Cystic Fibrosis-Related Diabetes: Screening and Diagnosis

In this Issue...

CFRD — Cystic fibrosis-related diabetes — is the most prevalent extrapulmonary complication of cystic fibrosis and is associated with decline in pulmonary function, poor nutritional status, and greater mortality. Although our general understanding of CFRD has increased, difficult areas remain in its pathophysiology, screening, diagnosis, and management that require further research and development.

In this issue, Dr. Andrea Granados from Washington University School of Medicine reviews key data on intrinsic abnormalities in insulin secretion related to the basic defect in the cystic fibrosis transmembrane conductance regulator (CFTR) and oxidative stress, mechanisms leading to the development of lung disease in the setting of hyperglycemia, and the value of alternative methods of screening for CFRD.

LEARNING OBJECTIVES

- Identify mechanisms that contribute to cystic fibrosis-related diabetes mellitus.
- Describe the complications of CFRD hyperglycemia in the CF lung.
- Explain alternative modalities for screening and diagnosing CFRD.

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Commentary & Reviews

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Guest Faculty Disclosure

Dr. Granados has indicated that she has no financial interests or relationships with any commercial entity whose products or services are relevant to the content of this presentation.

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Dr. Granados has indicated that there will be no references to unlabeled or unapproved uses of drugs or products.

IN THIS ISSUE

COMMENTARY

CFTR Silencing, Insulin Secretion, and Oxidative Stress
Impact of Hyperglycemia on the CF Airway

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Cystic fibrosis (CF) represents the most prevalent fatal hereditary disease in the Caucasian population. With improvements in clinical care, median predicted survival has risen fourfold, with people born with CF today expected to survive into their fifth decade of life. But as people with CF live longer, they experience more disease-related complications. Cystic fibrosis-related diabetes (CFRD) has become the most prevalent extrapulmonary complication of CF. Nearly 10% of patients with CF have the diagnosis of CFRD by the age of 10; among adults with CF 40%-50% are also affected, while another 35% have abnormal glucose tolerance. By the age of 50, 80% of those with severe CFTR mutations have diabetes. While we have learned much about CFRD over the past couple of decades, numerous questions remain.

The etiology of CFRD is complex and the mechanisms are not fully understood. It is characterized mainly by insulin deficiency, although insulin resistance is thought to play a secondary role in patients with CFRD, particularly in the settings of acute or severe illness. It is well recognized that the pancreatic damage observed in subjects with CF arises from fibrotic changes in the pancreatic architecture, resulting in exocrine pancreatic insufficiency, endocrine dysfunction, and insulin deficiency. However, further evidence suggests that an intrinsic defect in insulin secretion may exist, perhaps related to the basic defect in the cystic fibrosis transmembrane conductance regulator (CFTR). Ntimbane et al (reviewed herein) studied the role of the CFTR protein in combination with oxidative stress in beta cell function. Oxidative stress is a common feature in both CF and non-CF diabetes. The authors found that CFTR silencing is associated with impaired insulin secretion and this beta cell dysfunction is amplified by the presence of oxidative stress. These findings add evidence that abnormalities in CFTR and oxidative stress each contribute to development of CFRD.

The occurrence of CFRD has been associated with poor pulmonary function, decline in nutritional status, and increased mortality. The mechanisms leading to the decline in pulmonary function have not been completely elucidated, but studies have variously suggested that hyperglycemia could trigger a proinflammatory cascade, create oxidative stress, alter mucus viscosity, and/or promote the growth of respiratory pathogens. Bilodeau et al (reviewed in this issue) evaluated the impact of hyperglycemia on airway ion transport and epithelial cell repair. Hyperglycemia, altered ion transport, and impaired repair from lung damage all suggest an explanation for worse pulmonary function in patients with CFRD. A concerning finding was that hyperglycemia appears to hinder the ability of CFTR corrector drugs to effect airway repair.

