Natural history of glucose tolerance, beta-cell function and peripheral insulin sensitivity in cystic fibrosis patients with fasting euglycemia

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Abstract

Objective: The loss of pancreatic beta-cells is thought to be one of the principal causes of diabetes mellitus (DM) in cystic fibrosis (CF), but the role of peripheral insulin resistance (IR) in the pathogenesis of DM in CF remains unclear. The aim of this study was to evaluate whether eventual changes of glucose tolerance (GT) over time were associated with modifications of insulin secretion or sensitivity.

Methods: Plasma glucose and insulin responses to an oral GT test (OGTT) were investigated and reinvestigated 13 years later in 14 CF patients with initial and persistent fasting euglycemia and no history of insulin treatment. Insulin sensitivity (IS) at both tests was assessed on the basis of insulin and glucose levels both in the fasting state and during OGTTs.

Results: From the 1st to the 2nd OGTT: (a) the prevalence of DM responses significantly increased; (b) the areas beneath the respective glucose and insulin curves significantly increased and decreased respectively; (c) IR and IS indices decreased and increased respectively, even in the patients who developed DM; (d) pulmonary function significantly worsened in the entire series, especially in the patients who developed DM.

Conclusions: (i) the natural history of glyco-metabolic status in CF is characterized by deteriorating GT over time; (ii) insulinopenia plays a prominent role in the pathogenesis of GT worsening; (iii) IR does not play any significant part in the pathogenesis of DM development; (iv) deterioration of lung function tests is more severe in the subjects who develop DM over time.

European Journal of Endocrinology 149 53–59

Introduction

Cystic fibrosis (CF) is a multisystem and life-threatening recessive disease that affects about one in 2000 children in the Western world (1). Abnormal chloride channel activity in CF leads to hyperviscosity of ductular secretion, causing progressive obstructive damage to many organs, including the pancreas. Most patients have severe fibrosis and exocrine insufficiency of the pancreas, and pancreatic endocrine dysfunction is also common in the exocrine-insufficient individuals. The prevalence of glucose intolerance in CF is actually growing, probably due to these patients’ increasing life expectancy. Impaired glucose tolerance (IGT) affects up to 40% of patients with CF and 10% to 15% of CF adults develop overt diabetes mellitus (DM) with fasting hyperglycemia (2).

The loss of insulin-producing beta-cells, secondary to pancreatic fibrosis leading to progressive destruction of the pancreatic islet architecture, is thought to be one of the principal causes of CF-related diabetes (CFRD) (3, 4). CFRD also shares a number of clinical and pathological similarities with type 2 DM (5), a disease which is predominantly associated with a significant increase in peripheral insulin resistance (IR). The role of IR in the pathogenesis of CFRD, however, remains unclear, with several studies yielding inconsistent findings (6–13).

Thirteen years ago we started a longitudinal evaluation of glucose tolerance (GT), insulin secretion and peripheral insulin sensitivity (IS) in a cohort of CF patients originally selected on the basis of persistently normal fasting blood glucose levels (14), in order to evaluate the factors that can influence DM development in CF. The present paper reports the 13-year observational data of our prospective study, comparing this data with baseline results. The aim of this study is to evaluate whether eventual changes of GT over time are associated with modifications of insulin secretion or sensitivity.
Patients and methods

Study population and design

From a larger group of exocrine-insufficient patients included in the original studies (6, 14), 14 surviving subjects (eight males) were selected for the present study on the basis of the following inclusion criteria: (a) non first-degree family history of DM of any type; (b) age > 18 years; (c) adult pubertal status and bone age; (d) body weight (bw) within ± 15% of ideal bw; (e) persistently normal fasting blood glucose; and (f) no acute intercurrent disease.

At the time of both the original study and the present investigation none of the study population was taking drugs that can influence GT, such as steroids or salbutamol. The main clinical data of our patients at the time of both the original study and the present evaluation are given in Table 1. The study design was such that these 14 patients underwent an oral GT test (OGTT) for the assessment of GT, insulin secretion, and peripheral IS and that the results were compared with the ones obtained in the same 14 patients at the time of the original study that had been performed 11.8–14.7 years earlier (mean 13.7 ± 0.6 years).

Informed consent was always requested and obtained from all patients or their parents. This study design was approved by the Ethical Committee of our University Hospital.

