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Rapid communications

Increased proinsulin levels as an early indicator of B-cell dysfunction in non-diabetic twins of Type 1 (insulin-dependent) diabetic patients

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Summary. Glucose tolerance and insulin secretion were studied in two groups of non-diabetic identical twins of recently-diagnosed Type 1 (insulin-dependent) diabetic patients: (1) a group of 5 twins with islet cell antibodies, and (2) a group of 6 twins without. Despite similar fasting glucose, insulin and C-peptide concentrations both groups of twins had significantly higher fasting proinsulin concentrations than the control group ($p < 0.05$). The twins with complement-fixing islet cell antibodies had reduced glucose tolerance and clearance, whilst the twins without islet cell antibodies did not. Neither group of twins showed any abnormality in insulin, C-peptide or proinsulin response to oral or intravenous glucose. We conclude that increased fasting proinsulin levels precede abnormalities of insulin secretion, and are an early indication of minor B-cell damage in these twins irrespective of their risk of developing diabetes.

Key words: Proinsulin, insulin, C-peptide, identical twins, Type 1 (insulin-dependent) diabetes.

Type 1 (insulin-dependent) diabetes is due to the destruction of the insulin-secreting B cells of the pancreas. The disease is associated with immune changes in the peripheral blood, notably activated T lymphocytes and islet cell antibodies, and these changes can precede the clinical onset of diabetes [1, 2]. In addition, a decline in glucose-mediated insulin release has been reported some months before the diagnosis of diabetes [3]. The majority of identical twins of Type 1 diabetic patients do not develop the disease, despite evidence in some of immune changes and, even, B-cell dysfunction [2, 4]. In an attempt to identify changes in glucose tolerance or B-cell function that precede Type 1 diabetes we studied two groups of non-diabetic twins of recently-diagnosed diabetic patients; one group with complement-fixing islet cell antibodies, who are at high risk of developing Type 1 diabetes, and another group without any islet cell antibodies, in whom the risk of developing diabetes is low [3, 5].

Subjects and methods

We studied non-diabetic identical twins within five years of the diagnosis of their Type 1 diabetic twin. Monozygosity and the presence of islet cell antibodies was established as described previously [2, 5]. The twins were divided into two groups according to their risk of developing Type 1 diabetes, as defined by the presence of islet cell antibodies [3, 5]: group 1, five twins who had complement-fixing and cytoplasmic islet cell antibodies, most of whom will probably develop Type 1 diabetes; group 2, six twins without islet cell antibodies in whom the risk of developing the disease is small. The twins were studied a mean of 18 [22] months (group 1) and 17 [15] months (group 2) after the diagnosis of diabetes in there index twin. Each group of twins was compared with unrelated healthy control subjects sought from the local community who were not attached to the hospital or medical school. The five twins in group 1 were compared with 6 control subjects of similar age (mean 14.4 (SD 3.6) vs 14.8 (2.0) years, sex (three males in each group) and body mass index (18.4 (2.7) vs 19.2 (3.6) kg/m^2). The six twins in group 2 were compared with a further 6 control subjects who were also similar for age (20.4 (4.4) vs 19.6 (4.2) years), sex (2 males in each group), and body mass index (21.3 (1.1) vs 20.7 (4.4) kg/m^2). Insulin autoantibodies were measured by a modification of an ELISA method [6]. Two twins in each group had insulin autoantibodies but none of the control subjects did. Each participant had an oral and intravenous glucose tolerance test performed on a single occasion and none was ill or receiving drugs at the time of the study. Participants or their parents gave informed consent and the study was approved by the King's College Hospital ethical committee.

Oral glucose tolerance test

Following an overnight fast, subjects were studied supine at least 15 min after a venous cannula was inserted into the antecubital vein under local anaesthetic. Basal blood samples were taken at −10 and 0 min, after which 75 g of glucose (or 1.75/kg, whichever was the less) dissolved in 0.33 l water was consumed over 4 min. Further blood samples were taken at 10, 30, 60, 90, 120 and 180 min after oral glucose, for the measurement of whole blood glucose, serum insulin, C-peptide and proinsulin.

