Mechanisms of insulin resistance in cystic fibrosis

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Cystic fibrosis (CF) is a common inherited disease affecting one in 2,500 newborns. Diabetes and abnormal glucose tolerance are common in CF, affecting almost 75% of patients over the age of 25 (1, 16). The etiology of diabetes remains somewhat puzzling. Decreased insulin secretion has been well documented (19, 41) and occurs even in patients with normal glucose tolerance (NGT) (41, 42). The principal defects that cause decreased insulin secretion are fibrosis and fatty infiltration of β-cells. However, autopsy studies (36, 53) do not correlate percentage of damaged islets with development of diabetes. Thus other defects must be present to account for the high incidence of abnormal glucose tolerance in this population. Previous studies by our group (24, 25) and others (38, 43) have documented decreased insulin sensitivity in CF patients with frank diabetes [CF-related diabetes (CFRD)]. However, controversy exists regarding insulin sensitivity in nondiabetic CF subjects, with reports of normal (12, 49), enhanced (3, 43), or decreased (4, 24, 25) insulin action. One purpose of our study was to reexamine peripheral insulin sensitivity in CF subjects with impaired glycose tolerance (IGT).

Many potential factors can cause decreased insulin sensitivity in CF, and exploration of these factors may help us better understand the discrepancies in various studies. The glucose transporter protein GLUT-4 is the major glucose transport protein involved in insulin-stimulated glucose disposal. Translocation of GLUT-4 from the intracellular compartment to the cell surface is necessary for the normal transport of glucose into the cell, and abnormalities in GLUT-4 trafficking have been reported in type 2 diabetics (17). Translocation of GLUT-4 relies on normal endocytosis (50, 51). CF is caused by genetic alteration of a transport-linked gene, the CF transmembrane conductance regulator (CFTR), and past studies (27, 45) have suggested that abnormal CFTR may alter normal endosome fusion and exocytosis. We measured GLUT-4 translocation with the use of sucrose gradients and differential centrifugation to test our hypothesis that abnormal translocation of GLUT-4 from the intracellular compartment to the plasma membrane occurs in CF patients.

Patients with CF experience chronic long-term infection, even when not acutely ill. In normal volunteers, Yki-Järvinen et al. (56) have demonstrated decreased insulin sensitivity during acute illness. Therefore, it is highly possible that insulin sensitivity is decreased in CF patients, secondary to chronic low-grade infection. Elevation of cytokines occurs during acute and chronic illness, and elevation of the cytokine tumor necrosis factor-α levels. We also measured tumor necrosis factor (TNF-α) levels and FFA in 32 CF patients previously studied by our group. Results were that glucose disposal rate (GDR) was significantly lower in the CFIGT subjects than in controls, indicative of impaired insulin action. GLUT-4 translocation was impaired in CF and correlated with GDR. TNF-α levels were higher in all CF subjects than in controls and correlated with GDR. There was no difference in FFA between CF and control subjects. Modified NIH clinical status scores were inversely correlated with GDR and TNF-α levels. We conclude that IGTCF patients have decreased peripheral insulin sensitivity. Mechanisms include elevation of TNF-α and impaired translocation of GLUT-4.
factor-α (TNF-α) has been postulated to contribute to insulin resistance. Hotamisligil et al. (29) demonstrated increased TNF-α levels in insulin-resistant subjects, and Ofie et al. (47) demonstrated improved insulin sensitivity in type 2 diabetes by infusion of synthetic antibodies to TNF-α. Elevated TNF-α levels have been reported from bronchoalveolar lavage samples (8) and from plasma (39, 54) in CF patients. Given these previous reports, another purpose of our study was to correlate insulin sensitivity with plasma TNF-α levels in CF subjects and controls. Investigators (34, 49) have documented a close link between free fatty acid (FFA) levels and insulin resistance. Additionally, a relationship between FFA and TNF-α may exist independent of insulin sensitivity. One previous study (28) has described that elevated TNF-α levels result in decreased FFA levels. Levy et al. (39) reported a correlation between TNF-α levels and lipid and lipoprotein levels in CF. We therefore wished to evaluate total and individual FFA levels and their relationship to TNF-α and insulin sensitivity in CF subjects. Thus our study reexplores insulin sensitivity in CF patients with IGT and examines potential mechanisms of decreased insulin sensitivity.

METHODS

Subjects

We recruited nine CF patients (2 female, 7 male), ages 22–32 yr, for participation in this study. Seven were recruited from the CF center clinics at Texas Children’s Hospital and Methodist Hospital/Baylor College of Medicine. Two additional CF subjects were recruited from the University of Oklahoma, Tulsa, OK. All subjects were medically stable at the time of the study, with no hospital admissions for 6 wk and no home intravenous medications for ≥1 mo preceding the study. No subject had used oral or intravenous corticosteroids for ≥3 mo before the study. No subject was colonized with *Burkholderia cepacia*, and neither female subject was pregnant. All CF subjects had IGT, as determined by a 3-h oral glucose tolerance test (OGTT) and National Diabetes Data Group (NDDG) criterion (46) performed within 1 mo of the clamp study. Clinical status of the CF subjects was measured using a modified National Institutes of Health (NIH) scoring system (52).