Methods

Clinical parameters

Diagnosis of CF was based on clinical findings or positive family history and a sweat chloride concentration > 60 mmol/l, determined by pilocarpine iontophoresis.

Nutritional status was evaluated by body mass index (BMI), which was calculated as weight (kg)/m² and expressed in percentiles (BMP) according to the standards for white individuals assessed by Must et al. (15). The exocrine pancreas function was estimated indirectly on the basis of the daily intake of pancreatic enzyme capsules (pancaps). Clinical status was also monitored by means of pulmonary function tests, which included measurement of forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1).

Pubertal stages were assessed according to the criteria of Tanner for pubic hair (P) and breast (B) or genitalia (G). At the time of the 2nd OGTT pubertal status was B5/G5-P5 in all the subjects, according to the inclusion criteria. At the time of the 1st test it was B5/G5-P5 in patients 10–14 and B1/G1-P1 in patients 1–7 (see tables). In the remaining two subjects (nos 8 and 9) pubertal status at entry was B3/P3 and B3/P4 respectively.

Metabolic investigations

Both at the time of the 1st evaluation and at the present evaluation the OGTTs were performed at 0800 h, after an overnight fast, with a glucose load of 1.75 g/kg bw (max. 75 g), dissolved in 300 ml water and consumed within 3 min. Blood samples were taken prior to and 30, 60, 90 and 120 min after glucose ingestion. Plasma glucose was immediately measured (Glucose Analyzer 2, Beckman, Milan, Italy). Aliquots of plasma were stored at −20°C for insulin radioimmunoassay, which was performed with commercial kits (Diagnostic Product Corporation, Los Angeles, CA, USA; intra- and interassay coefficients of variation were ≤ 5%.

Table 1

| Age (years), body mass percentile (BMP), pulmonary function tests (FEV1, FVC; %) and daily intake of pancreatic enzyme capsules (pancaps) (number) in all the patients at the 1st (A) and the 2nd (B) evaluation. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Patient         | Age BMP         | FEV1            | FVC             | Pancaps         |                 |
|                 | A B             | A B             | A B             | A B             |                 |
| 1               | 5.4 19.2        | 16 17           | 91 72           | 97 76           | 9 9             |
| 2               | 7.1 21          | 16 16           | 82 70           | 97 78           | 12 12           |
| 3               | 8.1 21.8        | 16 26           | 95 80           | 97 84           | 20 28           |
| 4               | 8.4 22.3        | 17 16           | 71 29           | 85 34           | 14 12           |
| 5               | 8.5 22.3        | 15 12           | 92 29           | 92 28           | 18 12           |
| 6               | 9.1 22.8        | 17 24           | 98 111          | 101 118         | 12 12           |
| 7               | 10.6 24.5       | 18 20           | 89 46           | 93 54           | 6 8             |
| 8               | 12.5 26.2       | 20 16           | 78 52           | 75 53           | 9 9             |
| 9               | 13.5 27.4       | 17 17           | 64 78           | 82 71           | 18 16           |
| 10              | 14.1 28.1       | 20 22           | 100 70          | 100 72          | 20 18           |
| 11              | 14.1 28.8       | 17 21           | 82 68           | 88 59           | 14 12           |
| 12              | 15.7 29.1       | 17 14           | 100 37          | 100 51          | 9 15            |
| 13              | 16.7 30.6       | 24 31           | 100 43          | 100 53          | 15 18           |
| 14              | 22.6 34.5       | 21 17           | 100 37          | 100 51          | 9 15            |
| Mean            | 11.9 25.6       | 18 19           | 89 59           | 93 60           | 14 15           |
| s.d.            | 4.4 4.1         | 2.0 5.0         | 11 23           | 8.0 15          | 5.0 5.0         |

P n.s, not significant.
interassay coefficients of variations were 4.6 and 7.1% respectively at 360 pmol/l).

Areas under the curves were assessed according to the formula of Haffner et al. (16): 0.25 (baseline value) + 0.5 (30 min value) + 0.75 (60 min value) + 0.5 (120 min value).

GT was estimated according to WHO criteria (17) based on venous plasma glucose levels 2 h after load: < 7.8 mmol/l = normal (NGT); 7.8 – 11.1 mmol/l = impaired (IGT); > 11.1 mmol/l = DM.

PeripheIR IR was evaluated by the Homeostasis Model Assessment (HOMA) (18), according to the following formula: IR index = fasting insulin × fasting glucose/22.5.

