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Clinical Research Article

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Glucose Tolerance Stages in Cystic Fibrosis Are Identified by a Unique Pattern of Defects of Beta-Cell Function

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Abbreviations: AGT140, abnormal glucose tolerance 140; AUC_{ISR}, area under the curve of insulin secretion rate; AUC_I, area under the curve of insulin concentration; BMI, body mass index; CF, cystic fibrosis; CFRD, cystic fibrosis–related diabetes; CFRD-FH+, CFRD with fasting hyperglycemia; CFRD-FH-, CFRD without fasting hyperglycemia; DC, derivative (dynamic) control; FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; INDET, indeterminate glucose tolerance; ISPAD, International Society for Pediatric and Adolescent Diabetes; MRT_{Ins}, mean residence time of insulin; NGT, normal glucose tolerance; OGIS, Oral Glucose Sensitivity Index; OGTT, oral glucose tolerance test; PC, proportional (static) control.

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Abstract

Objective: We aimed to assess the order of severity of the defects of 3 direct determinants of glucose regulation—beta-cell function, insulin clearance, and insulin sensitivity—in patients with cystic fibrosis (CF), categorized according their glucose tolerance status, including early elevation of mid-level oral glucose tolerance test (OGTT) glucose values (>140 and <200 mg/dL), referred to as AGT140.

Methods: A total of 232 CF patients aged 10 to 25 years underwent OGTT. Beta-cell function and insulin clearance were estimated by OGTT mathematical modeling and OGTT-derived biomarkers of insulin secretion and sensitivity were calculated. The association between glucometabolic variables and 5 glucose tolerance stages (normal glucose tolerance [NGT], AGT140, indeterminate glucose tolerance [INDET], impaired

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glucose tolerance [IGT], cystic fibrosis–related diabetes CFRD]) was assessed with a general linear model.

Results: Beta-cell function and insulin sensitivity progressively worsened across glucose tolerance stages ($P < 0.001$), with AGT140 patients significantly differing from NGT (all $P < 0.01$). AGT140 and INDET showed a degree of beta-cell dysfunction similar to IGT and CFRD, respectively (all $P < 0.01$). Insulin clearance was not significantly associated with glucose tolerance stages ($P = 0.162$). Each stage of glucose tolerance was uniquely identified by a specific combination of defects of the direct determinants of glucose regulation.

Conclusions: In CF patients, each of the 5 glucose tolerance stages shows a unique pattern of defects of the direct determinants of glucose regulation, with AGT140 patients significantly differing from NGT and being similar to IGT. These findings suggest that AGT140 should be recognized as a distinct glucose tolerance stage and that reconsideration of the grade of glucometabolic deterioration across glucose tolerance stages in CF is warranted.

Key Words: cystic fibrosis, oral glucose tolerance test, glucose metabolism, β-cell function, insulin sensitivity

Cystic fibrosis–related diabetes (CFRD) is the most common comorbidity in patients with cystic fibrosis (CF) (1). The development of this complication is insidious, with symptoms strongly related to the clinical course of the disease. The presence of CFRD has, in fact, a relevant negative impact on respiratory infective exacerbations, pulmonary function, growth, weight gain and, consequently, on mortality (2, 3). Screening for CFRD through the standard oral glucose tolerance test (OGTT) is a key component of the clinical follow-up and current guidelines recommend performing this test annually from 10 years of age (1), although some authors have also demonstrated the presence of glucose metabolism alterations in CF patients under 6 years of age (4, 5). According to fasting and 2-hour plasma glucose values measured during OGTT, the following stages of glucose metabolism are defined in CFRD, as for the other more common forms of diabetes: CFRD with fasting hyperglycemia (CFRD-FH+), CFRD without fasting hyperglycemia (CFRD-FH-), normal glucose tolerance (NGT), impaired fasting glucose (IFG) and impaired glucose tolerance (IGT).

In addition, in 2010, the updated CFRD guidelines published by the American Diabetes Association and the Cystic Fibrosis Foundation have recognized the importance of also identifying CF patients with elevation of mid-OGTT glucose values above 200 mg/dL (>11.1 mmol/L), defined as affected by indeterminate glucose tolerance (INDET) (6). This last glucose tolerance class has been introduced after the recognition of its association with a consistent increased risk of subsequent develops of CFRD, especially in children (4). Moreover, in recent years, growing attention has been paid to early elevation of mid-OGTT glucose levels, herein named *abnormal glucose tolerance 140* (AGT140), characterized by mid-OGTT glucose value alterations (mid-OGTT glucose values ≥140 mg/dL [≥7.7mmol/L] or 155 mg/dL [≥8.6mmol/L]) for their impact on the clinical course of CF (7-10).

