CLINICAL STUDY

Identification of insulin secretory defects and insulin resistance during oral glucose tolerance test in a cohort of cystic fibrosis patients

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Abstract

Background: Cystic fibrosis (CF)-related diabetes is a leading complication of CF and is associated with pulmonary and nutritional deterioration, years before an evident hyperglycemia, possibly because of insulin deficiency and resistance.

Aim: To evaluate glucose tolerance, insulin secretion, and insulin sensitivity by a widely applicable method suitable for accurate and prospective measurements in a CF population.

Methods: A total of 165 CF subjects (80 females) aged 17 ± 5 years and 18 age- and sex-matched healthy controls (CON) received an oral glucose tolerance test with glucose, insulin and C-peptide determinations. Insulin sensitivity was defined on the basis of glucose and insulin concentrations using the oral glucose insulin sensitivity index, whereas β -cell function was determined on the basis of a model relating insulin secretion (C-peptide profile) to glucose concentration.

Results: Fifteen percent of CF patients had glucose intolerance and 6% had diabetes without fasting hyperglycemia and 3% had diabetes with fasting hyperglycemia. β -cell function was reduced in CF patients compared with CON (70.0 ± 4.1 vs 117.9 ± 11.6 pmol/min per m^2 per mM, $P < 0.001$) and decreased significantly with age by -2.7 pmol/min per m^2 per mM per year (confidence interval (CI) -4.5 to -0.82), i.e. almost 4% yearly. The early insulin secretion index was also reduced. Insulin sensitivity was similar to CON. CF patients who attained glucose tolerance comparable to CON had lower β -cell function and higher insulin sensitivity.

Conclusion: The major alteration in insulin secretion and insulin sensitivity of CF patients is slowly declining β -cell function, consisting of delayed and reduced responsiveness to hyperglycemia, that in CF patients with normal glucose tolerance may be compensated by an increased insulin sensitivity.

European Journal of Endocrinology 165 69–76

Introduction

Diabetes is an emerging issue in cystic fibrosis (CF) because its prevalence increases with advancing age, affecting the majority of the patients after the age of 40 (1, 2). In addition, CF-related diabetes (CFRD) has negative implications in terms of pulmonary deterioration, observed years before diabetes diagnosis, and mortality (3–10). CFRD prevalence and health consequences are expected to increase in the future as a result of prolonged survival of CF patients.

Alterations in glucose tolerance are characterized by marked glycemic peaks often followed by rapid normalization (11–13). Fasting glycemia is generally lower than in other forms of diabetes and fasting hypoglycemia has been reported (14). Therefore, the categories of 'diabetes without fasting hyperglycemia' (15) and of 'indeterminate glucose tolerance' (isolated hyperglycemia

at mid-oral glucose tolerance test (OGTT) or continuous glucose monitoring) were established (16). HbA1c is not sensitive in the diagnosis of CFRD (17, 18).

The classic diabetes diagnostic criteria, based on the detection of chronic hyperglycemia (19), were historically aimed at identifying the risk for microvascular complications (9). These are not the primary concern in CFRD. Rather, the impaired nutritional status and deterioration in pulmonary function preceding CFRD may be the consequences of insulin deficiency and insulin resistance (2, 20–23). It is currently believed that when the endocrine pancreatic defect progresses beyond a certain threshold, or when insulin resistance arises as a consequence of inflammation or simply because of the endocrine changes of puberty, the absolute or relative insulin deficiency may be responsible for clinical deterioration and eventual CFRD.

The gold standard technique to measure insulin resistance is the euglycemic insulin clamp. Studies performed with these techniques have shown variable results, with both normal insulin sensitivity (24–27) and insulin resistance for glucose (28–30), protein (31, 32), and lipid metabolism (33). These may be related to increased glucose production and gluconeogenesis during fasting (34, 35). Heterogeneous data on insulin sensitivity spanning from increased (36), normal (37, 38), and decreased (11, 39, 40) insulin sensitivity were obtained similarly using surrogate techniques.