IS was also investigated by a recently assessed index derived from the OGTT (19) and based on the assessment of plasma glucose and insulin concentrations both in the fasting state and during the OGTT, according to the following formula: IS index = 10 000/square root of (fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT) where 10 000 simply represents a constant that allows one to obtain numbers ranging from 0 to 12. Square root conversion was used to correct the non-linear distribution of value.

A weight-maintaining diet, with 50% carbohydrates, was consumed by each patient for 3 days before both the tests.

Peripheral IS was also evaluated in a control group including 12 healthy subjects (five males) with NGT in response to OGTT and no family history of DM. These control subjects were selected on the basis of age (10.5 ± 1.5 years, range 7.8 - 13.4 years), pubertal status (B1/G1-P1 in 6/12, B2/G2-P2 in 2/12, B3/G3-P3 in 2/12 and B5-P5 in the remaining two) and BMP (22 ± 4; range 15 – 30), which were similar to the results of the 14 patients at the time of the original investigation.

**Statistical analyses**

Data are given as means ± s.d. Comparisons between the 1st and the last OGTT results were performed by Student’s paired t-test and by the chi-square test. Student’s unpaired t-test was also used for statistical purposes, when appropriate. Correlations were made by Pearson’s test. A P value < 0.05 was considered significant.

**Results**

**Glucose tolerance**

At baseline OGTT, 10/14 patients (72.4%) were NGT, whereas the remaining four subjects (28.6%) were IGT and none exhibited a glucose response compatible with diagnosis of DM, according to the WHO criteria (20). At the time of the 2nd test two of the 10 subjects with initial NGT progressed to either IGT or DM and three of the four patients with initial IGT progressed to DM (Table 2). The remaining nine patients (eight with NGT and one with IGT) exhibited the same degree of GT in both tests. In summary, the prevalence of DM patients significantly increased over time, from 0 to 4/14, i.e. 28.6% (χ² = 4.7, P < 0.05), whereas the prevalence of both NGT (from 71.4% to 57.1%) and IGT subjects (from 28.6 to 14.3%) did not change significantly over time (Table 2).

**Table 2** Glucose tolerance, glucose (mmol/l) and insulin (pmol/l) areas under the curves, insulin sensitivity (IS) and resistance (IR) indices in all the patients at the 1st (A) and the 2nd (B) evaluation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Glucose tolerance</th>
<th>Glucose areas</th>
<th>Insulin areas</th>
<th>IS index</th>
<th>IR index</th>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
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<tr>
<td>5</td>
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<td>23</td>
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<td>192</td>
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</table>

\[ P < 0.0025 < 0.0005 < 0.0005 < 0.0005 \]
When the four patients who exhibited a DM response to the 2nd test were compared with the remaining 10 individuals, no significant differences were documented at the 1st evaluation as regards both clinical (age, BMP, pulmonary function tests, pancaps daily intake) and metabolic (glucose and insulin areas under the curves, IS and IR indices) parameters. At the time of the 2nd OGTT DM patients showed a more significant impairment of insulin output (Table 2) and a more significant worsening of lung function tests (Table 1).

**Glucose and insulin response to OGTT**

With respect to the 1st OGTT, at the 2nd test CF subjects exhibited significantly higher fasting blood glucose concentrations and also sustained glycemic responses, the average glucose levels being significantly higher at 30, 60 and 90 min (Fig. 1). The increased glycemic responses to the 2nd test resulted in greater average areas under the curves (Table 2).

In contrast, the overall insulin output significantly decreased from the 1st to the 2nd OGTT, as judged by the reduced average area beneath the insulin curve (Table 2). Moreover, when compared with those recorded at entry, the average insulin levels during the 2nd test were significantly lower at all times (Fig. 2). The overall insulin output in response to the 2nd OGTT was more severely impaired in the four patients with DM than in the remaining 10 subjects (130±44 vs 420±184 pmol/l, *P* < 0.0025). A significant reduction of insulin areas under the curve, however, was observed even in the 10 patients who did not show a diabetic GT at the 2nd test (from 979±407 to 420±184 pmol/l, *P* < 0.0025).

The insulin:glucose ratio significantly decreased over time, due to the severe deterioration of insulin output that was not completely counteracted by the concomitant enhancement of glucose levels from the 1st to the 2nd OGTT. During the last test the insulin:glucose ratio was, on average, distinctly lower at all times with respect to the original study (Fig. 3).

