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CLINICAL STUDY

Insulin-secretion abnormalities and clinical deterioration related to impaired glucose tolerance in cystic fibrosis

Santiago Tofé, José C Moreno, Luis Máiz[1], Milagros Alonso, Héctor Escobar[1] and Raquel Barrio

Pediatric Diabetes Unit, Department of Pediatrics, Hospital Ramón y Cajal, University of Alcalá, Crta. de Colmenar Km 9.1, 28034 Madrid, Spain
[1]Cystic Fibrosis Unit, Hospital Ramón y Cajal, University of Alcalá, Madrid, Spain

(Correspondence should be addressed to R Barrio; Email: rbarrio.hrc@salud.madrid.org)

J C Moreno is now at the Department of Internal Medicine of Erasmus Medical Center, University of Rotterdam, Rotterdam, The Netherlands

Abstract

Objective: To evaluate insulin-secretion kinetics and insulin sensitivity in cystic fibrosis (CF) patients with normal glucose tolerance (CF-NGT), impaired glucose tolerance (CF-IGT) or CF-related diabetes (CFRD), and the potential effects of moderate hyperglycemia on clinical and nutritional status.
Design and methods: Cross-sectional study including 50 outpatients with CF. Patients underwent both oral (OGGT) and intravenous (IVGTT) glucose tolerance tests in order to assess insulin secretion and peripheral insulin sensitivity. Homeostasis assessment model and OGGT were used to investigate insulin sensitivity. Forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) were measured to evaluate pulmonary function. Body mass index (BMI) was determined to assess nutritional status.
Results: Insulin secretion was significantly decreased (and delayed at OGTT) in the CFRD group ($n = 9$) versus the CF-IGT group ($n = 10$) and the CF-IGT versus the CF-NGT group ($n = 31$). Insulin sensitivity was significantly different in the CF-IGT and CFRD groups versus the CF-NGT group. FEV_1, FVC and BMI presented a significant linear correlation with plasma glucose value at 120 min at OGTT and were significantly lower in both CF-IGT and CFRD versus the CF-NGT group, whereas no differences were found between the CF-IGT and CFRD groups.
Conclusions: CF patients with IGT present diminished insulin secretion and increased peripheral insulin resistance, correlating with a worse clinical status, undernutrition and impaired pulmonary function. These findings open the question of whether early treatment of mild alterations of glucose metabolism with insulin secretagogues or short-action insulin may lead to improvement of clinical status in CF patients.

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Introduction

Cystic fibrosis (CF) is the most common life-threatening, autosomal-recessive disease among the Caucasian population worldwide (1). The incidence of this disorder is approximately one case in 2500–3000 live births (2). Mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, located on chromosome 7, have been shown to impair fluid and electrolyte composition of secretions from the lung, pancreas, intestine and hepatobiliary ducts, leading to progressive obstruction and fibrosis (3). More than 900 different mutations affecting the CFTR gene have been identified, but the loss of a phenylalanine residue at position 508 (ΔF-508 mutation) accounts for approximately 70% of them (1). Up to 4% of the general population is an asymptomatic carrier of this mutation (2).

As pulmonary and nutritional care have improved in CF patients, their median life expectancy has increased up to 31.3 years (4). As a consequence, the prevalence of morbid conditions associated with CF has also increased, especially those regarding alterations in glucose metabolism. CF patients are prone to developing impaired glucose tolerance (IGT) and diabetes mellitus referred to as CF-related diabetes (CFRD). These conditions affect up to 75% of the adult CF population (5). In the USA, 5–6% of CF patients are reported to have CFRD (4, 6), with increasing prevalence as patients become older (7). In Denmark, where oral glucose tolerance tests (OGGTs) are performed on a yearly basis among the CF population, up to 50% of patients older than 30 years are reported to have CFRD (8). Similarly, impaired glucose tolerance is found in 18–47% of CF patients, showing an age-related increase in prevalence (7, 9). Patients with IGT who are homozygous for the ΔF-508 mutation present with an increased risk for developing CFRD in the years following CF diagnosis (9, 10).

CFRD shares features of both type 1 and type 2 diabetes. The primary cause of CFRD is insulin deficiency,

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especially first-phase insulin secretion (11), but patients also show a variable degree of insulin resistance that may worsen in some clinical conditions, such as undernutrition, steroid therapy and/or chronic pulmonary infection (12–14). Recently, insulin resistance present in CF patients has been associated with increased levels of tumour necrosis factor-α and impaired membrane translocation of the glucose transporter GLUT-4 in skeletal muscle (15).