The pathophysiology of CFRD itself is complex and, to date, the pathophysiologic mechanisms responsible for alterations of glucose tolerance in CF patients are not completely understood. Some studies have measured pancreatic beta-cell function in CF patients, showing that insulin insufficiency was the predominant defect in these patients, whereas insulin sensitivity was not compromised in clinically stable patients (11, 12). A comprehensive and deep understanding of any change in glucose tolerance requires the simultaneous measurement of at least 3 direct determinants of glucose regulation: beta-cell function, insulin clearance, and insulin sensitivity (13-15). To date, this evaluation has not been performed in CF patients.

In addition, the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines identify a scale of progression in glucometabolic deterioration in CF patients with intermittent postprandial glucose excursions followed by INDET, then by IGT, and finally by CFRD (1). The pathophysiologic rational basis of this ascending order of severity of metabolic defects is unclear, because no studies have reported the simultaneous assessment of 3 direct determinants of glucose regulation in CF patients according to glucose tolerance status with inclusion of patients with early mid-OGTT glucose value alterations (16).

Therefore, in this study we measured 3 direct determinants of glucose regulation, specifically, beta-cell function (assessed as 2 components of glucose-stimulated insulin secretion: derivative and proportional control), insulin clearance, and

insulin sensitivity (14), in a large sample of patients with CF, categorized according to their glucose tolerance status.

The primary goal was to assess the order of severity of the defects of 3 direct determinants of glucose regulation across its whole spectrum, with special focus on INDET and AGT140.

Materials and Methods

Participants

A total of 232 children, adolescents, and young adults with CF in regular follow-up care at 2 Regional CF Care Centers (Verona and Napoli) and attending the 2 Regional Centers for Pediatric Diabetes of the same University Hospitals for a CFRD screening program between September 2017 and June 2019, were enrolled in this cross-sectional study.

Inclusion criteria were: age from 10 to 25 years (both inclusive); confirmed diagnosis of CF, including *CFTR* mutation analysis with 2 CF-causing mutations and positive sweat test; and exocrine pancreatic insufficiency, defined by fecal elastase <200 µg/g feces, adequately treated with pancreatic enzyme replacement therapy with no gastrointestinal symptoms (ie, steatorrhea, abdominal pain, diarrhea).

Exclusion criteria were: diagnosis of CFRD and/or insulin therapy; clinical history of pulmonary exacerbation and/or symptoms of acute infection and/or changes in antibiotics and/or steroids in the 6 weeks preceding the study enrollment; therapy with CFRD modulators; severe liver disease; liver and/or pulmonary transplantation. Informed consent was obtained from children, adolescents, and their parents. The protocol was approved by the Institutional Ethics Committees of the 2 participating Centers (Verona and Napoli, Italy).

Physical characteristics

At the time of the study enrollment, all study participants underwent a physical examination with the collection of anthropometric measurements (height and weight). Body mass index (BMI) values were standardized calculating age and gender-specific BMI percentiles using WHO child growth standards (17). Genetic data about CF related genotypes were collected from the revision of patients' clinical charts.

Oral glucose tolerance test

All participants underwent a standard OGTT (1.75 g/kg, max 75 g) at 8:00 AM, after overnight fasting. Blood samples for measuring plasma glucose, serum insulin, and C-peptide concentrations were taken at baseline and at times +30, +60, +90, and +120 minutes. A prolonged OGTT was performed in a subgroup of 54 subjects, with sampling at times of −10, 0, +10, +20, +30, +45, +60, +90, +120, +150, and +180 minutes. The same mathematical model (see below) can be applied to both OGTTs; since the latter provides a richer data set, the model-derived parameters of beta-cell function are estimated with greater precision.

Plasma glucose, insulin, and C-peptide levels were analyzed using standard procedures at the central laboratories of the 2 participating Centers. In particular, plasma glucose was measured with the glucose oxidase method. Insulin and C-peptide levels were analyzed by enzyme-immunoassay (Mercodia AB, Sweden).

Both laboratories belong to the Italian National Health System and are certified according to International Standards ISO 9000 (www.iso9000.it/), which involves undergoing semi-annual quality controls and inter-laboratory comparisons.

According to the current guidelines (1), participants were classified as having one of the following glucose tolerance stages: (i) normal glucose tolerance (NGT: fasting blood glucose [FPG] ≤126 mg/dL [≤7 mmol/L], 2-hour and mid-OGTT glucose level <140 mg/dL [<7.7 mmol/L]); (ii) indeterminate glucose tolerance (INDET: FPG ≤126 mg/dL [≤7 mmol/L], 2-hour glucose <140 mg/dL [<7.7 mmol/L] but OGTT glucose ≥200 mg/dL [≥11.1 mmol/L] at any mid-time between +30 and +90 minutes of the test); (iii) impaired glucose tolerance (IGT: 2-hour glucose level ≥140 [≥ 7.7 mmol/L] and <200 mg/dL [<11.1 mmol/L]); (iv) diabetes (CFRD: 2-hour glucose level ≥200 mg/dL [≥ 11.1 mmol/L]), with and without fasting hyperglycemia. In addition, patients with mid-OGTT glucose values ≥140 mg/dL (≥ 7.7 mmol/L) and <200 mg/dL (<11.1 mmol/L), who otherwise would be left out of the above classification, were defined as having abnormal glucose tolerance 140 (AGT140). This threshold was chosen by translating the IGT definition based on 2-hour glycemia to mid-OGTT values, analogous with the definition of INDET status based on the cutoff of 200 mg/dL used at 2 hours for CFRD diagnosis.

