BRAZILIAN INDIVIDUALS WITH IMPAIRED GLUCOSE TOLERANCE ARE CHARACTERIZED BY IMPAIRED INSULIN SECRETION

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SUMMARY - Background: To better understand the pathogenesis of type 2 diabetes mellitus, insulin secretion and insulin sensitivity (IS) were evaluated in white Brazilians with impaired glucose tolerance (IGT), using the oral glucose tolerance test (OGTT) and the hyperglycemic clamp technique.

Methods: Twenty-five IGT subjects were individually matched with normal glucose-tolerant (NGT) subjects for demographic characteristics. At first, they were submitted to the OGTT and plasma glucose and insulin were measured. Of the 25 pairs, 20 could participate in the hyperglycemic clamp procedures, at a second visit. All participants had their plasma glucose levels equally increased to 180 mg/dl; this was maintained for three hours by variable glucose infusion. During the procedure, plasma glucose and insulin were measured at established intervals.

Results: In the postabsorptive state, the IGT subjects presented higher levels of plasma glucose, blood HbA1c, and serum triglycerides, but similar plasma insulin levels. After the oral glucose load, early and total insulin release, in relation to glucose levels, were respectively, 43 and 67% lower in the IGT individuals. The index of whole-body IS was increased in IGT individuals, especially the first (82 ± 26 vs 215 ± 88 µU/ml; p < 0.05), and second (36 ± 19 vs 73 ± 44 µU/ml; p < 0.05) phases of insulin secretion was decreased in the IGT individuals, especially the first one. However, the groups did not differ in relation to the IS: IGT = 13.52 ± 7.27 and NGT = 9.96 ± 6.70 mg·ml/kg·µU·min−1; p > 0.05). Functional relationship of IS (y) on first-phase insulin release (x) showed a smaller (p < 0.05) regression coefficient for the IGT group.

Conclusion: Brazilians with IGT well-matched with NGT ones were characterized by impaired first- and second-phase insulin secretion (mainly the former), while defects in IS were not evident.

Key-words: impaired glucose tolerance, insulin secretion, insulin sensitivity, oral glucose stimulus, hyperglycemic clamp.

type 2 diabetes is characterized by two basic defects, impaired insulin secretion and insulin resistance. Although both of these defects are important, it has been controversial which is the major genetic factor [1]. In order to better understand this issue, it is important to evaluate individuals at risk of developing type 2 diabetes.

Most, if not all type 2 diabetic patients initially have impaired glucose tolerance (IGT) prior to the onset of diabetes, as shown by prospective studies [2-4]. In this stage, important metabolic defects for the development of type 2 diabetes may already be present, but without major glucose toxicity effects [5] caused by constant hyperglycemia.

In the past, several authors evaluated IGT subjects, observing either β-secretory function [6-9] or tissue insulin action [10-13] defects.

The 2-hour glucose challenge after an oral glucose load is a major predictor for the development of diabetes [4, 8]. Plasma glucose increases abnormally after glucose ingestion in individuals with IGT due to impaired suppression of endogenous glucose release [14, 15]. This defect of glucose dynamics results mainly from decreased early release of insulin [15].

Besides this β-secretory defect, people with IGT have often presented insulin resistance [7, 12, 16]. The latter, however, could be the result of acquired factors, since studies in which subjects have been carefully matched for them, such as obesity, have not found insulin resistance in IGT [6, 8, 9].

Then, to better understand the pathogenesis of type 2 diabetes this study was done. Its aim was to assess insulin secretion and tissue insulin sensitivity using the oral glucose tolerance test (OGTT) and the hyperglycemic clamp technique in IGT, white Brazilians. The white Brazilian population is characterized by a long history of miscegenation in variable proportions of the three races: Caucasian, Indian, and Black. In this population, prevalence of diabetes and IGT is 7.6 and 7.8%, respectively [17]. Group matching was carefully done as in our previous study with Caucasian people [9].

### SUBJECTS AND METHODS

After approval by the Ethical Committee of the School of Medicine, our study protocol was advertised in the hospital. Of the subjects that volunteered to participate, we selected nonathletic, healthy, white Brazilians between 20 and 70 years old, free from any medication, and with body mass index (BMI) lower than 30 kg/m². All participants received information about the study. After obtaining their written informed consent they were scheduled to the first visit.