**Peripheral insulin sensitivity**

In spite of the significant enhancement of glucose areas under the curves exhibited at the time of the 2nd examination, CF subjects showed on average a significant increase in the IS index with respect to the average value recorded at entry (Table 2). This increase was significant even in the four patients who developed DM (from 2.3±0.2 to 11.2±5.2, *P* < 0.05). At the 2nd OGTT this index negatively correlated with the insulin area under the curve in the whole series (*r* = −0.55, *P* < 0.05), whilst no relationship was found between this index and the glucose area under the curve. Moreover, the IS index was on average similar in the patients with either normal (8.6±3.4) or pathological GT (10.2±4.3).

On the other hand, at the time of the 2nd test CF patients exhibited a significant reduction in the HOMA model IR index (Table 2). This decrease was significant even in the 4 subjects who developed a diabetic GT (from 5.3±1.0 to 2.0±3.0, *P* < 0.05). At the time of the 2nd OGTT the IR index did not significantly differ in the subjects with normal (1.0±0.6) or pathological (1.7±2.4) GT.

At the last evaluation no significant correlation was found between either the IS index or the IR index and...
clinical parameters. Both the IS index (3.5±2.7 vs 3.4±2.4) and the IR index (3.8±2.8 vs 4.9±1.7) were very similar in control subjects and in the 14 patients at the time of the 1st examination.

**Clinical status**

Neither nutritional status nor exocrine pancreatic function changed substantially during the 13-year interval between the 1st and the 2nd OGTT, as demonstrated by the fact that BMP and intake of pancaps remained stable both in the patients who developed DM over time and in those who maintained a NGT (Table 1). On the other hand, pulmonary function tests significantly worsened over time in the entire series (Table 1). Moreover, the impairment of lung function was more severe in the patients who developed a diabetic GT at the time of the 2nd OGTT. At the 1st evaluation both FEV1 and FVC in these four individuals had been superimposable on the values recorded in the remaining 10 subjects. Afterwards, both these tests worsened significantly, concomitantly with the deterioration of GT, and were clearly lower than in non-DM patients at the time of the last examination (FVC 43.5±14.8 vs 74.5±20.0, P < 0.005; FEV1 42.2±18.4 vs 65.3±22.9, P < 0.05).

However, at the time of the last evaluation, no significant correlation was found between pulmonary function tests and either glucose or insulin areas under the curves in the whole patient cohort.

**Discussion**

The goal of the present study was to identify the natural history of GT in CF from childhood to adulthood and to investigate the roles of peripheral IR and pancreatic beta-cell dysfunction in the pathogenesis of CF-related GT abnormalities. In order to achieve this purpose plasma glucose and insulin responses to an oral glucose load were investigated and reinvestigated approximately 13 years later in 14 CF subjects with initial and persistent fasting euglycemia and no previous history of insulin or dietary treatment.

To our knowledge, this is the most prolonged study to longitudinally evaluate at the same time GT, beta-cell function, peripheral IS, exocrine pancreatic function and overall clinical status in CF patients. This study design enabled us to clarify the potential influences of all these factors on the natural course of GT and the reciprocal impact of metabolic and clinical changes during the long-lasting follow-up.

First of all, our results demonstrated that the natural history of glyco-metabolic status from childhood to adulthood in our patients was characterized by an evident enhancement of glycemic responses to the OGTT with a significant increase in the prevalence of subjects with DM tolerance during OGTT. According to our data, both metabolic and clinical parameters failed to predict the deterioration of GT: age, BMP, pulmonary function tests, daily intake of pancaps, glucose and insulin responses to the OGTT and peripheral IS were similar at the time of the 1st test in the patients who developed DM 13 years later and in those who did not.

In the entire study population, the overall insulin output elicited by OGTT significantly decreased after thirteen years, especially in the patients who developed a DM tolerance over time. The impairment of beta-cell function, however, was irrespective of GT, since it was observed even in the patients with persistent NGT. Together, these data strongly support, on the basis of a very prolonged follow-up, the theory that the impairment over time of beta-cell function may precede and probably lead to the onset of glucose metabolism abnormalities in CF (3, 20, 21).