Over recent years, many investigators have tried to find alternative screening methods to diagnose CFRD. According to current guidelines, the oral glucose tolerance test (OGTT) remains the recommended screening test. However, the OGTT is time-consuming and inconvenient for patients, and adherence to this recommendation is low. Special attention has focused on the performance of hemoglobin A1c (HbA1c) and continuous glucose monitoring (CGM) as screening tests for CFRD. HbA1c is generally felt to underestimate the degree of hyperglycemia in CF, since 70% of patients with CFRD have normal HbA1c values (< 6.0%). Underlying reasons for the lower glycation of hemoglobin have not been established, but increased red blood cell turnover has been suggested. As reviewed, Burgess and colleagues tested the accuracy of HbA1c as a screening tool and proposed a HbA1c ≥ 5.8%(40 mmol/mol) cutoff to identify subjects likely to have CFRD and thus need confirmatory OGTT. This study was significantly flawed, however, and a subsequent attempt...
Taylor-Cousar et al in a small, prospective, single-center, pilot trial, reported that CGM was a better predictor of future diabetes risk than the gold standard OGTT. They reported that 100% of patients who developed diabetes had abnormal CGM at baseline. While this was true, abnormal CGM was essentially universal at baseline (16 of 17 patients), and less than half of those with abnormal CGM actually developed diabetes; both factors limit the usefulness of CGM as a screening tool. Thus, while the OGTT is imperfect, no other test has yet been proved better as a screening tool for CFRD.

There is growing interest in the specific impact of the OGTT one-hour plasma glucose (PG1). The investigation by Sheikh et al found that, over five years, subjects with PG1 > 160 mg/dl were more likely to develop CFRD, and subjects with PG1 ≥ 200 mg/dl were 10 times more likely to develop CFRD than those with lower PG1 levels ($P = .005$).

Similarly, Coriati et al demonstrated that higher PG1 ($≥ 200$ mg/dl) was negatively associated with lower pulmonary function and that low insulin levels at one hour during the OGTT were associated with worse lung function and worse nutritional status. However, while much of the focus in the past has been on the two-hour OGTT time point, more attention should be paid to one-hour values.

Although our understanding of CFRD has increased in recent years, it is clear that much more research is needed. The articles discussed in this newsletter help improve our understanding of CFRD and emphasize the importance of early diagnosis of glucose abnormalities in patients with CF, allowing earlier intervention and prevention of poor clinical outcomes.

References:

CFTR Silencing, Insulin Secretion, and Oxidative Stress


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The relationship between glucose abnormalities and altered CFTR protein expression is not fully understood. Oxidative stress (OxS), which represents an imbalance between the generation of oxidant molecules and organ antioxidant systems, has been clearly demonstrated in patients with CF. Because of their poor antioxidant capacities, β-cells are particularly sensitive to OxS, a phenomenon that has been implicated in the occurrence of both type 1 and type 2 diabetes. The main objective of this study was to shed light on the role of CFTR in combination with OxS in insulin secretion from pancreatic β-cells.

The authors used the β-cell tumor line MIN6 as a model to study glucose-responsive insulin secretion. To generate a model similar to the condition of CF homozygous patients, the authors used a small shRNA to efficiently (90%) ablate CFTR mRNA and protein expression. MIN6 cells with and without CFTR silencing were then exposed glucose and to a Fe/Asc oxygen radical-generating system. Iron causes oxidative damage to biological macromolecules because it initiates oxygen radical formation and alters the intracellular redox environment, and ascorbic acid amplifies this effect.

The authors found a significant (70%) reduction of glucose-induced insulin production in the CFTR-silenced MIN6 cells compared to MIN6 cells with intact CFTR. They evaluated possible mechanisms for this effect and found altered mitochondrial oxidation of long-chain fatty acid production in CFTR-silenced MIN6 cells, leading to lower ATP levels (ATP is necessary for insulin secretion). Furthermore, CFTR silencing was accompanied by increased sensitivity to OxS, especially when iron and ascorbic acid were added to the cells, as evidenced by increased lipid peroxides, high levels of inflammatory cytokines (NK-kB, TNFα, IL-1β), and increased β-cell apoptosis. These defects were alleviated by the addition of an antioxidant suggesting a direct negative impact of OxS on CFTR-silenced cells.

These data suggest that the CFTR deficiency impedes normal glucose-stimulated secretion of insulin, and β-cell defects are aggravated by oxidative stress.

Impact of Hyperglycemia on the CF Airway


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CFRD is associated with increased morbidity and mortality, as well as with faster pulmonary function decline. While the mechanisms underlying the deleterious effect of hyperglycemia on lung function have not been clearly defined, elevated levels of oxidative stress and inflammatory mediators may be responsible for lung tissue injury in CF patients with diabetes. The aim of this study was to evaluate the impact of hyperglycemia on airway ion transport and tissue repair, both of which are crucial for maintaining lung function and integrity.