The subjects came to the laboratory at 7:00 a.m. after a 10-12-hour fast, and having observed three days before a diet containing at least 200 g of carbohydrate/day, no alcohol, and habitual physical activities. All the participants underwent a general clinical examination, routine blood and urine analyses, and OGTT according to the National Diabetes Data Group criteria [18]. Glucose and insulin were determined from plasma samples at 0, 30, 60, 90 and 120 min.

Of the 177 individuals, 149 had normal glucose tolerance (NGT); 25, IGT; and 3, diabetes mellitus. Each IGT subject was matched for sex, age, BMI, and waist-hip ratio with a NGT subject. Plasma glucose and insulin area under the curve (G AUC; I AUC) were calculated by numerical integration using the trapezoidal rule. Insulin release was evaluated by the ratio between incremental response of plasma insulin and plasma glucose at 30 min (Δ I30/Δ G30), and during 120 min (Δ I AUC/Δ G AUC). Insulin sensitivity index (ISI) was evaluated according to the equation proposed by Matsuda and DeFronzo [19].

Of the 25 pairs of individuals, 20 could participate in the hyperglycemic clamp procedure. The five subjects excluded from each group had the same clinical and biochemical characteristics of the participants. These subjects came to the laboratory a second time having observed the same conditions as for the first evaluation. The hyperglycemic clamp experiments were performed according to our previous study [20]. We used a primed-variable intravenous infusion of glucose to produce a square wave of hyperglycemia at the level of 180 mg/dl. Blood was collected at –30.0, –15.0, 0, 2.5, 5.0, 7.5, 10.0, 15.0, and 20.0 min for plasma glucose and insulin concentrations. Blood samples for glucose and insulin measurements were subsequently obtained at 5- and 20-min intervals, respectively, for the remaining period of 180 min. We evaluated both phases of insulin secretion and insulin sensitivity. First-phase insulin release was considered to be the sum of plasma insulin concentrations at 2.5, 5.0, 7.5, and 10.0 min of the hyperglycemic clamp experiment. Second-phase insulin release was taken as the average plasma insulin concentrations during the last hour of the hyperglycemic clamp when plasma insulin concentrations were expected to plateau. Insulin sensitivity, evaluated as an ISI, was calculated by dividing the average glucose infusion rate (GIR) during the last hour of the clamp by the average plasma insulin concentration during the same interval. Under the conditions of stable hyperglycemia (3rd hour), the necessary quantity of infused glucose gives the values of tissue metabolized glucose. This values divided by the endogenous insulin response reflects tissue insulin sensitivity in NGT [20-22] and IGT [9] individuals.

Plasma glucose was determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Glycohemoglobin (HbA1c) concentration in blood was measured by microcolumn affinity chromatography (Isolab, Akron, OH). Plasma insulin was
quantified using a solid-phase radioimmunoassay (Diagnostic Products Co., Los Angeles, CA). Serum cholesterol, HDL fraction, and serum triglycerides were measured by standard automated enzymatic techniques (Technicon Instruments Co, Tarrytown, NY).

Data are presented as mean ± SD, or median and 1st-3rd quartiles, or percentual frequency. Statistical significance was determined by Student-t and Mann-Whitney tests for independent samples [23]. Associations between first- and second-phase insulin secretion and ISI in both groups were evaluated by Pearson’s coefficient [23]. Evaluation of the relationship between plasma insulin and glucose levels during OGTT, and between first-phase insulin release and ISI by hyperglycemic clamp procedures was done by linear regression [23, 24]. P values of ≤ 0.05 were considered significant.

### RESULTS

#### Clinical characteristics

The main clinical and biochemical characteristics of IGT and NGT subjects are shown in Table I. The individuals in both groups were well-matched for sex, age, BMI, and body fat distribution (waist-hip ratio). In the IGT group, there was a higher frequency of type 2 diabetes family history, being the mother more frequently affected. In the postabsorptive state the IGT individuals had higher plasma glucose levels and blood HbA1c concentrations. Though plasma insulin levels were similar in both groups. Serum triglyceride levels were also significantly higher in the IGT subjects.

