Age- and Sex-Dependent Distribution of OGTT-Related Variables in a Population of Cystic Fibrosis Patients

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Context: Cystic fibrosis (CF) causes an exceptionally high prevalence of diabetes that increases with age, especially in females. The glucose tolerance defect is progressive, but a cystic fibrosis transmembrane conductance regulator-dependent insulin secretory defect cannot be excluded. The age and sex dependence of the secretory defect is unclear.

Objective: The objective of the study was to analyze the age and sex dependency of insulin secretory and sensitivity parameters in CF.

Design: This was a cross-sectional analysis in an observational ongoing cohort (mean follow-up duration 7.5 y).

Setting: The study was conducted at the CF Center of Milan.

Patients: The study included 187 patients aged 8-30 years.

Intervention: Interventions included 3-hour oral glucose tolerance tests (n = 478) with 30-minute insulin and c-peptide sampling.

Main Outcome Measures: Model-derived insulin secretory and sensitivity parameters were measured.

Results: Age was associated with a progressive decrement in insulinemia (at 30 min) and a subsequent increment in glycemia (at 60–90 min), returning at or below baseline (at 180 min). These changes are explained by a progressive reduction in β -cell sensitivity to glucose and a progressive increment in insulin clearance. Fasting and postprandial insulin sensitivity do not seem to be involved. Compared with males, females display higher glucose, insulin, and c-peptide responses with greater insulin secretion, β -cell sensitivity to glucose, insulin clearance, and equal insulin sensitivity.

Conclusions: A defect in β -cell sensitivity to glucose progressively develops with age, but it is not sex specific and does not explain the worse glucose tolerance reported in females. In contrast, insulin clearance increases with age, especially in females, contributing to the deterioration in glucose tolerance. The effects of age and sex should be considered when evaluating oral glucose tolerance test results in CF patients. (*J Clin Endocrinol Metab* 100: 2963–2971, 2015)

ISSN Print 0021-972X ISSN Online 1945-7197 Printed in USA Copyright © 2015 by the Endocrine Society Received February 25, 2015. Accepted June 2, 2015. First Published Online June 9, 2015 Abbreviations: AUC, areas under the OGTT curve; CF, cystic fibrosis; CFRD, cystic fibrosisrelated diabetes; CFTR, cystic fibrosis transmembrane conductance regulator; HOMA, homeostasis model assessment from basal levels; IGI, insulinogenic index; OGIS, OGTT-based index of insulin sensitivity; OGTT, oral glucose tolerance testing.

vstic fibrosis-related diabetes (CFRD) is the most fre- quent comorbidity of cystic fibrosis (CF) in patients who survive to adulthood (1, 2). Its prevalence increases with age, ranging from 2% in children to 19% in adolescents and 40%-50% in adults (3). This age dependency may imply that the causal or precipitating factors are worsening with age, although significant derangements in glucose tolerance have been reported already during the first decade of life (4, 5). Treatment with the cystic fibrosis transmembrane conductance regulator (CFTR) potentiator ivacaflor in CF patients carrying the G551D mutation has been reported to improve glucose tolerance (6, 7). Dysglycemia may therefore be intrinsic to CF and possibly congenital, which is somewhat counterintuitive, given the progressive course of glucose intolerance and CFRD shown in epidemiological studies. This issue is presently unclear because data exploring the age dependence of glucose tolerance in CF are presently limited.

Furthermore, sex differences in CFRD prevalence and outcomes have been reported (8) and debated (9-11), but their underlying mechanisms are unknown as well. A recent study was the first to show that CF women have a greater insulin secretory response to oral glucose (12), in contrast with the hypothesis postulating that their higher risk of developing CFRD is consequent to lower insulin response.

Large-scale assessment of CFRD determinants, ie, impaired β -cell secretory function and deranged insulin sensitivity (2), has been so far precluded by methodological issues. Even if these variables have been measured with a variety of techniques (2), their association with age and sex remains unclear, and reference values for CF patients are lacking, thus precluding interpretation of CFRD pathogenesis, natural history, prognosis, and adverse consequences.