The authors investigated exposure of non-CF and CF airway cells to normal glucose (90 mg/dl, low glucose [LG]), or high glucose (450 mg/dl glucose, [HG]) concentrations. K+ channel function is known to be critical to maintain Cl− transport through airway epithelia. Comparing K+ currents in non-CF and CF airway cell monolayers, the investigators report that under LG conditions, K+ currents were significantly higher in non-CF cells than in CF cells. HG reduced K+ currents in non-CF cells to the level seen in CF cells, but no further
reduction was seen under HG conditions in the CF cells.

To evaluate the impact of hyperglycemia on non-CF and CF airway epithelial repair, the authors employed a well-established wound-healing assay after mechanical injury. They confirmed that the wound-healing rates in CF epithelial cells were lower than in the non-CF monolayers. They observed that a 24-hour exposure to HG induced a significant decrease in wound repair rates in both non-CF and CF airway cell monolayers, compared to when the cells were grown under LG conditions. Importantly, CFTR correction (with VRT-325) accelerated the healing rates of CF cells monolayers under LG conditions, but this improvement was significantly reduced under HG conditions.

The authors conclude that hyperglycemia impairs both ion transport and epithelial repair function in airway epithelial cells. Interestingly, hyperglycemia appeared to disrupt the benefits of CFTR correctors on airway repair. This finding may assume clinical significance as more and more patients are treated with CFTR-modifying drugs.

HbA1c Screening for CFRD


The oral glucose tolerance test (OGTT) is the accepted method of screening for CFRD, and the American Diabetes Association, Cystic Fibrosis Foundation, Pediatric Endocrine Society, and International Society for Pediatric and Adolescent Diabetes all recommend that CF patients begin annual OGTT screening by age 10. However, the OGTT is inconvenient and time-consuming, making adherence to this recommendation low in the US. These investigators sought a screening test to determine which CF patients were likely to have diabetes and could then be referred for an OGTT to confirm the diagnosis. Their aim was to test the accuracy of the following measures as screening criteria:

- Hemoglobin A1c (HbA1c) ≥ 6.1
- Random blood glucose ≥ 200 mg/dL (11.1 mmol/L)
- Polyuria, polydipsia, and nocturia
- FEV₁: > 0% annual decline
- Weight: annual decline > 5%

The study included a primary investigation followed by a validation study, performed at a single large adult CF center in London, UK.

In the primary investigation, 94 adults were studied. The median age was 36 years, 62% were male, and 74% were treated with pancreatic enzyme supplements. Importantly, patients with known CFRD were excluded, as were patients who had received organ transplants. OGTT screening found 80 subjects with normal glucose tolerance, eight with impaired glucose tolerance, and only six with diabetes. Just three of those with diabetes had an HbA1c > 6.1%. When the authors dropped the HbA1c threshold to ≥ 5.8%, the sensitivity for predicting diabetes was 94% and the specificity was 52%. In the validation study, 335 consecutive OGTTs with simultaneous HbA1c levels were assessed in patients whose average age was 29 years; of those, 16 patients (4.7%) were found to have diabetes. The HbA1c threshold of ≥ 5.8% had a sensitivity of 94% and specificity of 53% for predicting a diabetic OGTT. Like previous investigators, the authors found that random blood glucose, symptoms of diabetes, FEV₁ decline, and weight decline were not predictive of diabetes.
The authors conclude that HbA1c ≥ 5.8% (40 mmol/mol) is an effective screening tool to identify patients who probably have diabetes and should proceed to OGTT screening.

However, this study has critical flaws. Most important, the investigators based screening guidelines for CFRD on a population that had very little diabetes. At an average age of 30, one would expect 40%-50% of the CF population to already have received diabetes diagnoses, but those patients were ineligible for their study. Not only is it questionable to justify screening guidelines based on findings in a handful of patients, but one could argue that CF patients who develop diabetes at a later-than-average age may not be representative of the larger CF population. Furthermore, their HbA1c threshold was not good at discriminating patients with abnormal glucose tolerance; emerging data suggest there is value in identifying these patients, who can only be found by OGTT, because they are at risk for nutritional and lung function decline.