### Oral Glucose Tolerance Tests

At all sampling times after oral glucose challenge, plasma glucose levels were greater in the IGT group, reaching peak level 30 min later (Fig. 1). Despite this, insulin response was delayed with lower plasma insulin levels at 30 min, and higher at 90 and 120 min in the IGT group (Fig. 1). The IGT subjects presented Δ G AUC two times greater (Table II). Despite much greater glucose stimulus, the IGT individuals showed Δ I AUC similar to that of the NGT individuals (Table II).

| Table I. Main clinical and biochemical characteristics of subjects with impaired glucose tolerance (IGT) and normal glucose tolerance (NGT). |
|----------------|----------------|----------------|
| N (F/M)         | 25 (20/5)      | 25 (20/5)      | –              |
| Age (yr)*       | 39 (36-48)     | 38 (34-45)     | NS             |
| Family history of type 2 diabetes (%) | 60              | 24              | < 0.05         |
| Caucasian race: pure/mixed (%)   | 44/56          | 40/60          | NS             |
| Smoking (%)     | 40             | 28             | NS             |
| Number of pregnancies*  | 3 (2-4)        | 3 (2-4)        | NS             |
| BMI (kg/m²)*    | 26.2 ± 3.0     | 26.2 ± 3.1     | NS             |
| Waist-hip ratio | 0.83 ± 0.06    | 0.82 ± 0.06    | NS             |
| Fasting plasma glucose (mg/dl)  | 99 ± 11        | 86 ± 9         | < 0.0001       |
| HbA1c (%)       | 6.57 ± 0.61    | 5.95 ± 0.78    | < 0.005        |
| Fasting plasma insulin (µU/ml)* | 10 (8-13)      | 9 (6-12)       | NS             |
| Cholesterol (mg/dl) | 197 ± 29      | 195 ± 47       | NS             |
| HDL – Cholesterol (mg/dl) | 40 ± 10        | 41 ± 10        | NS             |
| Triglycerides (mg/dl)        | 135 ± 48       | 107 ± 48       | < 0.05         |

* Data are expressed as median and 1st-3rd quartiles; the others as mean ± SD.
* BMI: Body mass index.
NS: non-significant. To convert the values for glucose to millimoles per liter, multiply by 0.05551; to convert the values for insulin to picomoles per liter, multiply by 7.175.
Hyperglycemic Clamp Experiments

During the last 2 hours of clamp experiments, the average plasma glucose concentrations was the same in both groups (180 ± 2 mg/dl), but with less variation in the IGT individuals than the NGT (CV = 2.2 ± 0.5% vs 3.0 ± 0.6%; p < 0.0001) (Fig. 3).

Assessment of Pancreatic β-Cell Function

OGTT

The ∆ I30/∆ G30 and the ∆ I AUC/∆ G AUC were always significantly lower in the IGT group (Table II).

Functional relationship of logarithmic transformation of plasma insulin (y) on plasma glucose or on the inverse of plasma glucose (x) (Fig 2), and ∆ I AUC (y) on ∆ G AUC (x) always showed a smaller (p < 0.05) regression coefficient (b) for the IGT group.

Hyperglycemic clamp experiments

Despite the comparable stimulus for insulin release, both first- and second-phases, as assessed by insulin concentrations, were decreased in the IGT subjects (Table III, Fig. 3). The first-phase insulin release
was more compromised than the second-phase in the IGT individuals (reduction of 61.9 and 50.7%, respectively).

### Assessment of Insulin Sensitivity

#### OGTT

The composite index of whole-body insulin sensitivity was increased in the IGT individuals (Table II).

#### Hyperglycemic clamp experiments

As expected in view of the lower plasma insulin responses, the GIR necessary to maintain the plasma glucose level of 180 mg/dl, was lower for the IGT group (Table III). However, ISI using endogenous insulin levels, was similar in both groups (Table III).

There was a significant and negative association between ISI and both first- and second-phase insulin secretion, being stronger in relation to the first-phase in the NGT than in IGT individuals (r = -0.73; p < 0.01 vs r = -0.48; p < 0.05), and similar in both groups in relation to the second-phase (r = -0.58; p < 0.01 vs r = -0.79; p < 0.01, for the NGT and IGT groups, respectively). Functional relationship of ISI (y) on first-phase insulin release (x) showed a smaller (p < 0.05) regression coefficient (b) for the IGT group (Fig. 4).

