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Insulin secretion, insulin sensitivity, and hepatic insulin extraction in first-degree relatives of type 2 diabetic patients

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Abstract

To identify early metabolic abnormalities in type 2 diabetes mellitus, we measured insulin secretion, sensitivity to insulin, and hepatic insulin extraction in 48 healthy normal glucose-tolerant Brazilians, first-degree relatives of type 2 diabetic patients (FH+). Each individual was matched for sex, age, weight, and body fat distribution with a person without history of type 2 diabetes (FH-). Both groups were submitted to a hyperglycemic clamp procedure (180 mg/dl). Insulin release was evaluated in its two phases. The first was calculated as the sum of plasma insulin at 2.5, 5.0, 7.5, and 10.0 min after the beginning of glucose infusion, and the second as the mean plasma insulin level in the third hour of the clamp procedure. Insulin sensitivity index (ISI) was the mean glucose infusion rate in the third hour of the clamp experiment divided by the mean plasma insulin concentration during the same period of time. Hepatic insulin extraction was determined under fasting conditions and in the third hour of the clamp procedure as the ratio between C-peptide and plasma insulin levels. FH+ individuals did not differ from FH- individuals in terms of the following parameters [median (range)]: a) first-phase insulin secretion, 174 (116-221) *vs* 207 (108-277) μU/ml, b) second-phase insulin secretion, 64 (41-86) *vs* 53 (37-83) μU/ml, and c) ISI, 14.8 (9.0-20.8) *vs* 16.8 (9.0-27.0) mg kg^{-1} min^{-1}/μU ml^{-1}. Hepatic insulin extraction in FH+ subjects was similar to that of FH- ones at basal conditions (median, 0.27 *vs* 0.27 ng/μU) and during glucose infusion (0.15 *vs* 0.15 ng/μU). Normal glucose-tolerant Brazilian FH+ individuals well-matched with FH- ones did not show defects of insulin secretion, insulin sensitivity, or hepatic insulin extraction as tested by hyperglycemic clamp procedures.

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• Type 2 diabetes
• Insulin secretion
• Insulin sensitivity

Introduction

Type 2 diabetes mellitus is a metabolic syndrome which is relatively common in most countries including Brazil (1,2), and is often the cause of severe micro- and macrovascular complications (3). Despite decades of research, its pathogenesis is poorly understood. It is generally agreed that it is a polygenic disorder characterized by varying degrees of impaired insulin secretion and insulin resistance (4,5), both of which can be affected by environmental and genetic factors (6,7). What remains controversial is

which of these abnormalities is the major genetic factor. One approach has been to determine which factor is first detectable in individuals genetically predisposed to develop type 2 diabetes. To do this it is necessary to study individuals with normal glucose tolerance to avoid secondary effects of glucotoxicity on insulin secretion and insulin sensitivity (8). However, at this stage, it is not possible to be sure who is a true prediabetic. Moreover, in these studies it is important to properly match experimental and control groups for acquired variables (e.g., obesity, age, physical fitness) which affect ß-cell function and insulin sensitivity (9). Finally, the findings for a specific ethnic group, such as the Pima Indians, the Nauruans, the Mexican- or African-Americans may be not valid for other type 2 diabetic patients.

In a previous study of European normal glucose-tolerant individuals who were first-degree relatives of type 2 diabetic patients and well-matched with the control group with no family history of diabetes we used the hyper- and euglycemic clamp techniques to assess insulin secretion and insulin sensitivity (10). We found that individuals with a first-degree relative with type 2 diabetes had impaired insulin secretion and were not insulin resistant. These findings were later supported by van Haeften et al. (11) in a similar study, while others observed decreased insulin sensitivity and apparently normal ß-cell function (12,13). However, in the study by Eriksson et al. (12), the subjects were not well-matched, and some of the participants in the study by Gulli et al. (13) who were Mexican-Americans probably had impaired glucose tolerance. With a less sensitive technique, i.e., the acute glucose infusion test associated with mathematical models, the results have also been controversial; ß-cell dysfunction (14), decreased insulin sensitivity (15), or no defect (16) has been observed. It should be noted, however, that in the study by Warram et al. (15) probands were markedly obese compared to the controls and none of these studies evaluated the appropriateness of ß-cell function in relation to insulin sensitivity (17). Johnston et al. (18) only observed decreased first-degree insulin release in offspring of type 2 diabetic patients when this variable was adjusted for their degree of insulin sensitivity.