This report led Boudreau et al to revisit the value of HbA1c as a tool to identify patients at risk of CFRD. Between 2004 and 2015, 207 patients in the Montreal Cystic Fibrosis Cohort underwent an OGTT for which an HbA1c value was also available. This cohort included 48.3% women and had an average age of 25.6 ± 8.0 years. Of these, 22 had newly diagnosed CFRD based on the OGTT results. A full 31.8% of the CFRD diagnoses would have been missed if the suggested HbA1c value of ≥ 5.8% was used as a screening tool. In this cohort, the sensitivity and specificity of the ≥ 5.8% threshold were, respectively, 68.2% and 60.5%, far lower than observed by Burgess et al. They recommended against using HbA1c as a screening method for CFRD.

In summary, because of clear flaws in the Burgess study and the inability of the Boudreau study to confirm the Burgess findings, HbA1c cutoff values cannot be recommended for CFRD screening.

Continuous glucose monitoring (CGM) has been postulated as an alternative screening tool for CFRD, and small studies have shown that early glucose abnormalities detectable by CGM in children and adults with CF may be associated with declines in nutritional status and lung function. The aim of this retrospective, single-center study was to determine whether abnormal glucose tolerance by CGM predicts subsequent CFRD in adults with CF. Patients underwent simultaneous three-day CGM and a two-hour OGTT at baseline, with development of diabetes assessed over the subsequent five years. Abnormal glucose tolerance by CGM was defined as fasting glucose > 100 mg/dl or random glucose > 140 mg/dl.

Eighteen subjects were initially studied, 72% female, all pancreatic sufficient, with average age of 34 years. One patient already had diabetes with fasting hyperglycemia at the time of the first OGTT. Of the remaining 17 patients, at baseline 10 had normal glucose tolerance by OGTT, but only one had normal CGM. Over time, seven of the 17 subjects developed diabetes. There was no difference in BMI, lung function, or pulmonary exacerbation rates between patients who developed CFRD and those who did not.

With regard to the predictive value of the baseline OGTT, an abnormal OGTT (seven of 17 subjects) correctly identified five of the seven (71%) who would develop diabetes, with a 29% false prediction rate (two patients with abnormal glucose tolerance did not develop diabetes during the study period). A normal OGTT correctly predicted no future diabetes during the study period in eight of 10 patients (80%), while two of 10 subjects with normal glucose tolerance developed diabetes (20%).

With regard to the predictive value of the baseline CGM, 16 of 17 patients had abnormal...
CGM, and seven of those (44%) developed diabetes while 10 (56%) did not. The four patients with the most abnormal glucose levels of > 200 (all of whom had abnormal glucose tolerance by OGTT), all developed diabetes. The sensitivity and negative predictive values were similar for both CGM and one-hour OGTT for subsequent development of CFRD, and both exceeded those values for the conventional OGTT measurements.

The authors conclude that in adult patients with CF, CGM identified a higher degree of impaired glucose metabolism than the gold standard two-hour OGTT. These findings should be interpreted with caution, given the very high false prediction rate with CGM. Essentially all the patients had abnormal CGM, so it is difficult to consider this an appropriate screening tool, particularly because a greater percentage of those with abnormal CGM did not develop diabetes compared to those who did.

**One-Hour Plasma Glucose During OGTT**


The North American CFRD Consensus Conference in 2009 defined glucose tolerance in individuals with a one-hour plasma glucose (PG1) > 200 mg/dl (11.1 mmol/L) as indeterminate glycemia (INDET). In people with CF, INDET is common, and cross-sectional data has previously suggested that higher PG1 may be associated with poorer clinical outcome.

The aim of the study was to identify the association of PG1 with FEV1 percent-predicted, CF exacerbations, and the development of CFRD. This retrospective study included clinically stable CF patients age > 8 years who underwent OGTT screening. Only subjects with a two-hour plasma glucose < 140 mg/dl (ie, having neither IGT nor CFRD) were included. Subjects were excluded if pulmonary function tests or OGTT PG1 were unavailable, if FEV1 percent predicted was ≤ 40%, or if the OGTT was performed during a CF exacerbation.