Life expectancy of CFRD patients is clearly reduced compared with the non-diabetic CF population (14, 16–19). The onset of CFRD is associated with nutritional failure, growth retardation in pubertal patients and worsening of pulmonary function due to frequent infections. These alterations can be observed 2–4 years before CFRD diagnosis (14). When overt diabetes is present, both insulin replacement and oral glucose-lowering agents have proven to reverse negative changes in weight, nutritional status and pulmonary function (20, 21). However, during pre-diabetic phases of this CF-related alteration of glucose metabolism, possible benefits of therapeutic interventions are not well established. Recently, a consensus report of the Cystic Fibrosis Foundation provided the guidelines for screening, diagnosis and treatment of glucose metabolism abnormalities related to CF (22). This document states that nutritional and metabolic consequences of IGT in CF patients are still unknown and thus no specific guidelines for management are given, except for a close follow up to detect the onset of CFRD. However, recent studies pointed towards mild glucose metabolism alterations as a cause of clinical deterioration in patients with CF (23, 24).

The aim of this study was to screen for glucose metabolism alterations in a cohort of CF patients on the grounds of standardized oral and intravenous glucose tolerance tests (OGTT and IVGTT), and to evaluate the kinetics of insulin secretion and peripheral insulin sensitivity. Furthermore, we investigated potential impact of pre-diabetic alterations of insulin secretion and insulin resistance on clinical well-being, nutritional status and pulmonary function of CF patients with glucose intolerance.

Subjects and methods

Subjects

A total of 50 out-patients who regularly attended our Cystic Fibrosis Unit were screened for glucose-metabolism abnormalities. Inclusion criteria included stable clinical situation, absence of clinical or radiological signs of concurrent pulmonary infection and absence of current steroid therapy. After informed consent was obtained from patients and/or parents, the formers were admitted to our Diabetes Day-Care Unit to undergo physical examination, lung-function testing and OGGTs and IVGGTs on two separate days. Pubertal state and nutritional status were respectively assessed by Tanner and body mass index (BMI). Patients with pancreatic exocrine insufficiency (45 out of 50) were under pancreatic enzyme-replacement therapy (lipase; mean dose, 4293±3660 IU/kg body weight). Genomic DNA was isolated from whole-blood samples to investigate the presence of the ΔF-508 mutation as a risk factor for CFRD development.

OGTT

All 50 patients were admitted to the Diabetes Day-Care Unit after 3 days of high carbohydrate intake (>50% of daily calories), prior to OGTT. After 1.75 g/kg body weight (maximum 75 g) of glucose was administered as 20% glucose solution, venous blood was sampled at baseline (times −10, −5 and 0 min) and then at 30, 60, 90 and 120 min. Plasma glucose level was immediately measured by the glucose-oxidase technique to avoid anaerobic glucose consumption. Plasma was then stored at −20° C for insulin determination. Patients were subsequently classified according to American Diabetes Association diagnostic criteria (22) into CF with normal glucose tolerance (CF-NGT), CF with impaired glucose tolerance (CF-IGT) or CFRD with or without fasting hyperglycemia. Maximal glucose excursion during OGGT (at time 120 min) was used to correlate severity of hyperglycemia to parameters reflecting clinical status of patients.

IVGTT

All 50 patients underwent IVGTT 24–48 h after OGTT was performed. After peripheral venous access was obtained, 50 ml of 50% glucose solution (25 g glucose) were administered in approximately 1–3 min. Venous blood was sampled at baseline and 1, 3, 5 and 10 min after glucose infusion and plasma glucose level was measured immediately by the glucose-oxidase technique. Plasma was stored at −20° C for determination of insulin levels.

Insulin-secretion and insulin-sensitivity measurements

First-phase insulin secretion was estimated upon oral ($F1_{OGTT}$) and intravenous ($F1_{IVGTT}$) glucose tolerance tests, according to previously reported validated models (25), as follows (where IC is insulin concentration, measured in pM, and GC is glucose concentration, measured in mM):

$$F1_{OGTT} = 1283 + (1.829 \times [\text{IC at } 30\,\text{min}]) - (138.7 \times [\text{GC at } 30\,\text{min}]) + (3.772 \times [\text{basal IC}])$$

$$F1_{IVGTT} = ([\text{IC at } 1\,\text{min}] + [\text{IC at } 3\,\text{min}] + [\text{IC at } 5\,\text{min}] + [\text{IC at } 10\,\text{min}]) - [\text{basal IC}]$$