Oral glucose tolerance testing (OGTT) is a good choice for longitudinal studies of CFRD, also because it should be performed annually according to most scientific societies (13–15). Simple modifications of the OGTT sampling protocol, including glucose, insulin, and c-peptide measurements, may produce valuable information beyond the assessment of glucose tolerance, ie, quantification of insulin sensitivity and measurement of several parameters related to insulin secretion and β -cell function (16, 17).

Over the last 10 years, we have extensively implemented this protocol at the CF Center of Milan (Milan, Italy) and found that the main defect related to glucose intolerance is β -cell dysfunction and specifically the sensitivity of insulinsecretion to a given glycemic stimulus (18). Although this parameter appeared to decline with age, in most of the patients, it was below the range found in normal subjects at any age and for each class of glucose tolerance.

The aim of the present study is to describe the age- and sex-related differences in the quantitative measures of the

determinants of glucose tolerance, ie, insulin sensitivity and secretory function, and to provide an age- and sexadjusted reference. The data presented here include insulin secretory and sensitivity parameters obtained by modeling OGTT data.

Research Design and Methods

Subjects

All CF patients aged older than 8 years and regularly followed up at our center with clinical and laboratory assessment, including an annual OGTT, were offered to participate in this study between 2003 and 2012. During a follow-up visit as outpatients, those who consented underwent a modified OGTT as described below and were included in the present study if they had been clinically stable in the previous 3 weeks (absence of major clinical events including pulmonary exacerbations, no change in their habitual treatment regimen including introduction of antibiotics or steroids) and if they had not received a CFRD diagnosis or treatment with insulin or oral hypoglycemic agents in the previous 6 months.

Table 1. Characteristics of the Study Subjects at Timeof Their First OGTT

Characteristics	Values
Subjects, n, %	187 (100)
Age, y, median (IQR)	15 (12; 19)
Male gender, n, %	93 (49.7)
CFTR mutations	
δF508 homozygous, n, %	55 (29.4)
δF508 heterozygous, n, %	72 (38.5)
Other, n, %	36 (19.3)
Unknown, n, %	24 (12.8)
Pancreatic insufficiency, n, %	146 (78.1)
Liver disease, n, %	46 (24.6)
Pseudomonas aeruginosa, n, %	112 (59.9)
<i>Burkholderia cepacia</i> complex, n, %	8 (4.3)
Liver transplantation, n, %	3 (1.6)
Lung transplantation, n, %	10 (5.4)
Permanent O_2 therapy, n, %	4 (2.1)
Height, SDS, median (IQR)	-0.60 (-1.25; -0.07)
Weight, SDS, median (IQR)	-0.46 (-1.22; 0.07)
BMI, SDS, median (IQR)	-0.30 (-0.99; 0.35)
CRP, mg/dL, median (IQR)	5 (1; 6)
HbA1C, %, median (IQR)	5.7 (5.4; 6)
HbA1C, mmol/mol, median (IQR)	39 (36; 42)
FEV1, % of predicted, median (IQR)	88 (72; 101)
FVC, % of predicted, median (IQR)	94 (81; 104)
Glucose tolerance	
Normotolerant, n, %	133 (/1.1)
Glucose intolerant, n, %	30 (16.1)
Diabetes without fasting	/ (3./)
hyperglycemia, n, %	
Diabetes with fasting	2 (1.1)
hyperglycemia, n, %	
Undetermined glycemia,	15 (8.0)
n, %	

Abbreviations: BMI, body mass index; CRP, C-reactive protein; FEV, forced expiratory volume; FEV1, forced expiratory volume expired in 1 second; HbA1C, glycated hemoglobin; IQR, interquartile range.