Using the hyperglycemic clamp, the reference method for glucose-mediated insulin secretion, defects in first- and second-phase insulin secretion were demonstrated (28, 29, 41). Similar results were obtained with the intravenous glucose tolerance test (IVGTT) technique (23, 39, 42–44). Both methods are unsuitable for large-scale and repeated assessment. The OGTT is the most common diagnostic test for CFRD and can be repeated yearly. Several studies sampled insulin concentrations during OGTT and analyzed insulin secretion using various algorithms and consistently found reduced and delayed insulin responses (8, 37, 45, 46), even in CF patients with normal glucose tolerance (NGT), supporting the concept that insulin secretory defects are inherent to CF.

Information on insulin secretion obtained during OGTT may be biased by increased hepatic first-pass insulin extraction (24, 25), consequent to insulin resistance and modulated by liver disease. Thus, single or integrated measures of insulin concentrations during OGTT may not reflect actual insulin secretion. Furthermore, insulin secretion indexes based on the ratio of insulin to glucose at 30 min after glucose ingestion could be influenced by a delayed but not absolutely defective secretion. A model-derived calculation of insulin secretion based on the C-peptide profile throughout the OGTT can overcome both difficulties (47).

The aim of this study was therefore to simultaneously evaluate glucose tolerance, insulin sensitivity, and insulin secretion in CF patients by means of insulin and C-peptide profiles measured during OGTT.

Methods

Subjects

All CF patients aged > 8 years in regular follow-up at our center were offered participation in this study during their annual routine care visits if they had been clinically stable in the previous 3 weeks (neither major clinical events including pulmonary exacerbations nor changes in antibiotic or other drugs). Other exclusion criteria were a CFRD diagnosis or insulin or oral hypoglycemic agents received in the previous 6 months. Overall, 165 patients (80 females) were considered in this analysis. Healthy volunteers of comparable gender and age distribution (8 females and 10 males) were

recruited as control group. The most relevant characteristics of the study subjects are reported in Table 1. The study protocol was approved by the Institutional Ethics Committee.

Oral glucose tolerance test

In all subjects, a standard 3-h OGTT (1.75 g/kg, max 75 g) was performed, sampling at baseline and at 30 min intervals plasma glucose, serum insulin, and C-peptide concentrations. Based on fasting and 2-h plasma glucose after OGTT, patients were assigned to one category of glucose tolerance (48): normal, impaired, diabetes without fasting hyperglycemia, or diabetes with fasting hyperglycemia.

Analytical methods

Plasma glucose was measured on fluoride plasma samples (Gluco-quant; Roche/Hitachi analyzer; Roche Diagnostics) and the other analytes were measured by commercial assays (ECLIA-Cobas C6000 – Roche Diagnostics).

Analysis and modeling of OGTT

Insulin sensitivity was calculated on the basis of glucose and insulin concentrations using the oral glucose insulin sensitivity (OGIS) index (49). This method is based on a model-derived equation that assesses glucose and insulin concentration at baseline and 90 and 120 min after glucose load.

β -cell function was evaluated according to a previously published model that represents insulin secretion as the sum of two components (50, 51). The first component describes the mean slope of the dose-response relating insulin secretion (calculated from the C-peptide profile) to glucose concentration during OGTT. This dose-response slope is denoted in the current document as β -cell function. This slope changes during the OGTT because it is modulated by a potentiation factor, because during an acute stimulation, insulin secretion is higher on the descending phase of hyperglycemia than at the same glucose concentration on the ascending phase. The potentiation factor encompasses several (potentiating) mechanisms (prolonged exposure to hyperglycemia, non-glucose substrates, gastro-intestinal hormones, and neural modulation). It is set to be a positive function of time, and is constrained to average unity during the experiment. In normal subjects, the potentiation factor typically increases from baseline to the end of a 2-h OGTT (47). To quantify this increment, the ratio between the 2-h and the baseline value was calculated. This ratio is denoted as potentiation ratio.

The second component describing insulin secretion represents the dynamic dependence of insulin secretion on the rate of glucose concentration change. This

Table 1 Clinical features and glucose tolerance during oral glucose tolerance test of the study groups. Height, weight, and BMI SDS: (SDS according to data published in Ref. (59)).