Another important point which has been investigated in the present study regards the hypothesis that the development of DM in CF may be conditioned by significant modifications of peripheral IS. As far as this point is concerned the data in the literature are very controversial, probably owing to the different techniques employed to assess IS: direct measurement of peripheral glucose utilization during stable hyperinsulinemia (clamp) in some investigations (6–12), as opposed to indirect evaluation by means of a numerical estimation of data in other reports (13, 22, 23). Moreover, in some studies where increased peripheral IR was reported, patients were generally known to have DM and were receiving exogenous insulin as part of their treatment (8, 11), which may have contributed to the development of IR.

In the present study none of our patients had received any hypoglycemic treatment before both OGTTs. Moreover, in all cases peripheral IS was evaluated with the same assessment technique both at the 1st and the last examination. Finally, the HOMA
model, which was used in our investigation, has been demonstrated to provide a reasonable estimate of tissue IS (24). The homeostatic model was very recently employed in the evaluation of CFRD by Yung et al. (13) who concluded, on the basis of a cross-sectional study, that IR does not have a significant role in the pathogenesis of CFRD. This conclusion is further supported by the results of the present study, based on a longitudinal assessment of the plasma glucose and insulin concentrations, not only in the fasting state but also during OGTT (19).

It might be questioned whether the use of IR and IS indices are suitable for the evaluation of insulin sensitivity in CFRD, a condition which differs considerably from both type-1 and type-2 DM. The similar results obtained in CF patients at the 1st OGTT and in healthy controls, however, suggest that these indices can also be appropriately used in CFRD. Moreover, the results of the present longitudinal investigation do not substantially differ from the results previously reported in a shorter prospective study based on the direct measurement of insulin sensitivity by the euglycemic clamp in CF individuals followed-up for 4 years (9).

In the whole cohort of our patients the IR index significantly decreased and the IS index significantly increased at the 2nd OGTT, in spite of the concomitant enhancement of glycemic responses. Moreover, the increase in the IS index was negatively correlated with the concomitant reduction in insulin output. These findings suggest that the progressive deterioration of beta-cell function over time represents the prominent event in the natural history of glyco-metabolic status in CF (9) and that the effects of this event may be only partly counteracted by the concurrent improvement in peripheral IS. The reduced insulin:glucose ratio found at the last OGTT supports this hypothesis.

Tissue IR decreased over time even in the four patients of our series who developed DM at the 2nd OGTT: this definitely excludes the intervention of IR in the pathogenesis of their DM tolerance. In these four patients insulin output impairment at the 2nd test was significantly more severe than in the remaining patients, which reinforces the view that insulinopenia plays a predominant part in the pathogenesis of their worsening GT.

It is well known that a physiological decrease in peripheral IS may be transiently observed during puberty and that such a physiological phenomenon is followed by an improvement in IS during adulthood (25). In our study population, however, 5/14 individuals at the 1st test and all of the patients at the 2nd test had already completed their pubertal evolution. Moreover, at the 1st evaluation seven subjects had not yet entered puberty. Therefore, in only two cases, both at the 1st OGTT, the evaluation of peripheral IS could have been affected by the physiological condition of IR which is known to be associated with puberty.

Finally, in our patients the deterioration over time of both GT and insulin secretion was accompanied by a worsening of their pulmonary function tests, which was more severe in the subjects who exhibited a diabetic GT at the 2nd test. The relationships between deteriorating GT and worsening clinical status in CF are not clear (1, 9, 10, 20, 26–29). Moreover, it is debatable whether the worsening of clinical conditions in these patients leads to metabolic derangements or whether the metabolic abnormalities precede and negatively influence the overall clinical course of the disease. Our present data seem to suggest that the impairment of beta-cell function in CF may be linked to changing overall health status. Nevertheless, all the patients of our series, even those who developed DM over time, maintained normal fasting plasma glucose and underwent no insulin or dietary treatment. Consequently, they do not constitute an appropriate sample to evaluate the potential impact of another chronic disease, such as DM, on the complex management of CF. It may be reasonable to suggest that an insulin-dependent state, even without significant hyperglycemic symptoms, can create a disadvantageous metabolic environment and negatively influence overall health status in CF (20). Another plausible hypothesis is that both deterioration of beta-cell function and worsening of lung function may be two unrelated events occurring in the natural history of the adult patients with CF, as suggested by the lack of any significant relationship between clinical and metabolic changes in our patients.

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Received 20 January 2003
Accepted 1 April 2003

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