# Glucose Tolerance and Insulin Secretion, Morbidity, and Death in Patients with Cystic Fibrosis

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**Objectives** To describe the history, mechanisms, and consequences of cystic fibrosis (CF)–related diabetes, from childhood to early adulthood.

**Study design** Pancreatic β-cell function was estimated from the plasma insulin/glucose ratios during oral glucose tolerance test (total area under the curve and $\Delta I_{30\text{-}0min}/G_{30min}$, homeostasis model assessment [HOMA]%B), insulin sensitivity with the HOMA%S index, in 237 children with CF (109 boys, 128 girls). Progression of glucose metabolism abnormalities was evaluated by analysis for interval censored data; rates of pulmonary transplantation and death by Kaplan-Meier analysis.

**Results** Impaired glucose tolerance was found in 20% of patients at 10 years, 50% at 15 years, 75% at 20 years, 82% at 30 years; for diabetes, >20% at 15 year, 45% at 20 years, 70% at 30 years; for insulin treatment, 30% at 20 years, 40% at 30 years. Early impairment was associated with lower survival rates and higher rates of lung transplantation. The area under the $curve_{glucose}$ correlated with decreased body mass index and height. Decrease in early insulin secretion ($\Delta I_{30\text{-}0min}/G_{30min}$) was associated with impaired glucose tolerance, in all estimates of insulin secretion with diabetes. HOMA%S did not differ between the groups. Increased inflammation correlated with insulin resistance and impaired glucose tolerance.

**Conclusions** CF-related diabetes, mainly because of β-cell deficiency, is frequent early in life and associated with impaired nutritional state and growth, increased rates of terminal respiratory failure, and death. *(J Pediatr 2008;152:540-5)*

Cystic fibrosis (CF) is a multisystem and life-threatening autosomic recessive disease mainly affecting the Caucasian population. Abnormal chloride channel function in CF leads to hyperviscosity of exocrine secretions, causing progressive obstructive damage to many organs, including the pancreas. As pulmonary and nutritional care have improved in patients with CF, their median life expectancy has increased.[1] As a consequence, the prevalence of comorbid conditions associated with CF has also increased, especially those alterations in glucose metabolism. Patients with CF are prone to development of impaired glucose tolerance (IGT) and diabetes mellitus, so called *CF-related diabetes* (CFRD), showing an age-related increase in prevalence.[2] In Denmark, where annual oral glucose tolerance testing (OGTT) has been performed in the CF population, up to 50 % of patients older than 30 years were reported to have CFRD.[3]

The primary cause of CFRD is insulin deficiency. The loss of insulin-producing β-cell function, caused by progressive destruction of the pancreatic islet architecture, is believed to be one of the main causes of CFRD.[4] Patients with CF also show variable degrees of insulin resistance,[5-7] but the role of insulin resistance in the pathogenesis of CFRD remains unclear, with several studies yielding conflicting findings.[5-10]

Patients with CF and diabetes are more likely to be undernourished and have significant alterations of pulmonary function and a greater mortality rate[11,12] than patients without diabetes.[12,13] Prediabetic state may also be deleterious because a greater decline in pulmonary function and nutritional state was found as early as 6 years before the diagnosis of diabetes.[14]

To evaluate the factors that can influence the development of CFRD and to assess its potential impact on clinical status, an evaluation of glucose tolerance and concomitant

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| ANOVA | Analysis of variance | HOMA | Homeostasis model assessment |
| AUC | Area under the curve | ICA | Islet-cell antibody |
| BMI | Body mass index | IGT | Impaired glucose tolerance |
| CF | Cystic fibrosis | NGT | Normal glucose tolerance |
| CFRD | Cystic fibrosis-related diabetes | OGTT | Oral glucose tolerance test |
| HLA | Human leukocyte antigen | SDS | Standard deviations |

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Submitted for publication Jun 4, 2007; last revision received Jul 17, 2007; accepted Sep 11, 2007.

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0022-3476/$ - see front matter



10.1016/j.jpeds.2007.09.025

clinical and biologic changes was conducted in a cohort of children with CF. Prolonged follow-up has led to a clear picture of the development of glucose tolerance abnormalities in youth with CF, the mechanisms underlying glucose metabolism derangements, and the consequences on morbidity and death in these children, adolescents, and young adults.

## METHODS

### Study Population and Design

The study included 237 patients with CF (109 boys, 128 girls), followed up at Necker-Enfants Malades Hospital between 1988 and 2005. The diagnosis of CF was based on typical clinical manifestations and a positive sweat testing result. The common CFTR mutation ΔF508 was present in 90% of the patients, and all had exocrine pancreatic deficiency.

