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Specific insulin and proinsulin secretion in glucokinase-deficient individuals

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Abstract

Glucokinase (GCK) is an enzyme that regulates insulin secretion, keeping glucose levels within a narrow range. Mutations in the glucokinase gene cause a rare form of diabetes called maturity-onset diabetes of the young (MODY). An early onset (less than 25 years), autosomal dominant inheritance and low insulin secretion stimulated by glucose characterize MODY patients. Specific insulin and proinsulin were measured in serum by immunofluorimetric assays (IFMA) during a 75-g oral glucose tolerance test (OGTT). Two kindreds (SA and LZ) were studied and compared to non-diabetic unrelated individuals (control group 1) matched for age and body mass index (BMI). In one kindred, some of these subjects were also obese (BMI >26 kg/m^2), and other family members also presented with obesity and/or late-onset NIDDM. The MODY patients were also compared to a group of five of their first-degree relatives with obesity and/or late-onset NIDDM. The proinsulin profile was different in members of the two MODY kindreds. Fasting proinsulin and the proinsulin/insulin ratio were similar in MODY members of kindred LZ and subjects from control group 1, but were significantly lower than in MODY members of kindred SA ($P<0.02$ and $P<0.01$, for proinsulin and proinsulin/insulin ratio, respectively). Moreover, MODY members of family SA had higher levels of proinsulin and proinsulin/insulin ratio, although not significantly different, when compared to their first-degree relatives and to subjects from control group 2. In conclusion, we observed variable degrees of proinsulin levels and proinsulin/insulin ratio in MODY members of two different kindreds. The higher values of these parameters found in MODY and non-MODY members of kindred SA is probably related to the obesity and late-onset NIDDM background present in this family.

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Key words
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- Obesity

Introduction

Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous syndrome that can be caused by heterozygous mutations in the glucokinase (GCK) gene in approximately 50% of MODY cases in France (1). This form is characterized by an early onset (<25 years old) of diabetes mellitus, mild hyperglycemia and autosomal dominant inheritance (1). Mutations in the GCK gene cause impaired insulin secretion in response to glucose (2), a reduction in postprandial hepatic glycogen synthesis and an increased rate of gluconeogenesis after meals (3).

Proinsulin, the precursor of insulin, is biologically less active than insulin and an increased secretion of these molecules is considered to reflect ß-cell dysfunction (4,5). A disproportionate increase of proinsulin and its conversion intermediates, and an increasing proinsulin-to-insulin ratio are associated with insulin resistance syndrome (6,7). Patients with increased insulin secretion without hyperglycemia, such as obesity, present hyperproinsulinemia without an increase in the proinsulin-to-insulin ratio (7). Proinsulin levels have not been extensively studied in glucokinase-deficient subjects. We report here the assessment of specific insulin and proinsulin in members of two Brazilian glucokinase-deficient MODY families.

Material and Methods

Individuals

We studied two MODY families (LZ and SA) with different mutations in the GCK gene. In the LZ kindred the E248X mutation was found in the proband and three of his sons with MODY (8). These patients were compared to six non-diabetic unrelated individuals (control group 1) matched for age and body mass index (BMI). In the SA kindred the V401del1 mutation was detected in 6 subjects with MODY (8). Some of these subjects were also obese (BMI >26 kg/m^2), and other family members also presented with obesity and/or late-onset non-insulin-dependent diabetes (NIDDM). The MODY patients were compared to a group of five of their first-degree relatives with obesity and/or late-onset NIDDM, and to eleven non-diabetic unrelated individuals (control group 2) matched by age and BMI.

The patients' blood glucose was controlled only by diet and they were not using any drug. Normal glucose tolerance (NGT), impaired fasting glucose (IGT) and diabetes mellitus (DM) were defined according to the new diagnosis and classification of diabetes mellitus by the Expert Committee Report (9). The control group consisted of outpatients seen at the Endocrinology Division of the Universidade Federal de São Paulo who showed NGT after the oral glucose tolerance test (OGTT). All individuals and patients gave informed consent to take part in the study. The study was approved by the University Ethics committee.

Analytical methods

All individuals underwent an OGTT after a 10-h fast and blood samples were collected at 0, 30, 60 and 120 min after 75 g glucose ingestion for plasma glucose, serum insulin and proinsulin measurement. Glucose was assayed by the glucose oxidase method. Serum insulin and proinsulin were measured by an immunofluorimetric assay. These assays detect 100% human insulin and proinsulin, respectively, and both show 100% cross-reactivity with 64,65 proinsulin and split 65,66 proinsulin and no cross-reactivity with des 31,32 proinsulin or split 32,33 proinsulin. Beta-cell function in response to the oral ingestion of 75 g glucose was quantified as the ratio of the incremental plasma insulin response (RIR) above basal levels at 30 min to that of plasma glucose, divided by basal plasma insulin levels ([Δ30-0-min in-

sulin/Δ30-0-min glucose]/basal insulin) (10).

Statistical analysis

Data are reported as mean ± SD, unless otherwise stated. Non-parametric tests were performed. The Mann-Whitney U and the Kruskal-Wallis tests were used when comparing two or three groups, respectively. Quantitative traits were compared by analysis of variance (ANOVA). When ANOVA was significant, comparisons between pairs were made by the Tukey-Kramer HSD test (11). Data were analyzed using the Statistical Package for Social Science for Windows, version 7.0 (SPSS Inc., Chicago, IL). A P value less than 0.05 was considered statistically significant.