Table 1. Mean (SD) fasting and total concentrations of glucose, insulin, C-peptide and proinsulin in groups 1 and 2 after oral glucose

	Group 1		Group 2	
	Twins	Control subjects	Twins	Control subjects
Glucose				
- Fasting (mmol/l)	4.2 (0.4)	4.3 (0.5)	4.1 (0.6)	4.1 (0.5)
- Total area (mmol/l per 180 min)	339 (70)[b]	146 (69)	311 (169)	177 (82)
Insulin				
- Fasting (pmol/dl)	6.6 (3.6)	7.0 (2.9)	7.1 (3.8)	6.1 (3.3)
- Total area (pmol/dl per 180 min)	2146 (606)	2292 (979)	2862 (504)	2406 (1169)
C-peptide				
- Fasting (pmol/dl)	36.7 (20)	33.3 (17)	33.3 (13)	36.7 (13)
- Total area (pmol/dl per 180 min)	9233 (6167)	8767 (4900)	12900 (6167)	13667 (5167)
Proinsulin				
- Fasting (pmol/dl)	2.8 (1.7)[a]	1.1 (0.2)	2.7 (0.8)[b]	1.4 (0.7)
- Total area (pmol/dl per 180 min)	296 (286)	325 (191)	311 (509)	272 (262)

[a] $p < 0.05$; [b] $p < 0.01$

Intravenous glucose tolerance test

At 180 min after oral glucose an intravenous glucose load of 0.5 g/kg was administered through the venous cannula over a period of 2 min. Further blood samples were taken at 183, 185, 187.5, 190, 195, 200, 205, and 210 min after the oral glucose load. Whole blood glucose was analysed by a glucose oxidase method (Yellow Springs Analyser, Ohio, USA). Serum insulin and C-peptide were measured by modifications of double antibody radioimmunoassay methods [7], while serum proinsulin was measured by a modification of a sensitive monoclonal two-site immunoradiometric assay [8]. Intravenous glucose clearance rates were calculated by a method of least squares, using the natural log of glucose concentration from 195 to 210 min. Total responses were calculated as areas under the curve above the basal valve, from time 0 to 180 min following oral glucose and from 180 to 210 min following intravenous glucose.

Statistical analysis

Results are expressed as the mean and standard deviation (SD) of the mean. The significant variables approximated to a normal distribution in that 66% of the values fell within one SD of their mean. Changes were compared using both a two-tailed Student's t-test for unpaired observations and a Wilcoxon's rank sum test; results were considered significant at $p < 0.05$.

Results

Fasting concentrations

Both groups of twins had similar mean blood glucose, serum immunoreactive insulin and C-peptide concentrations to the control subjects (Table 1). In contrast, the twins of both groups had significantly higher mean serum proinsulin concentrations (Table 1).

Responses to oral glucose

Glucose concentrations in the twins from group 1 were significantly higher at both 90 min (6.2 (1.0) vs 4.5 (0.9) mmol/l, $p < 0.01$) and 120 min (6.1 (0.6) vs 4.8 (0.9) mmol/l, $p < 0.02$) after oral glucose, and the mean total glucose area was also significantly increased (Table 1). The twins in group 2, however, had no significant differences from control subjects in either glucose area or concentrations. Following oral glucose the twins of both groups had similar concentrations and total responses of serum insulin, C-peptide and proinsulin to the control subjects (Table 1).

Responses to intravenous glucose

At 180 min blood glucose, serum insulin, C-peptide and proinsulin concentrations were similar in twins and the control subjects (Table 2). Following intravenous glucose, the glucose clearance rate (kg) was significantly worse in the group 1 twins (1.7 (0.2) vs 2.5 (0.6) %/min, $p < 0.02$) but not in the group 2 twins (2.4 (0.3) vs 2.4 (0.5) %/min. However, there was no significant difference between the twins and their controls in the concentrations and total responses of serum insulin, C-peptide or proinsulin to intravenous glucose (Table 2).