Our aim was to evaluate insulin secretion and insulin sensitivity in Brazilian glucose-tolerant first-degree relatives of type 2 diabetic patients. The Brazilian population is characterized by a long history of miscegenation, in variable proportions, of European, Black, and Indian ancestries (19), the latter two having an increased risk to develop type 2 diabetes (20). We used the hyperglycemic clamp technique. Each subject was carefully matched for age, sex, weight, body fat distribution, smoking history, and physical activity.

Subjects and Methods

Moderately active white Brazilians without a history of alcoholism, drug use or chronic diseases were admitted to the study. Each subject gave informed consent to participate in the study, which was approved by the Medical Ethics Committee of our Institution. The first visit was then scheduled when each subject was submitted to general clinical and laboratory evaluation and to the oral glucose tolerance test according to the National Diabetes Data Group criteria (21). We selected 56 subjects with (FH+) and 56 without (FH-) type 2 diabetic first-degree relatives. All were healthy and normal glucose-tolerant individuals at the time of evaluation. The two groups were individually matched for sex, age, body mass index, and waist-hip ratio.

Forty-eight pairs of individuals from the initial 56 ones were able to participate in the second evaluation after about 15 days. The eight pairs who were excluded because of personal or technical problems were not different from the participants in relation to the clinical and biochemical characteristics and to glucose tolerance. At this second visit

they underwent the hyperglycemic clamp study as described (10). Briefly, each volunteer came to the laboratory at 7:00 am after an overnight fast. A cannula was retrogradely inserted into a peripheral hand vein and kept patent by constant saline infusion. The hand was kept warm for blood arterialization. Blood samples were obtained from the hand vein every 15 min for half an hour under basal conditions and during glucose infusion, every 2.5 min for the first 10 min, and then every 5.0 min up to 180 min. Another cannula was inserted into an antecubital vein of the opposite arm for glucose infusion. For this infusion, we used a pump (Harvard Apparatus Co., Southnatick, MA, USA) beginning with the bolus dose (in ml = 2 {[weight (kg) x 1.5 x (180 mg/dl - basal plasma glucose (mg/dl))]/10^3}) which was followed by a variable velocity of glucose infusion depending on the plasma glucose level. This was done to obtain and maintain this level at 180 mg/dl. Glucose was measured in all blood samples; insulin and C-peptide were also measured at the same time as glucose under basal conditions and during the first 20 min, and then every 20 min.

Plasma glucose was determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA, USA). Glycosylated hemoglobin (HbA_1) was measured by affinity chromatography (Isolab, Akron, OH, USA). Plasma insulin and C-peptide were determined using the solid phase and the double antibody radioimmunoassay techniques, respectively (Diagnostic Products Co., Los Angeles, CA, USA). Serum cholesterol, its HDL fraction, and triglycerides were measured by standard automated enzymatic techniques (Technicon Instruments Co., Tarrytown, NY, USA).

The phases of insulin secretion were evaluated as follows: the first-phase insulin release was taken to be the sum of plasma insulin concentrations at 2.5, 5.0, 7.5, and 10.0 min of the hyperglycemic clamp experiment (10), and the second-phase insulin release was reported as the average plasma insulin concentration during the last hour of the hyperglycemic clamp, when plasma insulin concentrations were expected to plateau (10). Insulin sensitivity was assessed as insulin sensitivity index (ISI) and was calculated by dividing the average glucose infusion rate (GIR) during the last hour of the clamp, minus the occasional glucose urinary excretion, by the average plasma insulin concentration during the same interval (10). Under stable conditions of constant hyperglycemia (third hour of the clamp), the amount of glucose infused (GIR) gives an estimate of the glucose that is metabolized by the tissues since endogenous glucose production should be suppressed. This value divided by the plasma insulin response (second-phase insulin secretion) provides an estimate of tissue sensitivity (ISI) to endogenously secreted insulin (10) and has been shown to correlate with values for insulin sensitivity obtained in euglycemic/hyperinsulinemic clamp experiments (10,22).

Hepatic insulin extraction (HIE) under basal conditions was calculated as the ratio between mean basal plasma C-peptide and insulin (three determinations), and during glucose infusion, as the ratio between mean plasma C-peptide and insulin during the third hour of the hyperglycemic clamp experiment (23).

Data are reported as either the mean ± SDM or the median and 1st and 3rd quartiles or the percent frequency. The unpaired Student *t*-test was used to compare means, the Mann-Whitney test to compare medians, and the chi-square test to compare frequencies (24). Correlations were performed using linear regression (24). A P value equal to or less than 0.05 was considered to be statistically significant.

Results

The main clinical and biochemical characteristics of the two groups are shown in

Table 1. Clinical characteristics of individuals with (FH+) and without (FH-) a first-degree relative with type 2 diabetes.

