Acute Insulin Response to Glucose and Glucagon in Subjects at Risk of Developing Type I Diabetes

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OBJECTIVE — To determine if knowledge of characteristics of insulin response to various secretagogues during the preclinical phase of type I diabetes may facilitate the diagnosis of subjects at risk.

RESEARCH DESIGN AND METHODS — A test consisting of sequential intravenous challenge with glucose (0.3 g/kg) and glucagon (1 mg, 10 min after the end of glucose injection) was performed on 171 ICA^- relatives of type I diabetic patients, 18 ICA^+ relatives of type I diabetic patients, and 5 transiently hyperglycemic subjects. Acute response to glucose was expressed as the sum of plasma insulin at 2 and 5 min and response to glucagon as the increase in plasma insulin after 10 min.

RESULTS — Responses below the lower 95% confidence interval in the ICA^- population (40 and 43 μU/ml for glucose and glucagon, respectively) were considered abnormal. The two values were correlated ($r = 0.62$). Abnormalities coexisted in 2.3% of the ICA^- group, 11% of the ICA^+ group, and 100% of the transiently hyperglycemic group. All the relatives who subsequently developed diabetes or hyperglycemic subjects who required insulin exhibited combined abnormalities. Some ICA^- and ICA^+ relatives were tested repeatedly over a follow-up period of 1.5–4 yr. Although the intraindividual coefficient of variation for the two responses was high (28 and 30%), values tended to run parallel in both ICA^+ and ICA^- relatives. In 2 patients monitored for 2 and 4 yr before diabetes developed, both responses declined at the same rate. In terms of prediction of diabetes, sensitivity of combined abnormalities was high (100%). But compared with the intravenous glucose tolerance test, improvement of specificity by the double challenge was not statistically significant.

CONCLUSIONS — Both insulin responses to glucose and glucagon are related. They depend on the secretory capacity of β-cells and simultaneously become abnormal in the prediabetic phase.

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TYPE I DIABETES, INSULIN-DEPENDENT DIABETES MELLITUS; ICA, ISLET CELL ANTIBODY; AIR, ACUTE INSULIN RESPONSE; CI, CONFIDENCE INTERVAL; IVGTT, INTRAVENOUS GLUCOSE TOLERANCE TEST; CV, COEFFICIENT OF VARIATION; JDF U, JUVENILE DIABETES FOUNDATION UNIT; ANOVA, ANALYSIS OF VARIANCE; FPG, FASTING PLASMA GLUCOSE.

Epidemiological studies have demonstrated that type I diabetes is the result of a subclinical phase characterized by autoimmune destruction of insulin-producing cells (1). It has been proposed that screening of future diabetic individuals could be facilitated by associating these immunological markers with IVGTT to detect a low AIR to glucose (2). Although the sensitivity of this abnormality for screening pre–type I diabetes has been judged adequate (2–6), its specificity (4,9) and reproducibility (4,7–9) have been questioned. Moreover, it has never been demonstrated irrefutably that β-cells' insensitivity is limited to glucose alone in the latent phase of diabetes (10–12). In an attempt to deal with these problems, we designed a test measuring the response of β-cells to two stimuli, i.e., a physiological stimulus, glucose, followed in close succession by a pharmacological stimulus, glucagon. The test was performed in a high-risk population composed of first-degree relatives of type I diabetic patients and transiently hyperglycemic subjects. The evolution of these two parameters in the months or years before overt diabetes was compared.

RESEARCH DESIGN AND METHODS

— First-degree relatives of type I diabetic patients (80 offspring and 109 siblings, mean age 14.4 ± 6.7 yr) were recruited. Six were ICA^+ on at least three occasions, and 12 were either tested for being ICA^+ only once or twice or exhibited fluctuating ICA. The others were ICA^-. This distribution was not representative of the prevalence of ICA^+ in our overall population (5.86%). The follow-up of the ICA^- group was ≥4 yr in 90 subjects, 3 yr in 60 subjects, and 2 yr in 28 subjects (in the ICA^+ group, 14, 2, and 2 subjects, respectively). One permanently ICA^+ subject became diabetic 4 yr after inclusion, and 1 ICA^- subject developed transient postmeal hyperglycemia 2.5 yr after entry and typical type I diabetes 2 yr later. In addition, 5 ICA^+

subjects with transient hyperglycemia were studied. Four became insulin dependent during follow-up.