In addition, the glycated hemoglobin A1c (HbA1c) value was measured by high-performance liquid chromatography and standardized to the Diabetes Control and Complications Trial (DCCT) normal range (4.0%-6.0%, 20-42 mmol/mol) in the same day of the OGTT.

Assessment of the Determinants of Glucose Regulation during the OGTT

Beta-cell function and insulin clearance were reconstructed by mathematical modeling, as previously described (15),

with the aid of the SAAM 1.2 software (SAAM Institute, Seattle, WA) (18).

By this method, beta-cell function is described as the sum of 2 components:

1) Derivative (or Dynamic) Control (DC): Describes the sensitivity of beta-cells to the rate of increase of glucose concentration and it is quantified as the amount of insulin secreted in response to a rate of increase in glucose concentration of 1 mmol/liter per minute, occurring at the beginning of the OGTT and lasting for 1 minute [units: (picomoles × m^{-2} of body surface area) × (millimoles $^{\times}$ liter $^{-1}$ × minute $^{-1}$) $^{-1}$].
2) Proportional (or Static) Control (PC): Describes the sensitivity of beta-cells to glucose concentration; in this paper, it is presented as the steady-state stimulus-response curve relating insulin secretion rate (y-axis; units: picomoles × minute $^{-1}$ × m^{-2} of body surface area) to glucose concentration (x-axis; units: millimoles × liter $^{-1}$).

The derivative/dynamic component and the proportional/static component of this model account for first-phase insulin secretion and second-phase insulin secretion, respectively, during intravenous glucose administration, as previously demonstrated (19, 20).

Insulin clearance was computed according to the following formula: [$Clearance_{Ins} = AUC_{ISR}/[AUC_I + (I_{Final}-I_{Basal}) \times MRT_{Ins}]$ in which AUC_{ISR} is the area under the curve of insulin secretion rate (computed by the model), AUC_I is the area under the curve of insulin concentration, I_{Final} is insulin concentration at the end of the OGTT, I_{Basal} is insulin concentration at time 0′, MRT_{Ins} is the mean residence time of insulin, which was assumed to be 27 minutes in subjects with diabetes and 18 minutes in subjects without diabetes, as previously reported (21).

Insulin sensitivity was assessed by the Oral Glucose Sensitivity Index (OGIS) (22). We selected OGIS for several reasons. This index was derived from modeling analysis of the glucose-insulin relationship, based on established principles of glucose kinetics and insulin action, and it estimates insulin sensitivity as measured during a euglycemic insulin clamp (ie, the increase in glucose clearance brought about by an increase in insulin concentration). OGIS was validated with the euglycemic insulin clamp in both lean and obese nondiabetic subjects and in subjects with type 2 diabetes, thereby providing a robust prediction of insulin sensitivity (22). Additional advantages of this index are its simplicity and the possibility of being widely used for clinical investigation.

Several fasting and OGTT-derived biomarkers of insulin sensitivity/resistance and of beta-cell function also were computed:

- Homeostasis model assessment insulin resistance (HOMA-IR), as a marker of insulin resistance based on fasting glycemia and insulin: [(Insulin0′ (mU/L) × Glucose0′ (mmol/L))/22.5] (23);
- Insulinogenic index (IGI), as a marker of early insulin bioavailability in response to oral glucose: [insulin30′ (mU/L) – insulin0′ (mU/L)]/[glucose30′ (mg/dL) – glucose0′ (mg/dL)] (24);
- Matsuda index, as a marker of postprandial insulin sensitivity: 10 000/[(Glucose 0′ (mg/dL) × Insulin0′ (mU/L)) × (mean OGTT glucose concentration (mg/dL)) × (mean OGTT insulin concentration (mU/L))]1/2 (25);
- Oral disposition index (DI), a popular marker of the adequacy of insulin bioavailability to the prevailing insulin sensitivity: Matsuda index × IGI (26).

Statistical analysis

Patients' characteristics are reported as mean ± SD, unless otherwise specified. The Kolmogorov-Smirnov test was used to assess normal distribution of variables and variables with positively skewed distribution were log-transformed. The chi-squared test for categorical variables (ie, gender and genotype distribution) was used to test differences among OGTT categories. Generalized Linear Models, with or without repeated measures as appropriate, were run with modeling-derived beta-cell function indices and OGTT-derived indices as dependent variables and glucose tolerance stages as factors. For each index, the analysis was run entering as covariates the potential confounders, such as gender, age, and BMI z-score. Post hoc least square difference (LSD) analyses were performed to assess pairwise differences across categories.