Overall, 187 patients (93 males) were enrolled and underwent a total of 478 studies of OGTT; 62 patients underwent only one test, whereas 48, 34, 17, and 26 contributed 2, 3, 4, and 5 or more OGTTs, respectively. Main characteristics of the study subjects at the time of their first OGTT are reported in Table 1.

The study protocol was approved by the local ethical committee. Patients were informed about the purpose of the study and gave permission to include their clinical and laboratory data in this research.

Oral glucose tolerance test

All subjects received a 3-hour OGTT (1.75 g/kg, maximum 75 g) sampling at baseline, and at 30-minute intervals, the subjects had determinations of plasma glucose, serum insulin, and C-peptide concentrations. Based on the plasma glucose concentrations, patients were assigned to one of the following categories of glucose tolerance (13, 19): normal, impaired, diabetes without fasting hyperglycemia, diabetes with fasting hyperglycemia, and undetermined glycemia.

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 (Table 2)

 β -Cell function was assessed from the OGTT as previously illustrated in detail (20, 21), using a model that describes the relationship between insulin secretion and glucose concentration. The model expresses insulin secretion as the sum of two components. The first component represents the dependence of insulin secretion on absolute glucose concentration at any time point during the OGTT through a dose-response function relating the two variables. Characteristic parameter of the dose-response is the mean slope over the observed glucose range, denoted as β -cell glucose sensitivity. The dose-response is modulated by a potentiation factor, which accounts for the fact that during an acute stimulation insulin secretion is higher in the descending phase of hyperglycemia than in the ascending phase at the same glucose concentration. As such, the potentiation factor encompasses several potentiating mechanisms including prolonged exposure to hyperglycemia, nonglucose substrates, gastrointestinal 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-hour OGTT (17). To quantify this excursion, we calculated the ratio between the 2-hour and the baseline value. This ratio is denoted as potentiation ratio. The second insulin secretion component represents the dependence of insulin secretion on the rate of change of glucose concentration. This component is termed the derivative component and is determined by a single parameter, denoted as rate sensitivity. Rate sensitivity is related to early insulin release (17).

The parameters of the model were estimated from glucose and c-peptide concentrations by regularized least squares, as previously described (20). Regularization involves the choice of smoothing factors that were selected to obtain glucose and c-peptide model residuals with SDs close to the expected measurement error (~1% for glucose and ~4% for c-peptide). Insulin secretion rates were calculated from the model every 5 minutes. The integral of insulin secretion during the 3-hour OGTT was denoted as total insulin output.

Insulin clearance was calculated in the fasting state as the ratio between fasting insulin secretion and fasting insulin concentration and during the OGTT as the ratio between the integral of insulin secretion and that of insulin concentration.

The insulinogenic index (IGI), a commonly used index of β -cell function, was also calculated from the OGTT data: IGI = δ insulin (0–30 min) expressed in microunits per milliliter⁻¹ divided by the δ glucose (0–30 min) in milligrams per deciliter⁻¹.

	1								
Parameter	Unit	Description							
Insulin secretion	Picomoles per minute ⁻¹ per meter ⁻²	Pancreatic insulin secretion, calculated from c-peptide deconvolution and normalized to estimated body surface area							
Basal insulin secretion	Picomoles per minute ⁻¹ per meter ⁻²	Insulin secretion before the start of the OGTT							
Total insulin secretion β -Cell dose response	Nanomoles per meter ⁻²	Integral of insulin secretion during the whole OGTT Model-determined relationship between glucose concentration and insulin secretion							
β-Cell glucose sensitivity	Picomoles per minute ⁻¹ per meter ⁻² per millimole ⁻¹	Average slope of the eta -cell dose response (δ insulin secretion/ δ glucose)							
Rate sensitivity	Picomoles per meter ⁻² per millimole ⁻¹	Parameter quantifying early insulin secretion during the OGTT. The model assumes that when glucose is increasing, insulin secretion is augmented by a term proportional to the rate of change of glucose concentration (hence rate sensitivity)							
Potentiation factor	Dimensionless	A time-dependent parameter that accounts for the fact that insulin secretion is higher at the end of the OGTT compared with the beginning, at the same glucose level							
Potentiation ratio	Dimensionless	Ratio between the potentiation factor value at 2 hours and that at time zero, quantifying the potentiation factor excursion. A value of, eg, 1.5, means that, for the same glucose, insulin secretion is 1.5-fold higher at 2 hours compared with baseline							