Testing

Testing was performed on patients after an overnight fasting period. Every subject who reported an insufficient diet during the previous day, an infectious episode, or drugs interfering with carbohydrate metabolism was excluded. Glucose (0.3 g/kg, 30% glucose solution) was infused intravenously over a period of 2 min. Time 0 was the end of glucose infusion. Glucagon (1 mg, Novo Laboratories, Denmark) was intravenously injected 10 min later. Blood samples were collected before and 2, 5, 10, 15, 20, and 25 min after the end of glucose infusion. The glucose dose was lower and sample timing was different from that currently used in the literature (2–6) or recommended by the Icarus project. But we have previously demonstrated the good sensitivity of this investigation for the diagnosis of pre–type I diabetes (4). Insulin concentration was determined by radioimmunoassay (CIS Bio Industry, Gif-sur-Yvette, France). The intra- and interassay CVs were 5 and 8% for low values and 4 and 6% for high values, respectively. AIR to glucose was calculated as the sum of plasma insulin measured at 2 and 5 min (4). Insulin response to glucagon was expressed as the difference between the highest insulin value obtained after glucagon and the previous value at 10 min. Glucagon injection induced minor side effects (mainly nausea) in 20.1% of the population of relatives. Only 3 subjects vomited. The duration of the discomfort was 2–5 min. Cytoplasmic ICAs were detected by indirect immunofluorescence on a frozen section of human pancreas. The detection threshold of ICA was 10 JDF U.

Statistical analysis

Descriptive statistics were performed with the Statgraphics Statistical Graphics System. Means ± SD and CIs were established from log-transformed data.

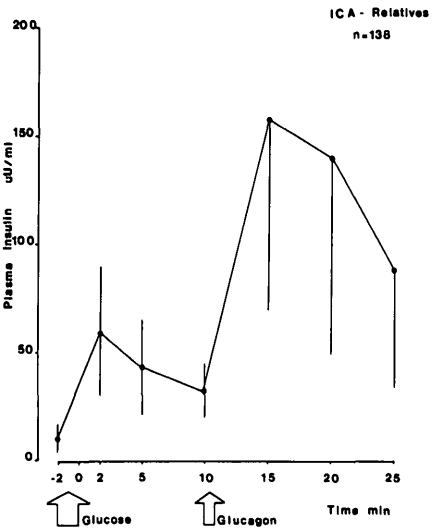

Figure 1—*Insulin response to glucose and glucagon in an ICA⁻ relative population that did not develop diabetes 3.5 yr later (n = 138). Glucose (0.3 g/kg) was infused in <2 min. Glucagon (1 mg) was administered intravenously 10 min later. Data are means ± SD.*

Comparisons between groups were performed by using ANOVA and Student's *t* test after log transformation.

RESULTS — Insulin responses to glucose and glucagon in ICA⁻ relatives are shown in Fig. 1. Insulin response to glucagon was higher than to glucose. Interindividual variations were also greater after glucagon than glucose. After analysis of the distribution of insulin responses to both secretagogues, the best fit was with a log-normal distribution. FPG was in the normal range (4.9 ± 0.34 mM). In contrast, plasma glucose before glucagon injection was elevated (10.3 ± 1.19 mM). During the second phase of the test, β-cells were submitted to two stimuli, i.e., mild hyperglycemia and glucagon. No correlation was seen between plasma glucose at 10 min and insulin response to glucagon ($r = 0.04$ ns). A subgroup of 138 permanently ICA⁻ relatives who did not develop diabetes during the follow-up (>3.5 yr) was used to define the mean and the lower 95 and 99% CIs for both responses. The mean AIR to glucose was 92 μU/ml, the lower 95% CI was 40 μU/ml, and the lower 99% CI was 28 μU/ml. The values for insulin response to glucagon were 113, 43, and 27 μU/ml, respectively. Values below lower 95% CI were defined as abnormal. The statistical correlation between both responses was highly significant ($r = 0.612$, $P < 10^{-7}$). Insulin response to both secretagogues tended to be lower in ICA⁺ subjects, but the difference did not reach a statistically significant level (response to glucose, 84.4 [46–156] vs. 92.3 μU/ml [55.6–153 μU/ml]); response to glucagon, 94.7 [60–149] vs. 113 μU/ml [63–208 μU/ml]).

In 5 patients with transient hyperglycemia, FPG was 5.25 ± 0.82 mM and preglucagon plasma glucose was 11.9 ± 1.78 mM. Insulin responses to glucose and glucagon were low (glucose, 22.6 ± 7.23 μU/ml; glucagon, 18.6 ± 9.6 μU/ml).