A P value < 0.05 was considered as statistically significant. All the analyses were performed using SPSS v.22.0 (SPSS, USA, Chicago IL).

Results

Clinical and metabolic characteristics of study participants categorized according to glucose tolerance stages are shown in Table 1. According to the OGTT results, 51 subjects (22.0%) had NGT, 85 (36.6%) patients had AGT140, 19 (8.2%) had INDET, 49 (21.1%) had IGT, and 28 (12.1%) had newly diagnosed CFRD. The distribution of the 5 stages of glucose regulation was similar in the 2 Centers. Glucose, C-peptide, and insulin values measured during the OGTT of study participants categorized according to glucose tolerance stages are available in the Supplemental material (27). As expected, the comparison of glucose values measured at times +30, + 60 and + 90 minutes showed significant

differences between all glucose tolerance stages, when each compared with each other (all $P < 0.001$), whereas at baseline and at +120 minutes, some stages were superimposable to each other (baseline glucose values: NGT vs AGT140, $P = 0.131$; AGT140 vs INDET, $P = 0.089$; INDET vs IGT, $P = 0.899$; glucose values at +120 minutes: NGT vs AGT140, $P = 0.042$; AGT140 vs INDET, $P = 0.62$).

No significant differences in age, BMI z-score, gender, and genotypes distribution were found between the 5 subgroups (all $P > 0.05$). Age was significantly higher in patients with CFRD compared with NGT patients ($P = 0.026$), whereas no significant differences were found comparing age between the other glucose categories subgroups (all $P > 0.05$).

The data for beta-cell function are shown in Fig. 1.

Beta-cell DC was highest in NGT, fell by more than 50% in AGT140 and IGT ($P < 0.01$ vs NGT), which, however, were comparable to each other, and by about 85% in INDET and CFRD ($P < 0.01$ vs AGT140 and IGT), which were similar to each other (Fig. 1A).

Beta-cell PC was highest in NGT, fell by about 36% in AGT140 ($P < 0.001$ vs NGT), by about 55% to 58% in IGT and INDET (IGT vs AGT140, $P < 0.001$; INDET vs AGT140, $P = 0.004$), which were superimposable to each other ($P = 0.706$). A further decrease of about 77% was found in CFRD patients, with significant differences with respect to IGT ($P < 0.001$) and INDET ($P < 0.001$) (Fig. 1B).

Insulin sensitivity, as assessed by OGIS, was highest in NGT, fell by only 9% in AGT140 ($P < 0.01$ vs NGT), by about 13% to 15% in IGT and INDET (IGT vs AGT140, $P < 0.01$; INDET vs AGT140, $P = 0.101$), and by about 20% in CFRD, falling short of statistical significance (CFRD vs IGT, $P = 0.079$; CFRD vs INDET, $P = 0.091$) (Fig. 2A).

Insulin clearance ranged between the highest value of 1.22 ± 0.56 $L \cdot min^{-1} \cdot m^{-2}$ body surface area [BSA] in NGT and the lowest figure of 0.99 ± 0.28 $L \cdot min^{-1} \cdot m^{-2}$ BSA in INDET, but on the whole, the changes fell short of statistical significance ($P = 0.162$) (Fig. 2B).

We qualitatively summarized the above findings by applying an arbitrary score to the severity of the defects observed in the 5 stages of glucose regulation, as shown in Table 2. Insulin clearance, apparently, played no major role. Both beta-cell DC and insulin sensitivity displayed 2 layers of defect, whereas beta-cell PC showed 3 layers of defects. Importantly, each class of glucose regulation was uniquely identified by the combination of the defects it displays. Furthermore, beta-cell data were necessary and sufficient to identify each of the 5 stages of glucose regulation.

The fasting- and the OGTT-derived surrogate indexes of insulin secretion and action failed to reproduce this picture of graded functional defects, demonstrating less pathophysiologic discriminant power for the 5 categories of glucose regulation (27).

Discussion

In this study, 3 direct determinants of glucose regulation, ie, beta-cell function, insulin clearance, and insulin sensitivity, were assessed simultaneously for the first time by model-based analyses of the C-peptide, insulin, and glucose curves during the OGTT in a large sample of children, adolescents, and young adults with CF. None of the past studies, even those that investigated insulin secretion components and insulin sensitivity in CF patients through

Table 1. Clinical and Metabolic Characteristics of the Study Subjects Categorized According to Glucose Tolerance Stages