Table 2. Summary of the β -Cell Model Parameters

	0 Minutes				30 Minutes			60 Minutes			90 Minutes			120 minutes			180 minutes		
	P25	P5	50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75
Glucose, mg/dL	1																		
Male	-1.5	0.	1	-0.0	-4.0	-7.0	-3.8	-3.6	-2.0	-2.0	-9.9	-10.7	-7.6	-10.2 ^a	-11.9 ^a	-17.2 ^b	-8.7 ^a	-10.3 ^c	-10.7 ^b
Age, y	0.2	0.	1	0.0	-0.1	0.0	-0.1	1.6 ^a	1.9 ^a	1.5 ^b	1.5 ^a	3.3 ^c	3.2 ^c	0.9 ^b	1.4 ^a	3.1 ^c	0.1	-0.1	0.2
Insulin, μU/mL ⁻	-1																		
Male	-0.5	1 –	0.44	0.03	-1.33	-2.83	-4.83	-4.10	-3.32	6.97	-5.70 ^b	-3.87	-1.78	-8.27 ^a	10.12 ^a	-9.93 ^b	-3.96 ^c	-7.25 ^c	12.55 ^a
Age, y	-0.09	9 ^b - I	0.11 ^a	-0.13 ^a	-0.41	^a –0.97	^c –1.31	c –0.47 ^t	0 –0.88 ^c	-1.01 ^a	-0.05	-0.27	-1.68 ^a	0.17	-0.05	-0.39	-0.09	-0.22	-0.45
c-peptide, ng/mL	1																		
Male	-0.19	9 ^a - 1	0.14 ^b	-0.03	-0.21	-0.59	^b -0.69	^b -0.38	-0.76 ^b	-0.88 ^b	-1.08 ^c	-0.90^{a}	-1.33 ^a	-1.50 ^c	-1.22 ^c	-1.75 ^c	-1.37 ^c	-1.70 ^c	-2.07 ^c
Age, y	-0.00) –	0.01	-0.01	-0.05	c -0.08	c −0.11	c – 0.05 ⁱ	-0.04	-0.07 ^b	0.03	0.02	0.00	0.07 ^b	0.08 ^a	-0.02	0.02	0.05 ^b	0.06
	Glucose AUC II			Insulin AUC			c-peptide AUC		НОМА		OGIS3, mL/min ⁻¹ \cdot m ⁻²			IGI, mg/dL ^{-1} · ng ^{-1} · mL					
	P25	P50	P7	5	P25	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75
Male	-0.44 ^b	-0.4	9 ^a –().57 ^b	-22.3 ^b	-27.9 ^b	-22.8	-0.93 ^c	-0.85 ^c	-1.35 ^c	-0.09	-0.09	0.02	20.0	18.5	8.4	0.0 2	0.00	-0.05
Age, y	0.04 ^b	0.08 ^c	0.0)9 ^c	-0.6	-2.7 ^b	-6.3 ^c	-0.00	-0.01	-0.05	-0.01	-0.02^{b}	-0.03 ^a	-0.48	-0.92	-1.53	-0.00^{a}	-0.01 ^c	-0.01 ^a
	Basal insulin secretion, To pmol/min ⁻¹ \cdot m ⁻² set			otal insulin cretion, nmol/m ⁻²			β-Cell glucose sensitivity, pmol/min ⁻¹ · m ⁻² · mM ⁻¹			Late Potentiation Ratio			Basal insulin clearance (L/min ^{-1} · m ^{-2}			OGTT insulin clearance (L/min ^{-1} · m ^{-2}			
	P25	P50	P75	P2	5	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75	P25	P50	P75
Male	-3.3	-2.4	0.3	'_	12.6 ^c	-15.8 ^c	′-20.8 ^c	-6.47 ^b	-9.80 ^b	-16.30 ^b	-0.16 ^b	-0.24 ^a	-0.32 ^c	-0.19 ^b	-0.23 ^b	-0.21	-0.19 ^a	-0.19 ^a	-0.27 ^a
Age, y	-0.5 ^b	-0.6^{a}	-0.9) ^a – (0.3	-0.4	-0.4	-0.93 ^a	-1.52 ^c	-2.38 ^c	0.02 ^a	0.02 ^a	0.04 ^c	0.02 ^b	0. 04 ^a	0.05	0.01	0.01	0.03 ^a