	CF	CON	P value*
No. of subjects	165	18	
Age (years) (mean $\pm$ s.d.)	17.0 $\pm$ 5.4	18.7 $\pm$ 4.9	0.119
Male gender <i>n</i> (%)	85 (51.5)	10 (55.6)	
Δ F508 homozygous <i>n</i> (%)	50 (30.3)	—	
Δ F508 heterozygous <i>n</i> (%)	73 (44.2)	—	
Other <i>n</i> (%)	28 (17.0)	—	
Unknown <i>n</i> (%)	14 (8.5)	—	
Pancreatic insufficiency <i>n</i> (%)	135 (81.8)	0	
Liver disease <i>n</i> (%)	33 (20.0)	0	
<i>Pseudomonas aeruginosa n</i> (%)	120 (72.7)	0	
<i>Burkholderia cepacia</i> complex <i>n</i> (%)	12 (7.3)	0	
Liver transplantation <i>n</i> (%)	5 (3.0)	0	
Pulmonary transplantation <i>n</i> (%)	3 (1.8)	0	
Permanent O2 therapy <i>n</i> (%)	6 (3.6)	0	
Height SDS (mean $\pm$ s.d.)	−0.67 $\pm$ 1.08	0.24 $\pm$ 1.40	0.002
Weight SDS (mean $\pm$ s.d.)	−0.54 $\pm$ 1.02	−0.04 $\pm$ 1.06	
BMI SDS (mean $\pm$ s.d.)	−0.26 $\pm$ 1.04	0.05 $\pm$ 1.14	
PCR (mg/dl)	10.1 $\pm$ 12.8	—	
HbA1c (%)	5.8 $\pm$ 0.8	5.4 $\pm$ 0.4	0.016
FEV1 (% of predicted)	79.8 $\pm$ 24.3	—	
FVC (% of predicted)	87.2 $\pm$ 19.3	—	
Pancreatic insufficiency <i>n</i> (%)	135 (82)	—	
Normo-tolerant <i>n</i> (%)	125 (76)	18 (100)	
Glucose intolerant <i>n</i> (%)	25 (15)	—	
Diabetes without fasting hyperglycemia <i>n</i> (%)	5 (3)	—	
Diabetes with fasting hyperglycemia <i>n</i> (%)	10 (6)	—	

*Only *P* values < 0.20 are reported.

component is related to early insulin release and is denoted as early insulin secretion index.

The model parameters were estimated from glucose and C-peptide concentrations by regularized least-squares, as previously described (50, 51). Regularization involves the choice of smoothing factors selected to obtain glucose and C-peptide model residuals with s.d. close to the expected measurement error ($\sim 1\%$ for glucose and $\sim 4\%$ for C-peptide). Insulin secretion rates were calculated from the model every 5 min. The integral of insulin secretion during the 2-h OGTT was denoted as total insulin output.

Statistical analysis

Statistical analysis was performed by means of PASW statistics 18 software. Comparisons of means were performed by means of Student's *t*-test for paired or unpaired data, or by non-parametric tests when appropriate. Data were log-normalized when necessary. The relationship among variables was performed by means of regression analysis. The comparison of proportions was performed by means of χ^2 test.

Results

Glucose tolerance distribution in CF and control subjects is shown in Table 1. The mean glucose concentrations during OGTT, providing a continuous

measure of glucose tolerance, and the insulin and C-peptide concentrations are reported in Table 2 as median and interquartile ranges.

Glucose concentrations of CF subjects were similar to controls (CON) at basal and increased at 2 h. The mean OGTT glucose concentration was increased in CF subjects (mean $\pm$ s.e.m. 128 $\pm$ 3 vs 90 $\pm$ 4 mg/dl, $P < 0.01$). In contrast, basal and 2-h insulin and C-peptide concentrations were similar to CON. The 2-h glucose, insulin, and C-peptide concentrations increased in the higher quartiles of mean OGTT glucose concentration.

Insulin secretion was delayed in CF patients, who achieved an insulin peak concentration similar to CON (436 $\pm$ 24 vs 314 $\pm$ 40 pmol/l, $P = \text{NS}$), but later after glucose ingestion 18, 28, 35, and 18% of CF subjects peaked at 30, 60, 90, and 120 min, respectively, compared with 39, 28, 0, and 33% of CON ($\chi^2 P < 0.01$).