The prevalence of abnormal responses in each group is shown in Table 1. In the ICA⁻ group, the prevalence of combined abnormalities (2.3%) was half that of abnormal response to each secretagogue analyzed separately. Indeed, in the whole group, abnormal response to glucose was observed in 5.9% (10 of 171 subjects) and to glucagon in 4.1% (7 of 171 subjects) of cases. In ICA⁺ relatives, the prevalence of combined abnormalities was higher, i.e., 17% in the subgroup with authenticated persisting ICA. Combined abnormalities were present in all transient hyperglycemic subjects. Furthermore, all relatives who developed diabetes and all transiently hyperglycemic subjects who became insulin dependent exhibited low insulin responses for both stimuli. In 4 relatives who did not develop diabetes during the follow-up, combined abnormalities were found in only one. Three of them, who were repeatedly tested, had recovered normal insulin response to glucose and glucagon. A glucose tolerance test was not performed in these subjects. Specificity

Table 1—*Frequency of isolated and combined abnormal insulin response to glucose and glucagon in the four groups studied. The persisting ICA$^+$ group is a subgroup of ICA$^+$ population and includes the 6 patients found to be ICA$^+$ on at least three occasions*

	ICA$^-$ RELATIVES	ICA$^+$ RELATIVES	PERSISTING ICA$^+$ RELATIVES	TRANSIENTLY HYPERGLYCEMIC PATIENTS
N	171	18	6	5
ISOLATED ABNORMAL RESPONSE TO GLUCOSE (*N*)	6	3	1	0
ISOLATED ABNORMAL RESPONSE TO GLUCAGON (*N*)	3	2	0	0
COMBINED ABNORMALITIES, *N* (%)	4 (2.3)*	2 (11.1)†	1 (16.7)†	5 (100)‡

*One developed type I diabetes.
†One persisting ICA$^+$ subject developed type I diabetes.
‡Four subjects developed type I diabetes.

of combined abnormalities was higher than that of insulin response to glucose alone (183 of 187 [97.8] vs. 174 of 197 [93%] subjects, but the difference was not significant [χ^2, 3.22]).

In ICA$^-$ relatives, 31 subjects underwent at least three functional tests during follow-up, lasting 1.5–4 yr (3.6 tests/patient, range 3–6). The CVs of insulin responses to glucose and glucagon were 28 ± 11.1 and 32.3 ± 16.1%, respectively. Figure 2 shows the course of the insulin responses in 6 subjects at high risk for diabetes. The ICA$^-$ subject and 1 ICA$^+$ subject developed diabetes. In these 6 subjects the evolution of insulin responses to glucose and glucagon were also parallel with time. In the 2 who developed type I diabetes, the two responses declined at the same rate. Similarly, in 3 subjects with transient hyperglycemia monitored under the same conditions, a parallel course of insulin responses was observed (results not shown). In our population of prediabetic subjects, the abnormality of insulin response to glucose did not seem to precede the alteration of response to glucagon. To evaluate the relationship between the two insulin responses more precisely, variations of the responses from one test to another were studied in both the 31 ICA$^-$ repeatedly tested relatives and 18 ICA$^+$ relatives. Both the responses decreased or increased in parallel from one test to another in the same subject on 49 occasions in the ICA$^-$ group (70%) and 22 occasions in the ICA$^+$ group (68.8%). The experimental data were significantly different from the theoretical distribution (χ^2, 37.3, $P < 5.10^{-4}$; and χ^2, 14.4, $P < 5.10^{-4}$, respectively). In addition to the qualitative evaluation, a quantitative relationship was found between the variation of each response from one test to another ($r = 0.48$, $P < 0.01$).

CONCLUSIONS— Ganda et al. (10) reported that although acute insulin response to glucose was specifically abolished in patients with transient hyperglycemia and during the honeymoon period, β-cells still responded to other secretagogues such as glucagon and arginine. With a different approach, we did not observe that the loss of insulin response to glucose precedes the abnormality of insulin response to glucagon. In fact, several of our findings strongly suggest that despite differences in amplitude, a relationship exists between these two responses. In the overall population, a highly significant correlation was noted between these two parameters. More importantly, in subjects at high risk for diabetes the synchronization between these two parameters was so close that it can be speculated that the responses depend on the same functional state of β-cells at a given time. Measurement of two responses over a period of only 30 min could result in interferences between the stimuli. Hyperglycemia, which persits until the 10th min of testing, could continue to stimulate insulin secretion. It also could cause glucose potentiation, as has been noted with numerous insulin secretagogues, including isoproterenol and arginine (13). In our population, we observed no correlation between plasma glucose at 10 min and insulin response to glucagon. The range of plasma glucose values at this point in the test agreed with the interval of acceptable baseline plasma glucose proposed by Masbad et al. (14) for the evaluation of insulin secretion in type I diabetes using the glucagon test. These two findings suggest that the correlation observed between the two responses is not an artifact attributable to the test design but rather corresponds to the same secretory response mechanism in β-cells activated by different stimuli. Identical results have been reported by Heaton et al. (11) in twins and by Bardet (12) in relatives of type I diabetic patients with tests performed on different days. In our 3 cases in which surveillance before the development of diabetes lasted 2–4 yr, the two responses decreased at the same rate and became patently abnormal 1–2 yr before the onset of overt diabetes. These observations are not in contradiction with those of Ganda et al. (10), who reported that although the insulin response was higher to glucagon than glucose in 4 prediabetic subjects, it was nevertheless overtly lower than the 5th percentile of the control population. Thus it appears that the difference in response to the two secretagogues is more quantitative than qualitative. The two responses became simultaneously abnormal at the end of the latent phase of type I diabetes. In terms of diagnosis of pre–type I diabetes, combined abnormalities seem to be a very sensitive marker. But some subjects