 Table 3.
 Quantile Regression With Intracluster Correlation Calculated in 479 OGTT Studies

For each quartile (P25, P50, P75) of the variables considered, the effects of male sex and 1 year of age are reported. To calculate punctual estimates of each variable, see the Supplemental Material for the complete parameters and confidence intervals.

^a P < .01.

^b *P* < .05.

^c *P* < .001.

Insulin sensitivity was determined in the basal state using the homeostasis model assessment from basal levels (HOMA) index and during the OGTT with the 3-hour oral glucose tolerance test-based index of insulin sensitivity (OGIS) index (OGIS 3) (16). These indices are surrogate methods of insulin sensitivity that are correlated with the gold standard clamp index, although they may not be entirely representative of peripheral insulin sensitivity as the clamp index.

Absolute areas under the OGTT curve (AUC) were calculated by trapezoidal integration over the entire OGTT.

Statistical analysis

Most variables had non-Gaussian distributions and are reported as 50th (P50), 25th (P25), and 75th (P75) percentiles. For comparison purposes we also calculated confidence intervals. Discrete variables are reported as counts and percentages. The associations of P50, P25, and P75 of the outcomes of interest (glucose, insulin, and c-peptide at 0, 30, 60, 90, 120, 150, and 180 min, glucose AUC, insulin AUC, c-peptide AUC, IGI, total insulin secretion, β -cell glucose sensitivity, HOMA, OGIS3, basal insulin clearance, and OGTT insulin clearance) with age and sex were evaluated using multivariable quantile regression models with intracluster correlation (22, 23). The response variable in such models was the continuous outcome of interest, and the predictors were age (continuous, rounded to the next year) and gender (discrete: 0, female; 1, male). Fractional polynomials of degree 1 were used to test whether there were nonlinear response-age relationships (24), and all were found to be linear. Each patient was treated as a cluster to relax the assumption of the independence of observations made by quantile regression (22). In this way, data collected longitudinally on the same individuals can be used to evaluate the cross-sectional associations of the responses of interest with age.

Results

Glucose tolerance

Of the 478 studies, 325 (68.0%) indicated normotolerance, 105 (22.0%) glucose intolerance, 40 (8.4%) diabetes without and eight (1.7%) with fasting hyperglycemia, and 43 (9.0%) undetermined glycemia. Glucose tolerance data in the 187 patients at their first study are reported in Table 1.

Fasting status (Table 3 and Figures 1 and 2)

Age

Age was not associated with fasting glucose and c-peptide but was inversely associated with fasting insulin and insulin secretion.

Sex

Males had slightly lower c-peptide levels than females, despite similar values of glycemia and insulinemia.

A normogram showing the quartiles of basal glucose concentrations for age and sex in CF is reported in Figure 2.



Figure 1. The median values of the p-glucose, insulin, and c-peptide profiles during OGTT are reported at 5-year intervals in both sexes. For numerical values including 25th and 75th percentile, refer to Table 3. *, Significant (P < .05) effect of age; §, significant (P < .05) effect of sex.

OGTT profiles (Figure 1 and Table 3)

Age

The glycemic profiles began to diverge with age at 60 minutes, with higher concentrations up to 120 minutes. The highest association between age and glycemia was seen at 90 minutes. At the third hour, no age-associated change was still evident.