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### Table II. Total incremental glucose (\( \Delta G \) AUC) and insulin (\( \Delta I \) AUC) responses to the oral glucose tolerance test (OGTT); insulin response to glucose stimulus at 30 min (\( \Delta I30/\Delta G30 \)), and during 120 min of the OGTT; insulin sensitivity index (ISI) of the two study groups: with impaired glucose tolerance (IGT) and normal glucose tolerance (NGT).

<table>
<thead>
<tr>
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<th>IGT</th>
<th>NGT</th>
<th>P</th>
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<tbody>
<tr>
<td>( \Delta G ) AUC (mg/100 ml x 120 min)</td>
<td>8.445 (7.508-10.373)</td>
<td>4.065 (2.655-4.868)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>( \Delta I ) AUC (( \mu U/)ml x 120 min)</td>
<td>6.254 (3.329-12.040)</td>
<td>5.445 (3.650-9.080)</td>
<td>NS</td>
</tr>
<tr>
<td>( \Delta I30/\Delta G30 ) (100 ( \mu U/)mg)</td>
<td>0.64 (0.31-0.93)</td>
<td>1.12 (0.70-1.87)</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>( \Delta I ) AUC/( \Delta G ) AUC (100 ( \mu U/)mg)</td>
<td>0.58 (0.46-1.47)</td>
<td>1.74 (0.89-2.91)</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>ISI (100 ml(^2);( \mu U^{-1}).mg(^{-1}))</td>
<td>4.36 (3.46-6.88)</td>
<td>3.61 (2.08-4.64)</td>
<td>&lt; 0.05</td>
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</table>

Data are expressed as median and 1\(^{st}\)-3\(^{rd}\) quartiles; to convert the values for glucose to millimoles per liter, multiply by 0.05551; to convert the values for insulin to picomoles per liter, multiply by 7.175.
DISCUSSION

In non-diabetic individuals, pancreatic β-cell function depends mainly on the ambient stimulus and tissue insulin sensitivity. The former is predominantly the arterial plasma glucose concentration. The latter depends on several factors such as gender, obesity, antecedents, diet, and physical fitness [25]. For a comparative evaluation of insulin secretion and insulin action, it was important to have two groups of healthy individuals from the same ethnic background, similar lifestyle, and individually matched for gender, obesity, and physical activity.

To better understand the pathogenesis of type 2 diabetes, an evaluation of IGT subjects is helpful because this stage is considered precursor of diabetes when the basic metabolic defects of type 2 diabetes may already be present [26]. Moreover, they do not present a constant hyperglycemia that may compromise insulin secretion and insulin action due to glucose toxicity [5].

We studied two well-matched groups of white Brazilians, one with NGT and one with IGT. The IGT
individuals had increased frequency of type 2 diabetes family history, suggesting its strong genetic component. In the prospective study with Swedish men by Ohlson et al. [27] a positive history of diabetes increased the risk of developing diabetes in 2.4 times. Mothers and their relatives would be more involved in transmitting this type of diabetes, as also reported in previous studies [28]. This may be due to additional effect of intrauterine diabetic environment on the offspring.

The IGT individuals presented higher fasting plasma glucose, blood HbA1c, and serum triglycerides however, within the normal reference levels. Higher fasting plasma glucose and HbA1c values were also observed in other ethnic groups [8, 9]. Under postabsorptive conditions, we found that IGT subjects had comparable plasma insulin levels to those with NGT, despite the fact that, they had greater plasma glucose levels. This suggests impaired insulin secretion in the IGT individuals, as is corroborated by the results of the OGTT and hyperglycemic clamp. In longitudinal studies involving IGT Japanese [2] and non-diabetic Caucasians [27], increased glucose and triglyceride levels were some of the risk factors for the development of type 2 diabetes.