Variables	NGT (n = 51)	AGT140 (n = 85)	INDET (n = 19)	IGT (n = 49)	CFRD (n = 28)	Total (n = 232)
Age, years	18.1 ± 6.9	20.6 ± 7.5	18.4 ± 5.7	18.7 ± 6.4	21.3 ± 5.9	19.6 ± 5.4
Gender (M/F), n (%)	24 (47.1) / 27 (52.9)	47 (55.3) / 38 (44.7)	12 (63.2) / 7 (36.8)	23 (46.9) / 26 (53.1)	17 (60.7) / 11 (39.3)	123 (53) / 109 (47)
ΔF508 homozygosis n (%)	6 (11.8)	22 (25.9)	7 (36.8)	15 (30.6)	6 (21.4)	56 (24.1)
ΔF508 heterozygosis, n (%)	25 (49.0)	42 (49.4)	9 (47.4)	21 (42.9)	16 (57.2)	113 (48.7)
Other mutations, n (%)	20 (39.2)	21 (24.7)	3 (15.8)	13 (26.5)	6 (21.4)	63 (27.2)
Height (Z-score)	−0.50 ± 0.98	−0.40 ± 0.91	−0.84 ± 0.86	−0.28 ± 0.88	−0.52 ± 0.74	−0.34 ± 0.91
Weight (Z-score)	0.20 ± 0.83	−0.33 ± 1.08	−0.63 ± 0.99	−0.65 ± 1.16	−0.73 ± 0.98	−0.46 ± 1.07
BMI, $kg \times m^{-2}$	20.3 ± 3.8	20.4 ± 4.1	20.2 ± 2.8	19.2 ± 3.3	20.6 ± 3.0	20.2 ± 3.7
BMI z-score	−0.06 ± 1.16	−0.15 ± 1.61	−0.12 ± 0.82	−0.41 ± 1.18	−0.52 ± 1.09	−0.40 ± 1.33
HbA1c, mmol/mol	38.0 ± 3.7	38.0 ± 4.01	39.2 ± 3.1	40.2 ± 4.3	43.0 ± 7.7	40.1 ± 5.5
HbA1c, %	5.62 ± 0.33	5.60 ± 0.36	5.75 ± 0.29	5.83 ± 0.39	6.10 ± 0.71	5.81 ± 0.50

Data are expressed as mean ± standard deviation, unless otherwise specified.

Abbreviations: AGT140, abnormal glucose tolerance 140; BMI, body mass index; CFRD, cystic fibrosis–related diabetes; INDET, indeterminate glucose tolerance; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