Integrated insulin secretion (IIS) was measured as the area under curve for plasma insulin after oral (IIS_{OGTT}) and intravenous (IIS_{IVGTT}) glucose tolerance

tests. β-Cell function (%β) was expressed as a percentage of theoretical normal values, and calculated according to the homeostasis assessment (HOMA) model (26), using mean values for plasma glucose concentration (in mM) and insulin concentration (in pM) obtained at baseline OGTT, as follows:

$$\%\beta = ([\text{mean basal IC}] \times 3.33)/([\text{mean basal GC}] - 3.5)$$

Insulin resistance was estimated upon the insulin sensitivity index (ISI) according to two validated models: OGGT (ISI_{OGTT}) (21), and the HOMA model (ISI_{HOMA}) (26) as follows:

$$ISI_{OGTT} = 0.226 - (0.0032 \times \text{BMI}) - (0.0000645 \times [\text{IC at } 120\,\text{min}]) - (0.00375 \times [\text{GC at } 90\,\text{min}])$$

$$ISI_{HOMA} = ([\text{mean basal IC}] \times [\text{mean basal GC}])/135$$

Lung-function testing

Lung function was assessed by spirometry, measuring forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC). FEV_1 and FVC were measured without bronchodilators on a VMax 20 Spirometer (Sensor Medics Corporation, Yorba Linda, CA, USA). Results are expressed as the percentage of predicted values for age, sex, ethnical background, weight and height, based on the standards of the European Community for Coal and Steel (27).

Assessment of nutritional status

BMI (= weight in kg/(height in m)^2 × 100) was used to assess nutritional status. BMI was corrected for age in pubertal patients. Patients with BMI ≥20 are considered to have optimal nutrition.

ΔF-508 mutation analysis

Whole blood was sampled and stored at −80°. ΔF-508 mutation was detected by PCR using allele-specific primers (28). Prevalence of patient genotype (homozygous for ΔF-508, heterozygous for ΔF-508 or homozygous wild-type) was compared between NGT, IGT or CFRD patients.

Statistical analysis

Quantitative continuous variables were compared using ANOVA. Qualitative variables (sex distribution and presence of ΔF-508 mutation) were compared using the chi-square test. Areas under the curve for glucose and insulin after OGTT and IVGTT were calculated using the polynomic transformation model. Linear regression coefficients were assessed using the Pearson correlation test. Significant differences were assumed at P values of <0.05 (SPSS statistical package, version 4.1; SPSS, Chicago, IL, USA).

Results

Categorization of patients: OGGT and CFTR genotype

Upon the results of OGTT, 31 patients (62%) were classified as having NGT, 10 (20%) showed IGT and 9 (18%) had CFRD. One patient with IGT presented a baseline plasma glucose level of 6.38 mM (115 mg/dl) and a 120-min plasma glucose level of 10.54 mM (190 mg/dl), compatible with the diagnosis of both impaired fasting glucose and IGT, according to ADA criteria. Fasting plasma glucose was normal in the rest of IGT patients.

Patients in the NTG, IGT and CFRD groups showed comparable sex ratios and mean age at the time of the study (Table 1). Prevalence of OGTT categories did not show significant differences upon age distribution of patients (results not shown).

Genotype analysis of the CFTR gene was performed in 41 out of 50 patients, showing increased incidence of the homozygous ΔF-508 mutation among patients with CFRD ($P < 0.05$; Table 1). Only two out of the 18 patients (11%) with glucose-metabolism alterations (either CF-IGT or CFRD) did not carry the ΔF-508 mutation, while eight (26%) patients with NGT presented other CFTR mutations in the heterozygotic state ($P < 0.05$).

Insulin-secretion kinetics and insulin sensitivity

Dynamics of insulin secretion, as studied by four different parameters (insulin peak, first-phase and integrated insulin secretion, and β-cell function) at OGTT and IVTTG, revealed different kinetic profiles among patients in the three categories of glucose metabolism (Table 2). As calculated from OGTT and IVGTT, peak insulin, first-phase secretion and β-cell function in CF-IGT and CFRD groups were significantly decreased compared with the the NGT group. IIS was also lower in CFRD versus NTG patients as calculated from both tests, while only the intravenous challenge could detect a significantly decreased IIS in CF-IGT patients versus NTG. As estimated by OGGT and HOMA models, insulin sensitivity was significantly reduced in CFRD patients with respect to NGT. CF-IGT patients presented lower, although not significantly different, levels of insulin sensitivity in comparison with CF-NGT (Table 2). Furthermore, at OGTT both CF-IGT and CFRD patients showed a time-delayed peak insulin secretion versus CF-NGT group (120 versus 60 min), while at IVGTT the insulin peak was significantly reduced in IGT and CFRD, but not delayed in time (3 min), with respect to NGT patients (3 min; Fig. 1).