Nine control volunteers (3 female, 6 male) were recruited by advertisement. They were matched to CF subjects for body mass index, age, and sex. No one was an endurance-trained athlete, a physical state known to enhance insulin sensitivity (21), and none had an eating disorder. All control volunteers had normal physical examinations, complete blood counts, and serum chemistries. None had any history of chronic illness, and all were normally glucose tolerant by NDDG criterion. In all subjects, lean body mass (LBM) was measured by dual-energy X-ray absorptiometry.

All protocols were approved by the Committee for the Protection of Human Subjects at the University of Texas Health Science Center. CF and control subjects all gave written, informed consent.

In addition to subjects specifically recruited for this study, cytokine and FFA levels were measured in frozen plasma samples from 32 CF subjects and 19 controls who had been previously studied (24, 25) with the use of similar techniques but who had not had a muscle biopsy. The purpose of includ-
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Weight, kg</th>
<th>LBM, kg</th>
<th>Hb A1c</th>
<th>FBG, mg/dl</th>
<th>2-h PP, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>26 ± 5</td>
<td>66 ± 12</td>
<td>47.0 ± 8</td>
<td>5.7 ± 0.3*</td>
<td>85 ± 6</td>
<td>176 ± 16*</td>
</tr>
<tr>
<td>Controls</td>
<td>23 ± 4</td>
<td>69 ± 10</td>
<td>54 ± 10</td>
<td>4.9 ± 0.2</td>
<td>96 ± 13</td>
<td>113 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SD. LBM, lean body mass; FBG, fasting blood glucose; PP, postprandial glucose; CF, cystic fibrosis group. *Significantly different from controls.

All CF subjects specifically recruited for this study had IGT, and control volunteers were normally glucose tolerant. Table 1 reviews the subject characteristics, including OGTT results. The data for serum cytokines and FFA include the previously collected samples (24, 25). These subjects were also categorized by OGTT. Eight had IGT, 11 had NGT, and 10 were diabetic. All previous control volunteers were normally glucose tolerant.

**RESULTS**

**Subjects**

Glucose levels were clamped at 5.0 ± 0.7 mM in all subjects. At an insulin infusion rate of 200 mU·m⁻²·min⁻¹, peripheral insulin levels were similar in controls (C) and CF subjects (C, 298 ± 16; CF, 303 ± 12 mU/ml; P = 0.1). Figure 1 compares the mean GDR, as normalized for LBM, in the CF subjects with IGT (IGTCF) and controls specifically recruited for this study. Even when not normalized for LBM, GDR was significantly less in IGTCF than in controls (CF, 11.4 ± 2.5; C, 16.2 ± 0.9; P = 0.002).

**GLUT-4 Subfractionation**

Sucrose gradient subfractionation of membranes results in three distinct layers, each corresponding to different subcellular locations. Although rodent studies (26, 35) suggest distinct compartments for plasma membrane-associated vesicles, human studies (17) report plasma membrane-associated proteins throughout each subfraction. Therefore, in humans, sucrose subfraction layers are best described by density (17). The 35% sucrose layer is consistent with dense membrane vesicles that are more closely located in the intracellular (plasma membrane-associated) compartment. With the assumption of normal translocation after insulin stimulation, less GLUT-4 should be found in this component according to the method of Pearson. Significance was assessed at the P < 0.05 level. All analyses were performed by a statistician using an SAS software package.

**Hyperinsulinemic Euglycemic Clamp**

**Statistical Analysis**

All results are given as means ± SD. Statistical significance between mean data in CF and controls was determined using Student’s t-test. The cytokine and FFA measures were analyzed by ANOVA. Linear regression analysis was used for correlations according to the method of Pearson. Significance was assessed at the P < 0.05 level. All analyses were performed by a statistician using an SAS software package.
partment. The 25 and 30% layers contain lighter vesicles, which are sarcolemma enriched. After insulin stimulation, more GLUT-4 should be in the lighter vesicle compartments. Our results demonstrate that, despite insulin stimulation, the GLUT-4 content of intracellular-associated layer was significantly higher in CF subjects compared with controls (Figs. 2A, 3), corresponding to significantly lower GLUT-4 content in cell surface-associated subfraction (Figs. 2B, 3). These findings are consistent with abnormal subcellular localization of GLUT-4. The 30% layer results are as follows: GLUT-4 content dpm/min: CF, 100 ± 41; C, 210 ± 50; P = 0.04. Additionally, the content of GLUT-4 in the 25% sucrose gradient positively correlated with GDR in all subjects (r = 0.61, P = 0.04). Protein recovery was similar between controls and CF for each subfraction. 5′-Nucleotidase activity was similar in CF and controls.