Pancreatic insufficient CF patients had similar distribution of glucose tolerance categories as pancreatic sufficient patients, despite increased mean OGTT glucose concentration (134 $\pm$ 6 vs 104 $\pm$ 6 mg/dl) and decreased fasting and 30-min insulin (41 $\pm$ 10 vs 72 $\pm$ 10 and 198 $\pm$ 49 vs 423 $\pm$ 49 pmol/l) and C-peptide (0.50 $\pm$ 0.07 vs 0.63 $\pm$ 0.03 and 1.20 $\pm$ 0.17 vs 2.00 $\pm$ 0.17 nmol/l) concentrations ($P < 0.001$ for all parameters).

β -cell parameters are reported in Table 2. β -cell function was reduced in CF compared with CON subjects (mean $\pm$ s.e.m. 70.0 $\pm$ 4.1 vs 117.9 $\pm$ 11.6 pmol/min

Table 2 Glucose, insulin and C-peptide concentrations and main model-derived parameters of β -cell function and insulin sensitivity during OGTT. Data are expressed as median and interquartile range. Mann–Withney *U* test was performed to compare CF against CON and Kruskal–Wallis test to analyze differences in quartiles of mean glucose during OGTT.

	All subjects			Quartiles of mean glucose during OGTT								Kruskal–Wallis test between quartiles <i>P</i> value	
				1st		2nd		3rd		4th			
	Median	25th	75th	Median	25th	75th	Median	25th	75th	Median	25th		75th
Basal glucose (mg/dl)	CF 77	68	84	72	63	78	75	69	80	86	77	95	<0.001
	CON 76	68	80	76	68	80	77	70	84	—	—	—	NS
120 min glucose (mg/dl)	CF 104*	88	135	82	67	93	95	87	102	124	143	212	<0.001
	CON 84	70	94	77	68	88	105	93	110	—	—	—	<0.001
Mean glucose (mg/dl)	CF 118*	101	145	90	84	94	105	102	109	132	153	193	<0.001
	CON 93	79	101	80	72	95	108	102	111	—	—	—	<0.001
Basal insulin (pmol/l)	CF 40	27	55	42	29	59	34	22	60	44	31	64	NS
	CON 42	30	50	40	30	44	50	46	63	—	—	—	NS
120 min insulin (pmol/l)	CF 213	117	335	140	98	276	173	90	264	226	183	579	<0.001
	CON 207	129	272	176	129	247	248	230	310	—	—	—	NS
Basal C-peptide (nmol/l)	CF 0.50	0.33	0.63	0.53	0.40	0.60	0.57	0.33	0.70	0.43	0.37	0.67	NS
	CON 0.47	0.43	0.67	0.47	0.43	0.53	0.60	0.43	0.83	—	—	—	NS
120 min C-peptide (nmol/l)	CF 1.83	1.40	2.40	1.57	1.17	1.96	1.63	1.20	2.33	1.86	1.76	3.00	<0.001
	CON 1.73	1.47	2.33	1.50	1.43	2.23	1.96	1.96	2.36	—	—	—	NS
β-cell function (pmol/min per m ² per mM)	CF 57*	32.4	89.4	86.2	55.9	140.5	80.5	51.4	98.6	45.7	22.3	58.7	<0.001
	CON 107.0	74.6	151.9	131.7	100.5	151.9	74.6	73.0	98.9	—	—	—	NS
Potentiation factor at 120 min dimensionless	CF 1.32	1.04	1.85	1.12	0.80	1.31	1.26	0.89	1.53	1.53	1.28	2.17	<0.001
	CON 1.35	1.11	2.24	1.35	1.02	2.24	1.35	1.23	2.13	—	—	—	NS
Early insulin secretion index (pmol/m ² per mM)	CF 28†	0	406	281	0	1609	94	0	446	1	0	219	<0.001
	CON 536	0	1651	507	0	1962	944	493	1136	—	—	—	NS
Insulin secretion at time 0 (pmol/min per m ²)	CF 74.4	53.9	97.1	76.5	61.0	92.9	80.1	54.5	100.7	66.0	53.5	100.8	NS
	CON 70.8	64.7	92.4	67.7	64.7	72.8	80.7	79.1	108.8	—	—	—	NS
Total insulin output (nmol/m ²)	CF 45.0	35.9	61.2	40.2	35.1	51.5	42.9	33.9	56.6	44.7	36.8	77.8	<0.001
	CON 42.9	34.4	48.2	42.6	33.6	44.3	53.4	42.2	63.6	—	—	—	NS
OGIS (ml/min per m ²)	CF 540	467	594	573	552	633	566	489	628	548	408	504	<0.001
	CON 540	506	573	558	530	599	506	501	529	—	—	—	NS

**P*<0.001; †*P*<0.05. OGIS, oral glucose insulin sensitivity index.

per m² per mM, $P < 0.001$). This tended to decrease with increasing quartiles of mean OGTT glucose concentrations. The frequency distribution of β -cell function (log-normalized data) is shown in Fig. 1 (panel A).