Table 1 Prevalence of NGT, IGT and CFRD and genotype in a Spanish cohort of CF patients. Age is expressed as mean values ±S.D. None of the 'other mutations' were found in compound heterozygosis with the ΔF-508 mutation. –, not found.

	NGT	IGT	CFRD	Total
Number of patients (%)	31 (62%)	10 (20%)	9 (18%)	50 (100%)
Age (years)	20.3±8.3	19.9±5.3	21.7±5.5	20.7±7.4
Gender				
Female (%)	15 (48.3%)	4 (40%)	5 (55.5%)	24 (48%)
Male (%)	16 (51.6%)	6 (60%)	4 (44.5%)	26 (52%)
CFTR genotype (% ΔF-508 mutation)				
Homozygous	7 (23%)	4 (40%)	5 (56%)*	16 (32%)
Heterozygous	10 (32%)	3 (30%)	2 (22%)	15 (30%)
Wild type	8 (26%)	2 (20%)	0 (0%)*	10 (20%)
Other mutations	8 (26%)	–	–	10 (10%)
Not determined	6 (19%)	1 (10%)	2 (22%)	9 (18%)

* Indicates level of significance with $P<0.05$.

Table 2 Insulin-secretion kinetics and insulin sensitivity in CF patients with NGT, IGT and CFRD. All values are expressed as mean ±S.D. Please note that numeric values of ISI_{HOMA} are inversely related to the actual level of insulin sensitivity.

	NGT ($n=31$)	**IGT** ($n=10$)	**CFRD** ($n=9$)
Insulin peak			
OGTT (pM)	58.6±40	48.9±25.4*	22.2±13.7**
IVGTT (pM)	45.9±17	26.5±14*	12.7±5**
First-phase insulin secretion			
$F1_{OGTT}$ (pmol)	360.29±179	155.6±150**	114.1±110**
$F1_{IVGTT}$ (pmol)	137±58	80.3±44.2**	35.5±12.6**
Integrated insulin secretion			
IIS_{OGTT} (pmol/l-h)	5.671±3.167	4.539±1.726	1.820±1.040**
IIS_{IVGTT} (pmol/l-min)	351±148	220.1±101.8*	100.5±34.6**
β Cell function			
β (%)	35.1±26.7	24.9±11.8*	10.3±9.3**
Insulin sensitivity			
ISI_{OGTT} (μmol kg^{-1} min^{-1} pM^{-1})	0.115±0.005	0.095±0.006	0.08±0.004**
ISI_{HOMA} (pM mmol l^{-2}	0.34±0.09	0.40±0.14	0.46±0.17*

*, **Indicate levels of significance in comparison with the NGT group: *$P<0.05$, **$P<0.001$.

Assessment of clinical status

Pulmonary function measured through spirometry presented significantly lower FEV_1 values (expressed as a percentage of normal values for sex, age, race, height and weight) in both CF-IGT and CFRD patients compared with CF-NGT patients (54.9±16.3 and 56.3±19.5% versus 73.5±14%; $P<0.05$). In addition, only IGT patients presented significantly lower FVC values (expressed as a percentage of normal values for sex, age, race, height and weight; 63.9±16.2 versus 81±17%; $P<0.05$). Figure 2A presents FVC and FEV_1 values for the three groups, expressed as a percentage±S.D. of predicted values for sex, age, race, height and weight.

Patients from CF-NGT showed a BMI (mean±S.D.) of 20.09 kg/m^2, in the lower limit of normality, whereas CF-IGT and CFRD patients presented a mean BMI under 20, indicating a certain degree of malnutrition. Both groups presented significant differences for BMI when compared with NGT patients ($P<0.05$; Fig. 2B).

Parameters reflecting clinical status and pulmonary function of patients were correlated against maximal glucose excursion at OGTT. BMI, FEV_1 and FVC showed statistically significant inverse correlations with plasma glucose level at 120 min in the OGGT; r values were as follows: BMI, −0.68; FEV_1, −0.61; and FVC, −0.64 (all $P<0.05$). Enzyme-replacement dosage did not show a significant correlation with plasma glucose at 120 min.

