Abnormal glucose tolerance in children with cystic fibrosis: the predictive role of continuous glucose monitoring system

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Abstract
A long pre-diabetic phase of abnormal glucose tolerance is described in subjects with cystic fibrosis (CF) since childhood.

Objective: The aims of the study were to compare oral glucose tolerance test (OGTT) and continuous glucose monitoring system (CGMS) in the diagnosis of altered glucose metabolism, and to longitudinally evaluate the role of CGMS in predicting glucose metabolism deterioration in children with CF.

Methods: Seventeen children with CF and 14 controls were enrolled (mean age 13.3 ± 3.0 years). All subjects underwent OGTT and CGMS registration. On the basis of OGTT, children were classified as normal glucose tolerance, impaired glucose tolerance (IGT), IGT plus at least one glucose value above 200 mg/dl at intermediate OGTT points (IGT+C200) and CF-related diabetes (CFRD). HbA1c, glucose area under the curve, insulin sensitivity, and insulinogenic and disposition indexes were also considered. Subjects with CF underwent another OGTT after 2.5 years.

Results: Baseline OGTT revealed 3/17 (7.6%) children with CF with at least one glucose value above 200 mg/dl (1 CFRD and 2 IGT+C200), while CGMS revealed 6/17 (35.3%) children with glucose excursions above 200 mg/dl (P = 0.010). None of the controls showed glucose over 200 mg/dl either at OGTT or at CGMS. At the 2.5-year follow-up OGTT, all the six subjects who had diabetic glucose excursion (i.e. > 200 mg/dl) at baseline CGMS presented IGT+C200 or CFRD. In logistic regression analysis, CGMS diabetic excursion was the strongest predictor of IGT+C200 and CFRD (P < 0.001).

Conclusions: CGMS could be a useful tool to predict glucose metabolism derangements in children affected by CF.

Introduction
The improved life expectancy of patients affected by cystic fibrosis (CF) has resulted in an increasing prevalence of CF-related diabetes (CFRD) (1–3). Subjects with CFRD have worse lung function, poorer nutritional status and decreased survival than non-diabetic patients with CF (3–5). Moreover, an insidious decline in clinical status can occur even during the pre-diabetic state, before the diagnosis of overt CFRD (6–8). This has led to the concept that insulin insufficiency, independent of its effect on blood glucose levels, has a negative impact on the clinical course of CF by compromising nutritional status, leading to increased protein catabolism and loss of weight (8).

Given all these factors, prompt diagnosis and aggressive management of glucose metabolism derangements appear to be fundamental to improve life expectancy (9).

The most accurate method with which to evaluate altered glucose metabolism in patients with CF is still controversial. Even if the annual oral glucose tolerance test (OGTT) by the age of 10 years is the recommended method, a diagnosis of ‘normal’ glucose tolerance during OGTT does not exclude abnormal postprandial glucose levels at home (9). There is now evidence that the OGTT method, evaluating fasting and 2-h postload glucose, may miss episodes of hyperglycaemia. It has been demonstrated recently that the continuous glucose monitoring system (CGMS) is a useful and valid tool in defining glucose metabolism in children and adults affected by CF with early glucose derangements (10–15).

Indeed, the CGMS allows monitoring of glycaemic profiles throughout a period of 72 h for a total of 288 glycaemic registrations per day. It identifies glycaemic excursions and constitutes a valid device to understand the 24-h glycaemic trend and profiles.

The aims of the present study were to compare the OGTT and CGMS methods in the diagnosis of altered glucose metabolism, and to longitudinally evaluate the
possible role of CGMS- and OGTT-derived parameters in predicting glucose metabolism deterioration in children affected by CF.

Subjects and methods

Subjects

Children and adolescents, followed at the CF Unit of the Bambino Geste Children’s Hospital, affected by clinically and genetically proven CF were consecutively enrolled in this study between January and December 2006, if they fulfilled the following criteria: i) below 18 years of age, ii) normal glucose tolerance, according to the ADA criteria (16), at the OGTT performed 12 months previously, iii) did not have any medical condition such as pulmonary exacerbation or acute infection that would interfere with glucose tolerance and iv) were off corticosteroid therapy in the last 3 months.