In contrast with this finding, the initial insulin response decreased with age despite similar levels of glycemia at 30 minutes. The highest association between age and insulinemia was seen at 30 minutes. After 90 minutes, there were no differences in insulin concentration with the exception of a lower value at 180 minutes.

The c-peptide profiles showed a slightly different pattern, with a decrement of the initial response at 30 minutes and an increased late response with increasing age. The glucose AUC increased with age, whereas the insulin AUC decreased and the c-peptide AUC remained unchanged.

Sex

Males showed lower glycemic, insulinemic, and c-peptidemic responses from 120 to 180 minutes and had lower values of glucose AUC, insulin AUC, and c-peptide AUC.

A normogram showing the quartile levels of the distribution of glucose concentrations at 60 and 120 minutes and those of insulin concentrations at 30 minutes in CF patients grouped by age and sex is given in Figure 2.

Insulin secretion and β -cell function during OGTT (Table 3)

Age

 β -Cell glucose sensitivity, ie, the slope of the dose response curve of insulin secretion vs glucose concentration,



Figure 2. The 25th, 50th, and 75th centiles of the distribution of glucose concentration at times 0, 60, and 120 minutes, insulin concentration at time 30 minutes, β -cell sensitivity to glucose, and insulin sensitivity (OGIS) during OGTT are reported above for both sexes.

decreased significantly with age. The IGI showed a similar relationship with age in all quartiles. Nonetheless, the potentiation ratio increased with age, and total insulin secretion in the 3 hours of the OGTT did not change with age. Fasting insulin clearance increased significantly with age, and during OGTT it increased in the highest quartile.

Sex

Males responded to the OGTT with a lower insulin secretion, β -cell glucose sensitivity, and potentiation ratio. Males also displayed a lower insulin clearance at baseline and during the OGTT.

A normogram showing the quartile levels of the distribution of β -cell glucose sensitivity in patients grouped by age and sex is reported in Figure 2.

Insulin sensitivity (Table 3)

Age

Insulin sensitivity assessed from basal levels (HOMA) decreased significantly with age. In contrast, the OGIS did not change with age.

Sex

There was no association between HOMA or OGIS and sex.

A normogram showing the quartile levels of the distribution of OGIS in patients grouped by age and sex is reported in Figure 2.

Conclusions

This is, to our knowledge, the first study providing reference values for OGTT-related parameters according to sex and age in CF patients. Similar works are limited in number (8, 10) and did not explore the combined effects of age and sex (8–12, 14, 19). We found that β -cell responsiveness to glucose decreases and insulin clearance increases with age. Both changes reduce postprandial insulin exposure of peripheral tissues. We also confirm that glucose tolerance is worse in females. However, this is not due to a reduced insulin secretory capability but rather to an increased insulin clearance. Postprandial insulin sensitivity, in contrast, seems unaffected by age and sex.

Fasting plasma glucose remained low and stable at all ages, whereas the fasting insulin secretory rate slowly decreased. It has been already shown in many populations including ours that fasting glycemia is not sensitive enough to diagnose or predict CFRD (2, 25). In a small prospective study from Lombardo et al (26), fasting glycemia increased minimally (<3 mg/dL in 13 y), and insulinemia

decreased similarly to what we have observed in the present study.

Fasting data therefore suggest a specific pathogenesis of CFRD, different from the general population in which age is associated with increasing fasting glycemia, inflammation, hepatic steatosis, and insulin resistance, initially compensated by decreased hepatic insulin clearance and later progressing to diabetes (27). In CF, increased inflammation (28) and fatty liver (29) are generally not associated with increasing insulin resistance (highly variable) or to decreasing insulin clearance (indeed, the opposite is true) and to increasing fasting glucose concentrations (stable with age).