After oral glucose challenge, the IGT group showed impaired β-cell function. Under greater glucose stimulus there was decreased initial and total insulin release. The lower early insulin response was followed by a late hyperinsulinemia. Also, the dose-response curve between plasma insulin response to plasma glucose levels was deviated to the right for IGT individuals. Compromised acute response by β-cells to glucose stimulus is known as one of the first secretory defects of type 2 diabetes [26] and was also observed in previous studies with other ethnic groups, such as Caucasians [9, 12, 15], Japanese [29, 30], and Pima Indians [31] with IGT. Some studies, however, have found no differences or even higher responses in those with IGT [13], that could be due to inadequate

**TABLE III. Evaluation of insulin secretion and insulin sensitivity using hyperglycemic clamp experiments in subjects with impaired glucose tolerance (IGT) and normal glucose tolerance (NGT).**

<table>
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<tr>
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<th>IGT</th>
<th>NGT</th>
<th>P</th>
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<tbody>
<tr>
<td>First-phase insulin release (µU/ml)</td>
<td>82 (61-113)</td>
<td>215 (126-301)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Second-phase insulin release (µU/ml)</td>
<td>36 (28-65)</td>
<td>73 (33-120)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>GIR (mg/kg.min)* - 5.38 ± 1.52</td>
<td>8.84 ± 2.98</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>ISI (mg.ml/kg.µU.min)** 13.52 (7.62-22.15)</td>
<td>9.96 (7.10-20.50)</td>
<td>NS</td>
<td></td>
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</table>

* Data are expressed as mean ± SD; the others as median and 1st-3rd quartiles. * GIR = glucose infusion rate; ** ISI = insulin sensitivity index. NS = non-significant. To convert the values for glucose to millimoles per liter, multiply by 0.05551; to convert values for insulin to picomoles per liter, multiply by 7.175.

**Fig. 4.** Regression model of first-phase insulin release on insulin sensitivity index from the hyperglycemic clamp experiments in individuals with impaired glucose tolerance (IGT) and normal glucose tolerance (NGT). *: P < 0.05 for IGT vs NGT. To convert the values for insulin to picomoles per liter, multiply by 7.175.
accounting for the underlying degree of insulin resistance; more variable insulin secretory responses presented by IGT people; somewhat arbitrary classification of glucose intolerance. Moreover, it has been demonstrated that early insulin release is a major determinant for glucose tolerance by experiments using brief intravenous somatostatin to suppress that response [26, 32]. This resulted in not only glucose intolerance but also late hyperinsulinemia. Thus, late hyperinsulinemia, often invoked as an indicator of insulin resistance, may actually merely be a consequence of impaired early insulin release that leads to greater hyperglycemia and thus a greater stimulus for insulin secretion [26]. We have shown an impaired β-cell response clamp procedure, similar in IDMs sub-IGT, that points to the importance of evaluating β-cell function in relation to the plasma glucose levels, as has been demonstrated by some authors [8, 33] since the studies by Perley and Kipnis [34]. Since this is a transversal study, we cannot consider this β-cell secretory defect as a risk factor for type 2 diabetes development. However, prospective studies [2, 30] have supported this conclusion.

The impaired β-cell secretion observed with the OGTT in the IGT subjects was supported by the results of the hyperglycemic clamp experiments. Under these conditions, we observed a decrease of both phases of insulin secretion, worse for the first one, in the IGT individuals. Previous studies in other ethnic groups, using the same technique, have also found impaired insulin secretion in IGT subjects [9, 12]. Whereas first-phase insulin release has been more frequently evaluated and its defect considered one of the main metabolic characteristic of the IGT stage, second-phase insulin release has been less evaluated. When this evaluation was performed it either was decreased [6, 9], as in our study, or unaltered [7, 12].

The inverse relationship between first-phase insulin release and ISI was weaker in the IGT individuals. Moreover, using the regression model for the functional relationship between first-phase insulin release (y) and ISI (x) we observed that for each insulin sensitivity level there was a lower acute insulin response in the IGT group. This is in agreement with the results obtained by other authors [32].

The composite index of whole-body insulin sensitivity obtained from OGTT and ISI, from the hyperglycemic clamp procedures indicated that the IGT subjects did not present insulin resistance. Also, in previous and similar study with Caucasian people, we did not observe insulin resistance in the IGT group by the two glycemic clamps [9].

The results of our study indicate that, when acquired causes of insulin resistance (i.e. age, physical activity, obesity) are controlled for, impaired β-cell secretory is present but not insulin resistance in subjects with IGT. This supports that impaired β-cell function may be the primary genetic defect leading to IGT. Previous prospective studies suggested that a decreased insulin response to glucose is the main risk factor for the development of type 2 diabetes in IGT individuals [2, 30].

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REFERENCES


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