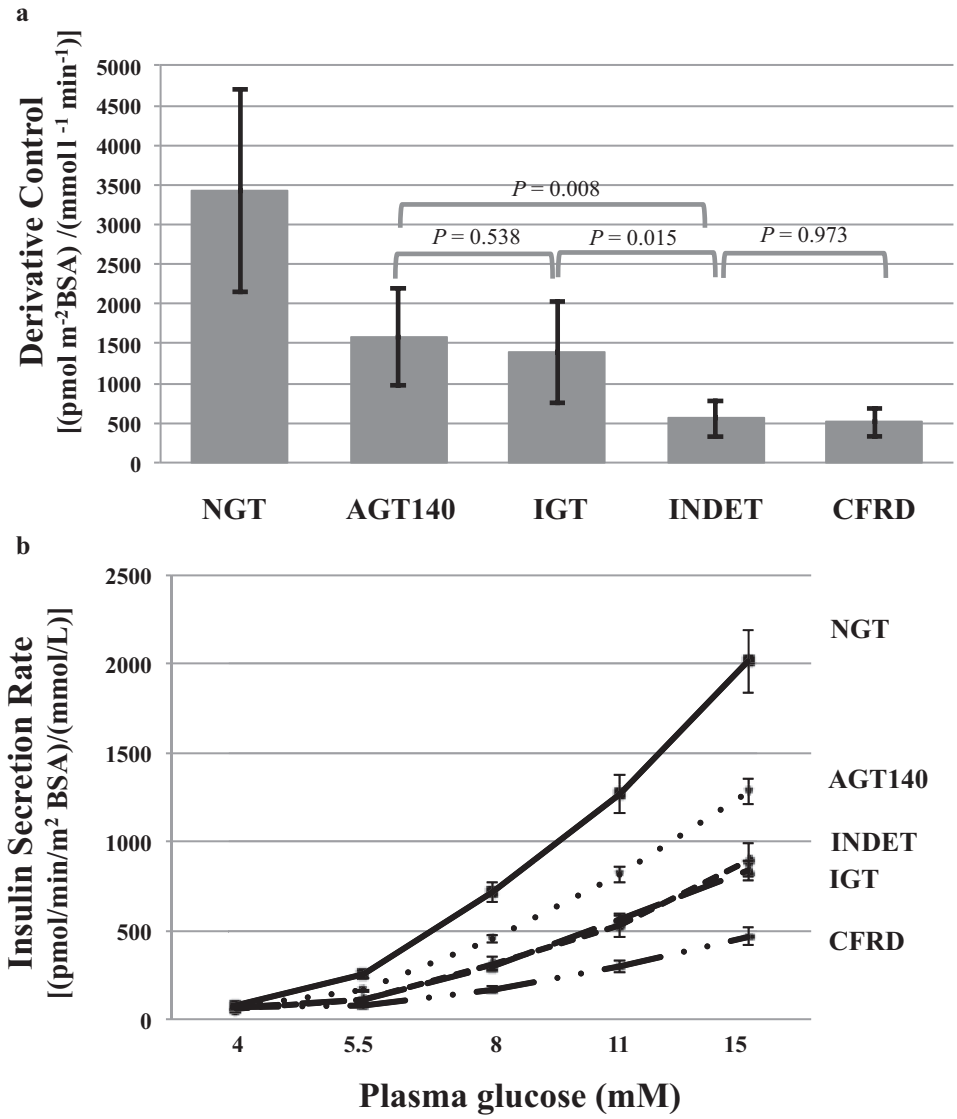

Figure 1. Derivative (a) and Proportional control (b) according to glucose tolerance stages. Proportional control is represented through insulin secretion rate values at growing glucose concentrations. The *P* values refer to the results of post hoc least square difference (LSD) analyses performed to assess pairwise differences between pairs of glucose tolerance classes. Abbreviations: AGT140, abnormal glucose tolerance 140; BSA, body surface area; CFRD, cystic fibrosis–related diabetes; INDET, indeterminate glucose tolerance; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

OGTT-derived indices and mathematical models (11, 12, 28-30), reconstructed these 3 components at the same time. In addition, for the sake of comparison with many earlier studies, we also report several surrogate indexes of insulin secretion and sensitivity.

Earlier studies reported that the primary glucometabolic impairment in CF involve insulin bioavailability, which is delayed and characterized by an attenuated peak compared with the healthy population, whereas insulin sensitivity were shown not to be compromised in clinically stable CF patients. Insulin clearance was not assessed, leaving unanswered the question whether any clearance-based compensation is at work in CF patients. Furthermore, complete data in each class of glucose tolerance (ie, NGT, INDET, IGT, and CFRD) are missing and it is unknown whether there is a specific pattern of defects for each class (16). In addition, an emerging class of impaired glucose regulation, ie, AGT140, was introduced recently in CF patients and it is unclear whether it is characterized by distinct pathophysiologic bases.

Thus, to the best of our knowledge, our study is the first attempt to answer the question whether the 5 recognized stages of glucose regulation in CF patients (NGT, AGT140, INDET, IGT, and CFRD) each display a specific combination of presence or absence of defects in the 3 direct determinants of glucose homeostasis assessed in the present study.

We report several novel results.