FEV1 over a five-year follow-up period was the primary outcome, and PG1 at baseline was the primary exposure of interest. Mixed effects models using a random slope and intercept were developed to consider the linear change in FEV1 as a function of time in the entire cohort. Separate models for males and females were examined. The associations of PG1 with FEV1 were assessed using PG1 as a continuous variable, as well as a binary variable using a cutoff of ≥ 160 mg/dl to define an elevated PG1. The investigators chose a PG1 ≥ 160 because it has been identified as a risk factor for T2DM development and cardiovascular disease in people without CF. An adjusted model considering several confounders such as BMI percentile, pancreatic insufficiency status, age at baseline, pseudomonas colonization, and CFTR mutation was used. A logistic regression analysis adjusted for sex, age at baseline, BMI percentile, and FEV1 percent predicted at study entry was used to assess association between elevated PG1 and the risk of developing CFRD over the subsequent five-year period.

The analyses included data from 80 subjects (43 male), age 5–20 years over a median study period of 4.8 years (range: 0.5–5.2 years). No significant difference in FEV1 between normal and elevated PG1 was found either at baseline or over time in males or females. However, subjects with PG1 > 160 mg/dl were more likely to develop CFRD (OR 4.5, 95% CI: 1.7, 18.7, *P* = .04), and subjects with PG1 ≥ 200 mg/dl were 10 times more likely to develop CFRD over the study period (*P* = .005). The CF exacerbation risk was similar in both groups.

This study is limited by a relatively small sample size. The researchers did not find an impact of the one-hour OGTT glucose level on lung function or pulmonary exacerbations. However, PG1 elevation, particularly when it reached the INDET threshold of > 200 mg/dl, strongly predicted future risk of diabetes.
Studies in pediatric subjects with CF have suggested that there is an association between elevated plasma glucose at one hour (PG1) during the OGTT and worse pulmonary function, and that this association appears stronger than that with the two-hour glucose test. Higher PG1 has also been associated with an increased risk of CFRD.

This study evaluated the relation between one-hour OGTT insulin and glucose values with clinical markers in adult patients with CF. The study was a cross-sectional analysis of data from the Montreal cystic fibrosis adult center and included 240 patients without known CFRD. Subjects underwent standard OGTT screening. Variables included in the analysis included CF genotype, pulmonary function (FEV1 percent predicted), BMI, and height. Of the 240 patients, 56% had normal glucose tolerance, 28% had impaired glucose tolerance, and 15% had de novo CFRD. Pancreatic enzyme supplementation was taken by 80% of the subjects, and 46% of those were women.

With regard to glucose levels, PG1 was negatively associated with pulmonary function ($r = -0.225$, $P = .001$). Patients with PG1 in the INDET range ($\geq 200$ mg/dl) had significantly lower pulmonary function than patients with PG1 below 200 mg/dl (FEV1: $70\pm22\%$ vs $76\pm21\%$; $P = .025$). There was no observed difference in BMI, pancreatic insufficiency or genotype profile between patients with high and low glucose levels at one hour.

Patients with one-hour insulin levels below the median of 43 IU/mL had both a significantly lower BMI ($21.0\pm2.7$ vs $22.4\pm3.1$ kg/m$^2$, $P = .003$) and significantly lower FEV1 status ($70 \pm 22$ vs $77 \pm 21\%$, $P = .022$) than those with insulin levels above the median. After adjusting for BMI, the FEV1 difference was no longer significant, suggesting the impact of low insulin on lung function was mediated by nutritional status. When they classified patients with CF according to the combination of one-hour glucose and insulin values, low insulin plus high glucose was associated with a 13% lower FEV1 value than the combination of high insulin and low glucose.

This study was limited by not differentiating subjects by oral glucose tolerance category, and 43.7% of the population had either impaired glucose tolerance or CFRD, which may have their own effects on lung function and BMI. This makes it difficult to tease out the significance of the one-hour glucose levels per se, although the data are highly suggestive that glucose tolerance is important.

### KEY TAKEAWAYS

- Mechanisms that contribute to CFRD include an intrinsic defect in insulin secretion that recent research indicates may be related to the basic CFTR defect.
- The hyperglycemic complications of CFRD include a proinflammatory cascade that alters ion transport, impairs repair from lung damage, creates oxidative stress, alters mucus viscosity, and promotes the growth of respiratory pathogens.
- For screening and diagnosing CFRD, while the two-hour OGTT is time-consuming and inconvenient for patients, recent investigations have found it more accurate than HbA1c and CGM measurements. However, some evidence indicates the one-hour OGTT may prove diagnostically valuable.
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