A control group was also included in the study. Normal, healthy children and adolescents, with one isolated episode of hyperglycaemia occasionally discovered and without chronic diseases, were enrolled in the study in the same period, after the exclusion of immune reactivity to pancreatic ß-cell autoantigens (no presence of autoantibodies to glutamic acid decarboxylase, tyrosine phosphatase and insulin).

Ethical approval was obtained by the local scientific committee. All parents/guardians provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Methods

All the subjects underwent a 4-day hospitalisation, during which they received an unrestricted diet. Height was measured without shoes to the nearest 0.1 cm using a wall stadiometer, and weight was measured in underwear to the nearest 0.1 kg using a medical balance beam scale. Body mass index (BMI) and BMI-SDS (17) were calculated. During the first 3 days, the CGMS (Gold Medtronic, MiniMed Inc., Sylmar, CA, USA) was applied for a 72-h period. CGMS data were excluded from statistical analysis if they did not reach the optimal accuracy criteria stipulated by MiniMed (i.e. a calculated mean absolute error of < 28%), or if they had been recorded for < 24 h during the 72-h period of CGMS application.

On the fourth day, after the removal of CGMS, OGTT was performed. Moreover, forced expiratory volume in 1 s (FEV1) was measured in children with CF.

Continuous glucose monitoring system

The CGMS comprises a pager-sized glucose monitor, a sterile disposable subcutaneous glucose sensor with an external electrical connector, a connecting cable and a communication device enabling data stored in the monitor to be downloaded to a personal computer. The monitor analyses the data every 10 s and reports an average value every 5 min. The system has been described in detail elsewhere (18). The sensor is composed of a platinum microelectrode with a thin coating of glucose oxidase beneath several layers of a biocompatible membrane. The sensor is inserted into the subcutaneous tissue of the anterior abdominal wall using a spring-loaded device and an introducer needle. An electrical current is generated by glucose oxidase catalysing the oxidation of glucose in the interstitial fluid. This current is stored as an electronic signal by the monitor, and the strength of the signal is proportional to the amount of glucose present. Capillary blood glucose measurements obtained with a Precision QID Blood Glucose Sensor (Abbott, MediSense, Baar, Switzerland) were used to calibrate the sensor readings. A minimum of four capillary blood glucose samples were entered into the monitor for calibration each day. The data were then downloaded using MiniMed Solutions Software (version 1.7a) onto a personal computer (the data set was not used directly from the MiniMed software).

Oral glucose tolerance test

OGTT was performed using 1.75 g/kg of body weight of a glucose solution to a maximum of 75 g. Baseline samples were taken at −15 and 0 min, and then blood samples were collected over a period of 2 h at 30-min intervals for glucose and insulin measurements. HbA1c was also determined.

Insulin sensitivity index (ISI) (19) was calculated to estimate insulin sensitivity. To assess ß-cell function, we used the insulinoegenic index, which was calculated as the ratio of the increment in the plasma insulin level to that in the plasma glucose level during the first 30 min after the ingestion of glucose. The disposition index (20) was defined as the product of ISI and insulinoegenic index. It reflects the capacity of pancreatic islets to compensate for lower insulin sensitivity. Glucose area under the curve (AUC) was calculated using the trapezoid rule.

Glucose tolerance categories

Given the frequent excursions of glucose above 200 mg/dl at intermediate OGTT points (i.e. at 30, 60 and 90 min) in subjects with CF (9, 10), in addition to fasting and 2-h glucose, intermediate points were also considered to classify children on the basis of the OGTT. Subjects were categorised as normal glucose tolerance (NGT; fasting plasma glucose < 100 mg/dl, 2-h glucose < 140 mg/dl and no intermediate points ≥ 200 mg/dl), impaired fasting glucose (IFG; fasting plasma glucose ≥ 100 and < 126 mg/dl, 2-h glucose < 140 mg/dl and no intermediate points ≥ 200 mg/dl), impaired glucose tolerance (IGT; fasting plasma glucose < 100 mg/dl, 2-h glucose ≥ 140 and < 200 mg/dl, and no intermediate points ≥ 200 mg/dl), impaired glucose tolerance (IGT; fasting plasma glucose < 100 mg/dl, 2-h glucose ≥ 140 and < 200 mg/dl, and no intermediate points ≥ 200 mg/dl).
and at least one intermediate point $\geq 200$ mg/dl), CFRD (fasting plasma glucose $\geq 126$ mg/dl and/or 2-h glucose $\geq 200$ mg/dl, and no matter what the intermediate values were).