Glucose tolerance was evaluated by use of serial OGTT. 517 OGTTs were performed in 206 patients (2.5 ± 1.3 per patient, range 1 to 7). 31 patients had diabetes diagnosed before a first OGTT was performed. Average age at the first OGTT or at diagnosis of diabetes for 31 patients was 12.6 ± 5.6 years (2 to 26 years), and the age at the end of follow-up was 18.5 ± 6.3 years (2 to 32 years). End of follow-up was either last visit at the hospital, death, or date of lung transplantation (because of the influence of immunosuppressive treatment on glucose metabolism).

Height and weight were measured at the time of each OGTT and body mass index (BMI: weight in kg/height$^2$ in meters) was expressed as Z-score.[15] Blood cell count, erythrocyte sedimentation rate (estimation of inflammation), and HBA1c were also measured at the same time. Human leukocyte antigen (HLA) class II typing and cytoplasmic islet-cell antibody (ICA) titers were studied when glucose tolerance was found abnormal. HLA DR3 or DR4 was present in 35% of the study group, and HLA DR 3-4 in 1.8%, which does not differ from the general population. ICAs were negative in all subjects.

### Metabolic Measurements

The OGTT was performed after an overnight fast, when the patient presented no acute pulmonary infection requiring antibiotics or any other infectious disease, and when no steroids or other drugs that might influence glucose metabolism were being administered. The oral glucose dose was 1.75 g/kg body weight (maximum 75 g) dissolved in water and consumed within 5 minutes. Blood samples were taken before and 30, 60, and 120 minutes after glucose ingestion to measure plasma glucose and insulin levels. OGTT results were classified according to the World Health Organization criteria: normal glucose tolerance (NGT), venous plasma glucose >7.8 mmol/L, 2 hours after glucose load; IGT, venous plasma glucose 7.8-11.1 mmol/L, 2 hours after glucose load; diabetic glucose tolerance, venous plasma glucose >11.1 mmol/L, 2 hours after glucose load and fasting plasma glucose ≥7 mmol/L. The criteria to start insulin treatment were the presence of clinical symptoms of diabetes (rarely), and, for patients with a diabetic OGTT, an increase of HBA1c (>7%) and weight loss or deterioration of pulmonary status.

Indexes of insulin secretion and insulin sensitivity were calculated from plasma glucose and insulin values during the OGTT. The area under the curve (AUC) relating glucose and insulin levels and time during the OGTT were calculated by the trapezoidal rule. Indexes of beta-cell function (insulin secretion) were computed as the ratios $AUC_{insulin}/AUC_{glucose}$ and $\Delta Insulin_{30\text{-}0min}/Glucose_{30min}$. Both indexes present good correlations ($R^2 \sim 0.7$) with insulin secretion determined during glucose clamps. Homeostasis model assessment (HOMA) indexes of beta-cell function (HOMA%B) and insulin sensitivity (HOMA%S) were computed from baseline fasting levels of glucose and insulin. HOMA is a mathematical model of insulin/glucose interactions that estimates the set of beta-cell function and insulin sensitivity, which is expected to give the fasting glucose and insulin concentrations observed in 1 individual.[16] HOMA results are expressed as a percentage of average values found in young fit subjects with ideal body weight, who were taken as an absolute reference population for constructing the model. HOMA estimations, expressed as percent of reference values, correlate well with glucose clamp measurements of beta-cell function and insulin sensitivity.[17]

Plasma glucose was measured by a glucose oxidase technique and plasma insulin by radioimmunoassay (Bi-Insulin IRMA Cis-Bio international, Gif sur Yvette, France). HbA1C was determined by high-pressure liquid chromatography (Tosoh Bioscience A1C2.2, standard NGSP), with normal values ranging from 4.2% to 5.6%. Cytoplasmic ICAs were detected by indirect immunofluorescence. HLA class II typing was performed by the standard microlymphocytoxicity technique and, more recently, by molecular biology.

### Statistical Analysis

The survival distributions for overall survival and for time to lung transplantation were estimated according to the method of Kaplan and Meier. The survival and the time to lung transplantation were compared between the groups with the log-rank test.

The onset of abnormalities of glucose tolerance (impaired tolerance, diabetic OGTT, or insulin treatment) was interval censored data. We used the hybrid iterative convex minorant estimator to determine the distribution function. The corresponding curves have been calculated for the whole sample and according to sex and to the "early" or "late" onset of abnormalities of glucose tolerance. Comparisons between curves have been made at age 10, 15, 20, and 25 years, calculating the confidence intervals of differences of proportions by use of bootstrap. Calculations were made on 2000 bootstrap samples. When the confidence interval did not include 0, it could be concluded that the difference was statistically significant.[18] As multiple comparisons were made (at different ages), we used 99% confidence intervals to set alpha level at 0.05, which is here an even more conservative