β -cell function declined significantly with age in CF but not in CON subjects. To avoid a selection bias due to diabetes-free long survivors, the regression analysis of β -cell function dependence on age was performed in patients younger than 25 years, as reported in Fig. 2 for both genders. The slope of β -cell function decline was -2.7 pmol/min per m² per mM (CI -4.5 to -0.82) each year, i.e. almost 4% per year. The potentiation factor for insulin secretion at 120 min, known to modulate β -cell function, was not different in CF and CON subjects. It increased with increasing quartiles of mean OGTT glucose concentrations, but did not show any dependence on age.

The early insulin secretion index was lower in CF, tended to decrease with increasing quartiles of mean OGTT glucose concentrations, and did not show any dependence on age.

Baseline fasting insulin secretion was similar in CF and CON subjects and between quartiles of mean OGTT

Figure 1 (Panel A) Frequency distribution of β -cell function (the slope of the dose-response relating insulin secretion to glucose concentration) during OGTT in CF and CON. The data have been log-normalized. The distribution in CF is skewed to the left compared with CON, consequently β -cell function is lower in CF than in CON. (Panel B) Frequency distribution of insulin sensitivity during OGTT in CF and CON. The distributions were not significantly different in the two groups.

Figure 2 Regression analysis of β -cell function dependence on age in CF individuals younger than 25 years. The data have been log-normalized. β -cell function declined significantly with age in CF by $\sim 4\%$ each year.

glucose concentration. Total insulin output was also similar between CF and CON subjects although it increased with increasing quartiles of mean OGTT glucose concentration.

Pancreatic insufficient CF patients had reduced β -cell function (62.2 ± 9.8 vs 104.8 ± 10.9 , $P < 0.001$), potentiation factor at 120 min (1.29 ± 0.08 vs 1.75 ± 0.07 , $P = 0.012$), and early insulin secretion index (268 ± 511 vs 1357 ± 508 , $P < 0.001$), but a similar total insulin output compared with those with pancreatic sufficiency.

Insulin sensitivity measured by OGIS was similar in CF and CON subjects (552 ± 10 vs 543 ± 17 ml/min per m², $P = 0.781$), but it decreased with increasing quartiles of mean OGTT glucose concentrations and it was unaffected by gender, age, and pancreatic status. The frequency distribution of insulin sensitivity in CF and CON subjects is shown in Fig. 1 (panel B).

The relationship of β -cell function and insulin sensitivity in determining glucose tolerance according to the quartiles of mean OGTT glucose concentration is shown in Fig. 3. CF patients who attained glucose tolerance comparable to CON (the lower 2 quartiles of mean OGTT glucose concentration) had lower β -cell function but similar or higher insulin sensitivity than CON.

Discussion

In a large population of CF patients, we applied an OGTT model to measure insulin secretion and insulin sensitivity. We also quantified the two defective components of insulin secretion in CF subjects, i.e. the delay and the reduction in β -cell responses to glucose, providing parameters suitable for comparison among research groups and evaluation for predictiveness of future diabetes development.

Our main findings are: i) β -cell function is impaired even in CF patients with NGT, specifically with regard to

Figure 3 Mean values of log-normalized β -cell function and insulin sensitivity were calculated in CF and CON for each of the quartiles of mean OGTT glucose concentrations (an index of glucose tolerance) in the two groups pooled. CON showed mean OGTT glucose concentrations exclusively in the two lowest quartiles of the distribution. The figure shows that corresponding degrees of glucose tolerance were obtained in CF with greater insulin sensitivity and lower β -cell function than in CON.

the insulin secretion increment for a given glucose concentration increment; early insulin secretion index is also impaired, with reduced ability to increase insulin secretion in response to the speed of glucose increment after glucose ingestion. Both defects contribute to deterioration of glucose tolerance in CF patients. ii) Despite defective β -cell function, during OGTT the insulin output may eventually increase to levels comparable to CON. iii) Insulin sensitivity may be increased and compensate for defective β -cell function in CF patients with NGT; when defective, however, it is a determinant of glucose intolerance. iv) The defect in β -cell function worsens with age at $\sim 4\%$ per year.