	FH+	FH–
N (females/males)	56 (46/10)	56 (46/10)
Age (years)	35 (29-40)	34 (30-40)
Ancestry: pure/mixed Caucasian (%)	36.0/64.0	37.5/62.5
Pregnancies	2 (2-3)	3 (2-4)
Smoking (%)	28.6	21.4
BMI (kg/m^2)	26.1 ± 3.3	25.7 ± 3.1
Waist-hip ratio	0.81 ± 0.06	0.81 ± 0.06
Fasting plasma glucose (mg/dl)	84 ± 8	85 ± 9
HbA_1 (%)	6.10 ± 0.61	5.93 ± 0.87
Fasting plasma insulin (µU/ml)	9 (6-13)	8 (6-12)
Fasting plasma C-peptide (ng/ml)	2.1 (1.8-2.6)	2.0 (1.5-2.4)
Cholesterol (mg/dl)	179 ± 36	177 ± 32
HDL cholesterol (mg/dl)	39 ± 9	39 ± 10
Triglycerides (mg/dl)	96 ± 42	94 ± 40
Fasting HIE (ng/µU)	0.269 (0.202-0.346)	0.272 (0.154-0.372)

Data are reported as medians (1st-3rd quartiles) or as means ± SDM. BMI: body mass index; HIE: hepatic insulin extraction. No significant differences were observed between the two groups (Student *t*-test, Mann-Whitney test or chi-square test).

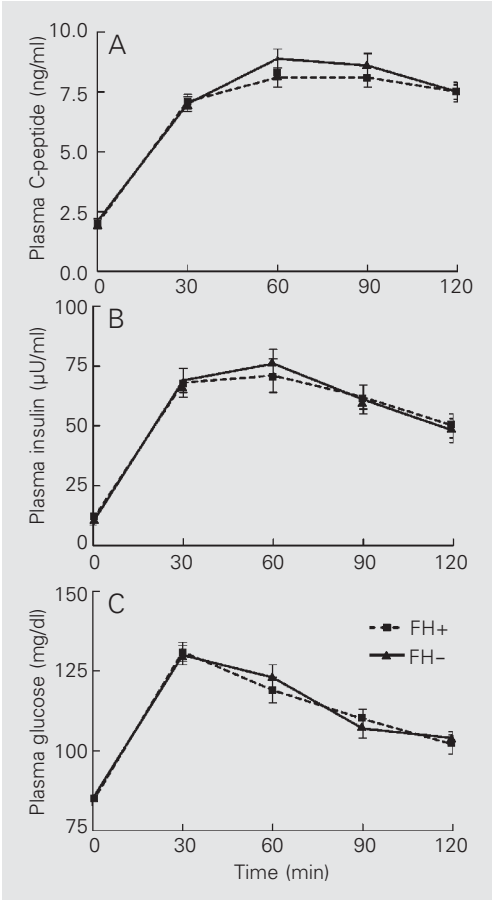

Figure 1. Plasma C-peptide (A), plasma insulin (B), and plasma glucose (C) responses during oral glucose tolerance tests of individuals with (FH+) and without (FH-) type 2 diabetic first-degree relatives. Data are reported as means ± SEM for 56 individuals in each group.

Table 1. Both groups were well-matched for sex, age, weight, and body fat distribution. Also they did not differ in terms of ancestry, number of pregnancies, or smoking habit.

In the FH+ group the mother alone was diabetic or other first-degree relatives were also diabetic in 50 and 66% of the cases, respectively, with the mother being the family member most frequently affected (P<0.01).

Prior to the clamp, plasma glucose, insulin and C-peptide levels, serum lipids, and HIE were comparable in both groups (Table 1). Moreover, both groups showed equal normal glucose tolerance as assessed by HbA_1 level (Table 1) and by plasma glucose concentrations after 75-g oral glucose, and presented similar plasma insulin and C-peptide responses during the oral glucose tolerance test (Figure 1). The linear regression coefficients of plasma insulin on plasma glucose with the oral glucose stimulus did not differ between FH+ and FH- individuals (P>0.05).

During the hyperglycemic clamp, mean plasma glucose concentrations were 179 ± 2 mg/dl (CV: 2.8 ± 0.9%) and 179 ± 2 mg/dl (CV: 2.7 ± 1.0%) (P>0.05) in the FH+ and FH- groups, respectively (Figure 2). In both groups, a biphasic plasma insulin response was observed (Figure 2). As shown in Table 2, first- and second-phase insulin secretion were comparable in the two groups. The average GIR necessary to maintain plasma glucose levels at 180 mg/dl during the last hour of the hyperglycemic clamp experiment was also not different between the two groups (Table 2). Consequently, ISI were similar in FH+ and FH- individuals (Table 2). During the third hour of glucose infusion, HIE showed a similar reduction from its basal value and reached a similar value in both groups (Table 2).