In contrast, remarkable age-related glycemic changes occur in the second hour after the glucose challenge. At 90 minutes plasma glucose increases more than 2 mg/dL yearly to reach values above 200 mg/dL, ie, undetermined glycemia (13, 19), in almost half of the patients older than 30 years. The glucose concentrations at 60 and 90 minutes show the highest dependence on age and should be monitored for the progression of CF tolerance defects.

OGTT data therefore suggest that the increasing prevalence of CFRD with age is due to a progressive dysregulation in the short-term tolerance to glucose. Most CF patients can return to baseline glycemia over a longer time period, which explains why CFRD diagnosis is more often established on the basis of OGTT results than on fasting glucose.

An insulin secretory defect must be involved because model-derived β -cell glucose sensitivity decreased 2% yearly. We also found a negative dependence on age of the IGI, a simpler parameter to describe the initial insulin response to glucose challenge. Similarly, Lombardo et al (26) noted a progressive decrement in the insulin to glucose ratio at 30 minutes during the OGTT. Reduced firstphase insulin response, a strong determinant of glucose tolerance, has been shown with the hyperglycemic clamp in CF (30-32). Our modeling approach is based on c-peptide profiles to quantify insulin secretion and responsiveness to avoid bias-related to changes in insulin clearance. We can therefore demonstrate that a true decrement with age in the early insulin responsiveness to glucose contributes to the progressive increment in glucose concentration in the subsequent hours of OGTT. We have shown that β -cell glucose sensitivity in CF patients is generally below that of control subjects (18). An important question to answer would be whether a congenital defect can give rise to the progressive decline in this parameter. Data in the first years of life are lacking; therefore, any consideration about β -cell secretory function at birth remains speculative. Based on our study (in which the youngest subjects were 8 y old), the median β -cell glucose sensitivity extrapolated to age 0 years would remain below that in control subjects (107 pmol/min⁻¹ \cdot m⁻² \cdot mM⁻¹) (18). In other words, if we assume that β -cell function decreases linearly across the life span, including the first 8 years of life, we may suggest that β -cell function is reduced already at birth, a hypothesis worth experimentally testing. It should be noted that the defect in glucose sensitivity and other β -cell function parameters, as well as their decrease with age, may reflect both a progressive functional impairment and a loss of β -cell mass because our methodology cannot distinguish between them.

In addition to possible congenital defects, β -cell dysfunction may be secondary to other metabolic or hormonal dysfunctions in CF. Perano et al (33) have suggested that a defective glucagon-like peptide 1 response, mediated by loss of exocrine pancreatic function, may underlie postprandial hyperglycemia and possibly β -cell dysfunction. Given the importance of glucagon-like peptide 1 in insulin secretion after an OGTT, this may be indeed a possibility.

Further analysis showed that the decreasing β -cell sensitivity to glucose increments is in part compensated with age by an increased potentiation to reach (more slowly) an adequate insulin secretion. The potentiating mechanisms have been related to prolonged exposure to hyperglycemia, gastrointestinal hormones, and neural modulation and have been previously described in glucose-intolerant groups (34). This issue deserves further investigation with an accurate evaluation of the counterregulatory and incretin responses to oral glucose.

A second defect, increased insulin clearance, adds to defective β -cell responsiveness in deteriorating glucose tolerance in CF with age. Insulin, and not c-peptide, undergoes a variable first-pass extraction in the liver. We found that 2 hours after the glucose challenge, c-peptide increased with age but insulin did not, which may be explained by an increment in insulin clearance. An increased insulin clearance in CF patients has been described (35, 36), but we are the first to show that this increase is progressive. In the general population, fasting insulin clearance changes with age in the opposite direction (37). Evidence for an increasing insulin clearance with age is stronger during the fasting state, and weaker during OGTT because it is significant only at the highest quartile of its distribution. The reasons are presently unclear and deserve further investigation targeting the liver as a site of insulin clearance.