Our results substantially agree with recent studies that assessed insulin secretion during OGTT using various algorithms and consistently found reduced and delayed insulin responses. However, quantitative aspects may not be comparable (8, 37, 40, 46). The largest available adult series (37) displayed an average β -cell function as 70% of control values in the whole CF population and 50% of control values in the subgroup with NGT. β -Cell function was measured by the insulinogenic index (ratio between the 30-min increments in plasma insulin and glucose concentrations), thus it could not discriminate between a secretion delay and absolute secretory defect. In our series, the insulin peak was significantly delayed in CF patients although one-third of CON subjects peaked in the second hour as well. Thus, delay alone was not an appropriate discriminatory factor from normal. Model-derived parameters provided quantitative measures capable to better characterize the defects inherent to β -cell function in CF patients and indicated that insulin secretion in response to glucose was consistently reduced to 60% of CON independently from the secretory delay.

In another study (40), the insulinogenic index of children with CF was 90% decreased, whereas the

insulin area under the curve (AUC) was only 30–50% decreased compared with CON. Thus, secretory delay and absolute secretion were considered distinct factors, both affecting glucose tolerance. However, in our series, hyperglycemia could stimulate total insulin output (and insulin and C-peptide AUCs) to levels similar or even greater than CON. This finding has practical implications, because the absolute insulin secretion rates or the insulin or C-peptide concentrations or AUCs may not accurately describe β -cell function defects.

We found that CF patients with NGT may compensate a decreased β -cell function with an intact or increased insulin sensitivity. Animal models showed that reduced insulin secretion increases insulin sensitivity until hyperglycemia arises (52, 53). In humans, we found that chronic hyperinsulinemia in insulinoma causes insulin resistance (54), whereas insulin secretory defects in pancreatic surgery initially increase insulin sensitivity (55). The results of the present study suggest that in many CF patients, an increased insulin sensitivity consequent to hypoinsulinemia may mask the insulin secretory defect, producing NGT. This unstable equilibrium may be easily disrupted when insulin sensitivity decreases because of infection or growth spurts-related endocrine changes, and causing initially transient and lately stable glucose intolerance and diabetes.

Insulin sensitivity measures in CF patients, although skewed towards lower values, were distributed over a wider range compared with CON and were not significantly related to age. Even though recent pulmonary exacerbations, IV antibiotics, steroid treatment, and insulin therapy were exclusion criteria, other factors such as insulinopenia, hypoxia secondary to lung disease (29), chronic inflammation (56), and puberty (57) could have affected insulin sensitivity.

In an earlier preliminary study, we suggested that the individual changes in glucose tolerance in repeated OGTT tests were due to changes in insulin sensitivity rather than changes in β -cell function, which appeared to be more stable over time (58). However, it is generally assumed that increasing CFRD prevalence with age is related to progressive deterioration of pancreatic function, but the formal demonstration is currently lacking. In the current, larger study, we were able to demonstrate that the decrement in β -cell function is age-related, fully supporting the above concept, and providing the rough estimate of a 3–4% yearly rate of decrement. Further validation will be needed from prospective assessments of β -cell function in studies with adequate sample size and duration, involving also older CF populations.

In conclusion, we quantified several defects in β -cell function, in both total and early insulin secretion in CF patients. Increased insulin sensitivity may compensate for the β -cell function defects, producing NGT. Hyperglycemia during OGTT may eventually stimulate a delayed insulin secretion to absolute rates comparable to CON. However, the secretory defects progress

with age. Accurate, large scale, and prospective measurements of β -cell function and insulin resistance can be obtained by modeling C-peptide and insulin concentrations during OGTT tests.

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.

Funding

The study is supported by a grant from Italian Cystic Fibrosis Research Foundation – ONLUS (Prog. FFC #16/2005).

Author contribution statement

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Received 12 April 2011

Accepted 18 April 2011