In the two study groups there was a similar and significant inverse relation between ISI and body mass index, first- and second-phase insulin secretion (r = -0.41, -0.42, and -0.59 *vs* r = -0.47, -0.57, and -0.66, for FH+ and FH- individuals, respectively; P<0.01).

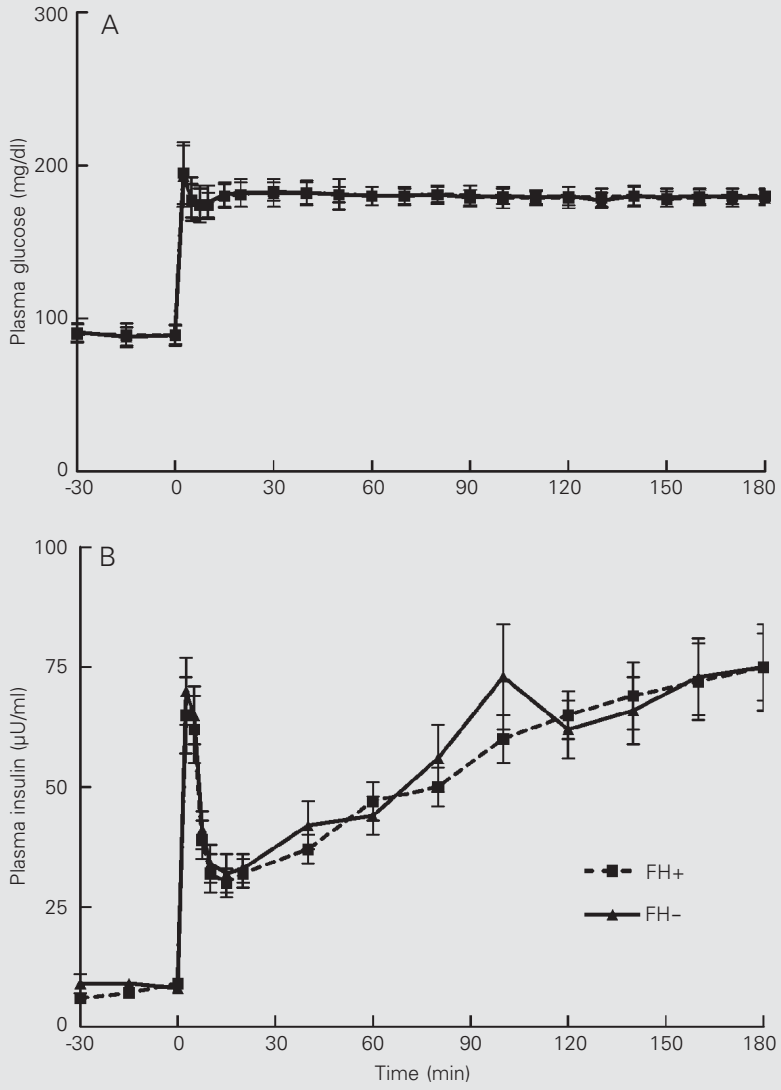

Figure 2. Plasma glucose (A) and plasma insulin (B) concentrations during hyperglycemic clamp experiments. FH+ and FH– indicate individuals with and without first-degree relatives with type 2 diabetes, respectively. Data are reported as means ± SEM for 48 individuals in each group.

Discussion

A comparative evaluation of ß-cell function, ISI, and HIE was performed between two groups of similar individuals but with (FH+) or without (FH-) first-degree relatives with type 2 diabetes. Both groups showed normal glucose tolerance and were well-matched for the main demographic characteristics. Under these conditions, the finding of a defect in any of the three evaluated variables could be considered to be genetically determined.

The fact that the mother was the most frequent relative affected by type 2 diabetes in the FH+ group, although possibly influenced by confounding factors and deserving more investigation, agrees with other studies with type 2 diabetic patients (25).

The ß-cell response to the oral glucose challenge of the FH+ group was similar to that of the FH- group. This finding was previously observed by us (10) and by others (12,14,26,27), whereas a decreased (28-30) or increased (13) insulin response to oral glucose has been less frequently reported. These divergent results in relation to ours may be due to the different ethnic groups studied (13) or to a small and specific group of prediabetic individuals that may have included future type 1 diabetic patients (28-30).