TNF-α

TNF-α levels were measured in plasma from subjects recruited for this study as well as from previous study subjects. Although our current study evaluated only CF subjects with IGT, our previous studies had also measured insulin sensitivity in CF subjects with NGT or diabetes. There was no significant difference between the interleukin (IL)-6 or IL-1β levels between controls and any CF subgroup.

TNF-α levels were significantly higher in all CF subgroups than in controls. Results averaged for all CF subjects and controls are demonstrated by Fig. 4. TNF-α levels were not significantly different in CF subgroups; however, CF subjects with IGT (IGTCF) and diabetes (DMCF) tended to have higher levels than CF subjects with NGT (NGTCF) (TNF-α pg/ml: IGTCF, 120 ± 5; DMCF, 130 ± 4; NGTCF, 90 ± 5; P = nonsignificant). There was no correlation between GDR and any of the cytokines measured.

FFA

The FFA palmitic, oleic, and stearic acid were measured in subjects recruited specifically for this study, as well as in previous study subjects (24, 25). Total FFA were similar between CF subjects and controls (Total FFA mmol/ml: CF, 405 ± 247; C, 374 ± 178; P = 0.6). Similarly, there were no differences in CF and control subjects in levels of individual FFA levels. There was no correlation between levels of either total FFA or individual FFA levels and GDR or TNF-α.

Clinical Status Scores

The mean NIH clinical status score for the IGTCF subjects specifically recruited for this study was 65 ± 8. Insulin sensitivity (GDR) correlated with NIH clinical status scores (r = 0.75, P = 0.042). There was no correlation between GLUT-4 content in any subfraction and NIH clinical status score. There was an inverse correlation between NIH clinical status score and TNF-α levels (r = 0.71, P = 0.03). There was no correlation between free FFA and clinical status scores.

Fig. 2. GLUT-4 translocation was measured using sucrose gradients (35, 30, and 25% wt/vol) and differential centrifugation. GLUT-4 in each sucrose subfraction was isolated and quantitated using SDS-PAGE immunoblot analysis, anti-GLUT-4 antibody, and 125I label. Mean GLUT-4 content (cpm) is depicted for the CF and control subgroups. A: GLUT-4 content is depicted in the 35% sucrose fraction (dense fraction associated with intracellular components) and demonstrates that more GLUT-4 is found in this subfraction in CF subjects than in controls. B: GLUT-4 content in the 25% sucrose fraction (lighter-density fraction associated with cell surface components) is depicted and demonstrates less GLUT-4 in CF subjects than in controls.

Fig. 3. Representative autoradiograph demonstrating differences in GLUT-4 content in the 35, 30, and 25% sucrose fractions from 2 individual CF patients and 1 control. GLUT-4 migrated as a 47-kDa protein.

Fig. 4. Tumor necrosis factor-α (TNF-α) levels from 38 CF subjects and 28 control volunteers. TNF-α levels are clearly elevated in the CF subjects.
DISCUSSION

CF patients have a very high incidence of diabetes (1), and insulin resistance plays a role in the high frequency of CFRD (24, 25). However, controversy remains regarding insulin sensitivity in CF patients with abnormal glucose tolerance who do not have CFRD. Our group (23–25) has reported decreased insulin sensitivity in CF subjects with NGT and IGT as well as in patients with CFRD. Similarly, Austin et al. (4) described decreased insulin sensitivity in CF subjects with IGT. However, Moran et al. (43) have reported normal peripheral insulin sensitivity in these patients. Other groups have not clearly distinguished results by glucose tolerance but have reported normal or enhanced insulin sensitivity in CF (3, 12, 38). It is with this controversy in mind that we recruited CF subjects with IGT for participation in these studies. Our current findings confirm previous reports (4, 24, 25) of decreased peripheral insulin sensitivity in CF subjects with IGT. The CF subjects participating in our previous studies, and those in this study, tend to have worse clinical status than CF subjects studied by others (43). We (24) have previously described the relationship between worsened clinical status and insulin resistance. Therefore, it seems likely that patients who have clinically worse disease, as described by worse pulmonary function or other parameters, are more insulin resistant than patients with less severe disease. To date, we have not found enhanced insulin sensitivity in any CF study subject, regardless of clinical status. Perhaps findings by others represent an early metabolic compensation in patients with mild disease. Treatment differences among the CF centers could also explain the discrepancy.

