administered to maintain plasma glucose at basal levels until time 120 min. Dextrose infusion was then reduced until plasma glucose could be maintained at 5 mmol.l⁻¹ during 30 min. Thereaf-

### ABBREVIATIONS

CNS : central nervous system  
MODY : maturity onset diabetes of the young
ter, dextrose infusion was gradually reduced to decrease plasma glucose concentrations by 0.6 mmol.l\(^{-1}\) every 30 min until a plasma glucose concentration of 3.3 mmol.l\(^{-1}\) was reached. In healthy controls, the experimental protocol was identical except that basal glucose concentrations were maintained until 180 min to minimize the differences of duration of insulin infusion between MODY2 patients or type 2 diabetic patients and healthy subjects at each glucose concentration step. During the whole procedure, the dextrose infused was mixed with 1.25% 6,6\(^{-2}\) H glucose to measure glucose kinetics using the “hot infusate” model [14]. An infusion of 0.14 µmol.kg\(^{-1}\).min\(^{-1}\) 6,6\(^{-2}\) H glucose was maintained when dextrose infusion was lower than 11 µmol.kg\(^{-1}\).min\(^{-1}\).

**Analytical procedures** – Plasma glucose was measured using a Beckman glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin (kit from Biodata, Guidonia Montecello, Italy), glucagon (kit from Linco Research, St Charles, MO) and cortisol (kit from Diagnostic Products Corporation, Los Angeles, CA) were measured by radioimmunoassays. Plasma growth hormone concentrations were measured by a chemiluminescence immunometric assay (Nichols Institute, San Juan Capistrano, CA). Plasma epinephrine and norepinephrine concentrations were measured by HPLC with electrochemical detection [15]. Plasma 6,6\(^{-2}\) H glucose was measured with GC-MS as described [8].

**Calculations**

Glucose rates of appearance (GRa) and disappearance (GRd) were calculated from 6,6\(^{-2}\) H glucose dilution analysis using the “hot infusate” model [14]. Endogenous glucose production was calculated by subtracting dextrose infusion from GRa. Glycemic thresholds for the secretion of counterregulatory hormones were determined as the first glucose concentration at which each hormone was statistically significantly increased compared to values obtained at basal glycemia.

**Statistical analysis** – All results in text, tables and figures are shown as mean ± 1 SEM unless stated otherwise. Comparisons between groups were done using Anova and post hoc tests. Determination of individual glycemic thresholds for counterregulatory

<table>
<thead>
<tr>
<th>Table I. Characteristics of the participants.</th>
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<tr>
<td><strong>Subjects</strong></td>
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<tr>
<td>MODY2 patients</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>5</td>
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<td>6</td>
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<td>7</td>
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<tr>
<td>Mean ± SD</td>
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<tr>
<td>Type 2 diabetic patients</td>
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<td>Healthy subjects</td>
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hormones secretion was performed using t-tests. The Statview® 4.5 statistical package (Abbacus Concepts, Berkeley, CA) was used for these calculations.

RESULTS

Basal hormones and glucose concentrations are shown in Table II. Plasma glucose concentrations were increased by 43% in MODY2 patients and by 133% in type 2 diabetic patients \((p < 0.01)\). Basal plasma insulin concentration was 90% higher in type 2 diabetic patients than in controls \((p < 0.05)\). Basal plasma glucagon, cortisol, and epinephrine concentrations were comparable in all 3 groups.

Insulin infusion resulted in similar increases in plasma insulin concentrations in MODY2 patients \((238 \pm 17 \text{ pmol.l}^{-1})\) type 2 diabetic patients \((281 \pm 35)\) and healthy subjects \((212 \pm 19 \text { pmol.l}^{-1})\). This relatively modest hyperinsulinemia did not significantly affect counterregulatory hormone concentrations when plasma glucose concentrations were maintained at basal levels. Glucose rates of utilization were similar in MODY2 patients \((19.8 \pm 3.0 \text { µmol.kg}^{-1}.\text{min}^{-1})\) and healthy controls \((19.8 \pm 1.8)\) but were reduced to \(12.4 \pm 1.3\) in type 2 diabetic patients \((p < 0.005)\). In the second part of the experiments, plasma glucose concentrations were allowed to decrease by reducing progressively the glucose infusion. The time course of plasma glucose and glucagon concentrations in MODY2 patients and in healthy subjects is shown in Figure 1. Even though the decrease in glycemia was started one hour earlier in MODY2 patients, the time required to decrease plasma glucose concentration to the target hypoglycemic values was higher in MODY2 patients than in healthy subjects due to their higher fasting plasma glucose concentration. In contrast, plasma glucagon concentration increased earlier in MODY2 patients than in healthy subjects, i.e., at a time when their average plasma glucose concentration was similar to normal fasting values.