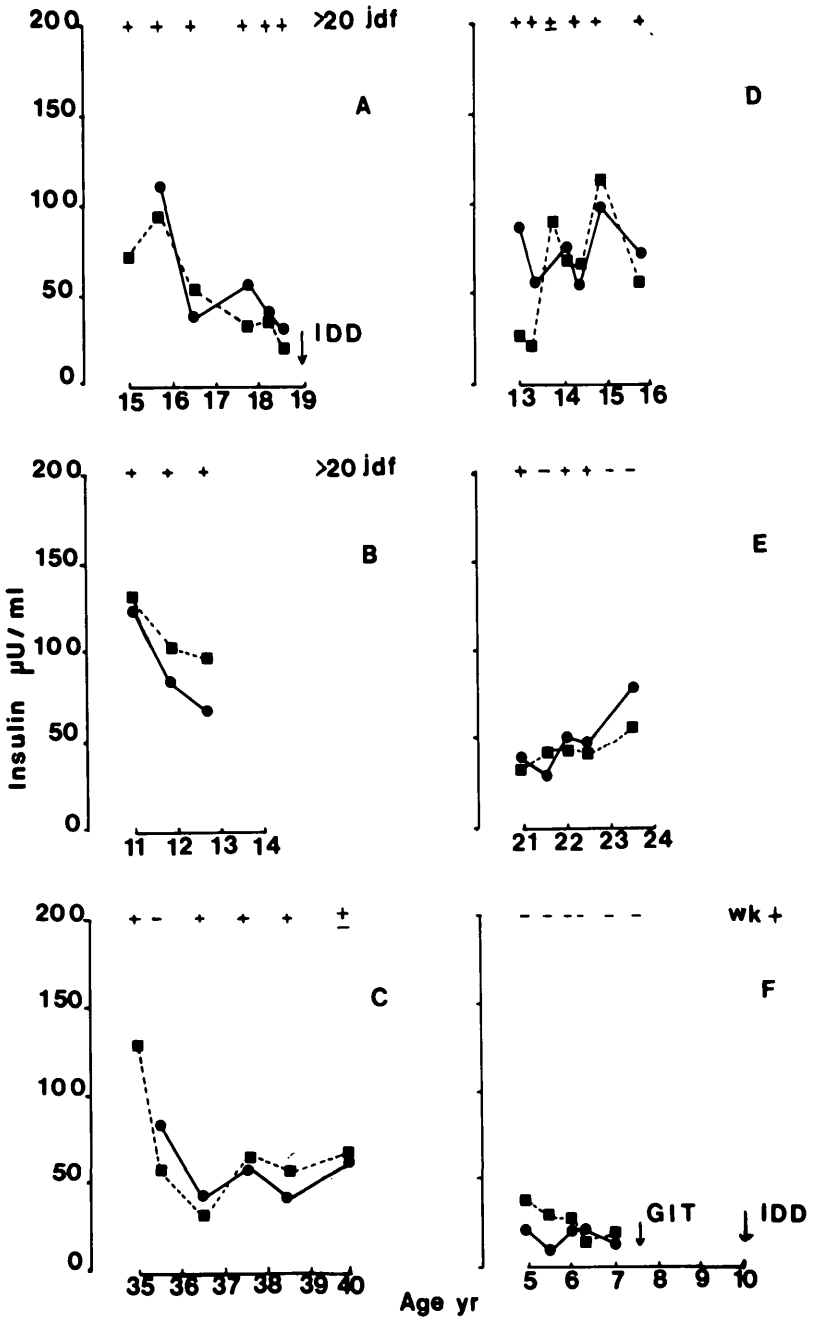

Figure 2—*Insulin response to glucose (■– – –■) and glucagon (●——●) in 6 relatives at risk of diabetes because of persistent ICA (titer >20 JDF U in subjects A and B) or occurrence of diabetes.*

can exhibit transitory abnormal responses without developing diabetes in the subsequent months. Despite a reduction by 3 of false-positive rate, no statistically significant improvement of the specificity of this test was observed compared with IVGTT alone.

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