On the basis of CGMS, children were considered at risk for diabetes (CGMS-DM) if two or more glucose random values, registered in two different days, were over 200 mg/dl.

**Follow-up**

OGTT was repeated after 2.5 years in children with CF. Similar to baseline, OGTT was repeated in the absence of concomitant illness, and in the absence of corticosteroid therapy during the last 3 months.

**Assays**

During OGTT, quantitative determinations of blood glucose were done by enzymatic method on Roche automated clinical chemical analyser (Hitachi 904 analyser, Roche). Glucose was assayed using a commercial kit (Glucose GOD-PAP, Roche). Measuring range was 2–450 mg/dl (0.11–25 mmol/l); intra- and inter-assay coefficient of variation (CV) values were 0.9 and 1.8% respectively. Serum insulin levels were measured by a chemiluminescent immunoassay method on ADVIA Centaur analyser using a commercial kit (ADVIA Centaur IRI). Lower and upper detection limits were 0.5 and 300 mU/l (3–1800 pmol/l) respectively. The intra- and inter-assay CV ranges were 3.3–4.6 and 2.6–5.9% respectively. HbA1c (%) was determined spectrophotometrically (Bio-Rad) in 3 ml of blood drawn into evacuated siliconised tubes containing EDTA. The non-diabetic range of HbA1c in our laboratory is 3.8–5.5%.

**Statistical analysis**

Data are expressed as means $\pm$ S.D. Differences between the subjects with CF and the controls were examined using an independent sample $t$-test, after controlling for variability of normality distribution. The group frequency was compared using the $\chi^2$ test.

To explore the factors predicting the presence of glucose values above 200 mg/dl at the follow-up OGTT, a logistic regression analysis was performed with the presence/absence of glucose values above 200 mg/dl at follow-up as the dependent variable. Age, gender, BMI-SDS, FEV1, HbA1c, glucose AUC, 2-h postload glucose and the presence/absence of CGMS-DM at baseline were entered as covariates. Insulinogenic index, ISI (19) and disposition index (20) were also inserted into the models to evaluate if $\beta$-cell function, insulin sensitivity and the degree of $\beta$-cell compensation to reduced insulin sensitivity respectively could affect the dependent variable.

A $P$ value of $< 0.05$ was considered statistically significant. SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used for all analysis.

**Results**

**Baseline data**

Twenty subjects with CF and 14 controls were enrolled in the study. Three subjects with CF did not produce at least 24 h of usable CGMS recordings, and were excluded from the present evaluation. Thus, 17 individuals with CF aged 7.8–17.7 years were included in the analysis. All the subjects who were included completed the 3-day CGMS registration (mean number of glycaemia registered 743 $\pm$ 161). CF patients and controls were not different according to age, weight, height, BMI, BMI-SDS (mean values in the entire population of CF patients and controls, 13.3 $\pm$ 30.0 years, 39.1 $\pm$ 11.6 kg, 143.5 $\pm$ 17.9 cm, 17.9 $\pm$ 3.0 kg/m$^2$, –0.6 $\pm$ 1.1 respectively) and gender distribution (50% males). All the patients with CF required pancreatic enzyme supplements. Five of them were homozygous for the $\Delta F508$ mutation, six were heterozygous and six had other mutations.

When OGTT results were considered, ten subjects with CF showed NGT (58.8%), one IFG (5.9%), three IGT (17.6%), two IGT + 200 (11.8%) and one CFRD without fasting hyperglycaemia (5.9%) (Fig. 1). General and metabolic characteristics of the CF subjects according to OGTT categories are reported in Table 1. Among controls, 13 of 14 had NGT; one child was diagnosed to have combined IFG and IGT.