Discussion

Non-diabetic identical twins of recently-diagnosed Type 1 diabetic patients have raised fasting proinsulin levels; this change occurred irrespective of whether their risk of developing diabetes was high or low. We have reported a similar increase in fasting proinsulin levels in non-diabetic twins of long-standing Type 1 diabetic patients, whose risk of developing diabetes was less than 3% [4]. Thus, increased fasting proinsulin levels can be a feature of non-diabetic twins of Type 1 diabetic patients irrespective of their duration of discordance or the presence of islet cell antibodies. We therefore conclude that minor changes in B-cell function in these twins, characterised by increased fasting proinsulin levels, need not progress to diabetes.

It has been suggested that a decreased insulin response to intravenous glucose in subjects with islet cell antibodies may be an early marker of B-cell damage [3]; however, most of the subjects reported had insulin responses within the normal range. The twins with islet cell antibodies that we studied also had normal insulin responses to both oral and intravenous glucose though their fasting proinsulin levels were raised. Because we

Table 2. Mean (SD) basal and total concentrations of glucose, insulin, C-peptide and proinsulin in groups 1 and 2 after intravenous glucose

	Group 1		Group 2	
	Twins	Control subjects	Twins	Control subjects
Glucose				
- Basal (mmol/l)	4.1 (0.8)	3.7 (0.9)	4.1 (1.6)	3.2 (0.9)
- Total area (mmol/l per 30 min)	241 (34)	214 (60)	204 (40)	222 (57)
Insulin				
- Basal (pmol/dl)	11.8 (5.5)	11.0 (5.0)	14.4 (10.8)	10.5 (5.7)
- Total area (pmol/dl per 30 min)	490 (214)	641 (149)	871 (209)	704 (80)
C-peptide				
- Basal (pmol/dl)	36.7 (23)	46.7 (33)	86.7 (57)	63.3 (30)
- Total area (pmol/dl per 30 min)	700 (367)	900 (967)	600 (667)	1933 (867)
Proinsulin				
- Basal (pmol/dl)	5.7 (2.8)	3.1 (2.2)	5.2 (3.8)	3.4 (2.4)
- Total area (pmol/dl per 30 min)	27.2 (50)	28.3 (33)	9.8 (14.4)	25.1 (39.4)

gave oral glucose before intravenous glucose, it is possible that we obscured any difference in insulin responses which might be observed to intravenous glucose alone. Nevertheless, in this study there was no difference in the insulin responses between the twins and control subjects. Thus, we conclude that changes in proinsulin can precede abnormalities of insulin response.

It remains possible that the immunoreactive insulin levels are artificially raised in these twins due to factors known to interfere with the insulin radioimmunoassay. Two such factors are relevant to this study; insulin autoantibodies and proinsulin. We believe that insulin autoantibodies are unlikely to account for the normal insulin levels since the C-peptide concentrations were also normal. Proinsulin binds to the insulin antibody used in the insulin radioimmunoassay and in our assay has 70% of the immunoreactivity of insulin. However, the increase in proinsulin could contribute no more than 1.5 pmol/dl to immunoreactive insulin levels; this would not significantly alter our observations.

The only factors which distinguish the twins with complement-fixing islet cell antibodies from those without such antibodies, are a minor impairment of both glucose tolerance and glucose clearance. Previous studies have suggested that glucose intolerance may precede the diagnosis of diabetes by many months [9, 10]. We do not believe such changes are specific for Type 1 diabetes since we have observed twins in whom glucose intolerance has improved and diabetes has not developed (unpublished data). Nevertheless, the present observations do suggest that changes in both glucose tolerance and fasting proinsulin may be earlier indicators of B-cell damage than a decreased insulin response to glucose challenge.

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