Plasma counterregulatory hormones concentrations during stepwise hypoglycemia are shown in Figure 2. The plasma glucose concentration threshold for stimulation of glucagon secretion was 22% higher in MODY2 patients compared to healthy controls \((p < 0.01)\) (Table III). As a consequence, the glucagon: glucose concentrations curve was shifted to the right in MODY2 patients (Fig. 2). No such alterations were present in type 2 diabetic patients. The response of

<table>
<thead>
<tr>
<th>Glycemia</th>
<th>Insulin pmol.l(^{-1})</th>
<th>Glucagon ng.l(^{-1})</th>
<th>Epinephrine pmol.l(^{-1})</th>
<th>Growth hormone µg.l(^{-1})</th>
<th>Cortisol nmol.l(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODY2 patients</td>
<td>(7.6 \pm 0.4^{**})</td>
<td>68.4 ± 8.4</td>
<td>77 ± 8</td>
<td>289 ± 109</td>
<td>1.48 ± 0.52</td>
</tr>
<tr>
<td>Type 2 diabetic patients</td>
<td>(12.4 \pm 2.3^{***})</td>
<td>119.8 ± 25.0(^*)</td>
<td>72 ± 7</td>
<td>288 ± 49</td>
<td>1.54 ± 0.62</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>5.3 ± 0.1</td>
<td>63.0 ± 9.0</td>
<td>81 ± 6</td>
<td>235 ± 33</td>
<td>2.76 ± 0.96</td>
</tr>
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</table>

\*\(p < 0.05\), \(^{**}\)\(p < 0.01\), \(^{***}\)\(p < 0.001\) vs healthy subjects.

Fig. 1. Time course of plasma glucose and glucagon concentrations in MODY2 patients (closed circles) and healthy subjects (open circle).
cortisol, epinephrine and growth hormone and the glycemic threshold for these hormones were not different in MODY2 and type 2 diabetic patients than in healthy controls (Fig. 2 and Table III).

**TABLE III.** Glycemic thresholds (mmol.l⁻¹) for counterregulatory responses to hypoglycemia.

<table>
<thead>
<tr>
<th></th>
<th>Endogenous glucose production</th>
<th>Glucagon</th>
<th>Epinephrine</th>
<th>Growth hormone</th>
<th>Cortisol</th>
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</thead>
<tbody>
<tr>
<td>MODY2 patients</td>
<td>5.0 ± 0.4**</td>
<td>4.5 ± 0.4*</td>
<td>4.3 ± 0.3</td>
<td>4.2 ± 0.4</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Type 2 diabetic patients</td>
<td>4.1 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>4.1 ± 0.2</td>
<td>4.2 ± 0.2*</td>
<td>3.9 ± 0.15</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>3.9 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>3.8 ± 0.2</td>
</tr>
</tbody>
</table>

*p < 0.02, **p < 0.01 vs healthy subjects.

Fig. 2. Plasma counterregulatory hormones concentrations as a function of glycemia in MODY2 patients (closed circles), type 2 diabetic patients (closed triangles) and healthy subjects (open circles). Values of hormones concentrations observed at each plateau of glycemia were averaged and plotted vs the average glycemia measured.
Endogenous glucose was suppressed to similar values in MODY2 patients and healthy controls during euglycemic hyperinsulinemia (Fig. 3). In contrast, insulin-induced suppression of glucose production was markedly impaired in type 2 diabetic patients. During hypoglycemia, endogenous glucose production rose in all 3 groups (Fig. 3). Glucose production started to increase at a significantly higher glycemia in MODY2 patients than in type 2 diabetic patients and in healthy subjects (Table III). As a consequence, the glucose production: glucose concentration curve was shifted to the right (Fig. 3) in MODY2 patients but not in type 2 diabetic patients.

**DISCUSSION**

MODY2 patients have impaired glucose-induced insulin secretion, secondary to decreased glucokinase activity in pancreatic β cells [7]. They also have impaired post-prandial suppression of glucose production [8] and a lower stimulation of hepatic glycogen synthesis [9] which can be explained by a decreased hepatic glucokinase activity. We extend these observations by showing that these patients have several abnormal counterregulatory responses to hypoglycemia, suggesting functional consequences of glucokinase mutations in the CNS and/or in pancreatic α cells.

Hypoglycemia-induced glucagon secretion was enhanced in MODY2 patients compared to healthy controls. This was documented by a significant increase in the glycemic threshold for stimulation of glucagon secretion, and by a shift to the right of the glucagon: glucose concentrations curve. This change cannot be attributed to the effects of chronic hyperglycemia, because it was not observed in a small group of type 2 diabetic patients. In the latter group, stimulation of glucagon secretion was observed at plasma glucose concentrations similar to those observed in healthy subjects. This is consistent with a report indicating a maintained glucagon response to hypoglycemia in type 2 diabetes [16].