CGMS revealed repeated glucose values above 200 mg/dl in six CF subjects, who were therefore classified as CGMS-DM (one with CFRD, two with IGT + 200 and three with NGTs at OGTT). Thus, three of ten subjects with NGT had diabetic glucose excursion at CGMS. Peaks of glucose were observed only in postprandial period. The number of glucose values over 200 ranged between 4 and 17 episodes. None of the control groups had CGMS-DM.

Overall, the prevalence of the CF subjects with glucose values above 200 mg/dl was higher at CGMS (6/17, 35.3%) than at OGTT (3/17, 7.6%; $P = 0.010$).

**Follow-up**

After 2.5 $\pm$ 0.6 years, OGTT was repeated in subjects with CF. Three individuals had CFRD without fasting hyperglycaemia (17.6%), three IGT + 200, three IGT and eight NGT (47.1%). Of note, among the subjects with NGT at baseline, two deteriorated to IGT + 200 and one to CFRD at follow-up. These three subjects had CGMS-DM at baseline (Fig. 1).
Predictability of altered glucose metabolism with glucose above 200 mg/dl at OGTT

Logistic regression analysis was carried out to explore baseline risk factors predicting glucose above 200 mg/dl at the 2.5-year follow-up OGTT (i.e the presence of IGT + 200 and CFRD). In this analysis, baseline factors significantly influencing the dependent variable were CGMS-DM ($P<0.001$), glucose AUC ($P=0.010$), FEV1 ($P=0.010$) and disposition index ($P=0.018$). Age, gender, BMI-SDS, HbA1c, 2-h postload glucose, insulinogenic index and ISI did not influence the dependent variable.

Discussion

In the present study, we have shown that CGMS could be a useful tool in detecting early glucose derangements in the subjects affected by CF since childhood. Indeed, CGMS revealed diabetic glucose excursions not only in children with altered glucose tolerance at OGTT, but also in individuals with NGT. Moreover, CGMS was the strongest predictor in a 2.5-year follow-up of the development of CFRD and IGT + 200: at baseline, all the patients who had glucose excursion above 200 mg/dl at CGMS developed frank altered glucose metabolism at follow-up. This is the first study to demonstrate the role of CGMS in predicting glucose metabolism deterioration in patients with CF.

Similar to our findings, relative to an entirely paediatric cohort, adult CF subjects with NGT at OGTT have been shown to have diabetic glucose excursions at CGMS in 33–36% of the cases (11, 13). Moreover, in selected high-risk adolescents with CF and NGT or IGT, pathological CGMS glucose excursions were reported in around 50–70% of the cases (10, 12).

Interestingly, in the present study, HbA1c and 2-h post-load glucose values were of no help in identifying the subjects at risk for deterioration of glucose tolerance. Furthermore, glucose AUC considering the whole increment of glucose during OGTT and disposition index expressing the degree of $b$-cell compensation to reduced insulin sensitivity (20) appear to be better metabolic indicators of future glucose tolerance status than the more often used 2-h glucose and HbA1c. As others have argued (11, 13), we believe that standard OGTT criteria considering fasting and 2-h post-load

Table 1 Characteristics of 17 children with cystic fibrosis according to oral glucose tolerance test (OGTT) results.