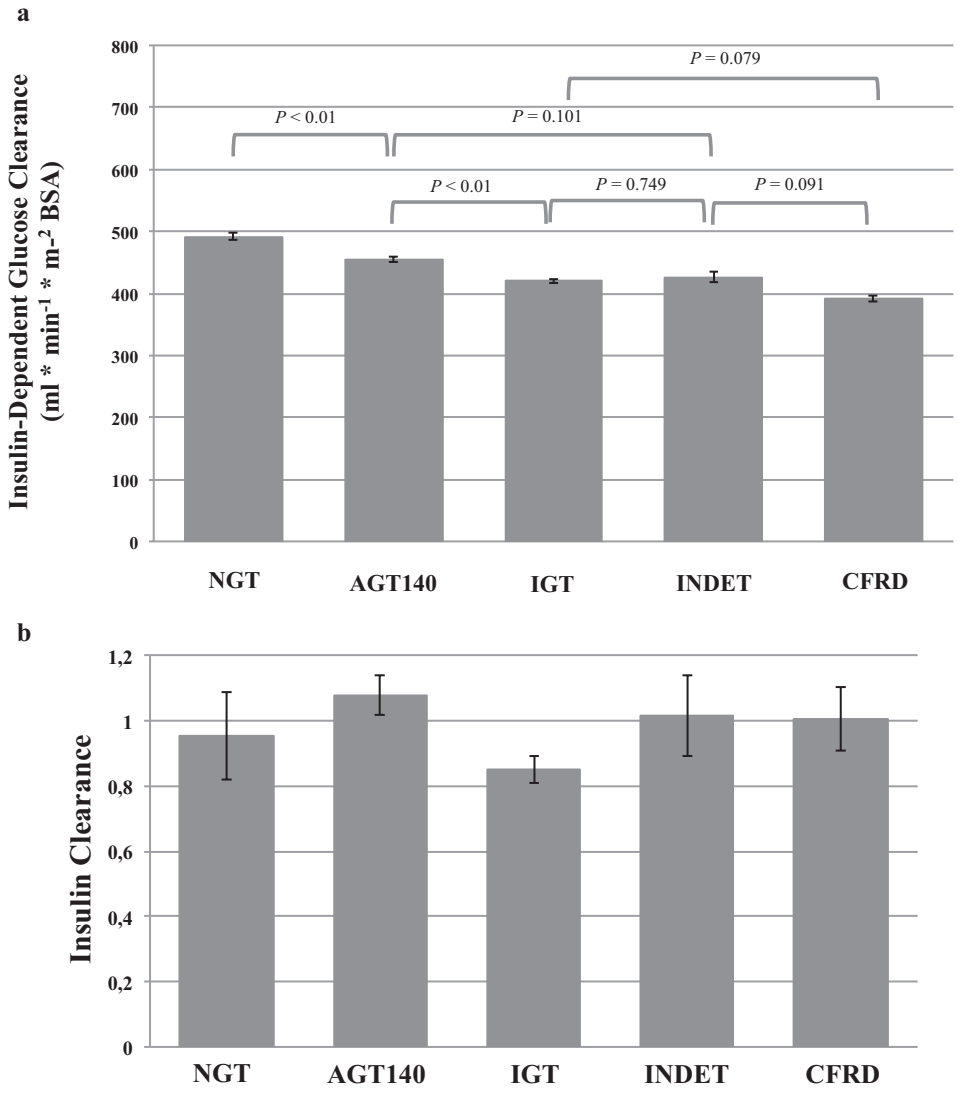

Figure 2. Insulin sensitivity (glucose clearance during a euglycemic insulin clamp) (a), as predicted by OGIS, and Insulin Clearance (b) during the OGTT across the 5 stages of glucose tolerance in patients with cystic fibrosis. The *P* values refer to the results of post hoc least square difference (LSD) analyses performed to assess pairwise differences between pairs of glucose tolerance classes.

First, even patients with early elevation of mid-OGTT glucose, defined as AGT140, have significantly worse DC and PC of beta-cell function and lower insulin sensitivity compared with NGT patients. Current guidelines do not call attention to these early abnormal glucose tolerance stages defined by mid-OGTT glucose values between 140 mg/dL (7.8 mmol/L) and 200 mg/dL (11.1 mmol/L). Thus, patients with this glycemic profile are usually considered to have normal glucose tolerance, but they show already detectable pathophysiologic alterations of glucose regulation.

Second, IGT patients, when compared with the AGT140 group, display a further decline in the PC of beta-cell function and in insulin sensitivity. The latter finding was not reported previously and, at variance with the current dogma, highlights the role played by insulin resistance in the early phases of the deterioration of glucose homeostasis in CF patients.

Third, INDET patients, in spite of being able to achieve lower than 140 mg/dL glucose concentration at 120 minutes in the OGTT, display identical defects as the IGT patients, but for DC of beta-cell function, which actually is worse than in IGT. As in other studies, the percentage of INDET patients was small, but the number of patients reported in this paper is greater than in previous studies, lending credence to our findings. Thus, INDET might be a condition of highest vulnerability to a further decline toward CFRD. Analogous to similar conditions, considering the small number of INDET patients usually reported, one might speculate that they belong to the subgroup of patients with particularly rapid evolution toward

Table 2. Arbitrary Scores of the Severity of the Defects of the 3 Direct Determinants of Glucose Regulation in NGT, AGT140, IGT, INDET, and CFRD Patients With Cystic Fibrosis

	Defect severity			
	Beta-cell function		Insulin sensitivity	Insulin clearance
	Derivative control	Proportional control		
NGT	0	0	0	0
AGT140	+	+	+	0
IGT	+	++	++	0
INDET	++	++	++	0
CFRD	++	+++	++	0

Abbreviations: AGT140, abnormal glucose tolerance 140; CFRD, cystic fibrosis–related diabetes; INDET, indeterminate glucose tolerance; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

diabetes mellitus. Further, prospective studies are needed to clarify this point.

Fourth, CFRD when compared with IGT and INDET, is hallmarked by a further decline in PC of beta-cell function. This additional defect appears to be the one heralding the onset of diabetic glucose intolerance. This result, although obtained via OGTT and not via the hyperglycemic clamp, is very similar to what has been previously reported in obese children/adolescents, in whom the decline in the PC of beta-cell function during the hyperglycemic clamp, but not the DC of beta-cell function nor insulin sensitivity, was the trait that differentiated children with type 2 diabetes from those with impaired glucose tolerance (31).

Fifth, insulin clearance seems to play a minor, if any, role in the pathophysiology of the alterations of glucose regulation in CF (Fig. 2). In spite of significantly graded increases in insulin resistance with worsening of glucose tolerance, this was not accompanied by statistically significant decreases in insulin clearance. Thus, this potentially relevant mechanism of compensation apparently is not at play in CF patients with glucose intolerance and might add to the deterioration in glucose homeostasis. Candidate mechanisms for the scant compensation of insulin resistance by insulin clearance in CF patients are unknown.