Beyond insulin secretion, insulin resistance is a major determinant of glucose tolerance, but the HOMA index, reflecting fasting insulin resistance, decreased with advancing age, ie, it changed in the direction opposite to what we would have expected in case of a contribution of insulin resistance to age-associated derangements in glucose tolerance. OGIS, reflecting insulin sensitivity during the glucose challenge, did not change with age and was not identifiable as a contributor as well.

We also measured the effect of sex on insulin secretory and sensitivity parameters. Recently Coriati et al (12) explored and then rejected the hypothesis that women may be at higher risk of CFRD and mortality because of reduced anabolic and glycemic-lowering insulin secretory responses. We found that females display the following: 1) similar fasting glucose but higher c-peptide; 2) higher glucose, insulin, and c-peptide responses with 3) greater insulin secretion and β -cell responsiveness to glucose; 4) greater insulin clearance; and 5) equal insulin sensitivity compared with males. Our results are not entirely consistent with those of Coriati et al (12) (who studied an older population than ours) with regard to glucose concentrations (lower initially and similar to males at the end of OGTT in their females, similar initially but higher at the end in ours), but they are consistent in the major conclusion: females do not display reduced insulin responses and responsiveness to glucose; rather, the opposite is true. The finding of Coriati et al that women's greater insulin secretion is counterbalanced by greater insulin resistance is not supported by our study. We found, however, that insulin clearance is increased in females, which should deteriorate glucose tolerance.

We are aware of some limitations of the present study. Data are derived from a single CF center because of the modifications introduced to the OGTT protocol. Such specificity prevents retrospective data from other centers from being used in modeling insulin secretion and sensitivity. Our population has a median age of 15 years (range 8-30 y), which may have limited the time span of glucose metabolism abnormalities. We had not enough power to analyze separately prepubertal and pubertal individuals. In addition, the gold standard methods for insulin secretion and sensitivity (hyperglycemic and euglycemic insulin clamp) were not feasible for a large study, whereas the clinical application of our modeling approach may represent a sound compromise with the need for a larger clinical applicability. We included repeat measuring from the same subjects in our analysis but we studied the crosssectional associations of interest by treating each patient as a cluster (22). Therefore, our study remains cross-sectional in its nature, which is ineludible, given the large age range investigated. Another potential limitation is the use of the c-peptide kinetic model by Van Cauter et al (38), required to calculate insulin secretion from c-peptide concentration. This model has been developed on a large number of subjects but has not been tested in CF because this would require complex testing with injection of exogenous biosynthetic c-peptide and administration of somatostatin. Nevertheless, a critical requirement for the use of this model, ie, lack of severe impairment of renal function, was satisfied in this population.

Among the strengths of this study there is the objective measurement of insulin secretory and sensitivity parameters based on a simple and repeatable technique as the OGTT. We also produced nomograms adjusted for sex and age, useful for the following: 1) to describe the natural history of glucose tolerance defects in CF, 2) to trace individual courses, and 3) simplify the generation and use of predictive models of CFRD and nutritional and respiratory decay. For example, using age- and sex-adjusted percentiles, we have recently found that defective β -cell responsiveness to glucose, 30 minutes of c-peptide, and 60 minutes of glucose concentrations are long-term predictors of CFRD (39). Assessment of the effects of CFTR mutation-targeted treatments on β -cells function may also take advantage of this instrument (40).

In conclusion, a defect in β -cell responsiveness to glucose in CF progresses with age, but it is not sex specific. In contrast, insulin clearance increases with age, particularly in females, and contributes to deteriorating glucose tolerance.

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Author contributions include the following: A.B. and C.C. designed the study, are the guarantors of this work, and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis; G.B. designed and conducted the statistical analysis of data; L.Z. contributed to the recruitment of the study population and the OGTT testing; A.M. contributed to the OGTT modeling to provide insulin secretion and sensitivity indexes; P.M.B. contributed to the study design and data collection organization; G.A. contributed to the data interpretation and manuscript drafting. All authors contributed to the data interpretation and gave their final approval of the manuscript.

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