<table>
<thead>
<tr>
<th></th>
<th>NGT</th>
<th>IFG</th>
<th>IGT</th>
<th>IGT + 200</th>
<th>CFRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Girls/boys</td>
<td>4/6</td>
<td>1/0</td>
<td>2/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.2±2.7</td>
<td>7.8</td>
<td>16.6±1.1</td>
<td>13.2±2.6</td>
<td>15.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.6±3.0</td>
<td>15.3</td>
<td>19.3±1.1</td>
<td>19.7±5.0</td>
<td>16.4</td>
</tr>
<tr>
<td>BMI-SDS</td>
<td>−0.9±1.4</td>
<td>−0.3</td>
<td>−0.6±0.8</td>
<td>0.2±1.2</td>
<td>−1.6</td>
</tr>
<tr>
<td>FEV1 (% of normal)</td>
<td>87.3±9.1</td>
<td>98.1</td>
<td>103.9±3.9</td>
<td>84.4±0.8</td>
<td>93.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.9±0.3</td>
<td>6.0</td>
<td>5.8±0.8</td>
<td>6.3±0.8</td>
<td>6.0</td>
</tr>
<tr>
<td>Glucose AUC (mg/dl)</td>
<td>14 720±3340</td>
<td>14 505</td>
<td>17 355±1986</td>
<td>20 250±1676</td>
<td>21 105</td>
</tr>
<tr>
<td>ISI</td>
<td>8.4±6.1</td>
<td>13.5</td>
<td>4.6±2.4</td>
<td>6.6±2.4</td>
<td>28.6</td>
</tr>
<tr>
<td>Insulinogenic index</td>
<td>0.81±0.78</td>
<td>0.01</td>
<td>0.70±0.63</td>
<td>0.27±0.29</td>
<td>0.01</td>
</tr>
<tr>
<td>Disposition index</td>
<td>4.02±1.68</td>
<td>0.1</td>
<td>2.5±1.3</td>
<td>1.5±1.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Glucose AUC refers to area under the curve during OGTT. FEV1 and ISI denote forced expiratory volume in 1 s and insulin sensitivity index respectively. NGT, IFG, IGT, IGT + 200 and CFRD denote normal glucose tolerance, impaired fasting glucose, impaired glucose tolerance, IGT plus at least one glucose value above 200 mg/dl at intermediate oral glucose tolerance test (OGTT) points and cystic fibrosis-related diabetes respectively.
glucose may not be representative of the overall glycaemia of these patients, and further episodes of hyperglycaemia had escaped detection. Thus, evaluating intermediate OGTT glucose points may be a more sensitive method than the 2-h values for detecting glucose abnormalities in children affected by CF.

Moreover, our findings on HbA1c are in accordance with previous studies on adult patients, showing HbA1c to be inappropriate for the diagnosis of glucose metabolism alterations in CF (11, 21–23).

It is worth noting that CGMS has been recently validated in CF paediatric patients, being reliable, reproducible and repeatable (14). In accordance with this, in the present study, CGMS did not over-estimate glucose peaks: in the control group glucose excursions above 200 mg/dl were not registered. The definition of CGMS-DM that we used (at least two glucose values above 200 mg/dl during CGMS registration) was derived by using a conventional threshold (the ADA random glucose value for diagnosis of diabetes (16)) evaluated by a non-conventional method (the CGMS). Of note, however, the ADA does not consider CGMS to be sufficiently sensitive to make a diagnosis of diabetes.

In line with current literature on low prevalence of CFRD in children (3), only one subject had CFRD, though altered glucose tolerance was quite common (30%). It is now clear that the identification of disordered glucose metabolism before major β-cell loss may be beneficial, since early insulin therapy improves lung function, reduces the number of acute respiratory infections and reverses chronic weight loss (24–28).

Despite some strengths of our study, including the longitudinal nature and the paediatric age of the entire cohort (this is the youngest group studied to date with the CGMS), we acknowledge one noteworthy limitation, which is the small sample size. Moreover, we admit that the category IGT + 200 is not validated or recognised as an individual entity to date, but in agreement with Dobson et al. (11), who underlined the relevance of high intermediate glucose levels during OGTT in subjects with CF, we believe that the patients with glucose values above 200 mg/dl during OGTT may present a more severe glucose metabolism derangement than individuals with simple IGT.

In conclusion, CGMS is a reliable and valid device for the evaluation of glucose metabolism in children with CF, in conjunction with OGTT. CGMS diabetic glycaemic excursions appear to indicate children at increased risk for glucose tolerance status deterioration in a short time.

Further studies with a larger sample size are needed in order to understand if this approach is the way to allow a more aggressive, intensive and early insulin treatment, and consequently to reduce the morbidity and mortality related to glucose homeostasis abnormalities in patients affected by CF.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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