To date, only 2 cross-sectional studies evaluated pediatric patients with CF with design and aim similar to our study (12, 16). Battezzati et al studied 165 patients with CF, 15% of whom had glucose intolerance and 9% had diabetes, with tools similar to those employed in the present paper. They found that both the parameters of beta-cell function and insulin sensitivity showed a decline across the spectrum of glucose tolerance. However, in their analysis they divided the patients by quartiles of mean glucose during the OGTT, and no information eventually is provided about the recognized stages of glucose regulation in CF. No data of insulin clearance are provided. Thus, their analysis does not address the pathophysiological bases of the stages of glucose intolerance/diabetes in CF (12).

Nyrjesy et al performed mixed-meal tolerance tests and intravenous glucose-potentiation arginine tests in 42 CF patients to measure postprandial glucose tolerance and beta-cell secretory capacity. They documented a graded fall in the parameters of beta-cell function across a spectrum of conditions, which included NGT, IGT, and CFRD (16). However, they studied neither AGT140 nor INDET patients, but patients with a peak glucose >155,mg/dL, a class of glucose intolerance translated from the non-CF adult subject to children. Thus, this study provided no direct data of beta-cell function in AGT140 and/or INDET patients, and it reported no data regarding insulin sensitivity and/or insulin clearance. Thus, also in this case the applicability to the recognized stages of glucose intolerance is questionable. In spite of these limitations, this was one of the first evidence of a significant alteration of beta-cell function in patients with CF with early mid-OGTT glucose alterations.

Our results suggest that the definition of an additional class of glucose regulation based only on early elevation of mid-OGTT glucose values is nowadays necessary, as previously done in 2010 with the definition of INDET glucose tolerance status. In addition to the relevance for glucose metabolism, 2 previous studies support our contention by documenting the association between early elevation of mid-OGTT glucose values and worse clinical course of CF. One cross-sectional study demonstrated that in pediatric CF patients, higher early glucose peak was significantly associated with a decline in both body weight and pulmonary function indices in the year preceding the OGTT (7). One 5-year retrospective cohort study focused on high vs low 1-hour plasma glucose (the threshold being 160 mg/dL) and reported a 4-fold higher risk of CFRD in the patients with high 1-hour glucose (9).

Our study also included the evaluation of OGTT-derived surrogate indices of insulin secretion and insulin sensitivity easily computed in clinical practice. Overall, they are unable to reconstruct the exact architecture of the severity of the defects in beta-cell function and insulin sensitivity herein reported. Furthermore, on the basis of our results (Table 2), the classification itself in the 5 stages of glucose intolerance can be used as a surrogate index to infer the severity of defects in beta-cell function and insulin sensitivity of CF patients.

This study has some limitations: (i) it was conducted in subjects with European ancestry, thus the results cannot necessarily be extended to CF children and youths with other ethnic backgrounds; (II) the cross-sectional design may not enable assessment of causality between

variables rather than just associations; (iii) no vector plots of insulin bioavailability per glucose stimulus vs insulin sensitivity nor the inverse relationship between insulin clearance and insulin concentration were investigated, thereby leaving unexplored these in-depth aspects of the direct determinants of glucose regulation; (iv) each patient was evaluated during a period of stable clinical condition, but limited data regarding the severity of CF and the pharmacologic—in particular steroid—therapy were collected, thereby leaving unexplored the potential impact of these factors on the determinants of glucose regulation.

In summary, when all 3 direct determinants of glucose regulation investigated in this study are assessed in CF patients, each of the 5 stages of glucose dysregulation shows a distinct, unique pattern of defects and, therefore, unique pathophysiology. In particular, the patients with 1-hour glucose values between 140 and 199 mg/dL (AGT140) have a significant impairment of beta-cell function and insulin sensitivity when compared with NGT patients. Although defects in insulin sensitivity are present and play a role in the glucose dysregulation of CF patients, the alterations in beta-cell function are sufficient to discriminate the 5 stages of glucose tolerance from each other, thereby implying a major causative role played by beta-cell dysfunction.

Our results suggest reconsideration of the grades in the ladder of glucometabolic deterioration of CF patients. The ISPAD guidelines define IGT as a worse class of glucose intolerance than INDET, identifying a progression that starts from the intermittent postprandial glucose and ends with CFRD. However, neither pathophysiologic nor longitudinal data support this contention. Our study, the first to thoroughly and simultaneously investigate 3 direct pathophysiologic determinants of glucose regulation, places IGT in an intermediate position between AGT140 and INDET, and highlights the role played by insulin resistance in the glucometabolic alterations of CF patients. In addition, our findings support the relevance of diagnosing AGT140 in clinical practice as a new class of glucose intolerance in order to improve the stratification of metabolic risk in CF patients.

Prospective studies are required to further corroborate our findings and to evaluate their implications for specific outcomes related to CF clinical course, such as nutritional status, lung function, and CF exacerbations, and to assess the possible benefits of new therapeutic interventions targeting early glucose abnormalities such as AGT140.

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Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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