Discussion

The present study was designed to evaluate the prevalence of CF-related abnormalities of glucose metabolism and to assess the potential impact of impaired glucose tolerance on clinical status. Prevalence of glucose metabolism abnormalities in this cross-sectional study was 38% (20% IGT and 18% CFRD), within the range of previously reported data in other European countries (18, 29, 30). Overall prevalence of the ΔF-508 mutation reached 75% (30 out of 40 patients), 50% of CFRD and 40% of IGT patients carrying the mutation in the homozygous state. These findings reflect the high risk of glucose metabolism

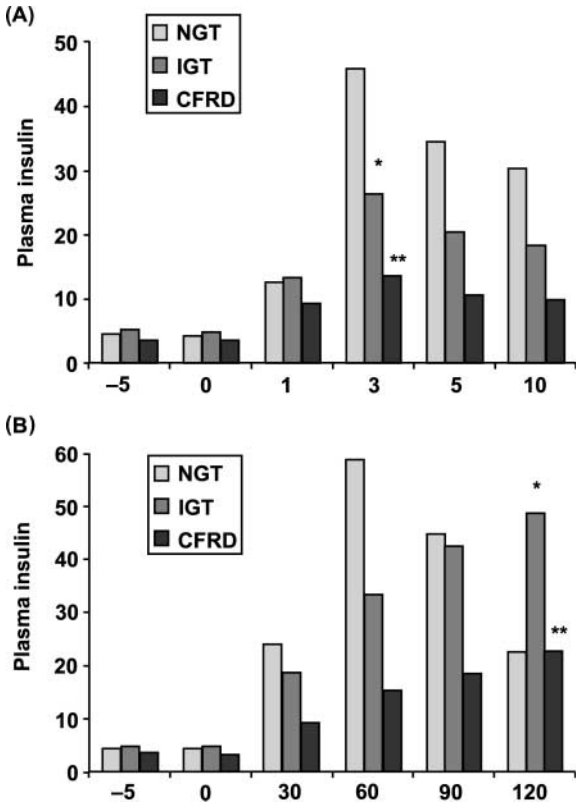

Figure 1 Insulin-secretion dynamics of CF patients at oral (OGTT) and intravenous (IVGTT) glucose tolerance tests. (A) At OGTT, peak insulin secretion is decreased and delayed in time in IGT and CFRD patients with respect to NGT patients. (B) At IVGTT, peak insulin secretion is decreased in IGT and CFRD patients (see also Table 2). The *x*-axes show times in minutes. $^{*}P < 0.05$; $^{**}P < 0.001$.

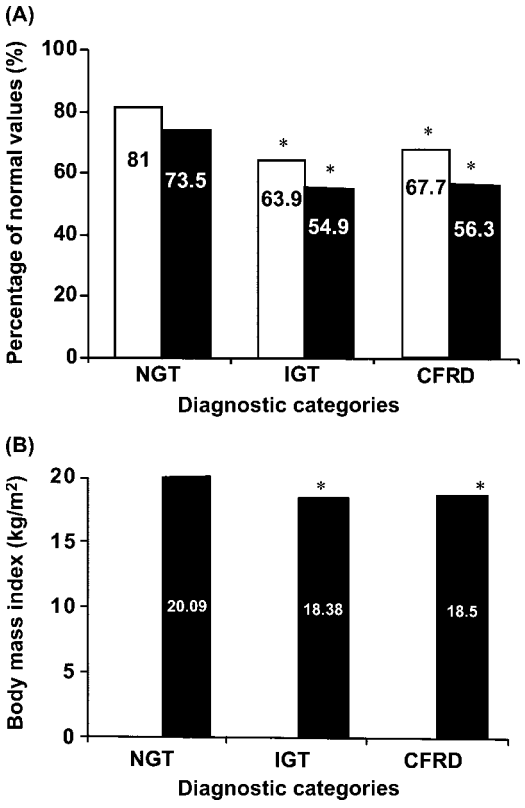

Figure 2 Pulmonary function and clinical and nutritional status of CF patients with NGT, IGT and CFRD. (A) Pulmonary function as determined by FEV_1 (black bars) and FVC (white bars). Results are expressed as percentages of predicted values for age, sex, ethnical background, weight and height, based on the standards of the European Community for Coal and Steel (27). (B) Nutritional status as assessed by BMI. $^{*}P < 0.05$.

impairment associated with this mutation, as well documented in the literature (6, 9). No differences were found for sex and age distribution among groups, and mean age at CFRD diagnosis (20.4 years) was within the reported range for CFRD onset (18–21 years) (6, 8, 16). According to these data, we consider our cohort of patients representative of the CF general population.