Results

The clinical and metabolic profiles of family members and control groups are shown in Table 1. The relative insulin response (RIR) was significantly decreased in MODY members of kindred LZ as compared to control group 1, as expected (2). RIR was significantly decreased both in MODY members and in the first-degree relatives of kindred SA compared to control group 2. The proinsulin profile was different in members of the two MODY kindreds. Fasting proinsulin and the proinsulin/insulin ratio were similar in MODY members of kindred LZ and subjects from control group 1, but were significantly lower when compared to those of MODY members of kindred SA ($P<0.02$ and $P<0.01$ for proinsulin and proinsulin/insulin ratio, respectively). Moreover, MODY members of family SA had higher levels of proinsulin and a higher proinsulin/insulin ratio, although not significantly different, when compared to their first-degree relatives and to subjects from control group 2.

Stepwise multiple regression analysis was performed with proinsulin or the proinsulin/insulin ratio as the dependent variable and sex, age, BMI, glucokinase mutation status, fasting and 2-h glucose, fasting insulin and RIR as independent variables. Data were log-transformed and all subjects were included in the analysis. The BMI and RIR (inverse correlation) accounted for 53%

Table 1 - Clinical and metabolic profiles of family members.

Data are reported as mean and interquartile range. The Kruskal-Wallis (rank sums) and Tukey-Kramer HSD tests with log-transformed data were used when the results of the Kruskal-Wallis test were significant ($P<0.05$). #$P<0.05$ compared to control group 2, and *$P<0.05$ compared to unaffected relatives. [a][Δ30-0-min insulin/Δ30-0-min glucose]/basal insulin. W, Women; M, men.

	Kindred LZ (MODY subjects)	Control group 1	P	Kindred SA (MODY subjects)	Control group 2	First-degree relatives	P
Subjects/sex (N)	4 (0 W/4 M)	6 (3 W/3 M)	–	6 (3 W/3 M)	11 (5 W/6 M)	5 (3 W/2 M)	–
Age (years)	21 (10-37)	27 (25-30)	0.24	21 (16-26)*	26 (23-31)	37 (29-47)	<0.02
BMI (kg/m^2)	19.8 (16.4-23.5)	22.0 (18.0-24.3)	0.34	26.5 (24.0-30.4)	27.8 (24.0-30.0)	27.9 (23.7-32.6)	0.97
Fasting glucose (mmol/l)	6.3 (5.4-6.7)	4.7 (3.4-5.3)	<0.02	6.8 (6.5-7.3)#*	5.1 (4.7-5.2)	4.8 (4.2-5.4)	<0.003
Fasting insulin (pmol/l)	18 (12-22)	39 (15-62)	0.20	39 (15-68)	35 (15-41)	47 (28-65)	0.45
Fasting proinsulin (pmol/l)	0.23 (0.10-0.48)	0.63 (0.10-1.20)	0.40	5.10 (2.45-7.78)	2.65 (0.4-4.2)	5.22 (1.55-9.85)	0.19
Fasting proinsulin/insulin (%)	1.6 (0.5-3.8)	1.1 (0.6-1.8)	0.59	17.7 (5.6-28.7)	9.5 (2.7-12.7)	10.5 (4.1-18.8)	0.43
2-h glucose (mmol/l)	10.3 (8.0-13.7)	4.4 (3.1-5.2)	<0.02	9.8 (7.3-13.1)#	5.5 (4.5-6.7)	7.7 (5.6-10.7)	<0.04
2-h insulin (pmol/l)	35 (8-69)	180 (64-297)	<0.03	174 (91-252)	161 (36-180)	155 (50-285)	0.61
2-h proinsulin (pmol/l)	9.2 (3.8-14.7)	12.5 (6.4-19.7)	0.46	30.1 (22.4-36.5)	19.3 (6.5-35.5)	22.7 (16.0-29.4)	0.22
Relative insulin response (l/mmol)[a]	0.71 (0.12-1.60)	3.61 (1.70-4.76)	<0.04	1.04 (0.10-1.58)#	3.28 (1.10-5.17)	0.60 (0.26-1.00)#	<0.04

(P<0.0001) and 8% (P<0.02) proinsulin variance, respectively. Moreover, BMI, fasting insulin (inverse correlation), the presence of a glucokinase mutation, and the sex (women) accounted for 24% (P<0.0002), 13% (P<0.004), 8% (P<0.04) and 7% (P<0.05), respectively, of the variance of proinsulin/insulin ratio in these subjects.

Discussion

Many reports have shown that GCK-deficient patients have a reduced early insulin response after a glucose load (1,4,12). In the present study we found abnormal ß-cell function in GCK-deficient individuals, even in the family with NIDDM patients (pedigree SA). In this family the proinsulin levels were increased maybe as a consequence of the stimulation of ß-cells to release immature granules (5). An increased proinsulin/insulin ratio can be observed in individuals with an NIDDM background (6). Patients from pedigree SA showed a trend towards high values for this ratio maybe as a consequence of their NIDDM background.

We found here a failure of insulin release after a glucose stimulus in GCK-deficient individuals. GCK-deficient individuals showed normal levels of proinsulin but when obesity was also present, ß-cells secreted more proinsulin. The proinsulin levels were increased in obese patients in spite of the low activity of the GCK enzyme. These results are in agreement with those of Hattersley and co-workers (13), showing normal proinsulin levels and proinsulin/insulin ratio in lean MODY subjects.

In conclusion, we observed variable degrees of proinsulin levels and proinsulin/insulin ratio in MODY members of two different kindreds. The higher values of these parameters found in MODY and non-MODY members of kindred SA are probably related to the obesity and late-onset NIDDM background present in this family. The BMI was the most important independent determinant of high proinsulin levels and proinsulin/insulin ratio in these subjects.

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