Skeletal muscle is the principal tissue utilized for glucose disposal (13), and GLUT-4 is the principal glucose transporter protein (6) found in skeletal muscle. Studies in diabetic rodents (22) report decreased GLUT-4 quantity in skeletal muscle; however, studies in humans with type 2 diabetes have described normal GLUT-4 quantity in plasma membrane samples from skeletal muscle (48). Investigators have now focused on decreased GLUT-4 function, rather than quantity, as the principal disorder associated with human insulin resistance (30, 31). Recently, Garvey et al. (17) have described abnormal subcellular location of GLUT-4 in type 2 diabetics and insulin-resistant volunteers without diabetes. They report that this abnormal distribution is associated with decreased translocation of GLUT-4.

We measured GLUT-4 translocation by use of methods similar to those of Garvey et al. (17), with muscle samples collected at maximum glucose disposal. Circulating insulin levels were higher than 300 μU/ml, thus allowing us to measure insulin-stimulated GLUT-4 translocation. Our results demonstrate preferential targeting of GLUT-4 to intracellular-associated sucrose gradient fractions in our IGTCP subjects and correspond to lower amounts of GLUT-4 in the lighter vesicle (cell surface-associated) layers. We also found a correlation between GDR and GLUT-4 levels in the cell surface layer (25% sucrose fraction). The differences in GLUT-4 levels between subfractions cannot be explained by differences in protein recovery or by recovery of plasma membranes. Thus our study suggests that one cause of insulin resistance in CF is abnormal translocation of GLUT-4.

One study (43) previously reported no difference between GLUT-4 quantity in CF subjects and controls studied under basal (non-insulin-stimulated) conditions; thus differences in translocation could not be discerned. Furthermore, biopsies were obtained from the exocrine-insufficient subgroup, which was composed of three NGT patients and four IGT subjects. All of these subjects had enhanced insulin sensitivity; thus GLUT-4 levels in the plasma membrane would not be expected to be low. The lack of basal difference in GLUT-4 quantity between CF subjects and controls suggests that our findings under insulin-stimulated conditions result from translocation differences rather than from inherent differences in GLUT-4 quantity.

CF is caused by mutation of a transport-related protein, the CFTR (33). Mutations of this protein have been associated with defects in translocation of ions in the gut (18, 32), and several groups (7, 45) have described abnormal endosomal packaging as a result of CFTR. GLUT-4 resides in a discrete cellular compartment (20, 40) and requires normal endosomal recycling for translocation to the cell surface (27, 51). Thus it is possible that the CFTR mutation, by altering endosomal function, could decrease GLUT-4 trafficking. However, other reasons for IGT, such as chronic elevation of cytokines, could also be responsible for this finding. Further research in this area will be helpful.

In our present study, we have found higher TNF-α levels in CF subjects than in controls. Similar findings have been reported by others (39, 54). TNF-α levels increase during acute illness, and previous studies indicate that TNF-α from sources such as fat (29) and serum (28) affects insulin sensitivity at the level of skeletal muscle. One group (11) has demonstrated downregulation of GLUT-4 in cultured muscle cells and adipocytes by administration of TNF-α.

Hotamisligil et al. (28) have demonstrated that TNF-α affects the phosphorylation cascade of insulin signaling at the receptor level, and Ofei et al. (47) demonstrated improvement in insulin sensitivity by giving TNF-α receptor antibodies. Although we could not find a correlation between TNF-α levels and GDR or GLUT-4 levels, we did find a correlation between clinical status scores and TNF-α levels. Thus it is likely that TNF-α levels either reflect or contribute to worsened clinical status. Without a direct link to glucose disposal, we are reluctant to state that TNF-α causes insulin resistance in CF; however, measurement of insulin resistance after use of TNF-α neutralizing antibodies would be interesting.

Studies in type 2 diabetes have described decreased insulin sensitivity when circulating FFA levels are elevated (34, 49). Elevation of FFA has been demonstrated in CF (10, 39); thus we wanted to determine a
possible relationship between elevated FFA levels and insulin resistance in our subjects. We did not find any difference between FFA levels in control and those in CF subjects, nor did we find differences in individual FFA levels between these two groups. Therefore, it is not surprising that we did not find a relationship between FFA levels and insulin sensitivity. FFA levels were measured at baseline only; thus we cannot rule out specific interactions between fatty acid metabolism and insulin resistance in the fed state. Future studies may elucidate such a relationship.

In conclusion, our current study has supported previous findings by our group (24, 25) and others (4) of decreased peripheral insulin sensitivity in CF patients with impaired glucose tolerance. Additionally, this study has elucidated potential mechanisms of decreased insulin sensitivity, specifically abnormal subcellular localization of GLUT-4 and higher-than-normal TNF-α levels. These studies suggest a considerable need for future research in the area of CF-related diabetes and insulin sensitivity.

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REFERENCES