CFRD patients presented a clear decrease of total insulin secretion after oral and intravenous glucose overload, reflecting the severe insulin deficiency that is a hallmark of the diabetic state in CF patients. Interestingly, IGT patients showed delayed and diminished insulin peak and first-phase insulin secretion at both tests, while the intravenous glucose load also detected decreased integrated insulin secretion. To our knowledge, this is the first report on significant decreases of peak, first-phase and total insulin secretion at IVGTT in CF patients with impaired glucose tolerance with respect to CF patients with normal glucose tolerance. Decrease of these three parameters reflect early alterations of insulin secretion well known to precede the onset of absolute insulin deficiency and CFRD (31, 32). In previous studies, Cucinotta *et al.* (33) found that all CF patients (irrespective to their different degrees of glucose tolerance; normal, impaired or diabetic) had significantly reduced basal and first-phase insulin secretion (1 + 3 min) at IVGTT compared with control subjects, but they could not detect significant differences between CF patient with normal and impaired glucose tolerance. Similarly, Austin *et al.* (13) studied first-phase insulin secretion in CF patients and healthy controls using the more time-consuming and technically complicated hyperglycemic clamp (13). As with Cuccinotta *et al.* (33), they found significantly lower first-phase insulin secretion in CF-NGT and CF-IGT patients compared with controls, but additionally, and consistently with our results, insulin sensitivity was also lower in CF-IGT compared with CF-NGT patients.

In our experience IVGTT (a test nowadays not routinely included in the screening of glucose metabolism alterations in CF patients) could thus be useful for the early detection of a subpopulation of CF patients with reduced first-phase insulin secretion who are prone to developing CFRD in the ensuing years.

Two validated mathematical models, based on the study of glucose/insulin ratios under the open-loop approach, were used to obtain an index of peripheral insulin sensitivity in CF patients, both in the basal state (HOMA) and after oral glucose overload (OGTT). All estimations showed increased peripheral insulin resistance in CFRD patients. HOMA- and OGTT-based estimations pointed in the same direction. Although controversial (12, 32–36), it is widely accepted that insulin resistance plays a variable but significant role in the pathogenesis of CFRD (37).

Clinical status of patients was assessed upon nutritional status and pulmonary function. In our study, both CFRD and CF-ITG patients scored significantly lower than CF-NGT patients with respect to all clinical parameters evaluated. Whereas onset of CFRD is associated with deterioration of pulmonary function and weight loss (complications that can reverse under insulin therapy (14, 20)), reports on the possible impact of IGT in clinical deterioration of CF patients are scarce in the literature (38, 39). In this study, we found that the severity of impairment of glucose metabolism is inversely correlated with all indices for CF clinical status. This finding suggests that even moderate hyperglycemia might have a negative effect on CF clinical status.

The well-established benefit of insulin replacement on reversion of clinical deterioration in CF patients (38, 40) is currently not well documented for the pre-diabetic state. Recent studies have demonstrated that early insulin therapy improves anabolism and clinical status in CF patients with IGT, providing good glycemic control with few episodes of severe hypoglycemia (38, 41). From the perspective of clinical practice, in a cross-sectional survey performed in USA up to 61% of physicians reported the use of insulin for IGT patients (36). In our study, a comparable degree of clinical deterioration was found in both CFRD and IGT patients, while, according to the inverse correlation between clinical status and severity of hyperglycemia found, the former were expected to present a worse clinical situation.

In conclusion, our results demonstrate that CF patients with impaired glucose tolerance present altered kinetics of insulin secretion, especially its first phase, and increased insulin resistance. They also scored significantly lower in overall nutritional and respiratory function parameters. Furthermore, the degree of hyperglycemia (plasma glucose levels after oral glucose load) inversely correlated with nutritional and pulmonary status, suggesting the involvement of pre-diabetic hyperglycemia in the onset of clinical deterioration. Finally, and based on our data, the possibility that early insulin therapy might contribute to a sustained clinical state in CF patients with initial impairment of glucose metabolism is inferred. In order to determine the possible benefits of early insulin therapy or oral insulin secretagogues in the CF population with IGT, controlled clinical trials would need to be implemented in the future.

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