Under similar conditions of ß-cell stimulation, the two groups were evaluated for insulin release and tissue sensitivity to insulin. The first and second phases of insulin secretion and the ISI did not differ between groups. Previous studies using hyperglycemic clamps in first-degree relatives of type 2 diabetic patients obtained the following results: unimpaired insulin secretion (12,18),

Table 2. Hyperglycemic clamp measurements of insulin secretion, insulin sensitivity, and hepatic insulin extraction of individuals with (FH+) and without (FH–) a first-degree relative with type 2 diabetes.

	FH+	FH–
First-phase insulin release (µU/ml)	174 (116-221)	207 (108-277)
Second-phase insulin release (µU/ml)	64 (41-86)	53 (37-83)
GIR (mg kg^{-1} min^{-1})	8.70 ± 3.03	8.94 ± 2.75
ISI (mg kg^{-1} min^{-1}/µU ml^{-1})	14.8 (9.0-20.8)	16.8 (9.0-27.0)
3rd-hour HIE (ng/µU)	0.151 (0.108-0.215)	0.151 (0.115-0.255)

Data are reported as means ± SEM, or medians (1st-3rd quartiles) for 48 individuals in each group. GIR: glucose infusion rate; ISI: insulin sensitivity index; HIE: hepatic insulin extraction. No significant differences were observed between the two groups (Student *t*-test or Mann-Whitney test).

decreased insulin secretion (10,11) or, in some cases, increased insulin secretion (13).

Many studies have evaluated insulin release by an intravenous glucose stimulus in offspring of type 2 diabetic patients who showed normal glucose tolerance. Although most of these studies found lower insulin secretion (14,31,32), many authors did not observe any difference in relation to control individuals (16,33,34), and in some cases increased insulin release was reported (15,35).

The reason the results of the present study differ from those of our previous one (10) may be due to the participation of different ethnic groups (36 *vs* 100% European ancestry, respectively), since type 2 diabetes inheritance is heterogenous. On the other hand, increased insulin release as a response to insulin resistance was only observed when the matching for weight was not good (15,35), or when the individuals come from ethnic groups characterized by insulin resistance, such as Mexican-Americans (13) and African-Americans (35).

Our results may be due to the fact that most first-degree relatives of type 2 diabetics with normal glucose tolerance, even when both parents are diabetic, would not show an insulin release defect, or this defect could not be detectable by the best techniques available, as proposed by Johnston et al. (18). Another reason may be that the distinction between the FH+ and FH- groups based on the presence or absence of type 2 diabetic first-degree relatives may not be sufficient to obtain two groups with a significantly different concentration of diabetes genes, even with a large number of individuals. Also, most of the volunteers of this study were 40 years old or younger and had a body mass index lower than 26.8 kg/m^2 (36), thus possibly having fewer effects of acquired factors that facilitate the expression of an insulin secretion defect (5).

We should point out that in this study and in previous ones, insulin was measured by radioimmunoassay using antibodies that significantly cross-reacted with proinsulin and its intermediates, causing an overestimation of true insulin release. For this reason, we measured plasma C-peptide during the hyperglycemic clamp procedures, a procedure that permitted us to evaluate the real second-phase insulin release. Both groups showed similar secretion (data not shown). However, the FH+ group may really present decreased first-phase insulin release since this is one of the initial defects of diabetes together with a disproportionate proinsulin release (37).

More definitive results about ß-cell function and insulin sensitivity before the development of type 2 diabetes were obtained from studies of discordant identical twins. Among these, Vaag et al. (38) performed clamp experiments during the stage of normal glucose tolerance and observed decreased first-phase insulin secretion with no change in insulin sensitivity. These results partially agree with ours and suggest that we may not have observed a ß-cell secretion defect because we did not have two significantly different groups with respect to the number of diabetes genes.

The similar insulin release displayed by the FH+ and FH- groups was not due to HIE differences under basal conditions or during glucose infusion. First-degree relatives of type 2 diabetic patients from southern Italy (39) and northern Europe (40) presented hyperinsulinemia due to decreased insulin clearance, a result which is not in agreement with our findings.

As already established, we observed a similar inverse relationship between body weight and insulin sensitivity and between insulin sensitivity and insulin secretion in both phases in the two study groups. These findings may be explained by the fact that the subjects were well-matched, and/or by the fact that the associations between the variables are not determined by genetic diabetes factors. Similar results were observed by Byrne et al. (16) for ISI and body mass index, and by Vaag et al. (38) for ISI and

first-phase insulin secretion.

Normal glucose-tolerant, white Brazilian first-degree relatives of type 2 diabetic patients did not show defects of ß-cell secretory function, ISI, or HIE as tested by hyperglycemic clamp procedures.

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