The mechanisms responsible for the early stimulation of glucagon secretion during hypoglycemia cannot be specified unequivocally from our experiments. A decreased activity of glucokinase in pancreatic α cells may be involved. However, it appears that a major portion of glucagon secretion during hypoglycemia is secondary to stimulation of the sympathetic nervous system, presumably arising from stimulation of glucose sensitive cells in the ventromedial region of the hypothalamus. In support of this concept, the stimulation of glucagon secretion by hypoglycemia is markedly blunted in several animal species and in humans by nicotinic receptor antagonists [17]. Similarly, it was recently observed that the glucagon response to hypoglycemia was defective in a patient with hypothalamic sarcoidosis in the absence of any known pancreatic abnormality [18]. These observations suggest therefore that the earlier glucagon response to hypoglycemia observed in MODY2 patients may rather be secondary to decreased glucokinase activity in glucose sensing CNS cells.

Activation of glucose sensing CNS cells involved in counterregulatory responses to hypoglycemia at a higher glycemia may be expected to lead to early responses of several counterregulatory hormones. Our data indicate a trend for a similarly increased threshold for growth hormone secretion in some of our MODY2 patients, which did not reach statistical significance due to marked interindividual variability. Alterations of growth hormone secretion during hypoglycemia may however be merely secondary to chronic hyperglycemia. A similar alteration was reported in poorly controlled type 1 diabetic patients. Interestingly, it could be reversed by intensive insulin treatment [19]. Cortisol data also failed to demonstrate a significantly increased glucose threshold, but these results are difficult to interpret because plasma cortisol concentrations depend on two simultaneous but opposite processes: plasma cortisol concentration tended to decline with time according to its circadian rhythm, and to increase as a consequence of progres-
sive hypoglycemia. Since it took more time to decrease their glycemia, cortisol values at similar glucose concentrations were recorded later during the day in MODY2 and type 2 diabetic patients than in healthy controls, which may have interfered with our statistical analysis. Epinephrine also showed a trend for an early response to hypoglycemia, but the sampling frequency may have been too small to detect glycemic thresholds accurately. For all these reasons, and since the number of subjects studied was small, we cannot discard the hypothesis that the secretion of counterregulatory hormones other than glucagon during hypoglycemia may be altered in MODY2.

In MODY2 patients, endogenous glucose production was normal at basal glycemia, but was stimulated at a higher glycemia than in healthy subjects when blood glucose was allowed to drop. The tracer used for calculation of glucose production (6,6-3H glucose) does not take into account the cycling between glucose and glucose-6-phosphate, and hence provides an estimate of net glucose production (i.e., glycolysis and gluconeogenesis). We have previously shown that glucose cycling is reduced in MODY2 secondary to glucokinase mutation [8]. Since glucose cycling has been proposed as a mean to limit net glucose production when total glucose output is increased [20], it is possible that inhibition of this process in MODY2 patients contributed to increase net endogenous glucose output.

The observation of an early stimulation of glucose production during hypoglycemia in MODY2 patients nonetheless substantiates alterations of counterregulatory responses to hypoglycemia as a consequence of glucokinase mutation. No such alteration was observed in type 2 diabetic patients who clearly had an impaired insulin-mediated suppression of glucose production, but whose endogenous glucose output increased at the same plasma glucose concentrations as in healthy controls. The early stimulation of glucose production in MODY2 may be secondary to the early secretion of glucagon. Two additional mechanisms may also be involved. First, it may be hypothesized that stimulation of sympathetic nerves targeted to the liver occurred at higher than normal glycemia, and resulted in stimulation of glucose producing pathways in liver cells. Second, it is recognized that endogenous glucose production is regulated not only by neuroendocrine factors, but also directly by plasma glucose concentration [21, 22]. In this process, hypoglycemia can elicit an increase in glucose production through autoregulatory hepatic mechanisms irrespective of increases in counterregulatory hormones. This autoregulatory response is known to occur only during severe hypoglycemia in healthy subjects [23]. We postulated however that glucokinase may be crucial in the autoregulation of glucose production [22], and it is possible that decreased glucokinase activity resulted in an early increase in glucose production through enhanced liver responsiveness to hypoglycemia.

In conclusion, our present data indicate that MODY2 patients have an early counterregulatory response to hypoglycemia, involving early stimulations of both glucagon secretion and glucose production. Both processes may be related to decreased glucokinase activity in glucose-sensitive cells in the CNS. In addition, decreased glucokinase activity in pancreatic α cells may be involved in alterations of glucagon secretion, whereas decreased glucokinase activity in liver cells may participate in alterations of glucose production. The presence of abnormal insulin secretion, hepatic glucose metabolism, and counterregulatory responses to hypoglycemia in MODY2 patients illustrate the central role played by glucokinase in integrative glucose homeostasis.

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REFERENCES

2 DeFronzo RA. The triumvirate: β-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes, 1988, 37, 667-687.


